

# **TRANSMUCOSAL NASAL DRUG DELIVERY**

## **Systemic Bioavailability of Nasally Applied Midazolam**

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## Abbreviations

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ACN	Acetonitril
CD	Cyclodextrin
CRTT	Computer-controlled self-adjusting reaction time test
EMA	European Agency for the Evaluation of Medicinal Products
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HP $\beta$ CD	Hydroxypropyl- $\beta$ -Cyclodextrin
HP $\gamma$ CD	Hydroxypropyl- $\gamma$ -Cyclodextrin
HPLC	High Performance Liquid Chromatography
i.m.	intramuscular
i.v.	intravenous
ICH	International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use
ISI	Interstimulus Interval
LC-MS	Liquid chromatography-mass spectrometry
MRI	Magnetic Resonance Imaging
MWCO	Molecular Weight Cut Off
Ph.Eur.	Pharmacopoeia Europaea
RM $\beta$ CD	Randomized methylated- $\beta$ -cyclodextrin
RT	Reaction Time
SD	Standard Deviation
VAS	Visual Analog Scale



## Summary

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Transmucosal nasal drug delivery is a drug delivery option for challenging clinical situations where common drug administrations (e.g., intravenous, intramuscular, or oral) are inapplicable. For drugs with constricted oral bioavailability, due to degradation in the intestinal tract or hepatic first-pass metabolism, transmucosal nasal delivery is a convenient alternative to intravenous and intramuscular drug administration. The considerable blood flow, actually responsible for breath conditioning, benefits efficient systemic drug uptake and provides direct access to the systemic circulation for transmucosal absorbed drugs.

Often, in nasal drug delivery the limited nasal capacity is disregarded and the instilled volumes exceed the limited capacity of the nose. Consequently, the administered preparations are partially swallowed and resulting pharmacokinetic characteristics refer to a combination of transmucosal nasal and gastrointestinal drug absorption. Due to low midazolam concentration, the commercially available preparations for intravenous administration (e.g. Dormicum<sup>®</sup>, Roche) is inappropriate for transmucosal nasal midazolam delivery. For the optimization of transmucosal nasal midazolam delivery minimized administration volumes are essential to prevent swallowing of nasally administered preparations. Therefore, nasal preparations with enhanced midazolam concentrations need to be provided.

In Project I different preparations for transmucosal nasal midazolam delivery were developed. The impact of vehicle and application modality on the pharmacokinetics of nasally applied midazolam was studied by administration of the developed preparations to healthy volunteers (Project II). The benefit of two nasal midazolam preparations for procedural anxiolysis in anxious patients undergoing MRI examinations was compared (Project III).

**Project I:** Midazolam solubilization with RM $\beta$ CD (randomized methylated- $\beta$ -cyclodextrin, a cyclodextrin derivative) facilitated compounding of midazolam preparations adjusted to the limited volumetric capacity of the nose. RM $\beta$ CD (added in equimolar or higher concentration to solubilize midazolam) reduced midazolam release in drug release studies with semi-permeable cellophane membranes (*in vitro*). Stability data affirmed shelf life of at least 6 months for RM $\beta$ CD containing nasal midazolam preparations. Addition of chitosan hydrochloride (penetration enhancer) affected midazolam stability; therefore shelf life of the chitosan containing nasal midazolam preparation was reduced. The developed preparations for transmucosal nasal midazolam delivery were the basis to study the influence of the vehicle and the application modality on pharmacokinetics and systemic bioavailability of nasally applied midazolam (Project II).

**Project II:** Pharmacokinetic characteristics following nasal application of 1 mg midazolam (Preparation 1, 2, and 3) and 3 mg midazolam (Preparation 4 and 5) were compared with pharmacokinetic characteristics of 1 mg i.v. administered midazolam (Dormicum<sup>®</sup>, Roche). The impact of RM $\beta$ CD (solubilizer), chitosan hydrochloride (penetration enhancer) and the application modality (one- versus two-sided nasal administration) was investigated in this open-label study with healthy volunteers. Volunteers were asked to describe nasal midazolam administration and to classify local irritation after nasal midazolam administration. Pharmacologic effects were assessed by computer-controlled self-adjusting reaction time test (CRTT, recording reaction time and interstimulus interval) and visual analog scale (VAS). Blood samples were serially obtained until 6 hours after midazolam administration. Serum concentrations of midazolam and two metabolites ( $\alpha$ -hydroxymidazolam and 4-hydroxymidazolam) were quantified by liquid chromatography-mass spectrometry (LC-MS). Non-compartment and two-compartment pharmacokinetic modeling was performed to estimate pharmacokinetic parameters. Bioequivalence testing was performed according to the requirements of EMEA (European Agency for the Evaluation of Medicinal Products).

Systemic bioavailability of nasally applied midazolam ranged from 78% (Preparation 5) to 93% (Preparation 2), differences of bioavailability were not significant. After nasal administration of 1 mg midazolam by Preparation 1, 2, and 3 mean  $C_{max}$  was,  $28.1 \pm 9.1 \mu\text{g/l}$ ,  $30.1 \pm 6.6 \mu\text{g/l}$  and  $28.9 \pm 5.4 \mu\text{g/l}$ , respectively. After nasal administration of 3 mg midazolam by Preparation 4 and 5  $C_{max}$  was,  $72.6 \pm 18.2 \mu\text{g/l}$ , and  $82.2 \pm 15.8 \mu\text{g/l}$ , respectively. Following nasal midazolam administration  $t_{max}$  was between  $7.1 \pm 0.6$  minutes (Preparation 5) and  $11.7 \pm 2.4$  minutes (Preparation 4). All tested nasal administration modalities to deliver 1 mg midazolam proved bioequivalence. For Preparation 4 and Preparation 5 bioequivalence was not confirmed. The serum concentration time profiles of the midazolam metabolites ( $\alpha$ -hydroxymidazolam and 4-hydroxymidazolam) demonstrated exclusive transmucosal absorption of nasally applied midazolam. Swallowing of nasally delivered preparations was prevented and hepatic first-pass effect successfully circumvented. Consequently, the assessed pharmacokinetic parameters characterized pure transmucosal nasal midazolam delivery.

Neither RM $\beta$ CD (equimolar to midazolam) nor application modality (one- or two-sided) changed absorption kinetics of nasally administered midazolam, whereas chitosan hydrochloride promoted absorption of nasally applied midazolam. Significant higher midazolam serum concentrations were achieved faster. The outcome of the pharmacokinetic study emphasizes the decisive role of minimized nasal application volume to prevent swallowing of nasally applied preparations and to provide for exclusive transmucosal midazolam absorption.

**Project III:** In this randomized multicenter trial with 110 anxious and/or claustrophobic patients undergoing MRI examinations, two nasal preparations for low-dose midazolam delivery, Midazolam MD Nasal Spray 5 mg/ml (MD) and Midazolam UD Nasal Spray 1 mg (UD), were compared. Nasal administration of 1 mg or 2 mg midazolam was provided before MRI examination. Within both groups anxiety reduction was significant, but there was no difference in anxiety reduction between the MD and UD group. Local irritation following administration of UD nasal spray was slightly more intense than local irritation after administration of MD nasal spray.

Nasal delivery of low-dose midazolam is a safe therapy to provide procedural anxiolysis in patients undergoing MRI examinations. The two compared low-dose midazolam preparations for transmucosal nasal delivery of midazolam proved therapeutic equivalence. Hence, anxious and/or claustrophobic patients equally benefit from procedural anxiolysis during MRI examinations following administration of low-dose midazolam by MD nasal spray or UD nasal spray. Concerning convenient handling, administration to laying patients, and hygienic aspects the new midazolam nasal spray (UD) is superior to the commonly used midazolam multidose nasal spray (MD).

Overall, the presented nasal preparations facilitated characterization of exclusive transmucosal nasal absorbed midazolam. *In vivo* neither RM $\beta$ CD (equimolar to midazolam) nor administration modality changed the pharmacokinetic profile of nasally applied midazolam. Chitosan hydrochloride promoted nasal midazolam absorption but clinical relevance (e.g., for the treatment of status epilepticus) is to be verified in further clinical investigations. High systemic bioavailability of nasally applied midazolam demonstrated the veritable potential of transmucosal nasal drug delivery as alternative to invasive drug administration.



## Background and objectives

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The most popular nasal drug delivery is nasal application of topical decongestants or anti-inflammatory drugs, to treat rhinitis or allergy related symptoms. In addition, for transmucosal nasal absorbed drugs the nasal route provides direct access to the systemic blood circulation. For drugs with constricted oral bioavailability, due to degradation in the intestinal tract or hepatic first-pass effect, transmucosal nasal delivery is an interesting alternative to intravenous and intramuscular drug administration. A challenge in transmucosal nasal drug delivery is the limited volumetric capacity of the nasal cavities. Therefore, the entire dose, preferably administered in solution, has to be instilled in a limited volume. Consequently, solubility of the drug is one of the critical parameters in transmucosal nasal drug delivery.

Midazolam is a benzodiazepine used for the treatment of anxious patients undergoing diagnostic or surgical procedures (procedural anxiolysis and/or sedation in adults and children) and the treatment of seizures (epileptic or febrile). These therapeutic indications are clinical situations, in which drug administration is potentially difficult and rapid onset of therapeutic effect absolutely desirable. Intranasal administration of midazolam has been of particular interest, because of the rapid and reliable onset of therapeutic effect and the convenience of administration. Due to low midazolam concentration, the commercially available preparation for intravenous administration (Dormicum<sup>®</sup>, Roche) is not optimal for transmucosal nasal midazolam delivery. For the optimization of transmucosal nasal midazolam delivery minimized administration volumes are essential to reduce the risk of swallowing the administered preparation. Therefore, nasal preparations with enhanced midazolam concentrations need to be provided.

The aim of **Project I** was the development and *in vitro* characterization of preparations for transmucosal nasal midazolam delivery. Several solubilization enhancers were evaluated to reduce the administration volume. For the developed midazolam preparations stability testing was performed.

The purpose of **Project II** was the pharmacokinetic characterization of the developed preparations for transmucosal nasal midazolam delivery. The influence of the vehicle, namely RM $\beta$ CD (solubilization enhancer) and chitosan hydrochloride (penetration enhancer) on pharmacokinetic profile of nasally delivered midazolam was assessed.

In a multicenter trial (**Project III**) two midazolam preparations for transmucosal nasal midazolam delivery were compared in anxious and/or claustrophobic patients undergoing MRI examination.

The objectives of this thesis were to provide preparations for transmucosal nasal midazolam delivery (Project I), to assess the pharmacokinetic characteristics of nasally applied midazolam (Project II), and to compare the benefit of two nasal midazolam preparations for procedural anxiolysis in anxious patients undergoing MRI examinations (Project III).





## THEORETICAL SECTION



# 1 Nasal drug delivery

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## 1.1 Topical and systemic bioavailability of nasally applied drugs

The rate and extent to which an active substance or active moiety is absorbed from the pharmaceutical preparation and becomes available at the site of action, is defined as **bioavailability** by the European Agency for the Evaluation of Medical Products [EMA 2001].

In general, drug administration is characterized by the **systemic bioavailability** (the rate and extent the active substance is absorbed into the systemic blood circulation, not taking in consideration the site of action). The reasons are ease of blood sampling contrary to the difficulties to assess the drug concentration directly at the site of action (e.g., central nervous system).

**Topical drug delivery** describes the application of a drug directly on the target organ (e.g., application of glucocorticosteroids for dermatological therapies). To study vehicle effects of topical drug delivery, both the assessment of topical bioavailability and systemic bioavailability are performed. Whereas **topical bioavailability** allows predicting the therapeutic effect and **systemic bioavailability** reflects the systemic exposure meaning the risk for systemic adverse drug reactions.

The term **nasal drug delivery** refers to **topical** and **systemic nasal drug delivery**. For diseases of the nasal mucosa, such as infectious rhinitis, allergic rhinitis, and nasal polyposis, the topical nasal administration delivers drug directly to the target organ [Salib and Howarth 2003]. Nasal applications of topical decongestants or anti-inflammatory drugs are therefore the most popular topical nasal drug deliveries.

Whereas nasal drug delivery for systemic effect means **transmucosal drug delivery**, and provides, for some compounds, direct access to the systemic circulation. **Transmucosal absorption** describes the uptake of a compound into the systemic circulation after application on a mucosa (e.g., nasal, buccal, or rectal mucosa). Transmucosal absorption subsumes following subsequent processes: **drug release**, **penetration** (entry into a layer), **permeation** (transition of a layer), and **absorption** (uptake into the vascular system).

The common term **nasal bioavailability** usually refers to the systemic bioavailability after nasal drug delivery and not to the topical bioavailability in the nasal mucosa.

## 1.2 Transmucosal nasal drug delivery

The relatively large surface area, the porous epithelial membrane, and the extensive vascularization are factors favoring transmucosal absorption of nasally administered drugs [Arora et al., 2002]. For transmucosal nasal absorption a surface of approximately 150 cm<sup>2</sup> (150 to 200 cm<sup>2</sup>) is available [Merkus 2004]. For efficiently transmucosal absorbed compounds therapeutic concentrations in the blood circulation are reached within few minutes. This is especially desirable for indications requiring fast onset of action (e.g., status epilepticus, acute pain). Furthermore, nasally absorbed compounds circumvent the first-pass elimination in the liver. Therefore, nasal drug delivery is an attractive alternative to i.v. or i.m. injections. The nose is easily accessible and nasal drug delivery is suitable for self-administration. Some of the major advantages offered by the nasal route are (modified after [Romeo et al., 1998]):

- rapid absorption (fast onset of therapeutic effect)
- high bioavailability (for drugs with good permeation abilities)
- circumventing of liver first-pass metabolism (resulting in higher and less variable bioavailability)
- no gastrointestinal drug degradation
- non-invasive, therefore reduced risk of infection
- ease of convenience and self-medication
- improved patient compliance

Transmucosal nasal drug delivery has been suggested as alternative route for drugs with poor systemic bioavailability after oral administration. For drugs extensively metabolized in the gastrointestinal tract or in the liver, such as proteins, peptides, and steroid hormones (estradiol, progesterone, and testosterone), nasal administration is a convenient alternative. Table 1-1 gives an overview of compounds tested for transmucosal nasal drug delivery.

Table 1-1: Compounds for transmucosal drug delivery

Compound	Indication	Reference
apomorphine	Parkinson's disease (on-off-symptoms)	[Kendirici and Hellstrom 2004]
buserelin	prostate cancer	[Arora, et al. 2002; Borrego et al., 1995; de Voogt et al., 1998; Larsen et al., 1987; Matsubara et al., 1995]
calcitonin	osteoporosis	[Denis et al., 1989; Matsuyama et al., 2006; Schmidt et al., 1998; Torres-Lugo and Peppas 2000; Wang et al., 2004]
cobalamin (vitamin B12)	substitution of vitamin B12	[Van Asselt et al., 1998]
desmopressin	diabetes insipidus centralis, enuresis nocturna	[Lopes et al., 2001]
diazepam	sedation, anxiolysis, status epilepticus	[Li et al., 2002]
estradiol	substitution of estradiol	[van den Berg et al., 2004]
fentanyl	analgesia, postoperative pain and agitation in children	[Borland et al., 2006; Finkel et al., 2001]
human growth hormone	growth hormone deficiency	[Hedin et al., 1993]
insulin	diabetes mellitus	[Dondeti et al., 1995] 112/270
ketamine, norketamine	analgesia	[Yanagihara et al., 2003]
L-dopa	Parkinson's disease	[Kao et al., 2000]
melatonin	jet-lag	[Merkus 2004]
midazolam	sedation, anxiolysis, status epilepticus	[Burstein et al., 1997; Burstein et al., 1996; Davis et al., 1995; Fösel 1996; Goepfert 1996; Griffith et al., ; Gudmundsdottir et al., 2001; Jaennet 1999; Jones et al., 1997; Knoester et al., 2002; Lejus et al., 1997; Ljung and Andreasson 1996; Loftsson et al., 2001; Malinovsky et al., 1993; Münte Sinikka 2002; Roelofse et al., 2000; Rose et al., 1990; Sonja Böttcher 2000; Tschirch et al., 2006; U.Haas 1998]
morphine	analgesia	[Dale et al., 2002; Illum et al., 2002; Merkus 2004]
progesterone	infertility, amenorrhea	[van den Berg, et al. 2004]
propranolol	hypertonia	[Hussain et al., 1980a; Hussain et al., 1980b; Hussain et al., 1979]
sildenafil	erectile dysfunction	[Merkus 2004]
testosterone	substitution of testosterone	[Lahat et al., 1998], [Hussain et al., 1984], [Hussain et al., 2002]

### 1.3 Challenges in transmucosal nasal drug delivery

The ideal drug for transmucosal nasal delivery show appropriate aqueous solubility (to deliver the entire dose in 25  $\mu\text{l}$  to at most 400  $\mu\text{l}$ ), good permeability, no nasal irritation, adequate potency (required dose of about 25 mg) and no offensive odor [Romeo, et al. 1998].

The physicochemical properties of the compound, nasal mucociliary clearance and penetration enhancers are the main factors providing drug absorption through the nasal mucosa. The enzymatic metabolism of the nasal mucosa, the physical barrier of the epithelium, the rapid mucociliary clearance limiting the time available for absorption, and the mucus layer covering the nasal mucosa are further challenges of transmucosal nasal drug delivery [Merkle et al., 1998]. Table 1-2 summarizes the most important characteristics to be considered in transmucosal nasal drug delivery. Chapter 2 reviews the impact of the anatomy and the physiology on transmucosal nasal drug delivery and in chapter 4 preparation related parameters are discussed.

**Table 1-2: Factors affecting nasal absorption**

<b>nasal cavity</b>	<p><b>anatomy</b> interindividual variability (surface) blood flow</p> <p><b>physiology</b> nasal secretion pH on intranasal surface mucocilliary clearance</p> <p><b>environment</b> temperature, humidity</p>
<b>nasal preparation</b>	<p><b>compound</b> molecular weight size solubility pKa concentration</p> <p><b>vehicle</b> pH irritability osmolality viscosity adhesion residence time</p>
<b>delivery device</b>	<p><b>drops, spray</b> nasal surface with drug contact size of droplets spreading</p>

#### 1.4 Nose to brain-transport

The blood-brain barrier provides an efficient diffusion barrier especially for polar drugs. Hence, limitations are evident in the treatment of diseases of the central nerve system, such as Parkinson's and Alzheimer's disease. It has been suggested that after intranasal administration, some drugs reach the targeted brain structures, circumventing the blood-brain barrier. Lipophilic compounds may be transported by neuronal transport of nervus olfactorius. Extend of neuronal transport is highly dependent on the physicochemical properties of the drugs, namely the molecular weight and the lipophilicity [Illum 2004]. The existence of the olfactory-pathway has been proven for some compounds in animal models, but not in human [Illum 2000].

Since central nervous bioavailability of drugs, transported by the olfactory-pathway is estimated to be 0.01% to 0.1%, only very potent drugs may reach therapeutic levels at the central nervous side of action by nose to brain-transport.

Pre-estimating the contribution of the olfactory pathway in drug transport to the brain, the differences between the species in respect to localization and extend of the olfactory epithelium has to be considered. Furthermore, Graff and Pollak suggested efflux transporters to impair drug concentration in the brain after transmucosal nasal administration [Graff and Pollack 2003].

In human the olfactory epithelium is situated in the superior conchae and therefore hardly moistened by common nasal sprays. To ensure the delivery of a drug to the olfactory epithelium some authors propose specially developed delivery devices or application techniques [Dhanda et al., 2005].

Assuming the existence of an olfactory-pathway to the brain in human, it remains unclear to what extent it contributes to central nervous availability of a nasally administered drug.





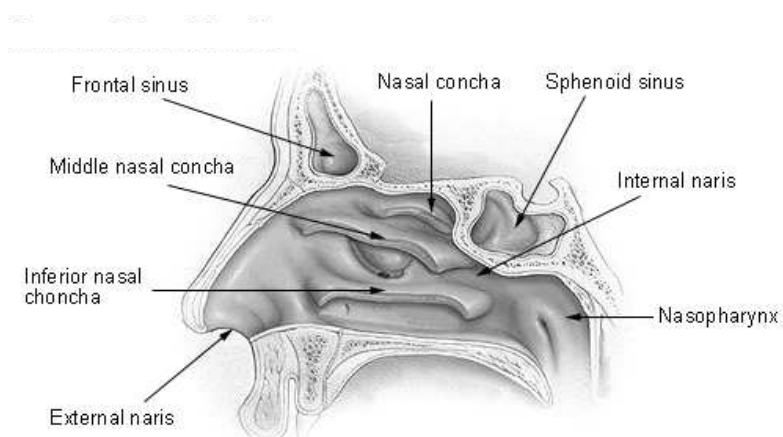
## 2 Impact of anatomy and physiology on transmucosal nasal drug delivery

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### 2.1 Anatomy and physiology of the nose

The surface of the nasal cavity is enlarged by three nasal conchae (superior, middle, and inferior), facilitating purification, humidification and temperature regulation of inspired air [Mygind and Dahl 1998] (see Figure 2-1). The total surface of both nasal cavities is about  $150\text{ cm}^2$  ( $150\text{-}200\text{ cm}^2$ ) [Merkus 2004] of which the respiratory epithelium covers about  $130\text{ cm}^2$  (large inferior turbinate) and the olfactory region about  $2\text{ cm}^2$  to  $10\text{ cm}^2$  (superior conchae).

The narrowest portion of the entire upper airway, the internal naris (nasal valve), is located about 1.5 cm from the external naris. The nasal valve accounts for approximately 50% of the total resistance to respiratory airflow from the nostril to the alveoli.



**Figure 2-1: Section of the human nasal cavity, from SEER's web-based Training Module [SEER, 2007]**

Breathing and olfaction are the prime functions of the nasal cavity. Beside these functions, the nasal cavity and the paranasal sinuses are important for the resonance of the voice.

The high vascularization of the submucosa facilitates warming up of the inspired air. The submucosal vascular plexus consists of arterioles, capillaries, semi-permeable post capillary sinusoids and venules. The post capillary sinusoids regulate resistance and secretory activity of the nasal mucosa. Vasodilatation and extravasation of plasma resulting in nasal congestion and rhinorrhoea, is induced by histamine, kinins, substance P, platelet-activating factor, and various leukotrienes [Foley and Hamid 2006].

## 2.2 Nasal mucosa and mucociliary clearance

### 2.2.1 Morphology of the nasal mucosa

All mucous membranes are linings of ectodermic origin and are involved in absorption and secretion processes. Mucous membranes cover various body cavities exposed to internal organs or external environment. At the nostrils, the lips, the ears, the genital area, and the anus, tissue changes in a smooth transition from skin (epidermis, dermis) to mucosa.

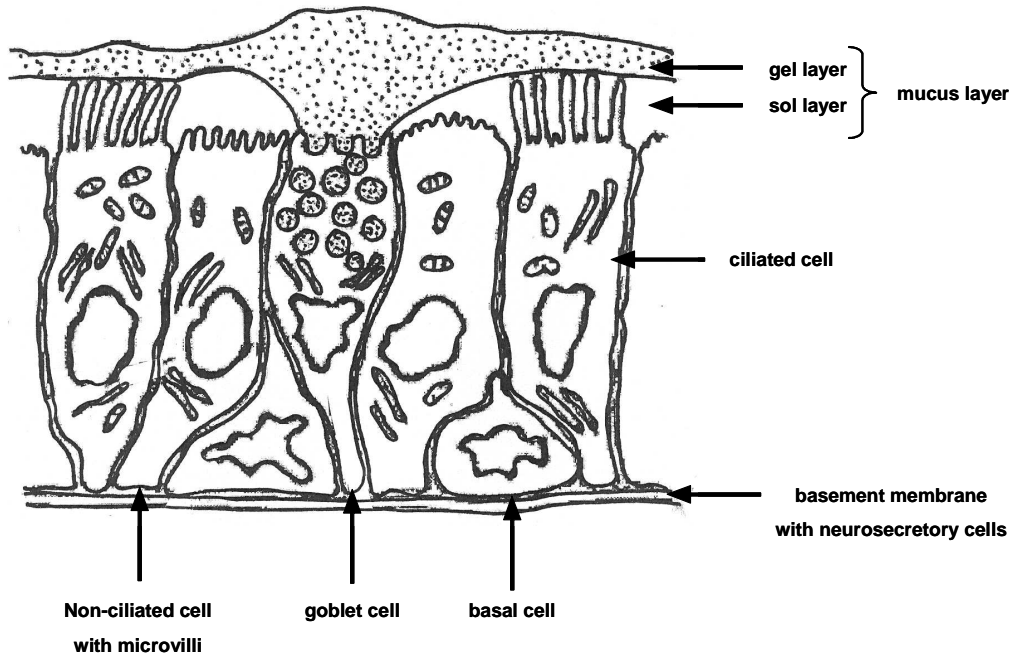
From the nostrils to the nasopharynx, the predominant cell types of the nasal mucosa lining are changing. Stratified squamous, respiratory (pseudostratified columnar), and transitional epithelium, are the different epithelia linings of the nasal mucosa. In the anterior third of the nasal cavity, stratified squamous and transitional epithelium precedes respiratory epithelium, which is predominant in the posterior two-thirds of the cavity.

The human olfactory region, situated in the superior conchae, covers only about 10% (about 10 cm<sup>2</sup>) of the nasal cavity, while in mice and rats about 50% of the nasal cavity is covered by olfactory epithelium [Gizurason 1993]. The olfactory epithelium is a pseudo stratified columnar structure. It consists of specialized olfactory cells, supporting cells, and serous and mucosal glands [Romeo, et al. 1998].

The respiratory epithelium is the major lining of the human nasal cavity and is essential for cleaning of nasal mucosa by the mucociliary clearance. The respiratory epithelium comprises of five cell types; ciliated and non-ciliated columnar cells, goblet cells, basal cells, and low numbers of neurosecretory cells in the basement membrane, see Figure 2-2. The apical side of the columnar and goblet cells reaches the lumen of the nasal cavity. Approximately 20% of the cells in the lower turbinate area are ciliated cells, with about 100-300 cilia on the apical surface. Cilia are hair-like projections (5-10 µm) on the apical surface of the columnar cells. All cilia beat in a coordinated fashion, transporting the mucus towards the nasopharynx.

Basal cells are adjacent to the basal lamina, on the basolateral side of the epithelium. The lamina propria is located beneath the basal lamina and contains many blood vessels, nerves and glands. The functions of epithelial cells in the nose can be summarized as follows: (1) building a physical barrier for particles and allergens; (2) secretion of mucus, to protect the mucosa and to provide efficient conditioning of the inspired air; and (3) responding to various stimuli by producing mediators to recruit lymphocytes, eosinophils, and mast cells to the nasal mucosa [Takeuchi et al., 2006].

The intense blood flow in the arteriovenous anastomosis and the large surface of the respiratory epithelium favors transmucosal nasal drugs absorption. Drug absorption in the olfactory region is possibly resulting in direct nose to brain-transport through the nervus olfactorius (q.v. chapter 1.4.) [Gizurason 1993].



**Figure 2-2: Respiratory epithelium.** The respiratory epithelium is covered by a mucus layer (gel and sol layer) and the cell types arising from the basement membrane are: ciliated and non-ciliated cells (with microvilli), goblet cell, and basal cell. Modified after [Sakane et al., 1991].

### 2.2.2 Mucus and mucociliary clearance

The goblet cells and the submucosal glands secrete about 20 ml to 40 ml mucus per day [Quraishi et al., 1998]. The mucus layer protects the underlying tissue from various environmental influences and the metabolic effects of enzymes. The mucus layer and hairs in the anterior nose filter 80% of particles larger than 12.5  $\mu\text{m}$  out from the inhaled air stream [Jones 2001]. The pH of the mucus layer varies from 5.5 to 6.5 in adults and from 5.0 to 7.0 in infants [Arora, et al. 2002].

The principal component of the mucus is water (90-95%) containing mucin (2%), electrolytes (1%), proteins (1%, mainly albumin, immunoglobulins, and lysozymes), and lipids [Merkus et al., 1998; Schipper et al., 1991]. Small molecules not interacting with the components of the mucus layer diffuse freely through the water network of the mucous gel. Khanvilkar et al. demonstrated that for many compounds, nasal mucus layer adds as little additional resistance as an unstirred water layer of equal thickness [Khanvilkar et al., 2001].

The mucus layer consists of two films with different rheological properties, a preciliary layer (sol layer) and a more viscous upper layer (gel layer) covering the tips of the cilia. Responsible for the rheological properties are two forms of mucin; soluble mucin and membrane bound mucin. Soluble mucin forms viscous gels by intermolecular disulfide bridges [Khanvilkar, et al. 2001].

The sol layer with low viscosity is slightly less thick than the length of an extended cilium (5  $\mu\text{m}$  to 10  $\mu\text{m}$ ). The extended cilia dip into the gel layer and, with effective beats, transports the layer to the nasopharynx. During the recovery stroke, the cilia move backward through the sol layer. In the

nasopharynx, the transported mucus layer with the trapped inhaled particles is swallowed or expectorated. Cilia in isolation are sensitive to temperature, optimally working at 35-40°C, and below these temperatures, the natural beat frequency drops [Jones 2001]. Increased mucus production in the respiratory tract is a symptom of many common diseases, such as the common cold.

Nasal mucociliary clearance describes the removal of inhaled particles, viruses and bacteria by the combined function of cilia and mucus layers. Nasal mucociliary clearance is important for the protection of the respiratory system [Merkus, et al. 1998]. It takes approximately 20-30 minutes to remove the whole mucus layer [Bommer 2002]. Mucociliary clearance is independent of age and sex [Kao et al., 1994].

Extend of transmucosal nasal drug absorption is related to contact time of the delivered drug with the nasal mucosa. Nasal mucociliary clearance limits the residence time of drugs administered into the nasal cavities, decreasing the time available for the drug to be absorbed [Merkus, et al. 1998]. In the nasopharynx, not absorbed compounds are swallowed, followed by gastrointestinal degradation, gastrointestinal absorption, and/or hepatic metabolization.

Mucociliary clearance can be modified by nasally administered drugs or components of the vehicle (viscosity enhancers, mucoadhesive excipients).

Evaluation of the effects of drugs and additives on nasal mucociliary clearance is an important issue, especially in developing preparations for long-term treatments. The measurement of ciliary beat frequency *in vitro* is a very accurate and reproducible technique to determine the effects on ciliated epithelium. Therefore, assessment of ciliary beat frequency is a screening method to estimate potential toxicity of drugs and excipients and to compare nasal preparations. However, ciliary beat frequency data have to be interpreted carefully, because effects of nasal formulations *in vitro* are usually stronger than effects *in vivo* [Merkus, et al. 1998]. *In vivo*, the mucus layer protects cilia and dilutes the nasally delivered preparations, whereas *in vitro*, the cilia are in direct contact with the formulations. Furthermore, the nasal mucosa continuously regenerates.

Consequently, it is difficult to predict the effects of chronic application on mucociliary clearance *in vivo* based on ciliary beat frequency assessment *in vitro*.

For the term 'ciliotoxic' no defined criteria exist and the classification depends on the performed *in vitro* assays. Furthermore, the cilioinhibitory effects of compound or vehicle depend on the applied concentration and are often additive [Merkus, et al. 1998]. To predict the safety of nasal drug formulations for human use, it is important to investigate the effects of therapeutic concentrations, both *in vitro* and *in vivo*.

### 2.3 Drug metabolism in the nasal mucosa

Nasal biotransformation enzymes are responsible for the metabolism of airborne xenobiotics. Geravasi et al. demonstrated the human respiratory epithelium of the nose containing a wide array of oxidative and non-oxidative enzymes, responsible for bioactivation or detoxication of inhaled xenobiotics [Gervasi et al., 1991].

Despite recent progresses in identification and characterization of numerous nasal biotransformation enzymes in animal models, metabolism in human nasal mucosa has not been conclusively characterized [Rahmel 2004; Zhang et al., 2005].

Some carcinogens or toxicants arise from metabolic transformation of originally nontoxic xenobiotic compounds. Most studies on metabolizing Cytochromes P450 monooxygenases (CYPs) in the respiratory tract focus on the role of these enzymes in metabolic toxicity [Ding and Kaminsky 2003]. CYPs, detected in extrahepatic tissues, usually, are also expressed in the liver at higher levels. Bereziat and Su characterized the sensitivity of tissues to a certain xenobiotic compound by the tissue-specific profile of exprimed CYP enzymes [Bereziat et al., 1995; Su et al., 2000]. However, the regulation mechanisms, responsible for tissue-specific CYP expression, have not been resolved.

In human nasal respiratory epithel, Rahmel identified different isoforms of CYPs (such as CYP 1A1, 1B1, 2A6/7, 2A13, 2B6, 2C8, 2C9, 2C18, 2C19, 2E1, 2F1, 2J2, 2S1, 3A4, 3A5, and 4B1) by RT-PCR<sup>1</sup>. Furthermore, traces of CYP 1A2, 2D6, 3A7, and 4A11 were detected and enhanced expression of CYP 1A1, 1B1, and 2S1 was demonstrated in nasal mucosa of smokers. But, only the translation of CYP 2A proteins has been confirmed by western blot [Rahmel 2004].

To estimate the metabolic capacity of nasal mucosa, the actual transcription level of the metabolic enzymes or even the metabolic activity should be specified. For Ioteprednol-etabonate (CYP 3A4 substrate), testosterone, and ethoxyresorufin (CYP 1A1 substrate) Rahmel demonstrated minimal biotransformation in nasal microsomal preparations [Rahmel 2004].

For nasally delivered substrates of enzymes, expressed in nasal mucosa, metabolism preceding systemic absorption is to be expected. To what extend this 'nasal first-pass effect' affects the systemic bioavailability of nasally delivered drugs, depends on actual enzyme expression.

Expression of CYP 3A4, mainly responsible for midazolam metabolism, has not been detected in human nasal mucosa. Therefore, for nasally delivered midazolam no nasal fist-pass metabolism has to be expected.

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<sup>1</sup> Reverse Transcription Polymerase Chain Reaction

## 2.4 Common cold and seasonal allergic rhinitis

Common cold is the most frequent of all human diseases, infecting adults at an average rate of up to 4 infections per year, and school aged children as many as 12 times per year.

By infecting the upper respiratory system numerous viruses (mainly rhinoviruses, coronaviruses, certain echoviruses, paramyxoviruses, and coxsackieviruses) cause common cold. Colds last typically three to five days, with residual coughing lasting up to three weeks. The nasal symptoms include sneezing, runny nose, and congestion. All these symptoms potentially affect transmucosal nasal drug delivery by diverse mechanisms.

Although common cold is a frequent affection, only little is known about the pathohistology and pathophysiology. *In vitro* experiments showed rhinovirus induced sloughing of epithelial cells [Mygind and Dahl 1998]. During a common cold, the number of ciliated cells decelerates and reduces mucociliary clearance [Hoorn and Tyrrell 1969].

Winther et al. described, that although many epithelial cells are sloughed, the epithelial lining is not destroyed, and scanning electron microscopy shows a continuous epithelial surface [Winther et al., 1983].

Little data on the effect of common cold on the pharmacokinetics of intranasal administered drugs is available [Mygind and Dahl 1998].

Allergic rhinitis is part of a systemic disease complex. Examination of nasal epithelial integrity by light microscopy and electron microscopy has not revealed any epithelial damage in allergic rhinitis [Takeuchi et al. 2006]. Furthermore, improved functional tightness of the airway mucosa in allergic rhinitis has been shown by Greiff et al. Consequently, luminal entry of plasma exudates in airway diseases is not necessarily coupled with an increased permeability for administered drugs [Greiff et al., 1997].

The efficient and rapid epithelial restitution *in vivo* may support the assumption that rhinitis (allergic or inflamed) does not mandatorily prime enhanced permeability of the nasal mucosa [Erjefalt et al., 1995]. The common notion that increased mucosal absorption characterizes allergic and inflamed airways is poorly supported by *in vivo* studies. The influence of nasal diseases on transmucosal nasal drug delivery has not conclusively been characterized [Mygind and Dahl 1998].

## 3 Midazolam

### 3.1 Physicochemical characterization

Midazolam base (white or light yellow crystalline powder) is a lipophilic compound with low solubility in water. Solubility of midazolam is highly pH dependent [Andersin 1991]. The alkaline nitrogen in position 2 of the imidazobenzodiazepine ring system enables midazolam to form water-soluble salts with acids (e.g., hydrochloric, maleic, or lactic acid), see Figure 3-1. Dissociation constant  $pK_{a1}$  is 1.7 (protonization of nitrogen in position 2). The  $pK_{a2}$  value for the protonization of the nitrogen in position 5 is about 6 (6.15 to 5.50 [DAV 2006]). Under acidic conditions, midazolam is present as an equilibrium mixture of the closed-ring form and an open-ring structure, formed by the acid catalyzed ring opening of the 4,5-double bond of the diazepine ring. Above pH 5, at least 99% of midazolam molecules are in the closed-ring form. Under physiologic conditions (pH 7.5, 37°C), the open-ring form reverts to the physiologically active, lipophilic, closed-ring form ( $t_{1/2}$  3.9 min [Loftsson, et al. 2001]).

For the present work (Project I – III) midazolam hydrochloride was used. The empirical formula of midazolam hydrochloride is  $C_{18}H_{13}ClN_3 \cdot HCl$ , and the molecular weight is 362 g/mol (midazolam base: 326 g/mol).

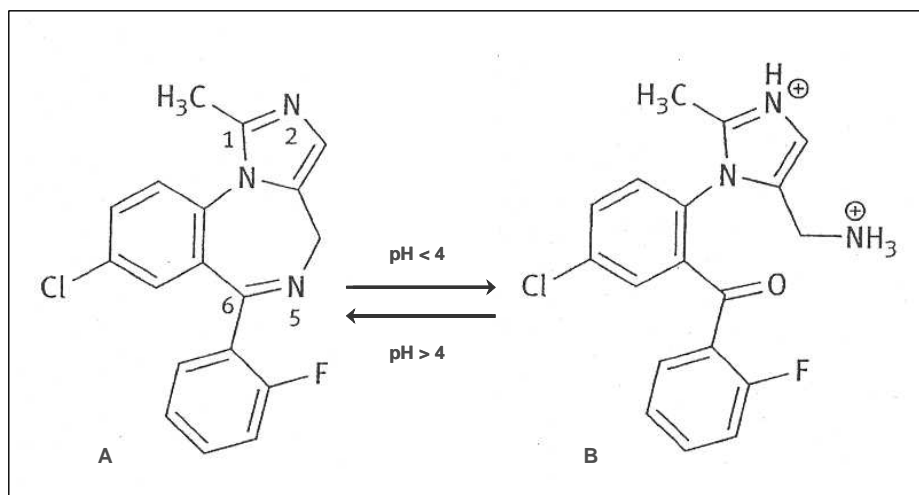


Figure 3-1: The closed (A) structure of midazolam and the corresponding open-ring form (B).

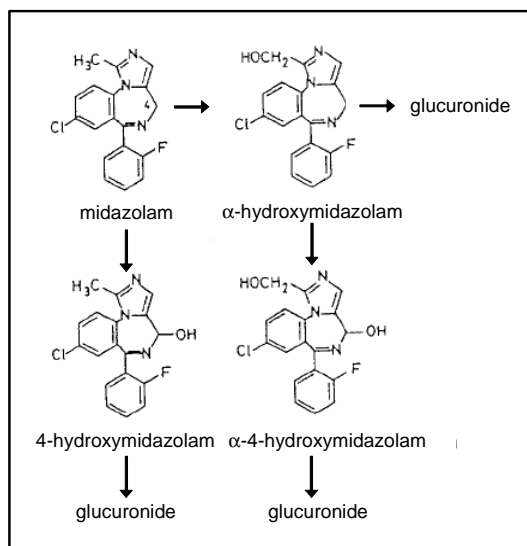
### 3.2 Pharmacologic effects, metabolism and elimination<sup>2</sup>

The pharmacologic effects of midazolam, an imidazobenzodiazepine, are basically the classic effects of all benzodiazepines. Midazolam has sedative and sleep-inducing effects of pronounced intensity. It also exerts anxiolytic, anticonvulsant, and muscle-relaxant effects. Anterograde amnesia<sup>3</sup> of short duration is reported, especially for administrations providing fast rising plasma concentrations (e.g., i.v. or i.m. injection). Midazolam stands out against the other benzodiazepines because of its rapid onset of clinical effects and shorter duration of action because of fast metabolic transformation as well as low activity and short half-life of the main active metabolite ( $\alpha$ -hydroxymidazolam).

The pharmacologic effects of benzodiazepines results from a reversible interaction with the gamma-amino butyric acid (GABA) receptor. GABA is the major inhibitory neurotransmitter in the central nervous system. The benzodiazepine receptor antagonist, flumazenil, readily reverses the action of midazolam. Midazolam is mainly metabolized by CYP 3A4 isoenzyme to major metabolite  $\alpha$ -hydroxymidazolam, see Figure 3-2.

The unconjugated metabolite  $\alpha$ -hydroxymidazolam is pharmacologically active, and contributes about 10% to the pharmacological effect of midazolam. The glucuroconjugated  $\alpha$ -hydroxymidazolam is excreted in urine (60-80% of injected midazolam). Less than 1% of the injected midazolam dose is recovered in urine as unchanged drug. The terminal elimination half-life of  $\alpha$ -hydroxymidazolam is about 1 hour. Due to the almost exclusive metabolism mediated by CYP3A4 isoenzyme, especially co-administration of CYP3A4 inhibitors and inducers result in pharmacokinetic interactions. Because of changed pharmacokinetics resulting in prolonged elimination half-life, in neonates, children, elderly, obese, patients with hepatic or renal impairment, critically ill patients, and patients with cardiac insufficiency,

midazolam application is to be performed with caution.



**Figure 3-2: Metabolism of midazolam. CYP 3A4 metabolizes midazolam to the main metabolite  $\alpha$ -hydroxymidazolam, 4-hydroxymidazolam, and to  $\alpha$ -4-hydroxymidazolam. All metabolites are urinary excreted as glucuronides.**

<sup>2</sup> [Documed 2006]

<sup>3</sup> The patient does not remember events that occurred during the maximal activity of the compound.



### 3.2.1 Indications and doses

The short-acting and sleep-inducing properties of midazolam facilitate the use of midazolam for different therapeutic indications. The Committee for Proprietary Medical Products (CPMP) of the EMEA summarizes the indications and doses of midazolam as displayed in Table 3-1.

**Table 3-1: Therapeutic indication and dosage of midazolam, published by the Committee for Proprietary Medical Products (CPMP) of the EMEA**

Indication	Children	Adults	
		< 60 years	≥ 60 years / debilitated or chronically ill
<b>Conscious sedation</b> before and during diagnostic or therapeutic procedures with or without local anesthesia	<b>6 months to 5 years (i.v.)</b> - initial: 0.05-0.1 mg/kg - total: < 6 mg	<b>i.v.</b> - initial: 2-2.5 mg - titration: 1 mg - total: <3.5-7.5 mg	<b>i.v.</b> - initial: 0.5-1 mg - titration: 0.5-1 mg - total: <3.5 mg
	<b>6 to 12 years (i.v.)</b> - initial: 0.025-0.05 mg/kg - total: <10 mg <b>&gt;6 months (rectal)</b> - 0.3-0.5 mg/kg <b>1-15 years (i.m.)</b> - 0.05-0.15 mg/kg		
<b>Anesthesia premedication</b>	<b>&gt;6 months (rectal)</b> - 0.3-0.5 mg/kg <b>1-15 years (i.m.)</b> - 0.08-0.2 mg/kg	<b>i.m.</b> - 0.07-0.1 mg	<b>i.m.</b> - 0.025-0.05 mg
<b>Anesthesia induction</b>	not used	<b>i.v.</b> - 0.012-0.2 mg/kg	<b>i.v.</b> - 0.01-0.2 mg/kg
<b>Sedation in ICU (Intensive Care Unit)</b>	<b>i.v. in neonates &lt; 32 weeks gestational age</b> - 0.03 mg/kg/h <b>i.v. in neonates &gt; 32 weeks gestational age &lt;6 months</b> - 0.06 mg/kg/h <b>i.v. &gt;6 months</b> - loading dose: 0.05- 0.3 mg/kg/h - maintenance dose: 0.06- 0.12 mg/kg/h	<b>i.v.</b> - loading dose: 0.03-0.3 mg/kg - titration: 1-2.5 mg - maintenance dose: 0.03-0.2 mg/kg/h	

### 3.2.2 Conscious sedation

The guidelines for conscious sedation of the Society for the Advancement of Anesthesia in Dentistry (SAAD) defines conscious sedation as: *“Technique in which the use of a drug or drugs produces a state of depression of the central nervous system enabling treatment to be carried out, but during which verbal contact with the patient is maintained throughout the period of sedation.*

*The level of sedation must be such that the patient remains conscious, retains protective reflexes, and is able to respond to verbal commands”.*

MRI (magnetic resonance imaging) examinations are common diagnostic procedures. But many patients refuse this helpful diagnostic proceeding because of claustrophobia or unspecific anxiety. Anxiety in patients undergoing MRI examinations is a multifaceted phenomenon involving fear of enclosed places (claustrophobia), pain, the unknown situation, as well as apprehension about what the test might reveal [Katz et al., 1994]. For clear diagnostic MRI images the cooperation of the patient is essential (breathing on command during investigations of the upper abdomen). Therefore, anxiolysis without sedating the patient is desired. Conscious sedation is one of the most important approaches to help claustrophobic and anxious patients to cope with MRI examinations [Tschirch, et al. 2006].

Before and during diverse diagnostic or therapeutic procedures with or without local anesthesia, conscious sedation facilitates completing unpleasant procedures and increases patient's comfort.

In a review de Visser et al. evaluated published testing methods to assess effects of anxiolytic benzodiazepines in healthy volunteers. Even numerous psychopharmacology tests have been described to assess anxiolytic effects in human, no validated testing method exists. Furthermore, tests to assess anxiolysis are usually not specific enough to distinguish anxiolytic effects from sedating effects [de Visser et al., 2003]. Because standardized assessments for anxiolytic effects are lacking, no correlation of midazolam blood concentrations and anxiolytic effect has been established. For the evaluation of the benefit of an anxiolytic therapy in the clinical practice, phase III studies with anxious patients have to be performed.

### 3.3 Undesirable effects

The characteristic undesirable effects of benzodiazepines are reported also for midazolam. Sedation, amnesia, impaired attention, and impaired muscular function may adversely affect the ability to drive or use machines.

Paradoxal reaction such as agitation, involuntary movements, hyperactivity, hostility, rage reactions, aggressiveness, paradoxal excitement, and assault, have been reported. This reaction occurs mainly with high doses and/or when the injection is given rapidly. The highest risk for paradox reaction has been reported for children and elderly patients [Weinbroum et al., 2001], as well as patients with actual or amnesic psychiatric diseases and patients with alcohol addiction [Mancuso et al., 2004]. Furthermore paradoxal reaction is often related to high midazolam doses [Litchfield 1981].

Administration of high midazolam doses, overdoses or in situations with impaired pharmacokinetics, the most vital symptoms to be monitored are areflexia, hypotension, cardiorespiratory depression, and apnea. Higher risk for cardiorespiratory depression, apnea, and areflexia is reported for co-administration of other central nervous acting drugs and inhibitors of CYP 3A4.

### **3.4 Midazolam delivery**

In Switzerland, midazolam (Dormicum<sup>®</sup> 1 mg/ml and 5 mg/ml) is approved for intravenous or intramuscular injection or oral administration as tablets (Dormicum<sup>®</sup> 7.5 mg and 15 mg). Rectal administration of Dormicum<sup>®</sup> (1 mg/ml and 5 mg/ml) is also compendial, but not widely used. Because of pain and distress, injections for drug administration are not popular and patients (adults and children) usually prefer alternative drug administration.

Oral midazolam delivery, the most popular drug administration route, is associated with considerable first-pass effect (bioavailability only 30-50% [Documed 2006]). Furthermore, oral midazolam administration is disposed to a delayed onset of action, additionally impaired by ingestion. Due to the fluctuant bioavailability, the pharmacological effect is hard to predict and the dose to be applied difficult to define.

The pharmacokinetic disadvantages of rectally applied midazolam are analog to the oral administration a slow onset of action and a low peak plasma concentration [Malinovsky et al., 1993].

Table 3-2 summarizes the characteristics of the different modes of midazolam delivery. Commonly, intravenous or intramuscular injection of midazolam is performed if rapid onset of action is desired [Burstein et al., 1997, Uygur-Bayramicli, 2002]. As a convenient and reliable alternative to intravenous and intramuscular administration, nasal midazolam delivery has received considerable attention not only for pediatric patients (see Table 3-4).

Intranasal drug delivery is painless, results in rapid drug absorption and circumvents hepatic first pass metabolism. Additionally, the convenient administration results in high acceptance by the patients. A number of studies report the beneficial effects of nasal delivered midazolam in patients, both children and adults (see Table 3-4).

Table 3-2: Benefits and limitations of different administration routes for midazolam application

Administration	Benefit	Limitation
<b>Oral (solid form)</b>	<ul style="list-style-type: none"> <li>- convenient, painless application</li> <li>- well accepted by patients</li> </ul>	<ul style="list-style-type: none"> <li>- dose: 7.5 mg or 15 mg</li> <li>- delayed onset of therapeutic effect</li> <li>- inappropriate for emergency care</li> <li>- only 30-50% bioavailability (relevant interindividual variability)</li> </ul>
<b>Oral (liquid preparation)</b>	<ul style="list-style-type: none"> <li>- individual dosing of syrup or drops</li> <li>- well accepted (if bitter taste of midazolam is masked)</li> <li>- convenient, painless application</li> </ul>	<ul style="list-style-type: none"> <li>- delayed onset of therapeutic effect</li> <li>- children sometimes refuse oral medication</li> <li>- inappropriate for emergency care</li> <li>- only 30-72% bioavailability (relevant interindividual variability)</li> <li>- no liquid oral midazolam preparation commercially available in Europe</li> </ul>
<b>Rectal</b>	<ul style="list-style-type: none"> <li>- no swallowing</li> </ul>	<ul style="list-style-type: none"> <li>- not well accepted (adults and children)</li> <li>- 10-20% of children refuse rectal administration</li> </ul>
<b>Intravenous</b>	<ul style="list-style-type: none"> <li>- fast onset of therapeutic effect</li> <li>- no swallowing</li> <li>- emergency care (if veins are accessible)</li> </ul>	<ul style="list-style-type: none"> <li>- painful</li> <li>- veins are not accessible during status epilepticus</li> <li>- children and some adults detest injections</li> </ul>
<b>Intramuscular</b>	<ul style="list-style-type: none"> <li>- fast onset of therapeutic effect</li> <li>- no swallowing</li> </ul>	<ul style="list-style-type: none"> <li>- painful</li> <li>- risk of tissue necrosis at the site of injection</li> </ul>
<b>Nasal</b>	<ul style="list-style-type: none"> <li>- fast onset of therapeutic effect</li> <li>- no swallowing</li> </ul>	<ul style="list-style-type: none"> <li>- individual dosing is limited</li> <li>- no nasal preparation commercially available</li> </ul>

#### 3.4.1 Pharmacokinetic parameters of transmucosal delivered midazolam

In Table 3-3 pharmacokinetic parameters of transmucosal delivered midazolam are summarized. It has to be taken in consideration, that for transmucosal nasal midazolam delivery different formulations were used.

Several clinical investigators have used midazolam solution for injection at a concentration of 5 mg/ml (Dormicum<sup>®</sup>, 5 mg/ml) for nasal delivery [Bjorkman et al., 1997; Burstein, et al. 1997; Connors and Terndrup 1994; Fösel 1996; Goepfert 1996; Ljung and Andreasson 1996; Ljungman 2000; Malinovsky et al., 1995; Münte 2002; Tolksdorf and Eick 1991; Uygur-Bayramicli et al., 2002]. In the cited studies, doses of 0.06 mg/kg to 0.6 mg/kg midazolam were administered by instilling Dormicum<sup>®</sup> (5 mg/ml) into the nose. The administered volume reached from 0.1 ml to 5 ml according to the bodyweight of patients. By exceeding the commonly recommended application volumes of 0.05 ml to 0.2 ml, the excess formulation tends draining off into the nasopharynx, where it is swallowed. The swallowed portion of the nasally delivered midazolam dose is absorbed in the

gastrointestinal tract and due to considerable first-pass effect bioavailability is impaired. Furthermore, the absorption is delayed and depends on ingestion [Burstein, et al. 1997].

Pharmacokinetic parameters of transmucosal nasally delivered drugs depend on many-sided interaction of different preparation and administration related parameters. Therefore, to compare the outcome of different investigations of nasal midazolam delivery, it is not enough to consider the administered doses. It is important to compare also the administered volume and the drug delivery device (spray or droplets), which determine the swallowed portion. In nasal drug delivery, the impact on pharmacokinetic parameters of the vehicle and other administration related characteristics (e.g., drug delivery device) is enormous. Therefore, the cited pharmacokinetic parameters are only of trend-setting character and cannot be generally assigned to transmucosal nasal midazolam delivery. In Appendix 10.1.1 published data on nasal midazolam delivery is summarized.

**Table 3-3: Dose, bioavailability, and onset of therapeutic effect of transmucosal administered midazolam. This parameters were assessed by nasal delivery of different preparations (in some publications the administered preparation was no specified).**

<b>Delivery</b>	<b>Dose per kg body weight</b>	<b>Bioavailability</b>	<b>Onset of effect</b>	<b>Reference</b>
<b>Oral</b>	0.3-0.6 mg	31-72%	10-20 min	[Allen 1991; Connors and Terndrup 1994; Cote et al., 2002; Gizurarson 1993; Goepfert 1996; Malinovsky, et al. 1995; Tolksdorf et al., 1989; Tolksdorf and Eick 1991]
<b>Nasal</b>	0.2-0.4 mg	50-83%	5-10 min	[Connors and Terndrup 1994; Fösel 1996; Goepfert 1996; Malinovsky, et al. 1993; Münte Sinikka 2002; Münte 2002; Roelofse, et al. 2000; Tolksdorf and Eick 1991]
<b>Rectal</b>	0.3-0.5 mg	no data	10-16 min	[Documed 2006; Goepfert 1996; Malinovsky, et al. 1995; Tolksdorf, et al. 1989; Tolksdorf and Eick 1991]

Table 3-4: Indications and clinical situations where nasal administration of midazolam is beneficial

Indication/clinical situation	Reference
<b>Premedication before anesthesia</b>	<b>children:</b> [Davis, et al. 1995; Geldner et al., 1997; Griffith, et al.; Jaennet 1999; Kogan et al., 2002; Lejus, et al. 1997; Ljung and Andreasson 1996; Rey et al., 1991; Rose, et al. 1990] <b>adults:</b> [U.Haas 1998]
<b>Emergency</b>	<b>children:</b> [Goepfert 1996] <b>adults:</b> [Wolfe and Bernstone 2004]
<b>Status epilepticus</b>	<b>children:</b> [Fisgin et al., 2000; Fisgin et al., 2002; Jaennet 1999; Kutlu et al., 2000; Lahat, et al. 1998] <b>adults:</b> [Gilat et al., 2003; Kendall et al., 1997; Lahat et al., 1992; Scheepers et al., 2000; Scheepers et al., 1998; Scott et al., 1999; Scott et al., 1997]
<b>(Febrile) childhood seizure</b>	[Johnson 2001; Koren 2000; Lahat et al., 2000]
<b>Short surgical procedures</b>	<b>adults:</b> [Ljungman 2000; Malinovsky, et al. 1993; Saint-Maurice et al., 1990]
<b>MRI examinations</b>	<b>adults:</b> [Moss et al., 1993; Münte Sinikka 2002; Schweizer et al., 1992]
<b>Upper gastrointestinal endoscopy</b>	<b>adults:</b> [Uygur-Bayramicli, et al. 2002]
<b>Dental surgery</b>	<b>children:</b> [Abrams et al., 1993; al-Rakaf et al., 2001; Fuks et al., 1994; Hartgraves and Primosch 1994; Kaufman et al., 1994; Kupietzky et al., 1996; Lloyd et al., 2000] <b>adults:</b> [Burstein, et al. 1996; Fukuta et al., 1993; Fukuta et al., 1994; Fukuta et al., 1997; Kaufman, et al. 1994; Roelofse et al. 2000]

## 4 Compounding of nasal midazolam preparations

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### 4.1 Regulatory requirements for nasal preparations [Ph. Eur. 5.5]

Nasal preparations are liquid, semi-solid, or solid preparations intended for administration to the nasal cavities for systemic or local effect. Nasal preparations are, as far as possible, non-irritating and do not adversely affect the function of the nasal mucosa and its cilia. Aqueous nasal preparations are usually isotonic and may contain adjuvants to modify the viscosity of the preparation, to adjust or stabilize the pH, to increase the solubility of the active compound, or for other technological purposes.

### 4.2 Excipients for nasal midazolam preparations

In nasal preparations, adjuvants have different purposes. Beside adjuvants to solubilize the active substance and excipients to adapt the preparation to the local physiological conditions, viscosity modifiers, bioadhesives, and penetration enhancers are added to modify bioavailability. Viscosity enhancers and mucoadhesive excipients transiently modify mucociliary clearance. But, the modification of the mucociliary function needs to be reversible and should not adversely affect the functions of the nasal mucosa and its cilia.

All drugs must possess some degree of aqueous solubility to be pharmacologically active, and most drugs need to be lipophilic to be able to permeate biological membranes by passive diffusion. How water-soluble a given drug needs to be is determined by its potency (i.e., the required dose) and type of formulation.

#### 4.2.1 Solubilizers

Due to restricted residence time (mucociliary clearance) and limited solvent (water) in nasal cavities, drugs are preferably administered in solutions. The ideal volume range is 25  $\mu\text{l}$  to 150  $\mu\text{l}$  with an upper limit of 200  $\mu\text{l}$  per nostril [Romeo, et al. 1998]. Therefore, the entire dose of a nasal delivered drug is to be administered in a volume of 50  $\mu\text{l}$  to 400  $\mu\text{l}$  (25  $\mu\text{l}$  to maximal 200  $\mu\text{l}$  per nostril). If the delivered volume exceeds the recommended volume, particularly liquid, little viscous preparations, tend to flow in the nasopharynx and are swallowed.

Different approaches have been explored to enhance drug solubility in nasal preparations. The use of solubilizers increases the aqueous solubility of insoluble compounds and some can even promote nasal drug absorption.

The challenge in compounding nasal midazolam preparations is the limited and highly pH dependent midazolam solubility (see chapter 3)

Due to the limited nasal capacity the whole midazolam dose has to be administered within 50  $\mu\text{l}$  to 400  $\mu\text{l}$ . To deliver therapeutic midazolam doses, nasal preparations with midazolam concentration, exceeding solubility of midazolam in water need to be developed. For example, to deliver the dose of 3 mg midazolam by instilling a volume of 100  $\mu\text{l}$ , concentrations of 30 mg/ml midazolam are required. Suitable solvents and solubilizers are needed to compound preparations for nasal midazolam delivery. Table 4-1 lists some solubilizers, which have been described in literature to solubilize midazolam for nasal administration.

**Table 4-1: Different approaches to solubilize midazolam for nasal administration**

Solubilizer	Midazolam concentration	Comment	Reference
Propylene glycol 26%, benzyl alcohol 1%	27.8 mg/ml	2% propylene glycol is isotonic with serum	[Knoester et al. 2002]
$\beta$ -cyclodextrin	10 mg/ml	Less nasal irritation compared with nasal administration of Dormicum <sup>®</sup> for injection 5mg/ml (Roche, Basel, Switzerland)	[Roelofse et al. 2000]
Sulfobutylether- $\beta$ -cyclodextrin (Captisol <sup>®</sup> , CyDex, USA)	30 mg/ml, pH 3	Different cyclodextrin derivatives were investigated to solubilize midazolam. Above pH 5 the maximal midazolam concentration is 5 mg/ml.	[Loftsson et al. 2001]

#### 4.2.2 Bio- and mucoadhesive excipients

Bioadhesion and mucoadhesion describe adhering of synthetic or natural macromolecules to biological or mucosal surfaces. Most biological surfaces of interest for drug administration are mucosal surfaces and the epithelial cells are covered with a mucus layer. Mucus is a complex hydrated gel-like material containing the large glycoprotein mucin. The term **mucoadhesion** describes molecules binding to a mucus layer, whereas **bioadhesion** describes the direct interaction of macromolecules with the cell surfaces. But, these two mechanisms of adhesion can hardly be differentiated; furthermore, many bioadhesives interact with biological surfaces by a combination of these two mechanisms. The term **specific bioadhesion** refers to specific interaction with defined structures. Specific bioadhesion is applied to target defined structures of the cell surface [Woodley 2001].

The mechanism of non-specific bioadhesion is supposed to result from electrostatic interaction of negatively charged cell surfaces and cationic macromolecules (e.g., chitosan see also chapter 4.4). For anionic bioadhesive molecules, the interaction with the biologic surface is composed of hydrophobic interaction, H-bonds, and Van der Waals interactions.

Addition of bioadhesive excipients to nasal preparation elongates the nasal residence time of nasally delivered preparations. The prolonged residence time favors, complete release of the drug from the preparation and thereby efficient transmucosal absorption [Dondeti et al., 1996].

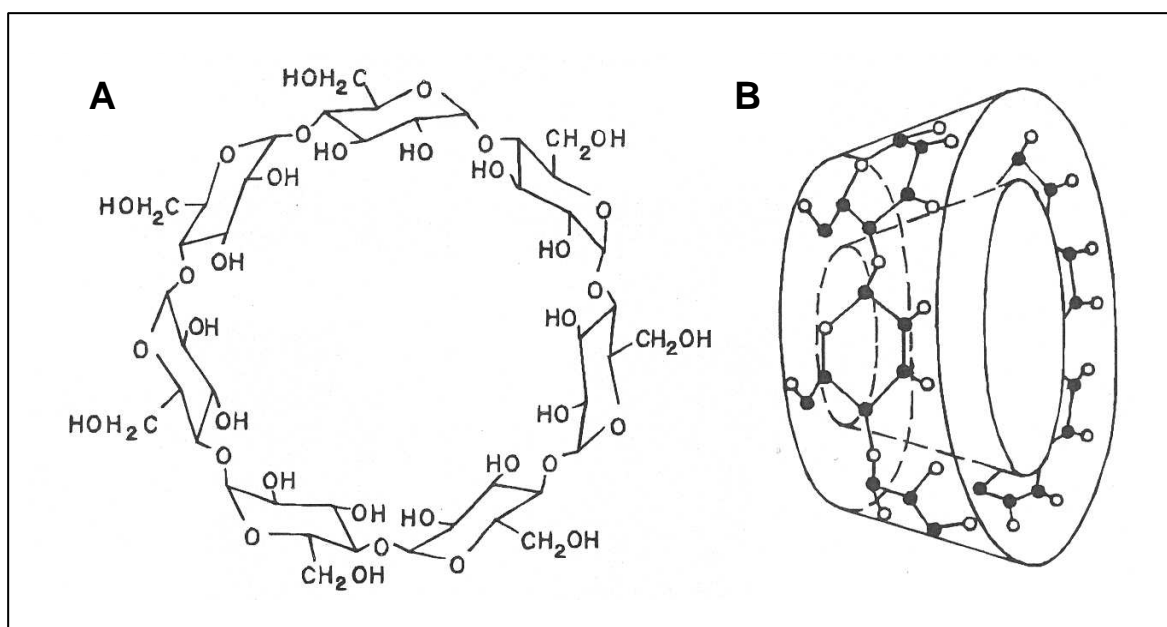


For ocular, gastrointestinal, vaginal, and nasal drug administration bioadhesives are popular excipients. In preparations for transmucosal drug delivery, bioadhesion enforces the contact of the administered preparation with the mucosa, promoting transmucosal drug absorption.

### 4.3 Cyclodextrins

#### 4.3.1 Chemical structure

Cyclodextrins are cyclic oligosaccharides, which are generated from starch by enzymatic cyclisation. There are several unsubstituted (native) cyclodextrins, the most common are  $\alpha$ -cyclodextrin (6 cyclo- $\alpha$ -(1,4)-anhydroglucose units),  $\beta$ -cyclodextrin (7 cyclo- $\alpha$ -(1,4)-anhydroglucose units), and  $\gamma$ -Cyclodextrin (8 cyclo- $\alpha$ -(1,4)-anhydroglucose units).



**Figure 4-1: Chemical structure (A) and toroidal shape (B) of  $\beta$ -cyclodextrin (7 cyclo- $\alpha$ (1,4)-anhydroglucose units). After [Loftsson and Brewster 1996]**

To change the physicochemical properties (particularly solubility) of native cyclodextrins, different derivatives have been synthesized (Table 4-2). The characteristics of the substituents determine the physicochemical properties of the modified cyclodextrin (lipo-/hydrophilicity profile).

Table 4-2: Unsubstituted cyclodextrins and some derivatives

Cyclodextrin	Abbr.	R	Substitution <sup>1</sup>	MW [Da]	Solubility in water [mg/ml]	Delivery
$\alpha$ -cyclodextrin	$\alpha$ CD	-H	-	972	145	oral, parenteral, topical
$\beta$ -cyclodextrin	$\beta$ CD	-H	-	1135	18.5	oral, topical
randomly methylated $\beta$ -cyclodextrin	RM $\beta$ CD	-CH <sub>3</sub>	1.7-1.9	1310	> 500	oral <sup>2</sup> , topical
2-Hydroxypropyl- $\beta$ -cyclodextrin	HP $\beta$ CD	-CH <sub>2</sub> CHOHCH <sub>3</sub>	0.65	1400	> 600	oral, parenteral, topical
$\beta$ -cyclodextrin sulfobutyl ether sodium salt	SBE $\beta$ CD	-(CH <sub>2</sub> ) <sub>4</sub> SO <sub>3</sub> <sup>-</sup> Na <sup>+</sup>	0.9	2163	> 500	oral, parenteral, topical
$\gamma$ -Cyclodextrin	$\gamma$ CD	-H	-	1297	232	oral, parenteral <sup>3</sup> , topical
2-Hydroxypropyl- $\gamma$ -cyclodextrin	HP $\gamma$ CD	-CH <sub>2</sub> CHOHCH <sub>3</sub>	0.6	1576	> 500	oral, parenteral, topical

<sup>1</sup> degree of substitution (per anhydroglucose unit)

<sup>2</sup> maximal 1 mg per kg body weight, Wacker Chemie AG, Burghausen, Germany

<sup>3</sup> limited amounts

#### 4.3.2 Pharmaceutical application of cyclodextrins

Due to the hydrophilic outer surface and the lipophilic central cavity, cyclodextrins are able to form inclusion complexes with lipophilic molecules in aqueous solutions.

Inclusion complexes are chemical complexes, where one molecule is enclosed within another. This kind of complexation enhances the apparent solubility of lipophilic molecules in aqueous solutions [Davis and Brewster 2004; Loftsson et al., 2002]. If any molecule entirely or at least partially enters into the cavity of a cyclodextrin, an inclusion complex is formed [Manosroi et al., 2005].

In pharmaceutical preparations cyclodextrins are added to improve the solubility and dissolution rate of hardly water soluble drugs, to enhance physical and chemical stability of the active compound, and to modify the bioavailability.

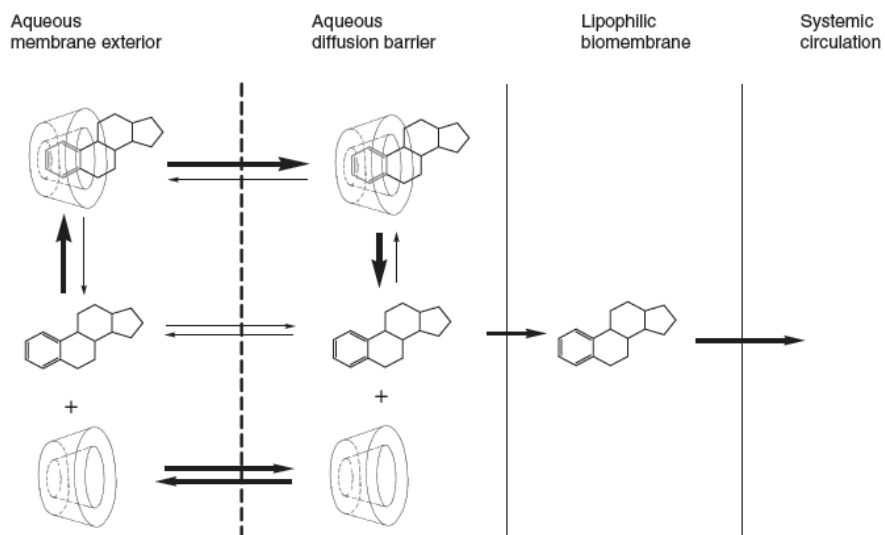
In aqueous solutions, cyclodextrins form inclusion complexes by taking up some hydrophobic moieties of drug molecules into the central cavity. No covalent bonds are formed or broken during the complex formation. In solution, drug molecules complexed with cyclodextrins are in dynamic equilibrium with free molecules [Irie and Uekama 1997; Loftsson and Brewster 1996; Rajewski and Stella 1996].

The driving force of complex formation is the release of enthalpy rich water molecules from the cyclodextrin cavity. The total energy of the drug-cyclodextrin complex decreases by displacing enthalpy rich water molecules by hydrophobic guest molecules [Loftson 1995]. Van der Waals forces, hydrogen bonds, and hydrophobic interactions stabilize the drug-cyclodextrin complex. The complexation ratio depends on the physicochemical properties, especially surface character and size of the guest molecule. Most of the drugs form 1:1 complexes with cyclodextrin [Szente and Szejtli 1999].

Generating of solid cyclodextrin-drug complexes is more sophisticated, 3 methods (complexation in solution followed by freeze drying, complexation as a paste, and complexation in organic solvents) are proposed by Wacker Fine Chemicals (Burghausen, Germany). Before applying solid drug-cyclodextrin complexes, for compounding of solid preparations (e.g., tablets) the complex formation needs to be verified (e.g., by X-ray diffractometry). Benefit of solid cyclodextrin-drug complexes in compounding of solid preparations are improved chemical stability, modified dissolution characteristics, or masking of unpleasant taste and/or odor.

Due to chemical structure of cyclodextrins (i.e., the large number of hydrogen donors and acceptors), the molecular weight and their low octanol/water partition coefficient cyclodextrins and the drug-cyclodextrin complexes do not readily permeate biological membranes. Only the free form of the drug is able to permeate the biological membrane.

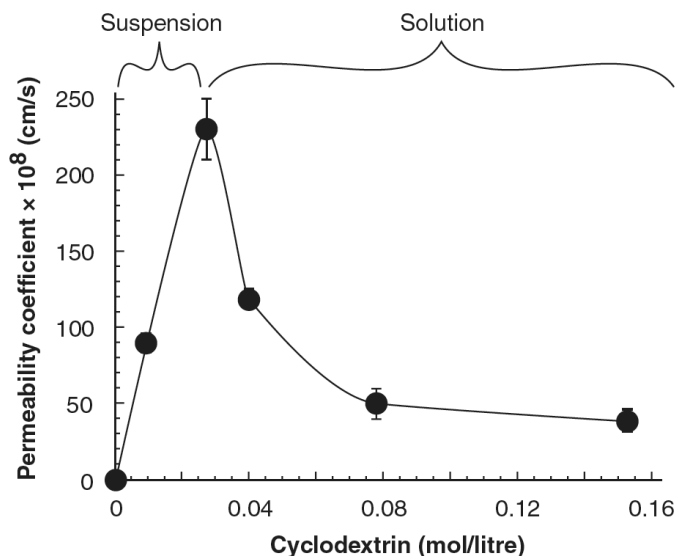
The drug-cyclodextrin complex diffuses to the lipophilic membrane, where the drug (without hydration) penetrates directly into the membrane, whereas the cyclodextrin remains in the aqueous phase [Gröger 2001]. Figure 4-2 shows the equilibrium drug complexation and drug release in the aqueous environment of a lipophilic biologic membrane. Competitive displacement of the drug molecule contributes to efficient release of the drug from drug-cyclodextrin complexes [Stella et al., 1999].



**Figure 4-2: Release of a lipophilic drug from a cyclodextrin-drug complex in the aqueous environment of a biological membrane. Form [Loftsson et al. 2005]**

The solubilizing effect of cyclodextrins is usually related to improved drug absorption [Illum 2002]. But the impact of cyclodextrins on bioavailability is controversially discussed in literature. Loftsson reduces the effect of cyclodextrins on drug transport through and into skin to true carriers keeping the hydrophobic drug in solution [Loftsson 1995]. In aqueous vehicles, cyclodextrins act as penetration enhancers by solubilizing lipophilic water-insoluble drug molecules.

For optimal permeation Loftsson and Jarho recommend to add exactly enough cyclodextrin to solubilize the drug [Jarho et al., 1996; Loftsson 1995]. This recommendation is based on the findings of *in vitro* studies from Jarho (Figure 4-3), demonstrating impaired permeation by excess of cyclodextrins [Jarho et al. 1996].



**Figure 4-3 : Optimal cyclodextrin concentration. Jarho et al. demonstrated exceeding cyclodextrin concentration to impair permeation of drug through cornea of pigmented rabbits. Figure modified after [Jarho et al. 1996].**

Permeation of drug molecules from aqueous cyclodextrin containing vehicles through semi-permeable membranes follows the same pattern as permeation through lipophilic biomembranes [Loftsson et al. 2002]. Determination of drug fluxes through semi-permeable membranes have been used to characterize release of drugs from cyclodextrin containing vehicles as well as to assess stability constants of drug-cyclodextrin complexes [Masson et al., 1999; Ono et al., 1999].

### 4.3.3 Cyclodextrins in transmucosal nasal drug delivery

In nasal formulations, cyclodextrins are used to increase the aqueous solubility of lipophilic drugs. However, cyclodextrins can also interact with biological membranes, acting as penetration enhancers, especially in nasal delivery of peptides [Marttin et al., 1998]. Numerous studies have demonstrated that methylated cyclodextrins are efficient absorption enhancers, and therefore well established in nasal drug delivery [Merkus 1999]. The first cyclodextrin-based nasal formulations contained steroidal hormones and peptides. The results were promising and the most effective cyclodextrins, methylated cyclodextrin derivatives, increased the bioavailability of progesterone threefold with suspension of the same compound [Hermens et al., 1990; Matsubara et al. 1995; van den Berg et al. 2004]. The estradiol nasal spray Aerodiol<sup>®</sup> (Servier S.A, Meyrin Switzerland). represents the successful use of cyclodextrins in nasal applications.

#### 4.3.4 Safety and toxicology of cyclodextrins

Irie and Uekama reviewed the toxicological profile of cyclodextrins. In general, orally administered cyclodextrins are non-toxic, due to lack of absorption from the gastrointestinal tract. Furthermore, a number of safety evaluations have shown that HP $\beta$ CD, SBE $\beta$ CD,  $\gamma$ CD, and HP $\gamma$ CD are suitable for parenteral as well as for oral administration [Irie and Uekama, 1997].

Asai et al. studied the effect of different cyclodextrin derivatives on the nasal mucosa of rats. Thereby, the nasal cavity of the rats was incubated for 60 minutes with 10% RM $\beta$ CD or 10% HP $\beta$ CD in water. No impairment of the nasal mucosa was detected by microscope. Repeated nasal application of RM $\beta$ CD (20%) had no toxic effect [Asai et al., 2002]. No histological changes were caused by repeated nasal or ocular administration of 7.5% RM $\beta$ CD, for 28 days twice daily in dogs, rabbits, and guinea pigs (Servier S.A, Meyrin Switzerland, Aerodiol<sup>®</sup> Nasal Spray). In animal models (rats) the acute histological effects of the lipophilic methylated cyclodextrins, were close to physiological saline [Martin et al., 1999].

Reeuwijk et al. detected only 2.5% to 4% of nasally administered dimethyl- $\beta$ CD in the urine [Reeuwijk et al., 1993]. Furthermore, the amount of cyclodextrins delivered by the nasal route is limited by the volume instillable to the nasal cavities.

## 4.4 Chitosan hydrochloride

### 4.4.1 Chemical structure and production

Chitosan is a linear polysaccharide, composed of glucosamine and N-acetylglucosamine, produced by partial deacetylation of chitin by alkaline or enzymatic hydrolysis. Chitin is the major component of the exoskeleton of crustaceans, insects, cell wall of fungi and yeast. For the commercial production, chitin from shells of prawns, crabs, or other crustacean is used [Shahidi and Abuzaytoun 2005]. The most remarkable difference between chitin and chitosan is their solubility characteristics. There are few solvents for chitin and most of them are toxic and therefore inappropriate for pharmaceutical applications. Chitosan is insoluble at neutral and alkaline pH, but forms positively charged salts with inorganic and organic acids (e.g., glutamic acid, hyaluronic acid, lactic acid, and acetic acid), whereas the positive charge of the protonated amine group promotes solubility in aqueous solutions. Chitosan is available in a wide range of different molecular weights (50 kDa-2000 kDa) and degrees of deacetylation (40%-98%). The most commonly used forms of chitosan salts are chitosan glutamate and chitosan hydrochloride [Illium 1998]. Chitosan features different conformations of the molecule for high and low deacetylated chitosan. At a high degree of deacetylation (highly charged) chitosan has extended conformation with a more flexible chain, whereas at low degree of deacetylation the molecule adopts a more coiled shape due to lower charge. The amino group in chitosan has a pKa value of ~6.5, thus, chitosan is positively charged and soluble in acidic to neutral solutions [Shahidi and Abuzaytoun 2005]. Chitosans with a relative low degree of deacetylation (40%) are soluble only at pH < 6.5 [Illium 1998].

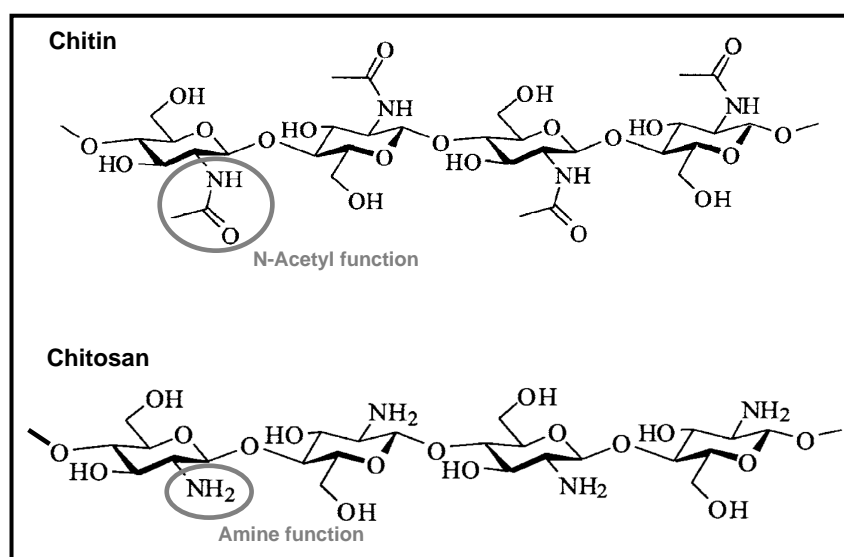


Figure 4-4: Linear polysaccharide chitin and the derivative chitosan resulting of deacetylation.

#### 4.4.2 Applications of chitosan

Chitosan is a multifunctional excipient with a wide range of industrial applications (e.g., flocculant for wastewater clarification, chelating of heavy metals, clarification of fruit juices and beers, and fungicide in agriculture) [Shahidi and Abuzaytoun 2005]. Further, in food industry, chitosan is a popular dietary supplement, particularly in Japan. In addition, chitosan is used for ophthalmic applications, such as contact lens coating or as the contact lenses material itself and in cosmetic industry chitosan is also a very popular excipient (e.g., for hair care products).

Chitosan is a versatile excipient for miscellaneous pharmaceutical applications. Chitosan is adjuvant in direct compressed tablets, for controlled release matrix tablets, in the process of wet granulation, in gels, films, and emulsions, as well as wetting and coating agent, and for the production of microspheres and microcapsules. Chitosan is used also in newer research areas like nasal vaccine delivery and DNA delivery [van der Lubben et al., 2001; Xu et al., 2004]. The European Pharmacopoeia describes the requirements for chitosan hydrochloride used in pharmaceutical products.

#### 4.4.3 Chitosan in transmucosal nasal drug delivery

Davis et al. were the first to demonstrate the cationic polymer chitosan to promote the transmucosal adsorption of challenging drugs, particularly with regard to improved nasal delivery. In animal and human models, chitosan generated a remarkably higher bioavailability [Davis and Illum 2003]. Illum et al found, that the addition of chitosan to a nasal formulation of insulin caused plasma glucose to fall to 43% of basal level compared to 83% without the addition of chitosan [Illum et al., 1994]. Similar effects on bioavailability have been achieved for polar low-molecular-weight drugs (e.g., morphine and antimigraine drugs). Chitosan is an effective nasal absorption enhancer in liquid and powder formulations. Chitosan improve bioavailability in nasal and pulmonary drug delivery [Illum 1998; Illum et al., 2002; Martinac et al., 2005; Yu et al., 2004].

In liquid formulations for nasal drug delivery, concentrations of 0.5-1.0% chitosan proved to promote absorption [Davis and Illum 2003].

Unlike common penetration enhancers, chitosan is neither membrane active nor a classical surfactant and is not transmucosal absorbed. The clearance of chitosan formulations from the nasal cavity of sheep and humans has been shown to be significantly slower compared with aqueous solutions [Soane et al., 1999; Soane et al., 2001].

The mucoadhesion results of the interaction between the linear cationic polysaccharide chitosan and the negatively charged mucosa. This mechanism decelerates the nasal clearance [Aspden et al., 1997].

The extended time of contact of the formulation and the nasal mucosa favors the transmucosal drug absorption [Pavis et al., 2002]. Studies with CaCo-2 cell monolayer suggest cationic polymers (e.g., chitosan) to modify permeability of the monolayer by interaction with the gating mechanism of tight junction, causing a transient opening [Artursson et al., 1994]. Dodane et al. demonstrated



complete reversibility of the transient opening of the tight junctions and showed the function of the nasal mucosa to be unimpaired [Dodane et al., 1999]. Consequently, the impact of chitosan on pharmacokinetics of transmucosal delivered drugs results of the prolonged drug mucosa contact by bioadhesion [Soane et al. 1999; Soane et al. 2001] and a transient completely reversible opening of tight junctions [Dodane et al. 1999].

#### **4.4.4 Safety and toxicology of chitosan**

In a review Dodane and Vilivalam characterized chitosan as safe and effective permeation enhancer for nasal drug delivery of systemic drugs [Dodane and Vilivalam 1998].

Concentrations of 0.5% chitosan hydrochloride improved absorption of different nasally administered compounds. The nasal mucosa was not affected.

Illum et al. demonstrated, that neither nasal nor intravenous delivered chitosan caused any humoral immune reaction [Illum 1998]. It has been shown that the absorption-promoting effect of chitosan on the mucosal membrane is transient, with the effect declining between 30 minutes and 45 minutes after application of a liquid formulation [Illum 1998].

In a clinical trial with 14 cancer patients, break-through pain was treated with morphine nose sprays containing chitosan. All observed adverse effects (bitter taste, tiredness) were correlated with morphine [Pavis et al. 2002].

Aspden et al. examined the effect of an aqueous chitosan solution (0.25%) on the nasal mucosa *in vitro* and *in vivo* with 10 healthy volunteers. After 7 days treatment with aqueous chitosan solution (0.25%) no markers of inflammation were detected neither by endoscopic investigation of the nasal mucosa (*in vivo*), nor by light-microscopic investigation (*in vitro*). All treated volunteers tolerated nasal application well. Furthermore, Aspden et al. showed that chitosan has a transient and completely reversible effect on mucociliary transport rate. Chitosan is bioadhesive and able to interact with the nasal mucus layer and with the nasal epithelial cells [Aspden, et al. 1997]

Chitosan is not absorbed, non toxic, biocompatible, and neither irritating nor allergenic [Dodane and Vilivalam 1998].



## EXPERIMENTAL SECTION



## 5 Project I: Development of preparations for transmucosal nasal midazolam delivery

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### 5.1 Introduction

The key issue in development of preparations for transmucosal nasal midazolam delivery is the relatively poor and highly pH-dependent solubility of midazolam. Solubilization enhancers ideally promote midazolam solubility independent of pH. Several approaches to enhance solubility of midazolam have been described in literature (see Table 4-1). Knoester et al. proposed a nasal midazolam preparation based on propylene glycol and benzyl alcohol [Knoester, et al. 2002] and other groups enhanced midazolam solubility by different cyclodextrin derivatives [Loftsson, et al. 2001; Roelofse, et al. 2000]. Loftsson et al. accentuated the importance of keeping the drug cyclodextrin ratio close to one and just adding as much cyclodextrin as required for drug solubilization. To investigate drug release from cyclodextrin complexes porous semi-permeable cellophane membranes with defined pore size (i.e., molecular weight cut off, MWCO) are used.

The residence time on the nasal mucosa of nasal delivered preparations is limited and therefore drug uptake has to proceed before the preparation is removed by mucociliary clearance.

Chitosan is reported to promote transmucosal nasal drug absorption by bioadhesion (prolongation of contact time) and transient opening of tight junctions (penetration enhancer). In a study with 12 healthy volunteers Illum et al. demonstrated penetration enhancing effect of chitosan in transmucosal nasal delivery of morphine [Illum, et al. 2002].

In order to discover eventual stability problems as early as possible, accelerated stability testing is accomplished already during development of pharmaceutical preparation. According to the current guideline of the International Conference on Harmonization (ICH), accelerated stability testing provides preliminary information about the compound and the vehicle. For the approval of a medicinal product, authorities generally require data from real-time stability testing [Gil-Alegre et al., 2001]. To determine the shelf life of a medicinal product, real time stability testing at the intended storage conditions has to be accomplished. As the determined shelf life is dependent of packaging, real time stability testing has to be generated with the bottled product.

This project comprises the development and *in vitro* characterization of midazolam preparations for transmucosal nasal midazolam delivery. Providing of stability data was prerequisite to use the developed preparations in clinical trials (Project II and Project III).

## 5.2 Materials and methods

### 5.2.1 Materials

Midazolam hydrochloride was purchased from Fährhauspharma (Hamburg, Germany), RM $\beta$ CD (methylated- $\beta$ -cyclodextrin, Cavasol<sup>®</sup> W7M Pharma), HP $\beta$ CD (hydroxypropyl- $\beta$ -cyclodextrin, Cavasol<sup>®</sup> W7HP Pharma), and HP $\gamma$ CD (hydroxypropyl- $\gamma$ -cyclodextrin, Cavasol<sup>®</sup> W8HP Pharma) from Wacker Chemicals (München, Germany), chitosan hydrochloride for preliminary testing from Kraeber GmbH & Co (Ellerbek, Germany), chitosan hydrochloride for pharmaceutical preparations from NovaMatrix FMC BioPolymer (Oslo, Norway).

For the preliminary experiments all chemicals were of analytical reagent grade and obtained from commercial sources. Preparations for stability testing, were produced with chemicals of pharmaceutical quality (see also Project II and Project III).

Semi-permeable cellophane membranes (Spectra/Por<sup>®</sup> Dialysis Tubing, MWCO 2000 from regenerated cellulose) were obtained from Spectrum Europe (Breda, The Netherlands). Unit dose nasal sprays, delivering 0.1 ml, were obtained from Ing. Erich Pfeiffer GmbH (Radolfzell, Germany) and multidose nasal sprays, delivering 0.1 ml per pump, from E. Anwander & Cie. AG (Oberwil, Switzerland).

### 5.2.2 HPLC-Method

The quantification of midazolam was performed by HPLC, Waters Alliance HPLC System (2690 Separation Module, 996 Photodiode Array Detector, Millenium<sup>32</sup> Software), Waters Corporation, (Millford, Massachusetts, USA). Table 5-1 shows the chromatographic conditions.

**Table 5-1: Chromatographic condition for midazolam analysis**

Parameter	Setting
column	ACE RP18 3 $\mu$ m, 4.0 x 75 mm
mobile Phase	buffer pH 5.5/ACN
flow rate	0.8 ml/min
running time	10 min
temperature	30°C
injection volume	10 $\mu$ l
midazolam quantification wavelength	223 nm

### 5.2.3 Solubility studies

To assess the pH-dependent solubility and the effects of different cyclodextrins, midazolam hydrochloride was added in excess amounts to Britton-Robinson buffer (BR buffer, 40 mM boric acid, 40 mM acetic acid, 40 mM phosphoric acid pH, adjusted to pH 3.0, 3.5, 4.0, 4.5, 5.0, and 7.4

with 1.0 M sodium hydroxide), containing no solubilization enhancer, 10% RM $\beta$ CD, 10% HP $\beta$ CD, or 10% HP $\gamma$ CD. The suspensions were equilibrated at room temperature for 3 days. The suspensions were filtered (0.45  $\mu$ m) and midazolam content assessed by HPLC. All samples were prepared in triplicates.

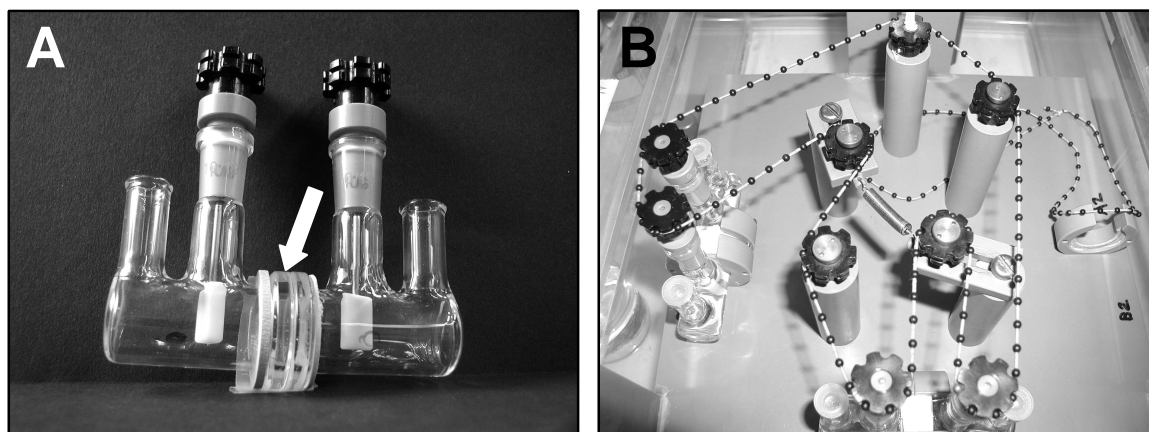
#### 5.2.4 Drug Release

For drug release of midazolam from RM $\beta$ CD-midazolam complexes aqueous solutions through semi-permeable cellophane membranes, diffusion cells (see Figure 5-1) were used. A semi-permeable cellophane membrane (Spectra/Por<sup>®</sup> Dialysis Tubing, MWCO 2000 from regenerated cellulose) was inserted into diffusion cells with a surface area of 2.3 cm<sup>2</sup>.

In a preliminary drug release study Dormicum<sup>®</sup> 5 mg/ml, 10 mg/ml midazolam solubilized with 20% RM $\beta$ CD, and 10 mg/ml midazolam solubilized with equimolar amount of RM $\beta$ CD (4%) were filled in donor compartments.

For drug release 5 mg/ml midazolam in water; 5 mg/ml, 15 mg/ml, and 30 mg/ml midazolam solubilized with equimolar amount of RM $\beta$ CD; and 5 mg/ml and 30 mg/ml midazolam with threefold molar amount of RM $\beta$ CD were filled into donor compartment. Osmolality of all preparations for midazolam release studies was adjusted to 300mOsmol/kg with sodium chloride.

The receiver compartment contained 0.9% sodium chloride in water. Both compartments contained 8.0 ml and were stirred during the release experiments (see Figure 5-1 A and B). The diffusion cells were immersed in a water bath maintained at 37°C. Samples were withdrawn from the receptor compartment replaced with aliquots of 0.9% sodium chloride. Midazolam release per minute was assessed from the slope of the midazolam concentration time plot.



**Figure 5-1 A and B: Picture of a diffusion cell (A) and 2 diffusion cells mounted, ready for drug release experiment. The arrow indicates the position of the cellulose membrane.**

### **5.2.5 Specification and stability testing**

#### **Specification and quality control of the investigational products**

For all investigational products (Preparation 1 to 5, see Table 5-4) GMP-conform specifications (see Appendix 10.2.4 to 10.2.9), instructions for manufacturing, and instructions for quality control were provided according to the quality management system of Hospital Pharmacy of the University Hospital Basel.

#### **Accelerated stability testing**

Accelerated stability testing was accomplished for the Preparation 3 and 4 (see Table 5-4). The preparations were bottled in sealed vials and stored protected from light at room temperature, 40°C, 50°C, and 60°C for 30 days. Midazolam content, pH, and aspect (color, precipitate) was assessed every 3 days.

#### **Real time stability testing**

For stability testing the nasal sprays for Project II (Preparations 1a, 2, 4, 5) were stored at room temperature for 6 months and the nasal sprays for Project III (Preparations 1b and 3) were stored at room temperature for 10 months.

Aspect (visual), pH (pH Meter 780, Metrohm AG, Herisau AR, Switzerland), tonicity (The Advanced™ Micro Osmometer Model 3300, Norwood, MA, USA), and midazolam content (HPLC, see Table 5-1), were analyzed according to the specifications (Appendix 10.2.4 to 10.2.9) of the investigational products.



## 5.3 Results

### 5.3.1 Solubility studies

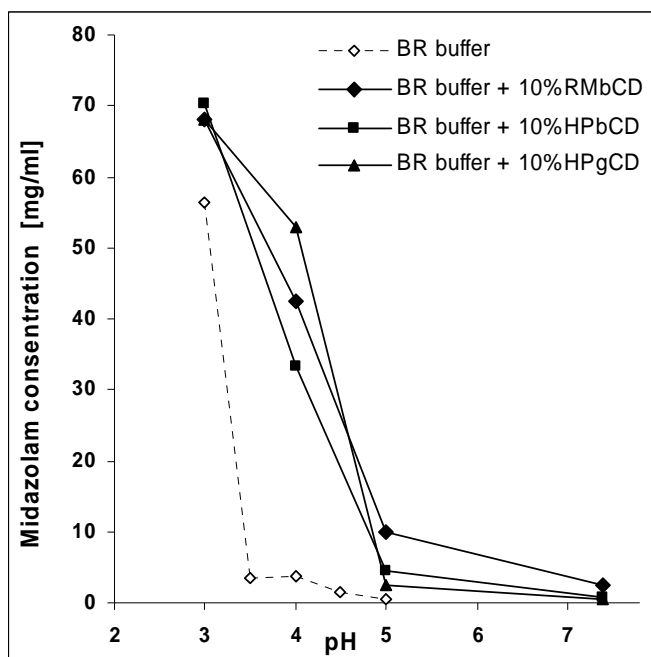


Figure 5-2: pH-dependent solubility of midazolam. In BR buffer (40 mM boric acid, 40 mM acetic acid, 40 mM phosphoric acid; pH, adjusted with 1.0 M sodium hydrochloride) with and without solubilizer (10% RMβCD, 10% HPβCD, and 10% HPγCD).

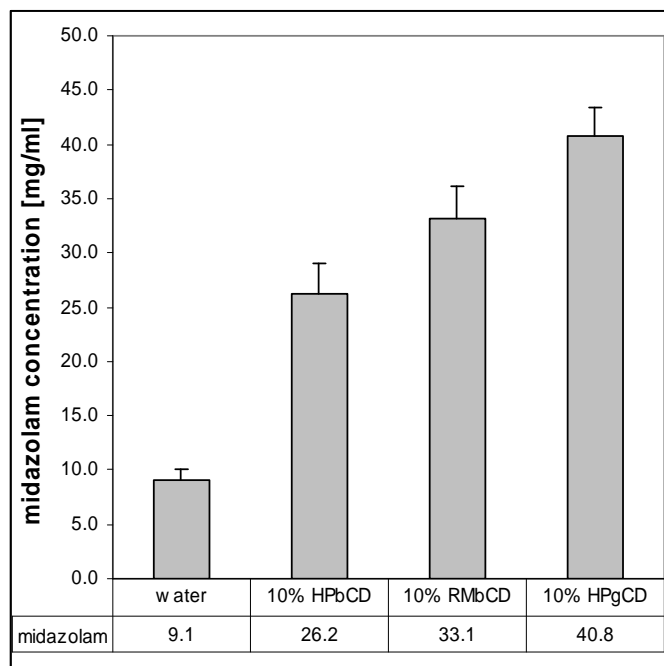
Figure 5-2 shows pH-dependent solubility of midazolam in BR buffer and in BR buffer with 10% RMβCD, 10% HPβCD, and 10% HPγCD.

All tested cyclodextrin derivatives enhance midazolam solubility, but not independent of pH.

In Table 5-2 maximal midazolam concentrations in BR buffer and BR buffer with 10% RMβCD, 10% HPβCD, and 10% HPγCD are listed.

Table 5-2: Midazolam solubilized with 10% RMβCD, 10% HPβCD, and 10% HPγCD.

pH	Midazolam hydrochloride solubility (mg/ml) in:			
	RB buffer (pH 3.5)	+ 10% RMβCD	+ 10% HPβCD	+ 10% HPγCD
3.0	56.4	68.1	70.3	68.0
3.5	3.4	---	---	---
4.0	3.8	42.4	33.3	52.8
4.5	1.5	---	---	---
5.0	0.5	10.0	4.4	2.6
7.4	---	2.53	0.8	0.6

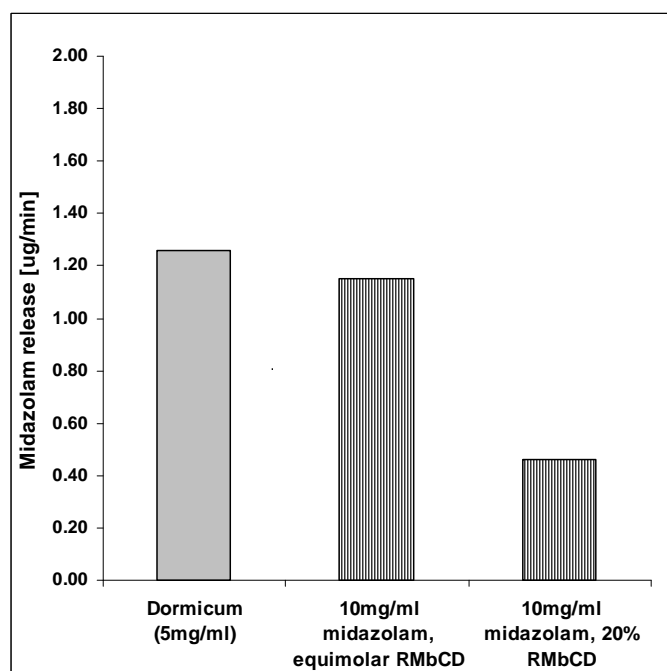


**Figure 5-3: Saturated midazolam concentration in water, 10% RMβCD, 10% HPβCD, and 10% HPγCD (n=3).**

Midazolam solubility was 9.1 mg/ml, 26.2 mg/ml, 33.1 mg/ml, and 40.8 mg/ml in water, 10% HPβCD, 10% RMβCD, and 10% HPγCD, respectively.

The calculated ratio of cyclodextrin and midazolam in the saturated solutions was 1 to 1.13, 1 to 1.33, and 1 to 1.97 for HPβCD-midazolam, RMβCD-midazolam, and HPγCD-midazolam complexes, respectively. Figure 5-3 displays the solubility of midazolam without and with solubilization enhancer (10% HPβCD, 10% RMβCD, and 10% HPγCD).

### 5.3.2 Drug Release



**Figure 5-4: Midazolam Release from Dormicum® (5 mg/ml midazolam), 10 mg/ml midazolam solubilized with equimolar amount of RMβCD (4%) and 10 mg/ml midazolam solubilized with 20% RMβCD.**

Midazolam release in preliminary drug release studies was 1.258 μg/min, 1.150 μg/min, and 0.461 μg/min from Dormicum® (5 mg/ml midazolam), 10 mg/ml midazolam solubilized with equimolar amount of RMβCD (4%) and 10 mg/ml midazolam solubilized with 20% RMβCD, respectively.

Midazolam release did not correlate with the total midazolam concentration. In Figure 5-4 the results of the preliminary drug release study is displayed.

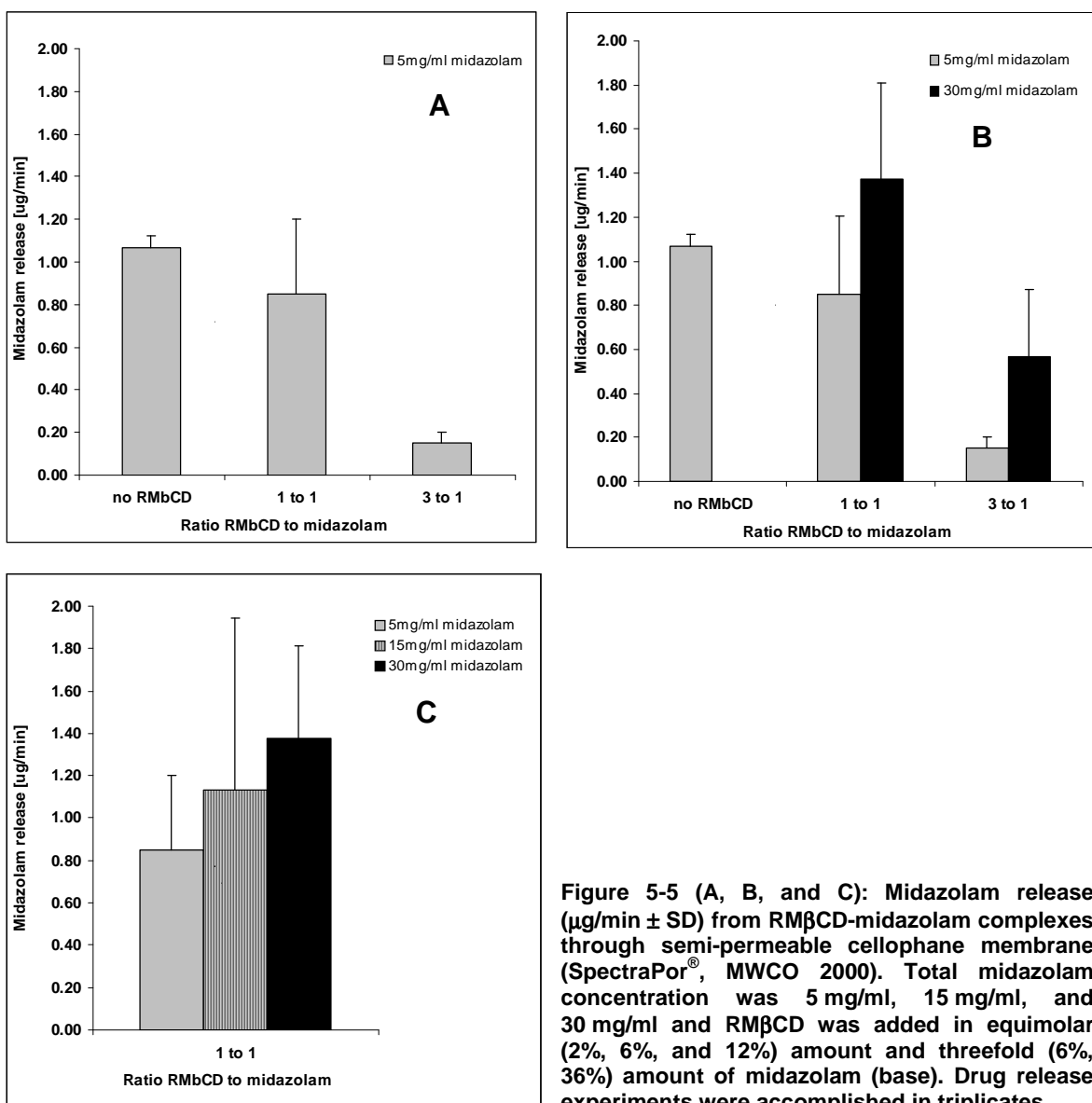


Figure 5-5 (A, B, and C): Midazolam release ( $\mu\text{g}/\text{min} \pm \text{SD}$ ) from  $\text{RM}\beta\text{CD}$ -midazolam complexes through semi-permeable cellophane membrane (SpectraPor<sup>®</sup>, MWCO 2000). Total midazolam concentration was 5 mg/ml, 15 mg/ml, and 30 mg/ml and  $\text{RM}\beta\text{CD}$  was added in equimolar (2%, 6%, and 12%) amount and threefold (6%, 36%) amount of midazolam (base). Drug release experiments were accomplished in triplicates.

Figure 5-5 (A-C) show the midazolam release from preparations with and without solubilization enhancer ( $\text{RM}\beta\text{CD}$ ). Midazolam release does not correspond to the total midazolam concentration available in the donor compartment. With increasing excess of  $\text{RM}\beta\text{CD}$ , midazolam release decreases.

Table 5-3 and Figure 5-5 summarize midazolam release from RM $\beta$ CD-midazolam complexes assessed by permeation experiments with semi-permeable cellophane membranes (SpectraPro<sup>®</sup>, MWCO 2000).

**Table 5-3: Midazolam release ( $\mu\text{g}/\text{min} \pm \text{SD}$ ) from RM $\beta$ CD-midazolam complexes. Total midazolam concentration was 5 mg/ml, 15 mg/ml, and 30 mg/ml and RM $\beta$ CD was added in equimolar amount and threefold amount of midazolam (base). Drug release experiments were accomplished in triplicates**

Total midazolam concentration	Ratio RM $\beta$ CD to midazolam		
	No RM $\beta$ CD	1 to 1	1 to 3
5 mg/ml	$1.067 \pm 0.058 \mu\text{g}/\text{min}$	$0.850 \pm 0.354 \mu\text{g}/\text{min}$	$0.150 \pm 0.050 \mu\text{g}/\text{min}$
15 mg/ml	---	$1.133 \pm 0.808 \mu\text{g}/\text{min}$	---
30 mg/ml	---	$1.375 \pm 0.436 \mu\text{g}/\text{min}$	$0.567 \pm 0.306 \mu\text{g}/\text{min}$

Midazolam release decrease with addition of RM $\beta$ CD and is  $1.067 \pm 0.058 \mu\text{g}/\text{min}$ ,  $0.850 \pm 0.354 \mu\text{g}/\text{min}$ , and  $0.150 \pm 0.050 \mu\text{g}/\text{min}$  for midazolam 5 mg/ml, 5 mg/ml with equimolar RM $\beta$ CD, and 5 mg/ml with threefold molar amount of RM $\beta$ CD, respectively.

For 30 mg/ml observed midazolam release was  $1.375 \pm 0.436 \mu\text{g}/\text{min}$  and  $0.567 \pm 0.306 \mu\text{g}/\text{min}$  for 30 mg/ml midazolam with equimolar RM $\beta$ CD, and 30 mg/ml with threefold molar amount of RM $\beta$ CD, respectively. The observed midazolam release was not proportional to the total midazolam concentration present in the donor compartment. Half of the midazolam concentration (15 mg/ml midazolam solved with equimolar amount of RM $\beta$ CD) did not result in half of midazolam release compared with the release of 30 mg/ml midazolam solved with equimolar amount of RM $\beta$ CD.

Midazolam release did not correlate with the total midazolam concentration in the donor compartment. Three fold amount of RM $\beta$ CD did considerably reduce midazolam release for both tested (total) midazolam concentrations 5 mg/ml and 30 mg/ml.

### 5.3.3 Specification and stability testing

Table 5-4 shows the composition of the preparations provided for Project II and III. All preparations are aqueous solutions and osmolality is adjusted with sodium chloride to 300mOsmol/kg. Table 5-5 displays the specification of all midazolam nasal sprays prepared for Project II and Project III. For all investigational products a shelf life of 6 months was proposed.

**Table 5-4: Preparations for nasal midazolam delivery provided for Project II and Project III. All preparations are aqueous solutions and osmolality is adjusted to 300mOsmol/kg with sodium chloride.**

Nr.	Preparation	Midazolam (base)	RM $\beta$ CD	Chitosan HCl	Preservatives	Dose (Volume)	Device <sup>1</sup>	Project
1a	Midazolam UD Nasal Spray 0.5 mg	5 mg/ml	---	---	---	1 mg (2 x 0.1 ml)	UD	II
1b	Midazolam MD Nasal Spray 5 mg/ml	5 mg/ml	---	---	Yes <sup>2</sup>	1 mg (2 x 0.1 ml)	MD	III
2	Cyclodex-Midazolam UD Nasal Spray 0.5 mg	5 mg/ml	2%	---	---	1 mg (2 x 0.1 ml)	UD	II
3	Cyclodex-Midazolam UD Nasal Spray 1 mg	10 mg/ml	4%	---	---	1 mg (0.1 ml)	UD	II / III
4	Cyclodex-Midazolam UD Nasal Spray 3 mg	30 mg/ml	12%	---	---	3 mg (0.1 ml)	UD	II
5	Chitosan-CD-Midazolam UD Nasal Spray 3 mg	30 mg/ml	12%	0.5%	---	3 mg (0.1 ml)	UD	II

<sup>1</sup> UD: unit dose system, MD: multidose nasal spray

<sup>2</sup> benzalconium chloride and sodium EDTA

**Table 5-5: Specification of the clinical test samples.**

Nr.	Preparation	Aspect	Identity Midazolam	Content [mg/ml]	pH	Osmolality [mOsmol/kg]
1a	Midazolam UD Nasal Spray 0.5 mg	Clear, colorless solution	conform	4.50-5.50	3.0-3.5	270-330
1b	Midazolam MD Nasal Spray 5 mg/ml	Clear, colorless solution	conform	4.50-5.50	3.0-3.5	270-330
2	Cyclodex-Midazolam UD Nasal Spray 0.5 mg	Clear, colorless solution	conform	4.50-5.50	3.5-4.5	270-330
3	Cyclodex-Midazolam UD Nasal Spray 1 mg	Clear, colorless solution	conform	9.00-11.0	3.5-4.5	270-330
4	Cyclodex-Midazolam UD Nasal Spray 3 mg	Clear, colorless solution	conform	27.0-33.0	3.0-4.5	270-330
5	Chitosan-CD-Midazolam UD Nasal Spray 3 mg	Clear, colorless solution	conform	27.0-33.0	3.0-4.5	270-330

### Accelerated stability testing

In Preparation 3 (10 mg/ml) midazolam content was constant and no precipitation or discoloring occurred during storage at room temperature 40°C, 50°C, and 60°C, for 30 days.

In Preparation 4 (30 mg/ml) midazolam content was constant and no precipitation occurred during storage at room temperature 40°C, 50°C, and 60°C, for 30 days. At storage temperature of 60°C Preparation 4, originally colorless solution turned slightly yellow. For all storage conditions pH was constant in both preparations (Preparation 3 and 4), the measured values are listed in Appendix 10.2.2 and 10.2.3.

### Real time stability testing

To perform real-time stability testing Preparation 1 and 3 were stored for 10 months and Preparations 2, 4, and 5 were stored for 6 months at room temperature. In Table 5-5 the specification of Preparation 1a to 5 are listed. To fulfill the stability requirements all analyzed parameters had to comply with the specified limits. In Table 5-6 and Table 5-7 the outcome of stability testing of 6 months and 10 months, respectively is summarized. Stability reports, including original data, are shown in Appendix 10.2.10 to 10.2.15.

Acquired stability data confirm specified shelf life of 6 months for Preparation 1a, 1b, 2, 3, and 4 but not for Preparation 5. After 10 months storage at room temperature Preparation 3 fulfills all specified quality requirements.

**Table 5-6: Outcome of stability testing: 6 months**

Nr.	Preparation	Aspect	Identity Midazolam	Content [mg/ml]	pH	Osmolality [mOsmol/kg]	Shelf life 6 months
1a	Midazolam UD Nasal Spray 0.5 mg	✓	✓	✓	✓	✓	<b>o.k.</b>
1b	Midazolam MD Nasal Spray 5 mg/ml	✓	✓	✓	✓	✓	<b>o.k.</b>
2	Cyclodex-Midazolam UD Nasal Spray 0.5 mg	✓	✓	✓	✓	✓	<b>o.k.</b>
3	Cyclodex-Midazolam UD Nasal Spray 1 mg	✓	✓	✓	✓	✓	<b>o.k.</b>
4	Cyclodex-Midazolam UD Nasal Spray 3 mg	✓	✓	✓	✓	✓	<b>o.k.</b>
5	Chitosan-CD-Midazolam UD Nasal Spray 3 mg	✓	✓	↓	✓	✓	<b>not o.k.</b>

✓ analyzed parameter fulfills specification  
↓ analyzed parameter below specified limits

**Table 5-7: Outcome of stability testing: 10 months**

Nr.	Preparation	Aspect	Identity Midazolam	Content [mg/ml]	pH	Osmolality [mOsmol/kg]	Shelf life 10 months
1b	Midazolam MD Nasal Spray 5 mg/ml	✓	✓	✓	↑	✓	<b>not o.k.</b>
3	Cyclodex-Midazolam UD Nasal Spray 1 mg	✓	✓	✓	✓	✓	<b>o.k.</b>

✓ analyzed parameter fulfills specification  
↑ analyzed parameter above specified limits

## 5.4 Discussion

Different cyclodextrin derivatives were evaluated to solubilize midazolam for development of preparations for transmucosal nasal midazolam delivery. Drug release studies were performed to assess the effect of the selected solubilizer (RM $\beta$ CD) on midazolam release *in vitro*. Furthermore, accelerated and long term stability testing was performed.

Besides presystemic drug elimination (intestinal and hepatic first-pass metabolism), the most important parameters affecting bioavailability are aqueous solubility and permeability of the drug. The Biopharmaceutical Classification System (BCS) was introduced by the FDA as binning system for oral drugs, and classifies all drugs into four groups based on their ability to permeate biologic membranes and their aqueous solubility. A given drug substance is considered 'highly soluble' when the highest dose strength is soluble in  $\leq 250$  ml water, and 'highly permeable' when the extent of oral absorption in humans is determined to be  $\geq 90\%$  of an administered dose (in solution). According to this definition, midazolam salts are rated as 'highly soluble', because the highest oral dose strength (15 mg midazolam) is soluble in  $\leq 250$  ml water. For transmucosal nasal drug delivery the definition of 'high' and 'poor' solubility has to be adjusted to the characteristics of the nasal cavities. Obviously, in the nose there is only very little solvent available to solubilize administered drugs. Therefore, drugs preferably are delivered as solutions. Since the capacity of the nasal cavities is limited, delivery of extended volumes results in swallowing of surplus liquid preparations. Consequently, minimizing of nasally instilled volumes is essential to provide for exclusive transmucosal nasal drug uptake. In transmucosal nasal drug delivery, drugs may be rated as 'highly soluble' if the highest dose to be nasally delivered is soluble in  $\leq 0.2$  ml aqueous solutions. According to this definition, for transmucosal nasal delivery midazolam is to be classified as poorly soluble. Transmucosal nasal drug delivery is particularly feasible for drugs with high potency. Furthermore, for efficient transmucosal nasal drug delivery the compound needs to be 'highly permeable'. Overall ideal characteristics of drugs for transmucosal nasal delivery are: high solubility, high permeability and adequate potency.

Since systemic bioavailability of transmucosal nasal absorbed midazolam is not affected by presystemic metabolization (hepatic first-pass effect), the doses to be nasally administered are lower than doses for oral application. Midazolam proved excellent permeation ability [Gudmundsdottir, et al. 2001], therefore, the key issue in compounding nasal preparations for transmucosal nasal midazolam delivery is the relatively poor and highly pH-dependent solubility of midazolam.

Different approaches to enhance midazolam solubility have been described in literature. Knoester et al. proposed a nasal midazolam preparation with 26% propylene glycol, but the administration of this hypertone preparation resulted in local irritation [Knoester, et al. 2002]. Less local irritation has been reported for nasal administration of midazolam preparations with cyclodextrins as solubilizer [Gudmundsdottir, et al. 2001]. Therefore, in the presented project different cyclodextrin derivatives were evaluated to solubilize midazolam in preparations for nasal midazolam delivery. To prevent

local irritation, preparations for nasal administration should as far as possible be adapted to the intranasal physiology concerning pH as well as tonicity.

In aqueous solutions cyclodextrin derivatives spontaneously form water-soluble complexes with a wide range of compounds. Since increased compound solubility stands for effectual complexation, verification of cyclodextrin-midazolam complexes was not performed. In order to successfully generate midazolam solutions, by complexation with different cyclodextrin derivatives (RM $\beta$ CD, HP $\beta$ CD, and HP $\gamma$ CD), it was essential to solubilize cyclodextrins first and then to add midazolam hydrochloride. Hardly soluble agglomerates resulted by inverting the procedure (adding cyclodextrins to midazolam suspensions).

All tested cyclodextrins (RM $\beta$ CD, HP $\beta$ CD, and HP $\gamma$ CD) improved the solubility of midazolam in aqueous solutions. Despite successful complexation with RM $\beta$ CD, HP $\beta$ CD, or HP $\gamma$ CD midazolam solubility remained pH-dependent and above pH 4 to 5 midazolam precipitated.

Despite superior solubilization ability of 10% HP $\gamma$ CD (midazolam concentration 40.8 mg/ml) compared to RM $\beta$ CD (midazolam concentration 33.1 mg/ml solubilized with 10% RM $\beta$ CD), RM $\beta$ CD was selected to compound preparations for nasal midazolam delivery, because RM $\beta$ CD is adjuvant in Aerodiol<sup>®</sup> (Servier, Meyrin, Switzerland) and approved by the authorities of several European countries.

The calculated ratio of different cyclodextrin derivatives and midazolam (1 to 1.13, 1 to 1.33, and 1 to 1.97 for saturated midazolam solutions with 10% RM $\beta$ CD, 10% HP $\beta$ CD, and 10% HP $\gamma$ CD, respectively) indicates that in saturated midazolam solutions not only 1 to 1 complexes are formed. The 1 to 1.97 ratio calculated for HP $\gamma$ CD-midazolam complexes implies the formation of 1 to 2 complexes (one HP $\gamma$ CD molecule complexes two midazolam molecules). But complex characteristics can not conclusively be described by calculated ratios of cyclodextrin and solubilized compound.

The effect of cyclodextrins on drug release, absorption kinetics, and consequently bioavailability of transmucosal delivered drugs is controversially discussed. Based on Fick's first law, the fundamental equation describing passive diffusion is represented by Equation 1:

$$J = P \cdot A \cdot (c_1 - c_2)$$

(Equation 1)

Where J is the resulting drug flux through the membrane, P is the permeability coefficient of the drug through the membrane (characterizing the ability of a given drug to permeate a specific membrane),  $c_1$  refers to the drug concentration in the donor compartment,  $c_2$  refers to the drug concentration in the receptor compartment, and A is the area of the membrane available for drug flux. As usually  $c_2 \ll c_1$ , only  $c_1$  is itemized (see Equation 2).

$$J = P \cdot A \cdot c_1$$

(Equation 2)



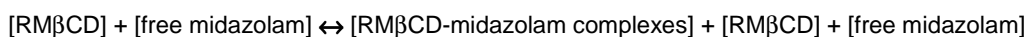
Assuming constant permeability coefficient (identical membrane and drug) and indifferent area, doubling the drug concentration in the donor compartment ( $2 \cdot c_1$ ) results in doubled drug flux ( $2 \cdot J$ ), according to Equation 2. Generally, Equation 1 and 2 describe passive diffusion (*in vitro* and *in vivo*) of any substance (e.g., drug, adjuvant) through any membrane (e.g., biomembrane or artificial membrane), for *in vitro* and *in vivo* situations.

Roelofse et al. published pharmacokinetic characterization of nasal midazolam (dose 7.5 mg) delivered by a test preparation (20%  $\beta$ CD to solubilize 10 mg/ml midazolam, nasal administration volume 0.75ml) or Dormicum<sup>®</sup> 5 mg/ml (nasal administration volume 1.5 ml) [Roelofse, et al. 2000]. The maximal midazolam plasma concentration and the systemic bioavailability were considerably lower for midazolam delivered by the test preparation (20%  $\beta$ CD, 10 mg/ml midazolam).

Based on the solubility study, the published data from Loftsson et Jarho [Loftsson 1995, Jarho, 1996], and the outcome of the *in vivo* study of Roelofse et al., the excess of cyclodextrin was supposed to reduce midazolam release. To confirm this assumption (*in vitro*) midazolam release studies were performed with preparations containing 20% RM $\beta$ CD and 4% RM $\beta$ CD (equimolar amount) to solubilize 10 mg/ml midazolam and compared with midazolam release from Dormicum<sup>®</sup> 5 mg/ml (no RM $\beta$ CD). Despite half midazolam concentration, midazolam release from Dormicum<sup>®</sup> 5 mg/ml was superior to midazolam release from RM $\beta$ CD containing preparations (total midazolam concentration 10 mg/ml). Furthermore, midazolam release from preparations with 20% RM $\beta$ CD (10 mg/ml midazolam) was lower as midazolam release from preparation with equimolar RM $\beta$ CD (4%) to solubilize 10 mg/ml midazolam.

In further drug release studies with semi-permeable cellophane membranes (*in vitro*) the effect of RM $\beta$ CD on midazolam release was investigated with 5 mg/ml, 15 mg/ml, and 30 mg/ml midazolam solubilized with equimolar and threefold amounts of RM $\beta$ CD. Due to limited midazolam solubility drug release studies without RM $\beta$ CD were performed only for 5 mg/ml midazolam.

Midazolam release from RM $\beta$ CD containing preparations did not correlate with the absolute midazolam concentration in the donor compartment (5 mg/ml, 15 mg/ml, and 30 mg/ml). The impaired midazolam release from preparations with higher RM $\beta$ CD concentrations (ratio RM $\beta$ CD to midazolam, 3 to 1) indicated that less free midazolam molecules are available for permeation. According to the law of mass action, high concentration of one educt (i.e., RM $\beta$ CD) promotes the formation of the product (i.e., RM $\beta$ CD-midazolam complexes), see Equation 3. In addition, huge excess of cyclodextrins is reported to promote non-conventional cyclodextrin complexes (i.e., non-inclusion complexes) and molecular aggregation [Loftsson, et al. 2005], consequently reducing free midazolam concentration available for permeation.



(Equation 3)

$$J = P \cdot A \cdot (C_{\text{free midazolam}})$$

**(Equation 4)**

Midazolam release (*in vitro*) did not correlate with the total midazolam concentration in the donor compartment; consequently Equation 2 does not exactly correspond with data assessed in the drug release studies. The results of the drug release studies suggest that only free midazolam concentration controls permeation through the artificial membrane (see Equation 4). The more RM $\beta$ CD is available for midazolam complexation, the more midazolam release was reduced. Consequently, to minimize the influence of RM $\beta$ CD on midazolam release *in vivo*, equimolar amount of RM $\beta$ CD was used to solubilize midazolam in preparations for nasal midazolam delivery. The pH of the developed preparations (Preparation 1 to 5) is not adjusted to physiologic pH. In the nasal preparation proposed by Roelofse et al. (ratio cyclodextrin to midazolam was 5 to 1, for 20%  $\beta$ CD to solubilize 10 mg/ml midazolam) pH was 5.8. The above discussed drug release studies are in accordance with the outcome of *in vitro* investigations of Loftsson et Jarho [Loftsson 1995, Jarho, 1996] and underline the importance of adequate cyclodextrin-compound ratio. Therefore, cyclodextrin excess to solubilize midazolam was considered as inappropriate; even pH could have been approached to physiologic pH.

Results of accelerated stability testing affirmed stability of midazolam solubilized with RM $\beta$ CD. Proven stability of midazolam enables the storage of the tested midazolam preparations at room temperature. For all midazolam preparations with RM $\beta$ CD (Preparation 2, 3, and 4) and without RM $\beta$ CD (Preparation 1a and 1b) stability data confirmed shelf life of 6 months. Preparation 5, additionally containing chitosan hydrochloride, was not stable concerning midazolam content. During 6 months storage at room temperature midazolam content decreased from originally 29.5 mg/ml to 25.1 mg/ml, not complying with the specified requirements for midazolam content. Apparently, chitosan hydrochloride impaired midazolam stability. The preparation has to be prepared immediately before use. Concerning pH, after 10 months storage Preparation 1b does not comply with the specified limits for pH (3.0 to 3.5). As with arising pH midazolam risks to precipitate, prolongation of shelf life for Preparation 1b is not reasonable. For Preparation 3 prolongation of shelf life from 6 months to at least 10 months is supported by long term stability data.

## 5.5 Conclusions

Nasal midazolam preparations, adjusted to the limited volumetric capacity of the nose, were compounded by addition of RM $\beta$ CD. In drug release studies with semi-permeable cellophane membranes (*in vitro*) RM $\beta$ CD reduced midazolam release. The reliability of *in vitro* drug release studies to predict drug release and absorption kinetics *in vivo* is to be verified (Project II).

The stability data acquired for all RM $\beta$ CD containing nasal midazolam preparations affirm a shelf life of at least 6 months. Addition of chitosan hydrochloride impaired midazolam stability and therefore shelf life of chitosan containing nasal midazolam preparations is reduced.

The developed preparations for transmucosal nasal midazolam delivery are the basis to study the influence of vehicle and application modality on the pharmacokinetics of nasally applied midazolam (see Project II).



## 6 Project II: Pharmacokinetic of transmucosal nasal delivered midazolam – impact of adjuvants

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### 6.1 Introduction

Fast onset of action, complete independence of ingestion, and the needle-less application render nasal application of midazolam to a convenient alternative to common application modes [Bjorkman, et al. 1997; Goepfert 1996; Malinovsky, et al. 1995; Münte 2002; Tschirch, et al. 2006; Uygur-Bayramicli, et al. 2002]. Although no nasal midazolam preparation is commercially available, numerous authors report on successful nasal delivery of midazolam. In most studies and case reports, Dormicum® for i.v. application is instilled nasally. Often the volumetric capacity of the nose (0.1 ml to 0.4 ml per nostril [Penkler 2001]) is exceeded and therefore, a portion of the nasal administered preparation is swallowed. Consequently, the resulting pharmacokinetic profile is a combination of nasal and gastrointestinal drug absorption.

To minimize the volume to be applied for nasal delivery of midazolam, preparations with higher concentration than 5 mg/ml midazolam need to be provided. Addition of solubilization enhancers (e.g., cyclodextrins) facilitates compounding of midazolam preparations with increased midazolam concentrations; this allows delivering therapeutic doses by instilling minimized volumes into nasal cavities.

The solubilization enhancing effect of cyclodextrins, by forming inclusion complexes, is well established (see chapter 4.3 and Project I). However, the impact of cyclodextrins on pharmacokinetics of transmucosal delivered drugs is controversially discussed [Illum 2002] and is assumed to depend on the drug cyclodextrin ratio, as well as on the solubility and the permeation ability of the compound.

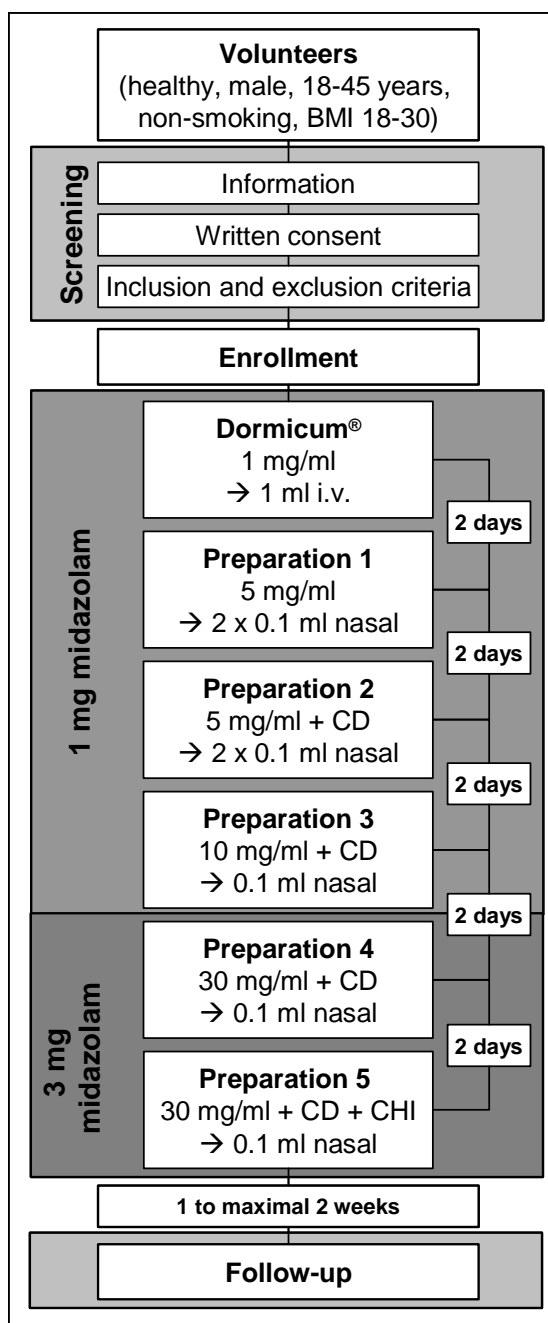
Mucociliary clearance restricts contact time of nasal administered preparations and nasal mucosa; consequently the period for transmucosal nasal drug absorption is limited. For efficient drug uptake, absorption promoting or bioadhesive excipients are added to preparations for transmucosal nasal drug delivery. Chitosan features both, bioadhesion and absorption promoting (see chapter 4.4.3). In addition, Zerrouk et al. showed the synergistic effect of cyclodextrins and chitosan on drug absorption [Zerrouk et al., 2006].

The aim of this clinical trial was to characterize the impact of the vehicle (RM $\beta$ CD and chitosan hydrochloride) and the application modality (e.g., one-sided versus two-sided nasal administration) on pharmacokinetics of transmucosal nasal delivered midazolam. Pharmacokinetic and pharmacodynamic parameters of i.v. delivered midazolam (Dormicum®, Roche) and nasally applied midazolam (5 different preparations) were assessed in healthy volunteers.

## 6.2 Methods

According to the study protocol (see Appendix 10.3.1) non-smoking, healthy volunteers (all men) age 18 years to 45 years and body mass index of 18 kg/m<sup>2</sup> to 30 kg/m<sup>2</sup> were recruited for this open-label trial accomplished at the University Hospital of Basel (for study design see Figure 6-1). Before enrollment, volunteers were conclusively informed about the study protocol and all subjects gave written consent.

All interested volunteers then underwent a medical screening procedure, including physical examination, medical history, clinical laboratory testing, and electrocardiogram, according to the protocol (see Appendix 10.3.2 to 10.3.4). Subjects with acute or chronic nasal symptoms, clinically significant previous nasal surgery, nasal trauma or polyps, or any systemic illness were excluded from participation. Only volunteers complying with all inclusions criteria were enclosed. The volunteers were fasting and abstaining from beverages containing caffeine from midnight and until four hours after administration of study medication. Sugar free beverages were allowed. Volunteers received six different treatments (i.v., Dormicum®, Roche and nasal Preparation 1 to 5). All preparations for nasal administration (Preparation 1 to 5) are described in Chapter 1. For nasal delivery unit dose nasal spray (Pfeiffer, GmbH Radolfzell), delivering 0.1 ml, were used. In Table 6-1 the applied preparations, midazolam concentrations, vehicles, the administered doses, and the application modality are specified. Two days washout was scheduled between midazolam administrations.



**Figure 6-1: Study design for pharmacokinetic characterization of nasally applied midazolam. Administration of study medication was separated by two days washout (CD: RMβCD, CHI: chitosan hydrochloride).**

**Table 6-1: Specification, midazolam concentrations, vehicles, administered doses, and application modality of the preparations administered to healthy volunteers for pharmacokinetic characterization of nasally applied midazolam.**

Preparation Nr.	Specification	RM $\beta$ CD	Chitosan HCl	Midazolam (base)	Dose	Delivered volume, one-sided (0.1 ml) or two-sided (2 x 0.1 ml)
---	Dormicum <sup>®</sup> , Roche	---	---	1 mg/ml	1 mg	1.0 ml i.v.
1	Midazolam UD Nasal Spray 0.5 mg	---	---	5 mg/ml	1 mg	2 x 0.1 ml nasal, two-sided
2	Cyclodex-Midazolam UD Nasal Spray 0.5 mg	2%	---	5 mg/ml	1 mg	2 x 0.1 ml nasal, two-sided
3	Cyclodex-Midazolam UD Nasal Spray 1 mg	4%	---	10 mg/ml	1 mg	0.1 ml nasal, one-sided
4	Cyclodex-Midazolam UD Nasal Spray 3 mg	12%	---	30 mg/ml	3 mg	0.1 ml nasal, one-sided
5	Chitosan-CD-Midazolam UD Nasal Spray 3 mg	12%	0.5%	30 mg/ml	3 mg	0.1 ml nasal, one-sided

Hospital Pharmacy of University Hospital Basel produced the nasal preparations for midazolam delivery according to current GMP guidelines. A clinician administered Dormicum<sup>®</sup> 1 mg/ml i.v. (1 ml), on the opposite arm of the indwelling catheter used for blood sampling. Venous blood samples of 7.5 ml were obtained predose, 1, 2.5, 5, 10, 15, 20, 30, and 45 minutes and 1, 2, 3, 4, and 6 hours after midazolam administration. The blood samples were centrifuged at 3000 G for 10 minutes, the resultant serum was stored at -20°C until analysis. Quantification of midazolam and midazolam metabolites ( $\alpha$ -hydroxymidazolam, and 4-hydroxymidazolam) was performed at the Institute of Forensic Medicine of the University Basel, according to the method published by Dussy et al. [Dussy et al., 2005].

Volunteers were asked to describe nasal midazolam administration (indifferent, tolerable, disagreeable, or painful) and to classify local irritation (no irritation, very slight, slight, intermediate, strong, or very strong) 1, 5, 15, 30, and 240 minutes after nasal midazolam administration. In addition, volunteers rated subjective fatigue and drowsiness on a visual analog scale (VAS), a 100 mm non-graduated line, the left end referring to “no pharmacological effect” (no fatigue and/or drowsiness) and the right end referring to “very intense pharmacological effect” (falling asleep). Furthermore, reaction time (RT) and interstimulus interval (ISI) was assessed by a computer-controlled self-adjusting reaction time test (CRTT, BonnDet), developed by Langewitz et al. [Langewitz 1987]. Flashing colored lights had to be answered by pushing a corresponding button. ISI (i.e., working speed) and RT were registered. The computer controlled a fast on-line feedback loop between performance and work speed as to yield a constant failure rate of 50% over 540 stimuli.

Blood pressure, oxygen saturation, and heart rate were monitored. To treat severe adverse reactions flumazenil (Anexate<sup>®</sup>, Roche, Basel) was available.

The local Ethics Committee and the national authorities (Swissmedic) approved the protocol of this pharmacokinetic trial. The study was carried out at Clinical Research Unit of University Hospital Basel according to national regulation and the current Declaration of Helsinki.

Serum concentration time profiles were analyzed with WinNonlin (Version 3.1, Pharsight Corporation, Mountain View, CA, USA) and pharmacokinetic parameters were assessed by applying non-compartmental (for all midazolam applications) and two-compartmental (for nasal administration of 3 mg of midazolam) models. Terminal elimination constant was assessed by logarhythmic-linear regression of terminal part of the concentration time profile. Secondary pharmacokinetic parameters were derived from assessed primary parameters according to standard proceedings.

Bioequivalence was accepted, for mean and 90% confidence interval of the pharmacokinetic parameters (test preparation) lying within the boundaries of 80% (lower boundaries) and 125% (upper boundaries) of the corresponding pharmacokinetic parameter of the reference preparation according to the requirements of EMEA [EMEA 2001].

Normal distribution of parameters was tested, and if adequate analyzed by repeated measures ANOVA, not normal distributed parameters by not parametric testing (Friedman's Test). Statistical analysis was performed by SPSS (Version 13.0 for Windows Microsoft). As level of significance  $p=0.05$  was accepted.



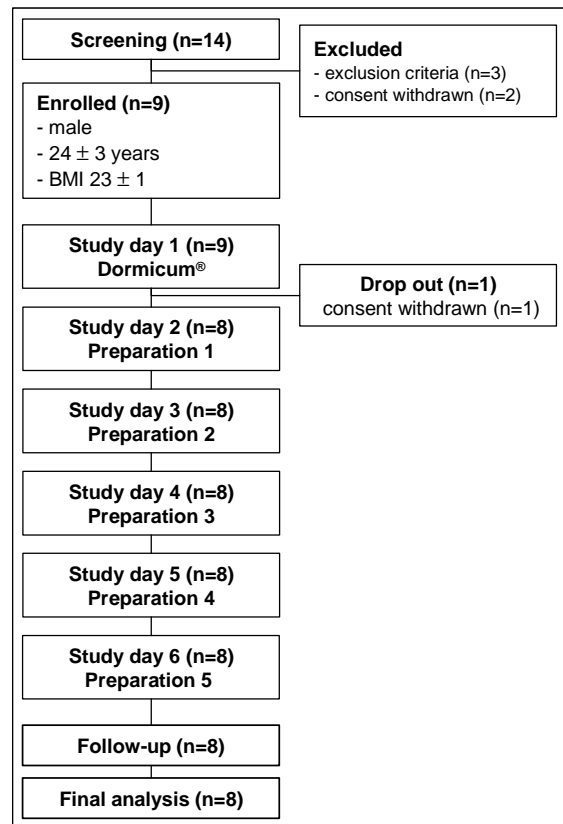
### 6.3 Results

From 14 screened subjects, 3 did not meet the inclusion criteria, and two subjects had withdrawn their consent before participating on a study day. Finally 9 subjects were enrolled into the study. After attending the first study day one volunteer had withdrawn his consent and dropped out.

Demographic characteristics of all volunteers who completed the study are presented in Table 6-2. Figure 6-2 displays subject proceeding during Project II.

**Table 6-2: Demographic characteristics of subjects completing the study (n=8).**

Characteristics	(n=8)
Age, years (SD)	24 (3.2)
Weight, kg (SD)	74 (5.4)
Body height, cm (SD)	180 (4.0)
BMI, kg/m <sup>2</sup> (SD)	23 (1.3)



**Figure 6-2: Subject proceedings during clinical study; screened n=14, excluded (exclusion criteria n=5, withdrawal of consent n=2), enrolled n=9, drop out after study day n=1, completed study n=8.**

Immediately after nasal administration of Preparation 1 to 5 (overall 40 nasal applications), volunteers classified the nasal administration as indifferent, tolerable, disagreeable, or painful. Overall 4 times nasal administration of study medication was classified as indifferent, 26 times as tolerable, and 8 times as disagreeable (see Figure 6-3). Nasal administration of the study medication never was classified as painful. Nasal administration of Preparation 5 most often (4 times) was classified as disagreeable. Local irritation of Preparation 5 set in about 30 seconds to 60 seconds after administration.

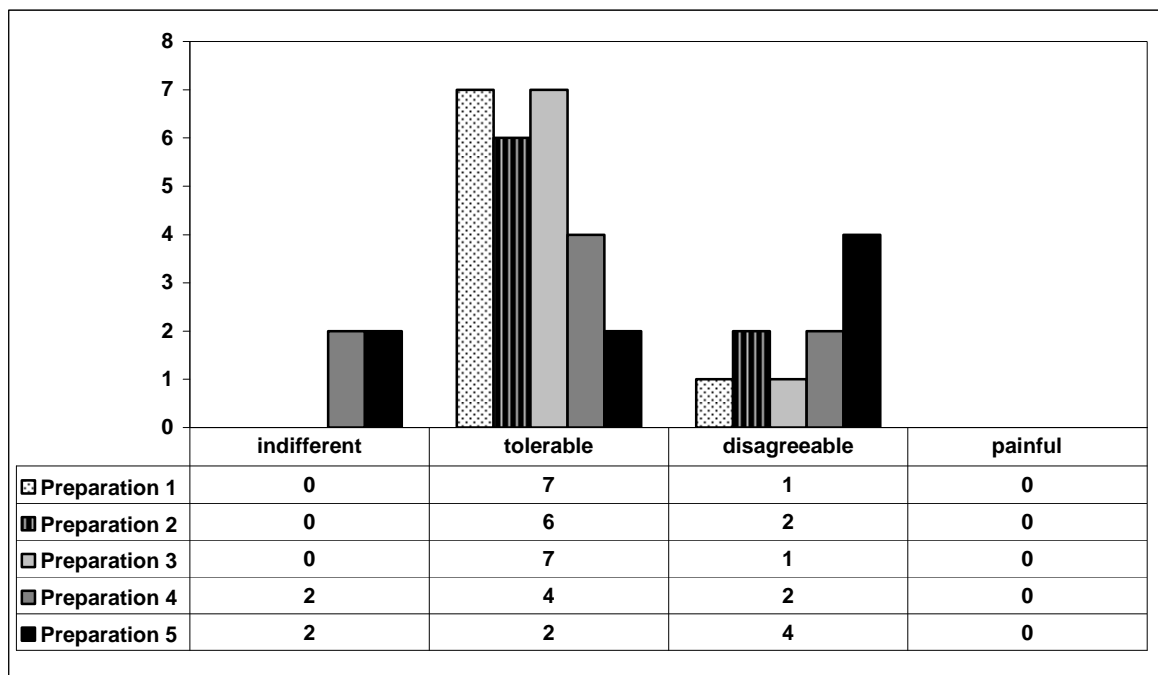


Figure 6-3: Tolerability of nasal administration of Preparation 1 to 5 to 8 healthy volunteers. Immediately after administration volunteers classified the nasal administration of Preparation 1 to 5 as indifferent, tolerable, disagreeable, or painful.

Nasal administration of midazolam (Preparation 1 to 5) caused some irritation in the nose or throat area immediately after administration (see Figure 6-4).

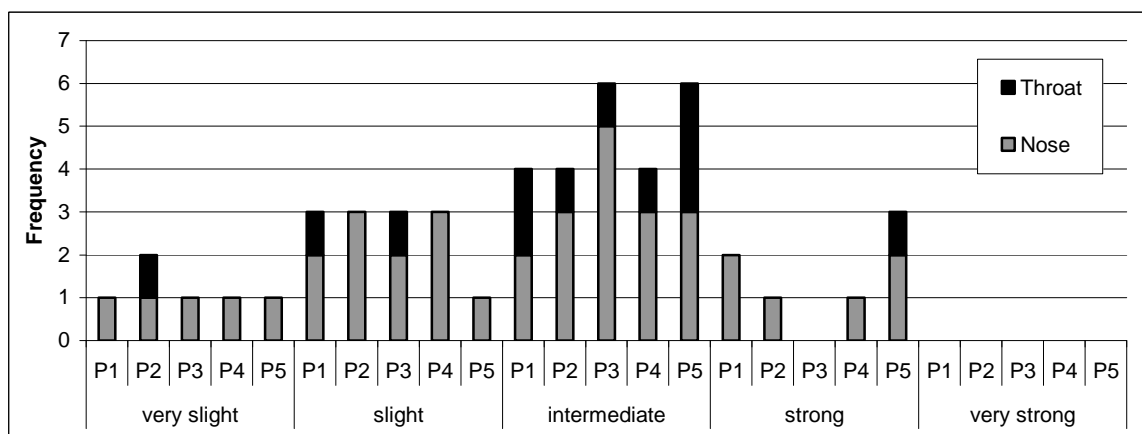


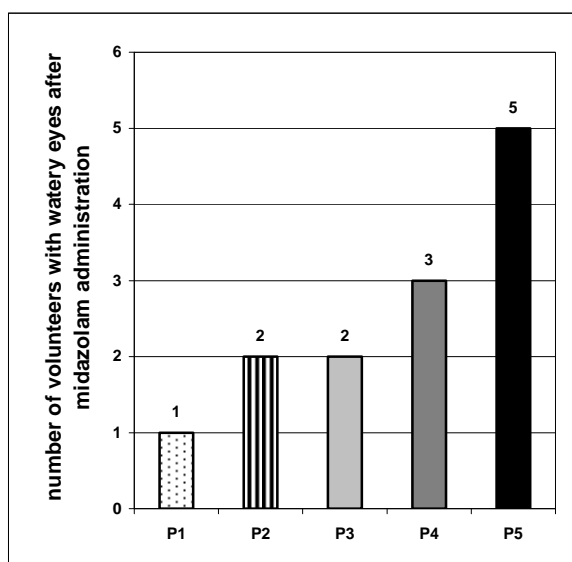
Figure 6-4: Local irritation immediately after nasal administration of Preparation 1 to 5 (P1, P2, P3, P4, and P5). Volunteers (n=8) rated the intensity immediately after nasal administration of the study medication.

Changes of local irritation assessed immediately to 4 h after nasal administration of study medication is shown Appendix 10.3.5. and 10.3.6.

Within 30 minutes after nasal administration (Preparation 1 to 5) nasal irritation was completely reversible. Nasal irritation after administration of Preparation 1, 2, and 3 (nasal delivery of 1 mg

midazolam) decreased within 5 minutes after administration (very slight to slight) and 15 minutes after administration of Preparation 3 only one volunteer indicated very slight nasal irritation.

Irritation in the throat after administration of Preparation 1, 2, and 3 (nasal delivery of 1 mg midazolam) was never rated as strong immediately after administration (very slight, slight or intermediate). Irritation in the throat was once described as strong (scratching in the throat) 15 minutes after administration of Preparation 3. Generally, 15 minutes after nasal administration of 1 mg midazolam local irritation has almost disappeared and later (30 minutes and 4h) volunteers indicated no local irritation at all. Nasal irritation after administration of Preparation 4 and 5 (nasal delivery of 3 mg midazolam) decreased within 15 minutes after administration (very slight to slight) and lasted until 30 minutes after administration of Preparation 4. Preparation 5 caused more irritation in the throat area which lasted until 30 minutes after nasal administration. Four hours after nasal administration of study medication no local irritation was left.

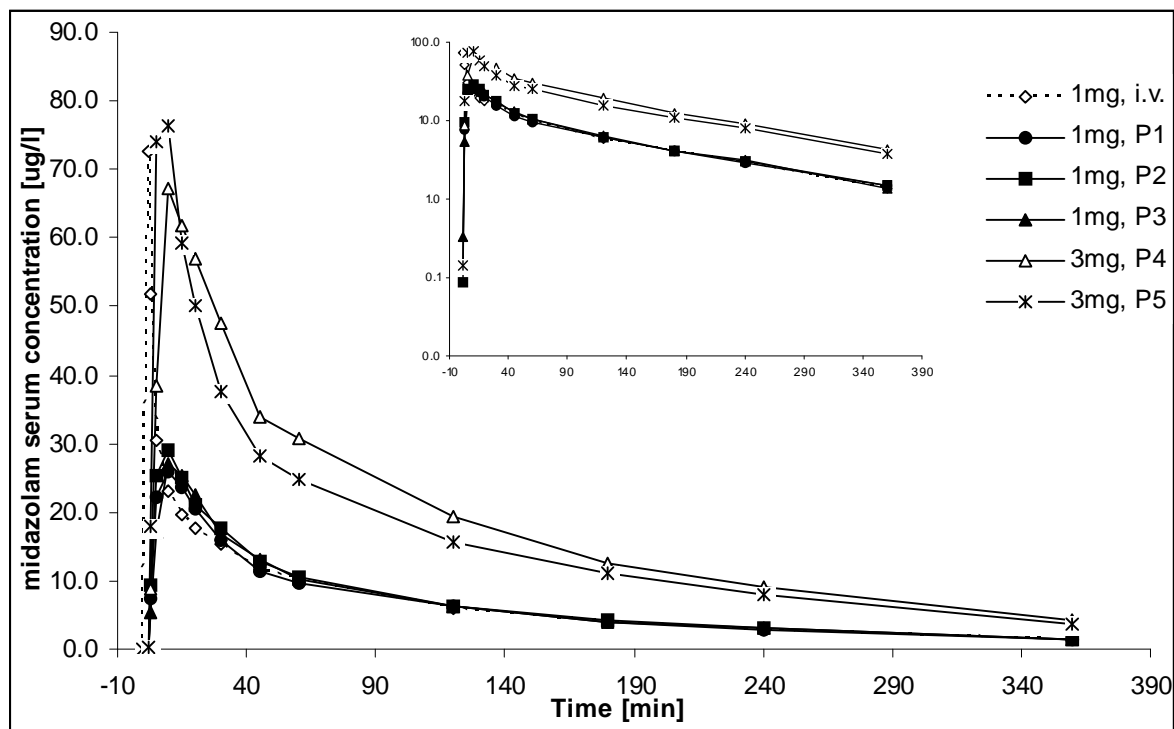


**Figure 6-5: Teary eyes after nasal administration of Preparation 1 to 5 (P1, P2, P3, P4, and P5) to 8 Volunteers (n=8) rated. One-sided nasal administration (P3, P4, and P5) caused teary eye only on the side of administration.**

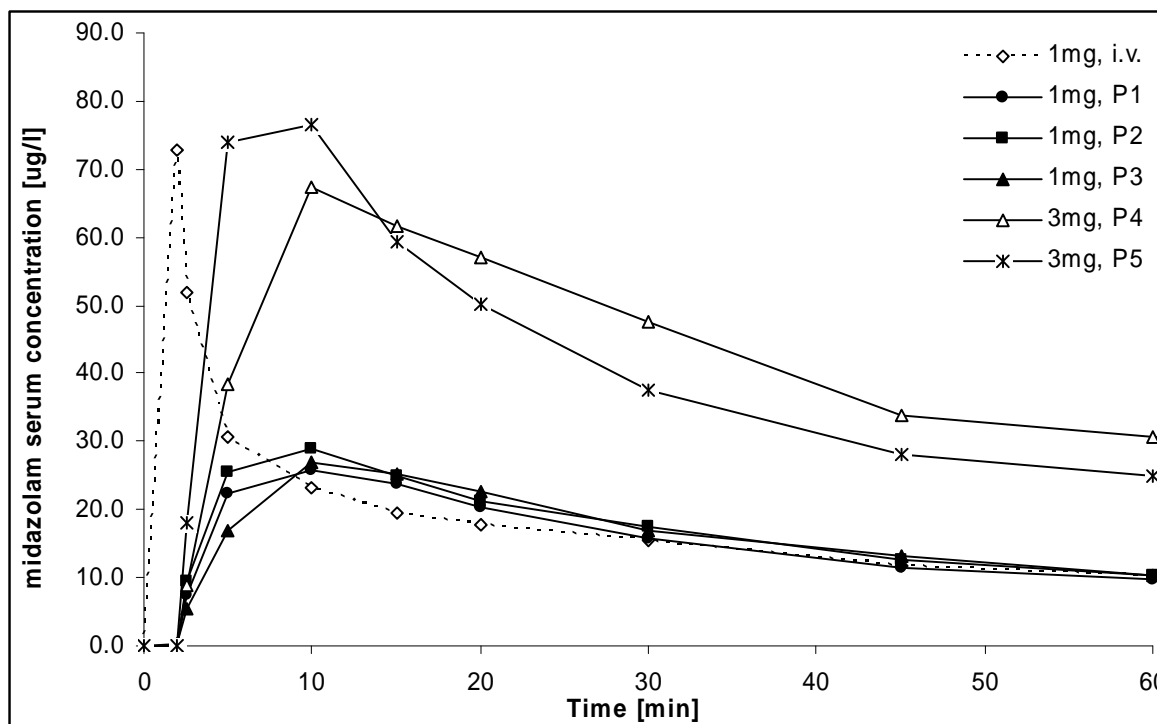
Some subjects complained about teary eyes (see Figure 6-5), bitter or unpleasant taste, or nasal prickling immediately after nasal administration of study medication. One-sided nasal administration of midazolam (Preparation 3, 4, and 5) caused teary eye only on the side of administration.

No relevant changes in blood pressure, heart rate, and oxygen saturation were observed after i.v. and nasal administration of midazolam (1 mg and 3 mg).

Figure 6-6 shows the midazolam serum concentration time profile following i.v. administration of 1 mg midazolam (Dormicum®), nasal administration of 1 mg midazolam (Preparation 1, 2, and 3) and nasal administration of 3 mg midazolam (Preparation 4 and 5). In the elimination phase all midazolam time profiles are parallel (see inserted semilogarithmic concentration time profile in Figure 6-6). Figure 6-7 focuses on midazolam serum concentration time profile until 60 minutes after midazolam administration. Nasal administration of 3 mg midazolam resulted in higher serum concentration levels than nasal administration of 1 mg midazolam. Maximal midazolam serum concentrations following nasal administration of 3 mg midazolam were in the same range as maximal midazolam serum concentrations following i.v. administration of 1 mg midazolam. Midazolam serum concentration following i.v. and nasal application were approximating 10 minutes after administration of 1 mg midazolam. Original data and midazolam serum concentration time profiles of all subjects are displayed Appendix 10.3.7 to 10.3.10.



**Figure 6-6: Midazolam serum concentration (mean) following i.v. and nasal administration of 1 mg midazolam (Preparation 1, 2, and 3) and nasal administration of 3 mg midazolam (Preparation 4 and 5) to 8 volunteers. Midazolam concentration was assessed predose and 1, 2.5, 5, 10, 15, 20, 30, 45, 60, 180, 240, and 360 minutes after midazolam administration. Inserted figure shows semilogarithmic concentration time profile.**



**Figure 6-7: Midazolam serum concentration (mean) following i.v. and nasal administration of 1 mg midazolam (Preparation 1, 2, and 3) and nasal administration of 3 mg midazolam (Preparation 4 and 5) to 8 volunteers. This figure displays midazolam concentrations assessed predose, 1, 2.5, 5, 10, 15, 20, 30, 45, and 60 minutes after midazolam administration.**

Table 6-3 summarizes the assessed pharmacokinetic parameters following i.v. and nasal midazolam delivery. Time to reach maximal midazolam serum concentration ( $t_{max}$ ) after nasal administration of Preparation 1 to 5 ranged from  $7.1 \pm 0.6$  min (Preparation 5) to  $11.7 \pm 2.4$  min (Preparation 4). Mean systemic bioavailability (F) of midazolam after nasal administration of test preparation varies from 93% (Preparation 2) to 78% (Preparation 5).

Differences of systemic midazolam bioavailability following nasal midazolam administration were not significant. Clearance (Cl) and half life ( $t_{1/2}$ ) were constant and independent of midazolam administration modality.

**Table 6-3: Pharmacokinetic parameters (mean and SD) following i.v. (Dormicum® 1 mg/ml, 1 mg) and nasal application of midazolam (1 mg by Preparation 1 to 3 and 3 mg by Preparation 4, 5); n=8.**

Preparation Nr.	Specification	t <sub>max</sub> [min]	C <sub>max</sub> [µg/l]	AUC <sub>0-∞</sub> [µg*min/l]	Cl [l/min]	t <sub>1/2</sub> [min]	F		
non-compartment model 1 mg i.v.	---	Dormicum®, Roche	2.1 (0.8)	87.6 (58.7)	2798.5 (508.9)	0.37 (0.07)	113.5 (25.9)	100%	
	1	Midazolam UD Nasal Spray 0.5 mg	10.6 (5.0)	28.1 (9.1)	2461.0 (627.8)	0.43 (0.11)	114.2 (23.0)	88% (17)	
	2	Cyclodex-Midazolam UD Nasal Spray 0.5 mg	9.4 (3.2)	30.1 (6.6)	2596.4 (680.4)	0.41 (0.13)	113.0 (22.8)	93% (15)	
non-compartment model 1 mg nasal	3	Cyclodex-Midazolam UD Nasal Spray 1 mg	11.3 (4.4)	28.9 (5.4)	2510.5 (541.4)	0.42 (0.11)	105.4 (17.7)	90% (16)	
	two-compartment <sup>1</sup> 3 mg nasal	4	Cyclodex-Midazolam UD Nasal Spray 3 mg	11.7 (2.4)	72.6 (18.2)	7324.8 (1599.5)	0.43 (0.10)	120.9 (32.9)	86% (8)
		5	Chitosan-CD-Midazolam UD Nasal Spray 3 mg	7.1 (0.6)	82.2 (15.8)	6580.6 (1358.4)	0.48 (0.12)	113.9 (20.8)	78% (10)

<sup>1</sup> for two-compartment modeling data from Subject 1 had to be excluded

Mean serum concentration time profiles following nasal administration of 1 mg were congruent and demonstrated equal absorption kinetics independent of the delivered preparation (Preparation 1, 2, and 3) and the administration modality (one- versus two-sided), see Figure 6-8 A, B, and C. Intra-individual variability of midazolam serum concentration profiles following nasal administration of 1 mg midazolam (Preparation 1, 2, and 3) is shown in Appendix 10.3.9.

Midazolam serum concentration time profile following nasal administration of Preparation 5 is shifted compared to the profile resulting of nasal administration of 3 mg midazolam by Preparation 4, see Figure 6-8 D.

Faster increasing midazolam serum concentrations were detected in all subjects after nasal administration of Preparation 5 (3 mg midazolam plus chitosna) than after nasal administration of Preparation 4 (3 mg midazolam without chitosan), see Appendix 10.3.10. Modeling of midazolam serum concentration time profiles following nasal administration of Preparation 4 and 5 was performed by applying a two-compartment pharmacokinetic model (see Figure 6-9). Range (interindividual variability) of AUC (4349.7 µg\*min/l), C<sub>max</sub> (38.8 µg/l), and t<sub>max</sub> (1.9 min) following

nasal administration of Preparation 5 were closer than the range of AUC ( $4668.5 \mu\text{g}\cdot\text{min/l}$ ),  $C_{\text{max}}$  ( $52.5 \mu\text{g/l}$ ), and  $t_{\text{max}}$  (6.7 min) following nasal administration of Preparation 4.

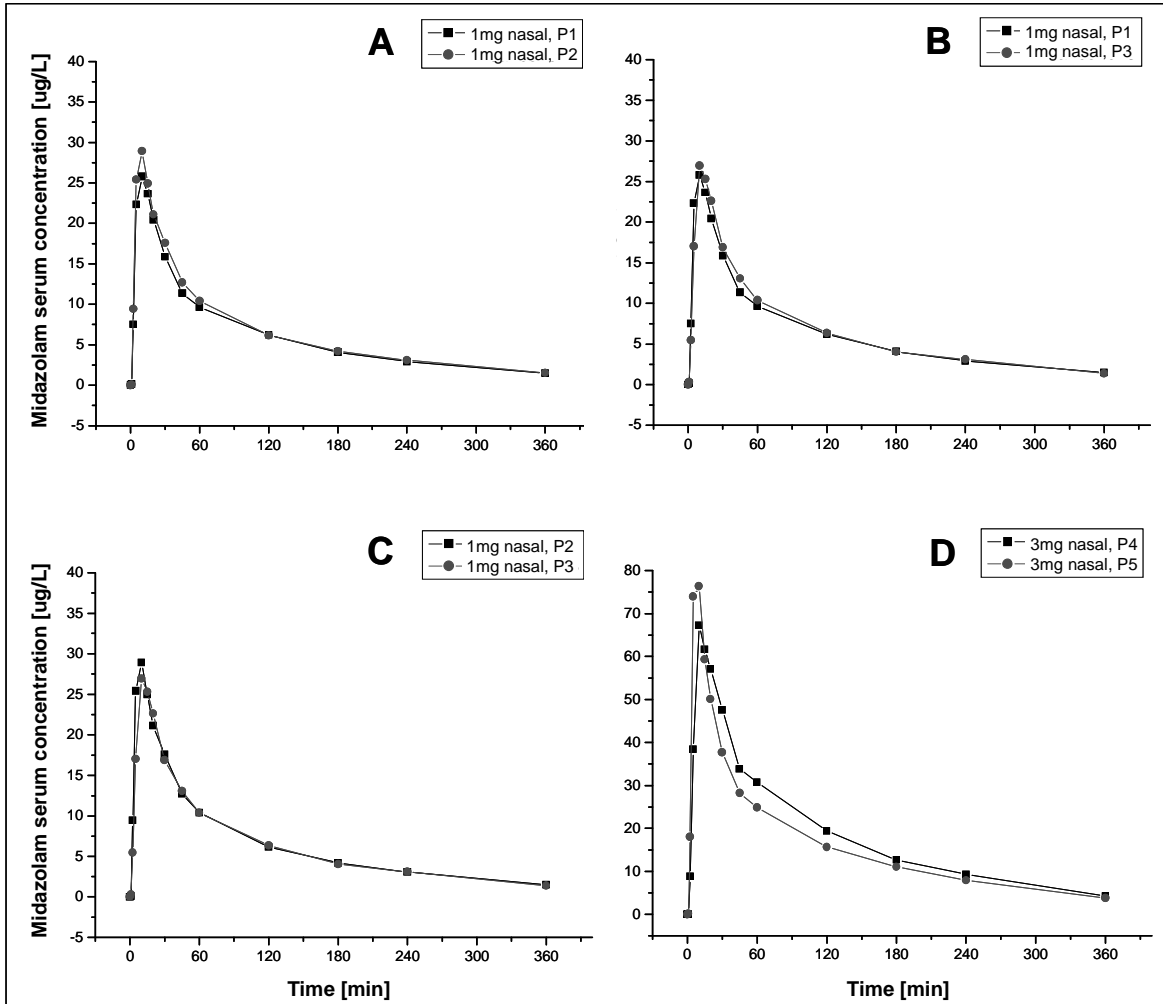


Figure 6-8 (A-D): Overlaid profiles of midazolam serum concentrations following administration of Preparation 1 and 2 (A), Preparation 1 and 3 (B), Preparation 2 and 3 (C), and Preparation 4 and 5 (D). The displayed midazolam serum concentrations refer to the mean serum concentrations ( $n=8$ ).

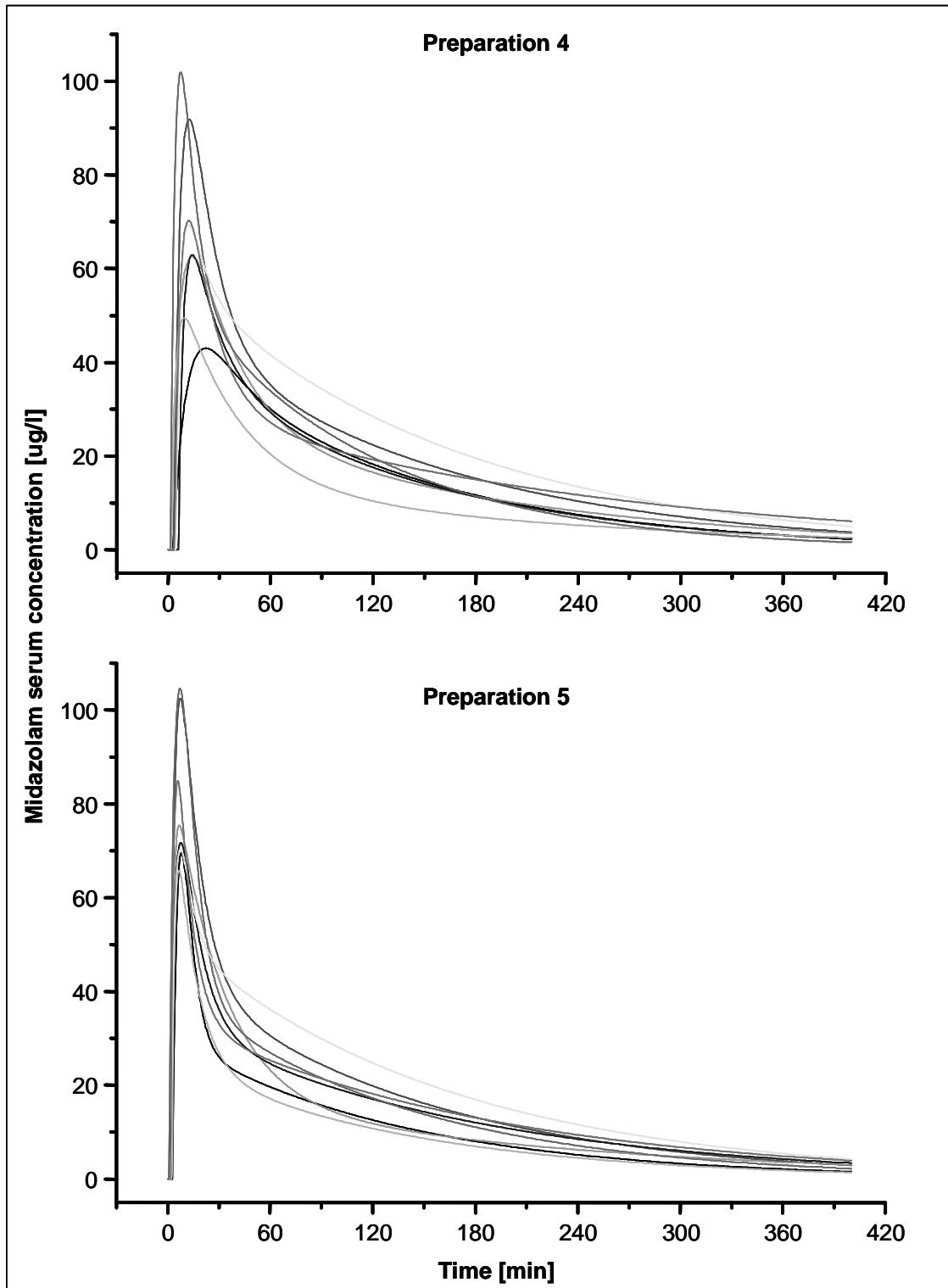


Figure 6-9: Modeling of the midazolam concentration time profile was performed by applying a two-compartment pharmacokinetic model for midazolam serum concentrations after administration of Preparation 4 (n=7) and Preparation 5 (n=7).



Bioequivalence testing was performed for nasal delivery of 1 mg (Preparation 2 and 3) and 3 mg (Preparation 5) with reference Preparation 1 and Preparation 4, respectively (Table 6-4 and Table 6-5). Nasal delivery of 1 mg midazolam by administration of Preparation 1, 2 and 3 was bioequivalent, according to the requirements for bioequivalence defined by the EMEA [EMEA 26. July 2001] (see Table 6-4).

**Table 6-4: Bioequivalence testing for nasal delivery of 1 mg midazolam. Boundaries (lower 80% and upper 125% for  $AUC_{0-\infty}$ ,  $C_{max}$ ) and mean are indicated for reference (Preparation 1), 90% confidence intervals and means of  $AUC_{0-\infty}$ ,  $C_{max}$  of Preparation 2 and 3 are listed. wb: within boundaries, ✓: 90% confidence interval and mean of test preparation fulfill the requirements of EMEA for bioequivalence.**

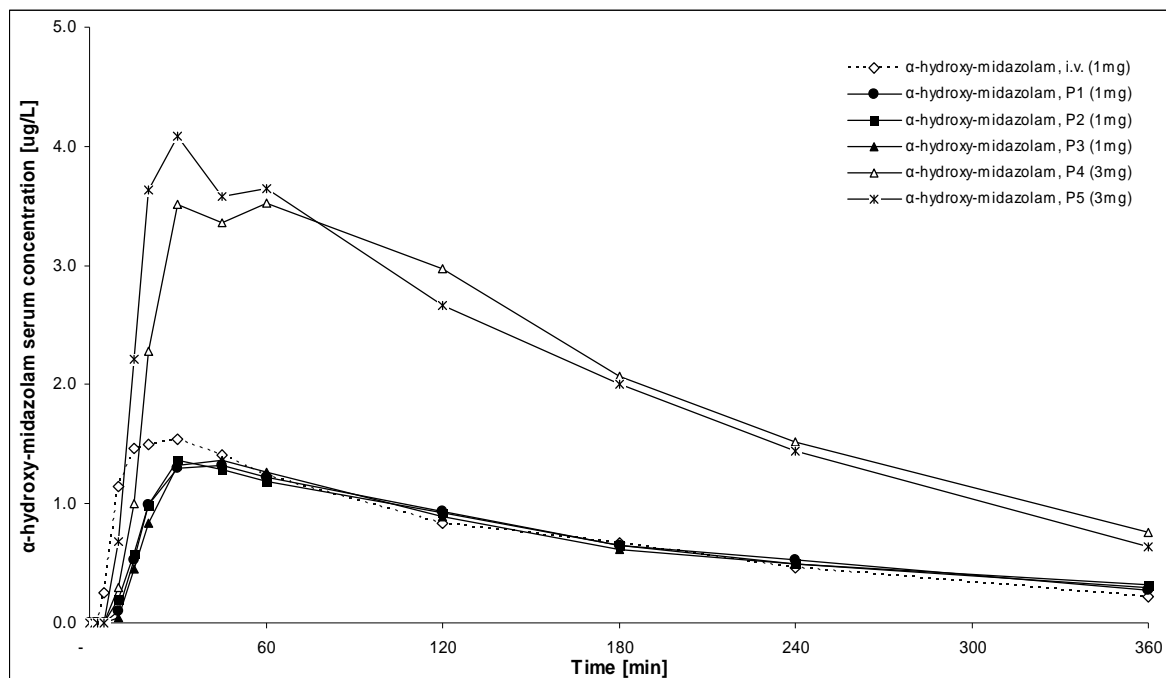
Parameter	Boundaries of reference (mean)	90% confidence interval (mean)			
	Preparation 1	Preparation 2	wb	Preparation 3	wb
$AUC_{0-\infty}$ [ $\mu\text{g}\cdot\text{min/l}$ ]	1969 – 3076 (2461)	2140 – 3052 (2596)	✓	2148 – 2873 (2510)	✓
$C_{max}$ [ $\mu\text{g/l}$ ]	22.5 – 35.2 (28.1)	25.6 – 34.5 (30.5)	✓	25.3 – 32.5 (28.9)	✓

None of the tested pharmacokinetic parameters ( $AUC$ ,  $C_{max}$ , and  $t_{max}$ ) Preparation 4 and 5 did fulfill the requirements for bioequivalence, see Table 6-5. The difference between  $AUC$  following administration of Preparation 4 and 5 was not significant, whereas differences of  $C_{max}$  ( $p=0.008$ ) and  $t_{max}$  ( $p=0.008$ ) were significant. The range of  $AUC$  (4349.7  $\mu\text{g}\cdot\text{min/l}$ ),  $C_{max}$  (38.8  $\mu\text{g/l}$ ), and  $t_{max}$  (1.9 min) following nasal administration of Preparation 5 were closer than the range of  $AUC$  (4668.4  $\mu\text{g}\cdot\text{min/l}$ ),  $C_{max}$  (52.5  $\mu\text{g/l}$ ), and  $t_{max}$  (6.7 min) following nasal administration of Preparation 4.

**Table 6-5: Bioequivalence testing for nasal delivery of 3 mg midazolam. Boundaries (lower 80% and upper 125% for  $AUC_{0-\infty}$ ,  $C_{max}$ ) and mean are indicated for reference (Preparation 4), 90% confidence intervals and means of  $AUC_{0-\infty}$ ,  $C_{max}$  of Preparation 5 are listed. wb: within boundaries, no: 90% confidence interval and mean of test preparation does not comply with the requirements of EMEA for bioequivalence.**

Parameter	Boundaries of reference (mean)	90% confidence interval (mean)	
	Preparation 4	Preparation 5	wb
$AUC_{0-\infty}$ [ $\mu\text{g}\cdot\text{min/l}$ ]	5860 - 9156 (7325)	5583 – 7578 (6581)	no
$C_{max}$ [ $\mu\text{g/l}$ ]	58.1 – 90.8 (72.6)	68.3 – 88.6 (78.5)	no
$t_{max}$ [min]	9.3 – 14.6 (11.7)	6.6 – 7.5 (7.1)	no

Figure 6-10 shows the serum concentration time profiles of  $\alpha$ -hydroxymidazolam (main metabolite of midazolam) following i.v. and nasal delivery of 1 mg midazolam (Preparation 1, 2, and 3) and nasal delivery of 3 mg midazolam (Preparation 4 and 5).



**Figure 6-10: Serum concentration of  $\alpha$ -hydroxymidazolam after i.v. (Dormicum®) and nasal delivery of 1 mg midazolam (Preparation 1, 2, and 3) and nasal delivery of 3 mg midazolam (Preparation 4 and 5); P: Preparation.**

Table 6-6 summarizes pharmacokinetic parameters of  $\alpha$ -hydroxymidazolam following i.v. (Dormicum®, 1 mg) and nasal application of midazolam (1 mg by Preparation 1, 2, and 3 and 3 mg by Preparation 4 and 5).

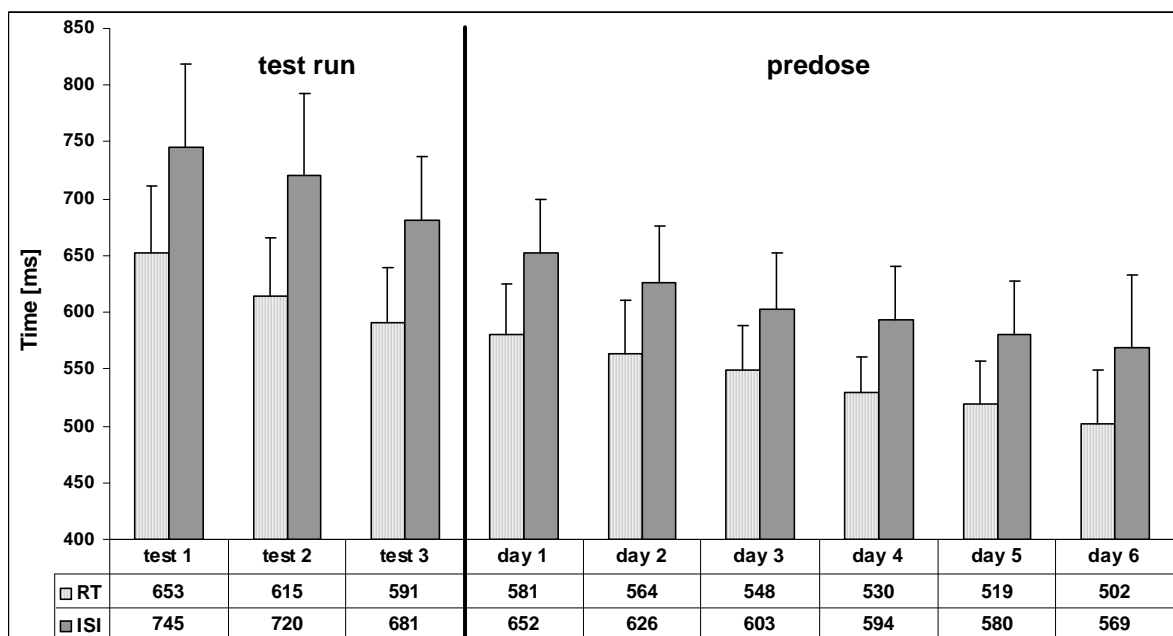
There is no significant difference between dose adjusted AUC of  $\alpha$ -hydroxymidazolam following i.v. and nasal delivery of 1 mg midazolam (Dormicum®, Preparation 1, 2, and 3) and nasal delivery of 3 mg midazolam (Preparation 4 and 5). Following nasal midazolam administration, maximal  $\alpha$ -hydroxymidazolam serum concentrations were reached after  $33.3 \pm 13.3$  min (Preparation 5) and  $41.3 \pm 13.3$  min (Preparation 4). Following i.v. midazolam administration maximal  $\alpha$ -hydroxymidazolam serum concentrations were reached  $22.5 \pm 11.6$  min after midazolam injection. Maximal 4-hydroxymidazolam (second midazolam metabolite) concentration detected were  $0.6 \mu\text{g/l}$ ,  $0.2 \mu\text{g/l}$  following nasal delivery of 3 mg midazolam and administration of 1 mg midazolam (i.v. and nasal), respectively. Often, 4-hydroxymidazolam was detected, but below LOQ ( $0.2 \mu\text{g/l}$ ), see Appendix 10.3.7.

**Table 6-6: Pharmacokinetic parameters of  $\alpha$ -hydroxymidazolam following i.v. (Dormicum® 1 mg/ml, 1 mg) and nasal application of midazolam (1 mg, Preparation 1 to 3 and 3 mg, Preparation 4 and 5); n=8, (SD): standard deviation**

Preparation Nr.		$T_{max}$ [min]	$C_{max}$ [ $\mu$ g/l]	$AUC_{0-\infty}$ [ $\mu$ g *min/l]	$T_{1/2}$ [min]	Cal [l/min]	F
1 mg i.v.	---	22.5 (11.6)	1.7 (0.8)	293.8 (73.0)	118.0 (23.4)	3.6 (0.8)	100%
1 mg nasal	1	39.4 (11.2)	1.4 (0.4)	305.0 (67.0)	131.0 (27.6)	3.4 (0.8)	104% (9.0)
	2	35.6 (7.8)	1.4 (0.6)	316.6 (129.5)	146.7 (33.1)	3.6 (1.3)	106% (11.5)
	3	41.3 (10.6)	1.5 (0.6)	311.0 (81.6)	142.2 (41.8)	3.4 (0.8)	106% (11.5)
3 mg nasal	4	41.3 (13.3)	3.8 (1.2)	292.5* (81.6)	122.8 (41.4)	3.7 (1.0)	100% (18.2)
	5	33.3 (13.3)	4.4 (1.3)	279.5* (53.0)	112.3 (9.8)	3.7 (0.6)	97% (14.5)

\* dose adjusted AUC

Reaction Time (RT) and Interstimulus Interval (ISI) were assessed by Computer-controlled self-adjusting reaction time test (CRTT). Figure 6-11 displays RT and ISI assessed during the test runs (day 1 before predose run) and predose (all study days). Mean inter test difference of RT and ISI was  $19 \pm 9$  ms and  $22 \pm 10$  ms, respectively. Delta RT and delta ISI refer to the difference of the assessed RT and ISI at 20 min, 120 min, and 240 min after midazolam administration and the corresponding parameter assessed previous to midazolam administration.



**Figure 6-11: Three test runs and predose RT and ISI assessed by CRTT. Overall mean inter test difference of RT and ISI was  $19 \pm 9$  ms and  $22 \pm 10$  ms, respectively (n=8).**

Delta RT and delta ISI refer to the difference of the assessed RT and ISI at 20 min, 120 min, and 240 min after midazolam administration and the corresponding parameter assessed previous to midazolam administration. In Figure 6-12 and Figure 6-13 delta RT and delta ISI assessed after administration of study medication are displayed.

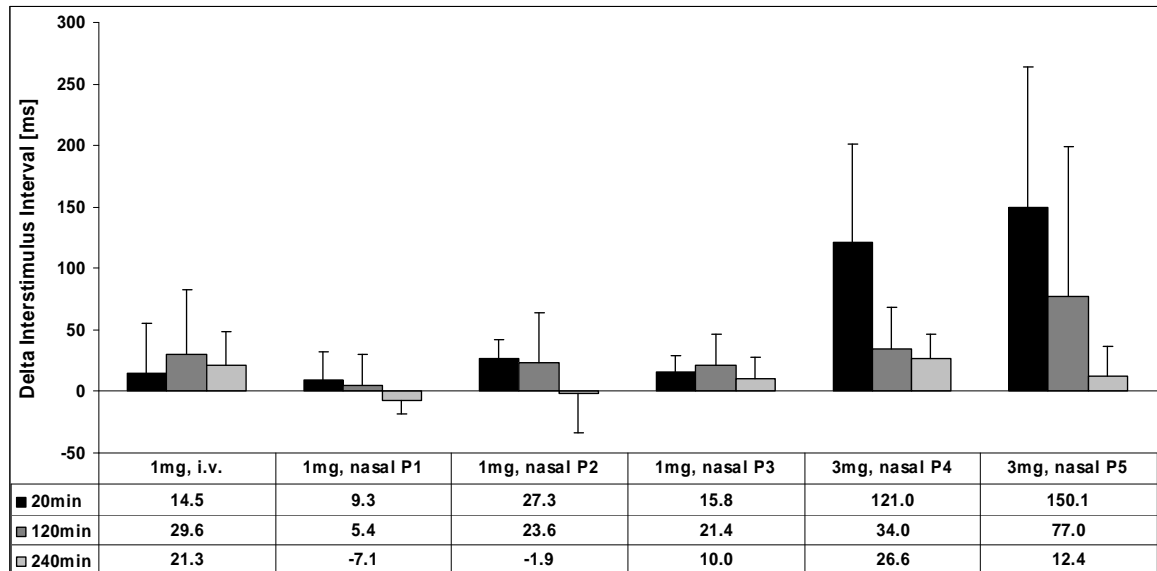


Figure 6-12: Delta interstimulus interval (ISI) assessed by CRTT. Delta interstimulus interval refers to the difference between the ISI at 20 min, 120 min, and 240 min after midazolam application and the corresponding ISI.

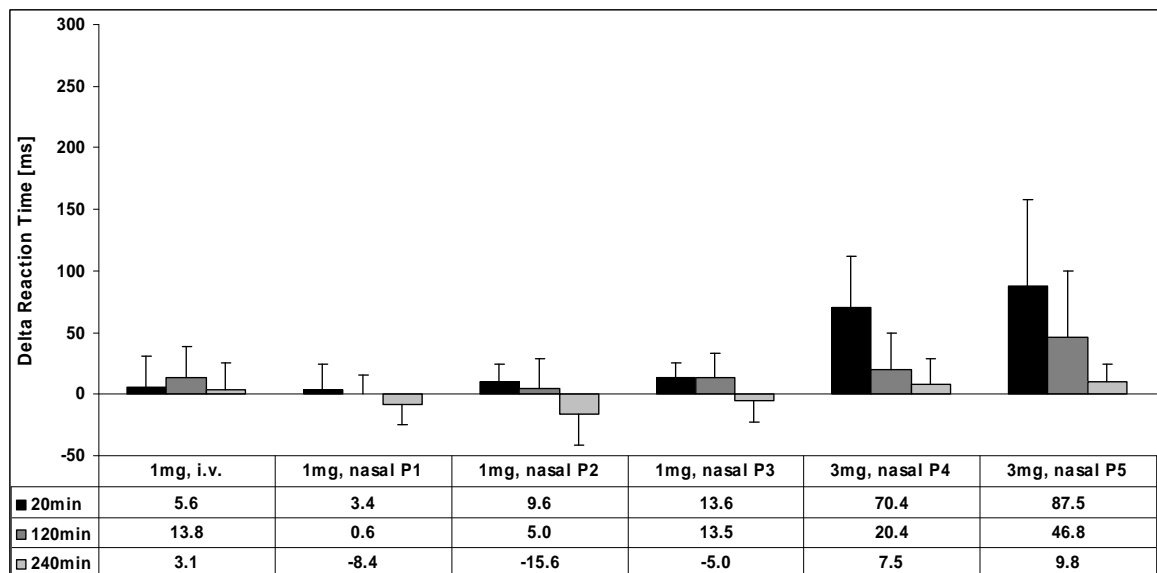


Figure 6-13: Delta reaction time (RT) assessed by CRTT. Delta RT refers to the difference between the RT assessed 20 min, 120 min, and 240 min after midazolam application and the corresponding RT assessed predose.

Figure 6-14 shows the correlation of midazolam serum concentration time profile and performance of CRTT (delta RT and delta ISI).

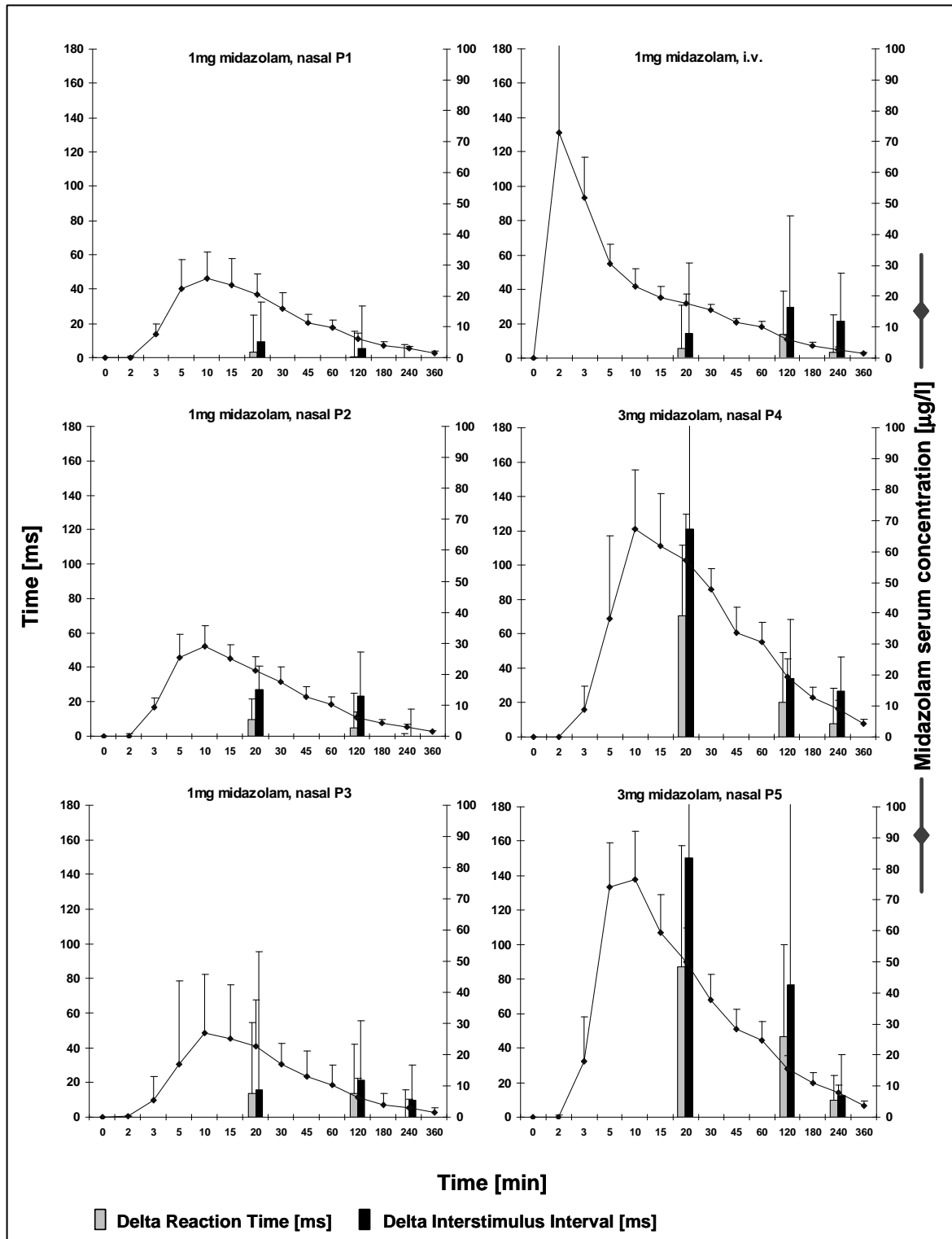


Figure 6-14: Correlation of midazolam serum concentration and outcome of CRTT performance (delta reaction time and delta Interstimulus interval), n=8.

Figure 6-15 shows correlation of midazolam serum concentration time profile and subjective rating of fatigue and drowsiness on VAS<sup>4</sup>. Maximal VAS score was reached within 30 minutes after midazolam administration.

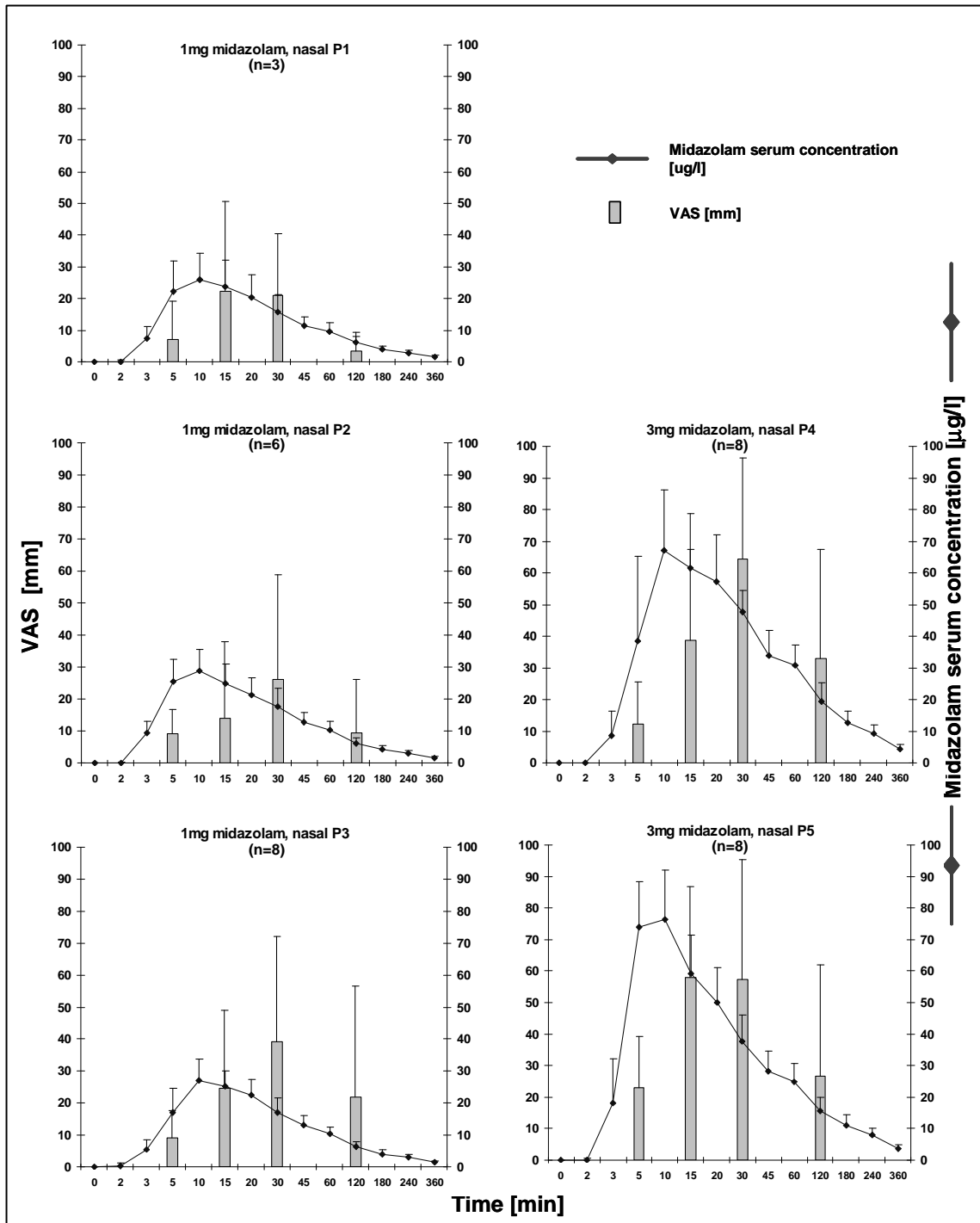


Figure 6-15: Subjective rating of fatigue and drowsiness on VAS (mean). Rating was provide 5 min, 15 min, 30 min and 120 min after nasal midazolam administration of Preparation 1 (n=3), Preparation 2 (n=6), Preparation 3 (n=8), Preparation 4 (n=8), and Preparation 5 (n=8).

<sup>4</sup> The missing data of assessed fatigue and drowsiness score is due to delayed implementation of this score. Therefore, only 3 and 6 volunteers rated their fatigue and drowsiness for Preparation 1 and Preparation 2, respectively. For Preparation 3, Preparation 4, and Preparation 5 all volunteers rated their fatigue and drowsiness (n=8)

## 6.4 Discussion

Five different nasal midazolam preparations and i.v. midazolam (Dormicum®) were administered to healthy volunteers to provide pharmacokinetic characterization of nasally applied midazolam. Preparation 1, 2, 3, 4, and 5 were nasally applied to assess the impact of RM $\beta$ CD (solubilizer), chitosan hydrochloride (penetration enhancer), and the application modality (one- versus two-sided) on systemic bioavailability and pharmacokinetics of nasal delivered midazolam. Pharmacologic effects were assessed by visual analogue scale (VAS) and computer-controlled self-adjusting reaction time test (CRTT, measuring reaction time and interstimulus interval).

Nasal midazolam administration was well tolerated and never rated as painful. Nasal irritation was reversible within 30 minutes. Local irritation (nose, throat area, and teary eyes) was more intense following nasal administration of chitosan containing preparation (Preparation 5).

All tested nasal administration modalities for nasal delivery of 1 mg midazolam proved bioequivalence. The administration volume of the unit dose system is predetermined (0.1 ml). To deliver total dose of 1 mg midazolam by one- and two-sided administration, midazolam concentration had to be adapted (concentration was halved and consequently the application volume was doubled by spraying 0.1 ml into each nostril). Congruence of midazolam serum concentration time profiles, assessed pharmacokinetic parameters, and bioequivalence testing proved equal pharmacokinetic characteristics resulting of following administration modalities for nasal delivery of 1 mg midazolam:

- two-sided administration of 0.5 mg midazolam without solubilizer (Preparation 1)
- two-sided administration of 0.5 mg midazolam with equimolar RM $\beta$ CD (Preparation 2)
- one-sided administration of 1 mg midazolam solubilized with equimolar RM $\beta$ CD (Preparation 3)

Consequently, neither RM $\beta$ CD (equimolar to midazolam) nor application modality (one- or two-sided) changed absorption kinetics of nasally administered midazolam.

Systemic bioavailability of midazolam following one-sided administration of escalated doses (3 mg midazolam, Preparation 4, and Preparation 5) was equal to bioavailability following nasal administration of 1 mg midazolam. This demonstrated nasal residence time to be long enough for complete midazolam uptake. Consequently, midazolam dose can easily be doubled to 2 mg and 6 mg nasal midazolam by two-sided administration of Preparation 1 for 2 mg and Preparation 4 and Preparation 5 for nasal administration of 6 mg midazolam.

Since systemic bioavailability of nasally applied midazolam was 86% to 93%, nasal administration of 6 mg midazolam correspond to about 5 mg midazolam i.v. administered and to about 10 mg to 16 mg orally applied midazolam (systemic bioavailability of oral midazolam is 30% to 50% [Documed 2006]).

Transmucosal nasal midazolam absorption was promoted by chitosan hydrochloride. Chitosan hydrochloride modified absorption kinetics ( $C_{max}$  and  $t_{max}$ ) of nasally applied midazolam (3 mg), but overall systemic bioavailability was not enhanced.

Furthermore, smaller ranges for AUC,  $t_{max}$ , and  $C_{max}$  observed after nasal administration of 3 mg (Preparation 5) refer to less interindividual variability and therefore reproducible pharmacokinetics.

Bioequivalence testing was performed for all assessed pharmacokinetic parameters (AUC,  $t_{max}$ , and  $C_{max}$ ) following nasal administration of Preparation 4 (reference preparation) and Preparation 5 (test preparation). None of the tested parameters (AUC,  $t_{max}$ , and  $C_{max}$ ) following nasal administration of Preparation 5 fulfilled the requirements of the EMEA for bioequivalence. Accordingly, bioequivalence was not confirmed for Preparation 4 (3 mg midazolam, no chitosan hydrochloride) and Preparation 5 (3 mg midazolam, 0.5% chitosan hydrochloride).

Chitosan hydrochloride (Preparation 5) promoted absorption kinetics of nasally applied midazolam and significant higher midazolam serum concentrations were faster achieved. Chitosan hydrochloride proved valuable penetration enhancer abilities in transmucosal nasal midazolam delivery. Chitosan hydrochloride potentially facilitates optimization of nasal midazolam preparation for indications requiring fast onset of therapeutic effects (e.g., status epilepticus). Chitosan containing nasal midazolam preparations are a promising therapeutic alternative to i.v. delivered midazolam for the treatment of status epilepticus. Providing reliable anticonvulsive therapy the more intense nasal irritation, of chitosan containing nasal midazolam preparation (Preparation 5) is of secondary importance compared to the benefit of needle-free drug delivery.

The congruent metabolite profile after nasal and i.v. administration of 1 mg midazolam supports the presumption of direct midazolam uptake through nasal mucosa into the systemic circulation. Apart from slightly earlier peak concentration after i.v. administration, the metabolite profiles after nasal and i.v. administration of 1 mg midazolam are congruent. In addition, the constant AUC (dose adjusted) of  $\alpha$ -hydroxymidazolam following nasal and i.v. administration of midazolam attests effective bypassing of the first-pass metabolism. Thus, serum concentration time profiles of the midazolam metabolites ( $\alpha$ -hydroxymidazolam and 4-hydroxymidazolam) demonstrated exclusive transmucosal absorption of nasally applied midazolam.

Nasal administration of 0.1 ml prevented swallowing of the nasally delivered preparation, gastrointestinal midazolam uptake, and reduction of systemic bioavailability by hepatic first-pass metabolism. Consequently, the assessed pharmacokinetic parameters characterized pure transmucosal nasal midazolam delivery.

This interpretation of metabolite profiles is in accordance with the findings of Heizmann et al., who demonstrated higher plasma levels for  $\alpha$ -hydroxymidazolam following oral administration of midazolam compared to i.v. midazolam delivery [Heizmann et al., 1983]. Illum et al. were the first who proved direct transmucosal nasal drug uptake based on the characterization of drug and metabolite concentration time profiles [Illum, et al. 2002].



Constant decreasing of the reaction time (RT) and interstimulus interval (ISI) assessed predose demonstrated the continuous learning effect of repeated performance of CRTT (computer-controlled self-adjusting reaction time test). Due to this constant learning effect delta RT and delta ISI was calculated to estimate the pharmacological effect. Following nasal administration of 1 mg midazolam improved performance (negative difference for delta ISI and RT) refers to the learning effect emerging of repeated performances of CRTT 120 minutes after midazolam administration. RT and ISI assessed 20, 120, and 240 minutes after nasal administration of 3 mg midazolam was reduced compared to RT and ISI assessed predose. Consequently, performance of the volunteers was still impaired 4 h after nasal administration of 3 mg midazolam. This finding is of clinical relevance assuming that fitness to drive needs more than 4 h to be restored after nasal administration of 3 mg midazolam.

Maximal score for fatigue and drowsiness was achieved within 30 minutes after midazolam administration and was delayed compared to maximal midazolam serum concentration.

Generally, maximal midazolam serum concentrations preceded maximal pharmacologic effects assessed by CRTT and VAS.

## 6.5 Conclusions

Neither RM $\beta$ CD (equimolar to midazolam) nor application modality (one- or two-sided) changed absorption kinetics of nasally administered midazolam. Chitosan hydrochloride promoted absorption kinetic of nasally applied midazolam and significant higher midazolam serum concentrations were faster achieved. Nasal delivery of midazolam in a minimized volume provided exclusive transmucosal nasal midazolam uptake and thus direct access to the systemic blood circulation. Consequently, gastrointestinal midazolam uptake was avoided and first-pass metabolism circumvented.

Pharmacokinetic profile and high systemic bioavailability following transmucosal nasal midazolam delivery distinguish this drug administration option as veritable alternative to invasive midazolam administration.



## **7 Project III: Transmucosal nasal delivery of low-dose midazolam – evaluation of two preparations for procedural anxiolysis**

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### **7.1 Introduction**

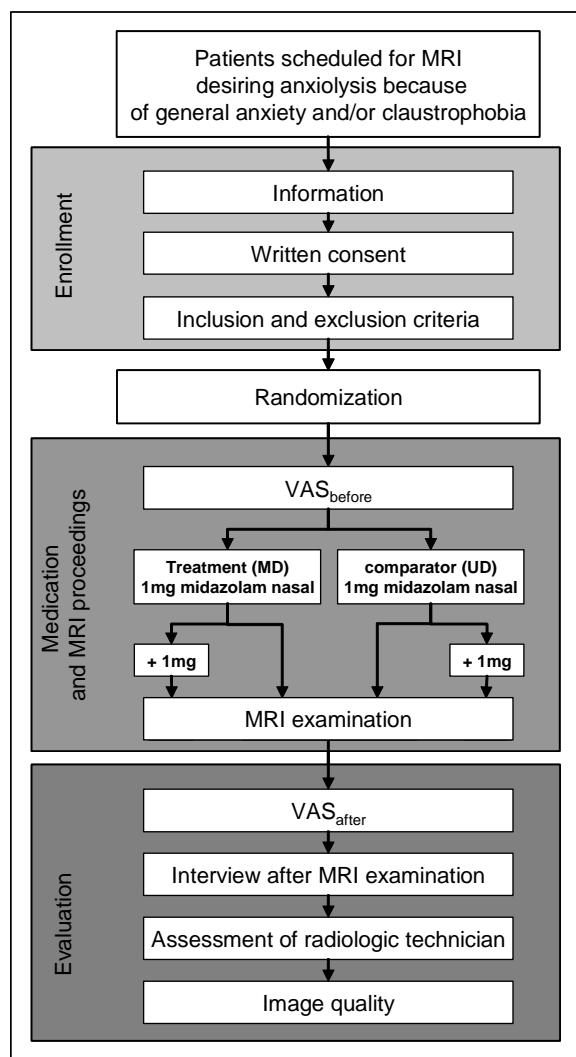
During MRI (magnet resonance imaging) examinations up to 30% of patients experience moderate to severe anxiety and 10% of all MRI examinations cannot successfully be completed because of claustrophobia [Münste Sinikka 2002].

For radiological investigations anxiolysis usually is provided by oral or intravenous application of benzodiazepines (e.g., lorazepam or midazolam). Claustrophobic patients who are afraid or even panic before or during MRI examination profit from conscious sedation, which helps them to cope with the situation in the narrow MRI tunnel. Conscious sedation refers to anxiolysis and only slight sedation, not impairing patient's ability to maintain his airways and to respond appropriately to physical stimulation and verbal commands [Merrick and Ramsby 1994]. This is required for procedural anxiolysis during MRI examinations because some examinations require the cooperation of the patients (e.g., in- and exhaling on command). Nasal application of low-dose midazolam has been reported to be effective in procedural anxiolysis (conscious sedation), furthermore the rapid onset is considered beneficial for MRI workflow [Moss, et al. 1993; Münste 2002]. Compared to oral application of 7.5 mg midazolam, the benefit of nasal delivered low-dose midazolam (1-2 mg) was faster onset of anxiolysis and the absence of deep sedation. Therefore, patients were able to follow the instructions during MRI examinations [Tschirch, et al. 2006]. For nasal application of low-dose midazolam, no preparation is commercially available. Usually, midazolam formulations for i.v. application are administered nasally or local Hospital Pharmacy provides nasal midazolam preparations according to the monograph of NRF [NRF 2002].

The aim of this multicenter trial was to compare the benefit of two different preparations for nasal delivery of low-dose midazolam to provide procedural anxiolysis during MRI examinations.

### **7.2 Methods**

The ethics committees of Zurich and Basel and the national authorities (Swissmedic) approved the clinical protocol of this multicenter trial, that was carried out at Center A (Institute of Diagnostic Radiology, University Hospital Zurich), Center B (Institute of Radiology, Kantonsspital Winterthur), Center C (Imamed Radiologie Nordwest, Basel), and Center D (Department of Radiology, Orthopedic University Hospital Balgrist, Zurich). Coordination and monitoring was performed in collaboration with Akroswiss AG, Zurich.



**Figure 7-1: Study design of multicenter trial with nasal low-dose midazolam. Anxious and/or claustrophobic patients, scheduled for MRI examinations at the study centers (A, B, C, and D), were invited to participate in this multicenter trial with nasal low-dose midazolam. MD: multidose nasal spray (treatment), UD: unit dose nasal spray (comparator).**

## Patients and study design

Patients, scheduled for MRI examinations at the study centers and desiring anxiolysis, because of general anxiety and/or claustrophobia, were invited to participate in this clinical trial with nasal low-dose midazolam. Radiologists conclusively informed the interested patients and handed out written information about the clinical study protocol. Before enrollment, patients gave written consent. Figure 7-1 displays the study design of the multicenter study with nasal low-dose midazolam.

Exclusion criteria were age <18 years, general contraindications for MRI (e.g., cardiac pacemaker, neurostimulators, or ferromagnetic implants), pregnancy, breast feeding, drug or ethanol abuse, general contraindications for midazolam or other benzodiazepines (e.g., myasthenia gravis, known allergic or previous paradox reaction after benzodiazepine administration), simultaneous therapy with midazolam or other benzodiazepines, simultaneous participation in another clinical trial, and otorhinolaryngeal diseases (e.g., status post surgery, rhinitis, nasal polyposis), see case report form in Appendix 10.4.1 and 10.4.2. When consent had been obtained and all inclusion criteria were met, patients were randomly allocated to treatment or comparator group.

## Treatment (MD) and Comparator (UD)

Midazolam MD Nasal Spray 5 mg/ml (MD) and Midazolam UD Nasal Spray 1 mg (UD) were produced at the Hospital Pharmacy, University Hospital Basel, according to the current guidelines for Good Manufacturing Practice (GMP). Midazolam hydrochloride was purchased from Fährhaus Pharma (Hamburg, Germany), randomly methylated- $\beta$ -cyclodextrin (RM $\beta$ CD, CavaSol<sup>®</sup> W7M Pharma) from Wacker (Munich, Germany), sodium chloride and 0.1 N hydrochloric acid were of pharmaceutical quality according to Ph.Eur. 5.4.

Midazolam MD Nasal Spray 5 mg/ml was produced according to the NRF monograph for nasal midazolam preparation [NRF 2002]. Midazolam MD Nasal Spray 5 mg/ml (preservative: benzalconium chloride and sodium ethylenediaminetetraacetic acid) was provided in a multidose nasal spray, delivering 0.1 ml per pump (0.5 mg midazolam base per pump). To prepare the multidose nasal spray for exact dosing, the pump had to be pressed five times previous to first nasal administration. The application had to be provided in sitting patients, because the upright position of the multidose nasal spray is essential for exact dosing.

Patients were administered 1 pump (0.1 ml) per nostril of Midazolam MD Nasal Spray 5 mg/ml immediately prior to MRI examination (total administered dose: 1 mg midazolam base). If the administered dose of 1 mg midazolam was not enough to provide sufficient anxiolysis to perform MRI examination, administration was allowed to be repeated (1 pump per nostril, additional administration of 1 mg midazolam).

Midazolam UD Nasal Spray 1 mg (preservative-free, solubilizer: 4% RM $\beta$ CD) was provided in unit dose nasal sprays (Pfeiffer GmbH, Radolfzell, Germany), delivering 1 single pump of 0.1 ml (1 mg midazolam base per pump), independent of the actual position. Therefore, the application was provided to the patients lying on the MRI table. For detailed information about the nasal midazolam preparations see Table 5-4, Table 5-5 (Project I), and Appendix 10.2.4 to 10.2.9.

#### **Additional dose and therapy failure**

If anxiolysis of the initial treatment with 1 mg midazolam was not enough to provide MRI examination an additional dose of 1 mg midazolam was offered to patients of both groups (MD and UD). Administration of the additional dose was no exclusion criteria.

#### **Anxiety score and subjective experience**

Patients were asked to rate their anxiety on a visual analog scale (VAS) before medication (VAS<sub>before</sub>) and after completing MRI examination (VAS<sub>after</sub>). VAS to rate anxiety was a non-graduated 100 mm line, the left end labeled 'not anxious at all' and the right end labeled 'extremely anxious'.

In addition, after completing the MRI examination patients were interviewed about their experiences and whether they would repeat the MRI examination again with analog anxiolytic treatment receiving during the study.

#### **Assessment of radiologic technician**

The radiologic technician rated the comportment and cooperation of the patient during the proceedings (calm and good cooperation, agitated insufficient cooperation, patient felt asleep, slightly anxious, and very anxious) and classified the proceeding as normal proceedings, interruption of the examination, or MRI examination not feasible and named the reasons for incomplete examinations. If MRI examination was not feasible or had to be interrupted, the therapy was judged as failed (therapy failure).

### **MRI image quality**

The radiologists assessed the image quality of each MRI examination, according to following five-grade scale: (1) excellent image quality (ideal image quality for diagnosis), (2) good image quality (very little motion artifacts), (3) satisfactory image quality (diagnosis possible, despite some motion artifacts), (4) poor image quality (diagnostic information is affected by motion artifacts), and very poor image quality (no diagnosis because of extensive motion artifacts).

### **Local irritation and adverse drug reactions**

Radiologic technicians were instructed to register all spontaneous reports on local irritation and adverse effects. Patients were asked to classify the intensity of the local irritation (mild, intermediate, strong) and the time for recovery was noted. The radiologists were instructed to treat serious adverse reactions with flumazenil (Anexate®).

### **Analysis and statistics**

Two tailed t-tests for differences in continuous variables and  $\chi^2$ -tests (image quality) of trends for ordinal variables were performed. Statistical significance was accepted for  $p < 0.05$ . The anxiety scores were compared by means of the Mann-Whitney test. Analysis of the questionnaire concerning the willingness to repeat the MRI examination was made by Fisher's exact test.

### **Monitoring and study material supply**

Before delivering the clinical study material to the study centers all collaborating radiologic technicians and radiologists were instructed (see also instruction material in Appendix 10.4.4.). To assure consistent application of the nasal sprays the handling of the nasal sprays (multidose and unit dose) was explained and demonstrated.

On a colored flowchart (see Appendix 10.4.3) the different forms and questionnaires were displayed. All forms (questionnaires) were of different colors, corresponding to the color code of the flowchart (patient information: white, consent form: green with white carbon copy, questionnaire to check the inclusion and exclusion criteria and the assessment of image quality: blue, and the questionnaire for the assessment by the patient and radiologic technicians: yellow). The duties and responsibilities of radiologic technicians and radiologists were labeled.

To monitor the rate of recruitment and the compliance with the guidelines for Good Clinical Practice the study centers were visited once per months.

## 7.3 Results

### Study population

Between May 2006 and February 2007, overall 110 patients were recruited and randomly assigned to either treatment (MD, n=56) or comparator group (UD, n=54). There were no significant differences between patients recruited in the study centers A, B, C, and D.

Regarding age (MD  $51 \pm 16$  years, UD  $51 \pm 13$  years), weight (MD  $72 \pm 15$  kg, UD  $77 \pm 16$  kg) and sex (MD m18/f38, UD m19/f35) was no significant difference between treatment (MD) and comparator group (UD).

The MRI examinations were classified into three groups: investigations of a limb (body and head outside of the MRI tunnel), investigation of head and neck (only head and neck inside the MR tunnel), and whole body investigations (whole body inside the MR tunnel). There was no significant difference between the classification of MRI examinations of treatment and comparator group.

**Table 7-1: Demographic and clinical characteristics of the study population (n=110).**

Characteristics	Treatment group MD (n=56)	Comparator group UD (n=54)
Age, y (SD) <sup>1</sup>	51 (16)	51 (13)
Weight, kg (SD)	72 (15)	77(16)
Sex (m/f)	18/38	19/35
VAS <sub>before</sub>	0.72 (0.23)	0.66 (0.25)
Center A	15	15
Center B	13	12
Center C	13	12
Center D	15	15
Duration of MRI examination, min (SD)	31 (13) <sup>3</sup>	31 (11) <sup>4</sup>
Acquired sequences (SD)	7 (4) <sup>3</sup>	6 (2) <sup>4</sup>
Total body examination	41 <sup>3</sup>	37 <sup>4</sup>
Head/neck examination	12 <sup>3</sup>	12 <sup>4</sup>
Limb examination	3 <sup>3</sup>	3 <sup>4</sup>
Contrast agent, i.v.	14 <sup>3</sup>	7 <sup>4</sup>
Arthroscopy <sup>2</sup>	14 <sup>3</sup>	11 <sup>4</sup>

<sup>1</sup> a 16 year-old patient was enclosed, after her mother signed consent form. This protocol deviation was tolerated and the acquired data entered the final analysis

<sup>2</sup> intra-articular injection of contrast agent

<sup>3</sup> n=53, no data for therapy failures or dropout

<sup>4</sup> n=52, no data for therapy failure and protocol deviation

### Additional dose and therapy failure

All patients treated only with the initial dose of 1 mg midazolam (MD n=52, UD n=49) completed MRI examination. Additional administration of 1 mg midazolam (total administered dose of 2 mg) was required of total 17 patients (MD n=13, UD n=4). This additional midazolam dose was not judged as therapy failure, but if MRI examination was not possible or had to be interrupted the

therapy was declared as failed. Overall, therapy failed in 3 patients (MD n=2, UD n=1). All therapy failures occurred within patients receiving 2 mg midazolam. Acquired data of one patient of the treatment group was excluded from final analysis, because of protocol deviation (after nasal administration of 1 mg midazolam the patient has withdrawn his consent and desired intravenous anxiety). Due to protocol deviation (application of false medication) one patient of comparator group had to be excluded from final analysis.

Final analysis was performed with data acquired from 105 patients, completing MRI examination (MD n=53, UD n=52). Figure 7-2 displays the number of enrolled, randomized, and excluded patients.

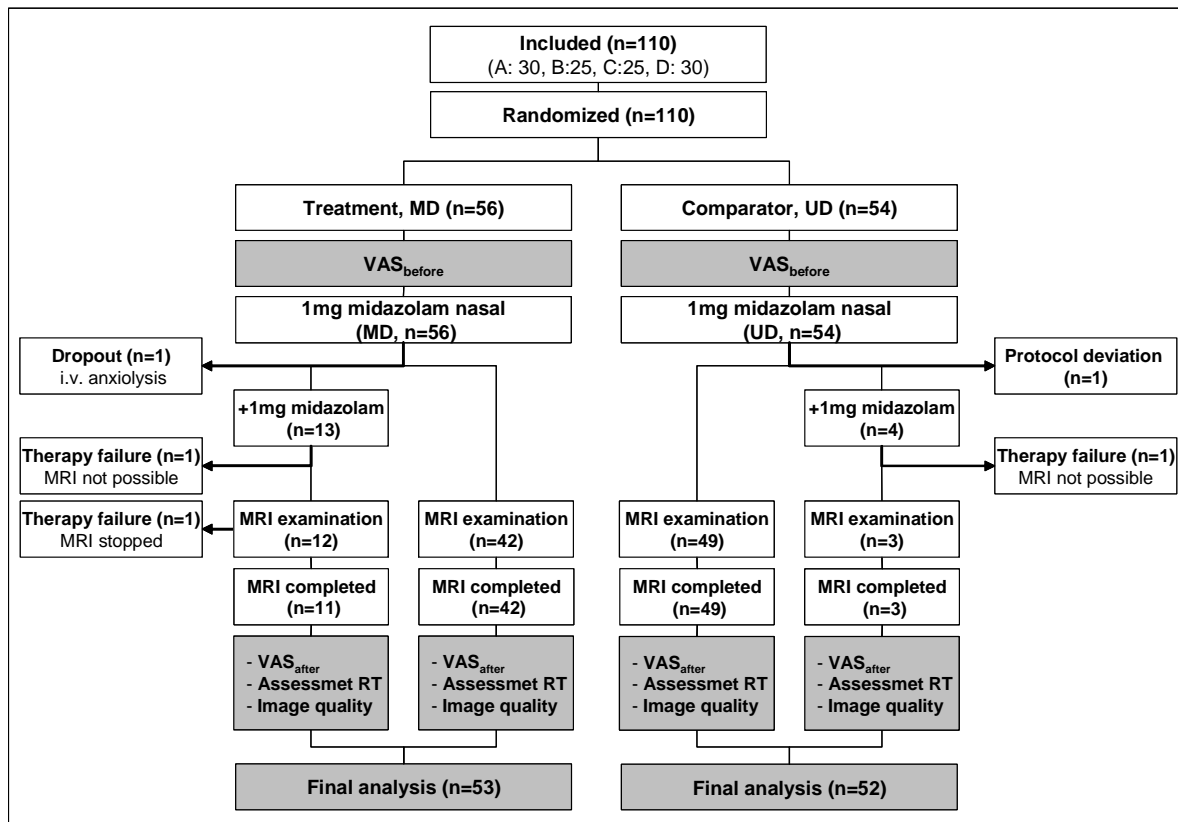
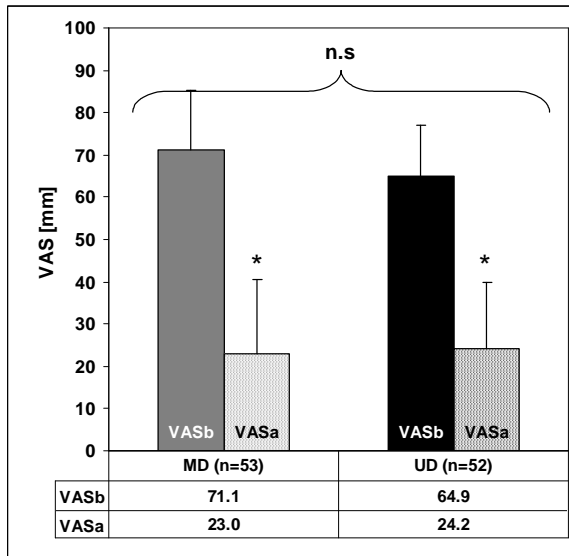


Figure 7-2: Flow diagram of subject process through randomized study – evaluation of two preparations for nasal delivery of low-dose midazolam; enrolled n=110 (center A: 30, B: 25, C: 25, and D: 30); randomized n=110 (MD n=56, UD n=54); dropout n=1 (MD); protocol deviation n=1 (UD); therapy failure n=3 (MD n=2, UD n=1); additional dose n=17 (MD n=13, UD n=4); MRI completed n=105 (MD n=53, UD n=52), and final analysis n=105 (MD n=53, UD n=52). MD: multidose nasal spray (treatment), UD: unit dose nasal spray (comparator), VAS<sub>before/after</sub>: Visual analog scale before and after MRI examination, RT: radiologic technician.



**Patient experience and anxiety reduction**



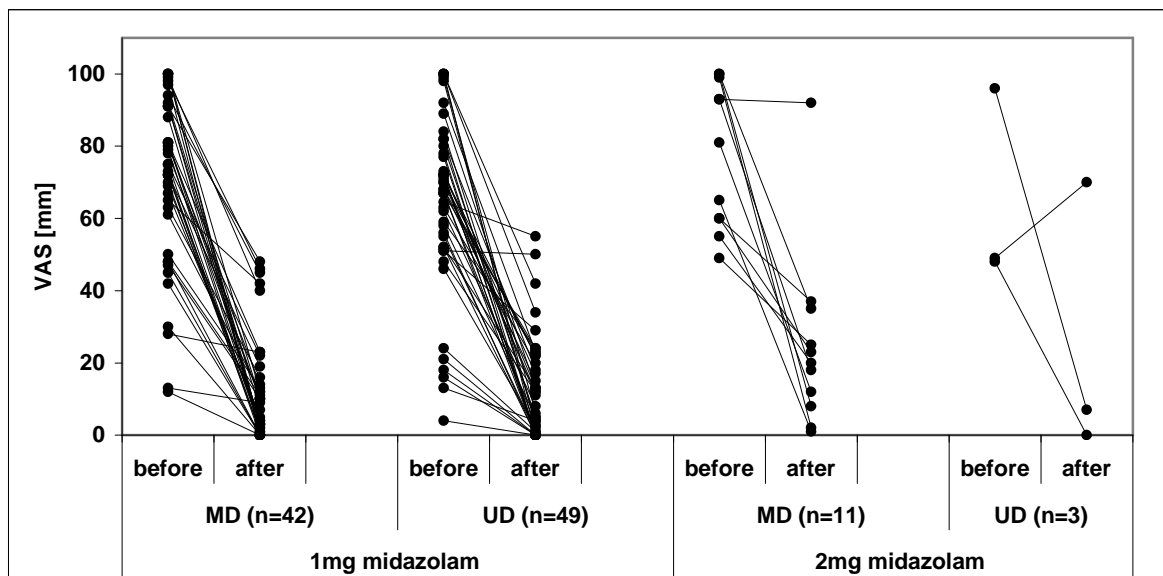
**Figure 7-3: Reduction of anxiety in treatment (MD, n=53) and comparator group (UD, n=52). For both groups anxiety score assessed before medication was significant higher than score assessed after MRI examination. There was no difference in anxiety reduction between the two groups. VASb: VAS<sub>before</sub>, VASa: VAS<sub>after</sub>, (-): significant (p<0.05), n.s: not significant (p>0.05),**

Anxiety score of all patients, who completed the MRI examination (n=105), are displayed in Figure 7-3.

Between treatment and comparator group, there was no significant difference in anxiety score assessed before midazolam administration (VAS<sub>before</sub>).

In both groups (MD and UD) reduction of anxiety (VAS<sub>before</sub>-VAS<sub>after</sub>) was significant (p<0.05). But, there was no significant difference of anxiety reduction between treatment (MD) and comparator group (UD).

From 105 patients, who completed MRI examination, all but one indicated in the interview after the MRI examination less anxiety. Figure 7-4 displays anxiety score of all patients, receiving 1 mg or 2 mg midazolam by MD or UD application.



**Figure 7-4: Anxiety score before midazolam administration (VAS<sub>before</sub>) and after MRI examination (VAS<sub>after</sub>) of patients receiving 1 mg midazolam (MD n=42, UD n=49) and patients receiving 2 mg midazolam (MD n=11, UD n=3). Difference of anxiety reduction of all groups was not significant.**

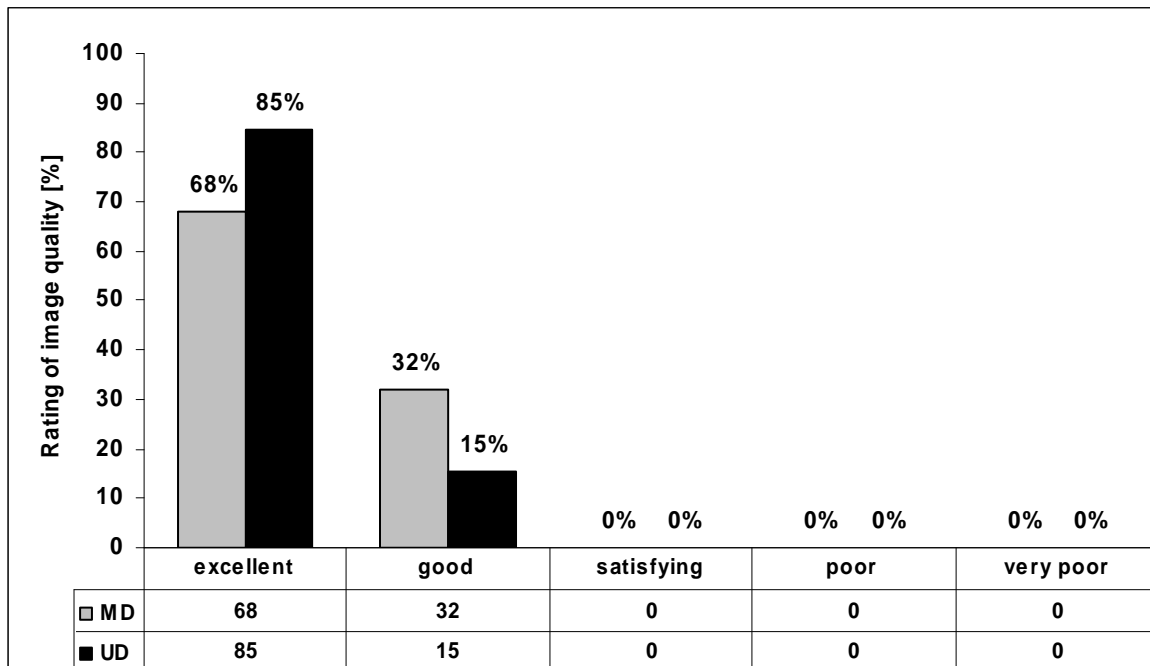
From 105 patients, who completed the MRI examination 94% (n=99; MD n=50, UD n=49) would agree to repeat the MRI examination with analog anxiolytic medication, they received as study medication. From patients, who would refuse to repeat the MRI examinations with the same anxiolytic medication, 2 were treated with 2 mg midazolam.

**Assessment of radiologic technician**

All completed MRI examinations (n=105, MD n=53, and UD n=52) were rated as normally accomplishable. In general, patients were judged as calm and cooperative during MRI proceedings (MD n=44 [85%] and UD n=47 [92%]), 8 patients were very anxious (MD 5 [9%], UD 3 [6%]) and no patient has fallen asleep during MRI examination.

**Image quality**

All completed MRI examinations (n=105, MD n=53, and UD n=52) generated image sets of excellent quality (MD n=36 [68%] and UD n=44 [85%]) or good quality (MD n=17 [32%]) and UD n=8 [15%]). No MRI image was of satisfying, poor, or very poor quality. All generated image sets were suitable for diagnosis. The difference of image rating between MD and UD group was not significant, see Figure 7-5.



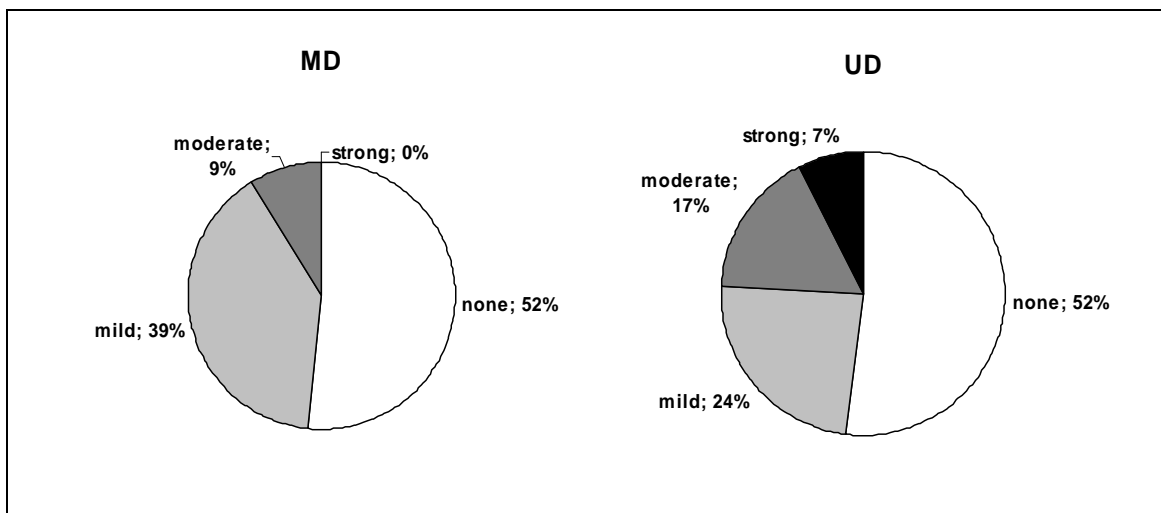
**Figure 7-5: Assessment of image quality (n=105, MD n=53, UD n=52). All MRI images were of excellent (MD n=36 [68%]) and UD n=44 [85%]) or good quality (MD n=17 [32%]) and UD n=8 [15%]). MRI image quality of all image sets (n=105) was suitable for diagnostics.**

### Local irritation and adverse drug reactions

Of total 110 patients receiving study medication, 53 patients (MD n=27 [48%], UD n=26 [52%]) spontaneously reported local irritation. Intensity of local irritation was rated as mild (MD n=22 [39%], UD n=13 [24%]), moderate (MD n=5 [9%], UD n=9 [17%]), or strong (MD n=0, UD n=4 [7%]), see Figure 7-6.

Besides burning (burning in the nose MD n=14 [52%], UD n=15 [58%] and burning in the throat area MD n=3 [11%], UD n=1 [4%]), patients sporadically reported on watery eyes and coughing. Local irritation was reported to vanish within few minutes and never had to be treated.

No severe adverse reaction occurred and hence flumazenil never had to be administered. Other mentioned discomfort (e.g., noise, pain in the back or hip) were related to the MRI examination or disease of the patient.



**Figure 7-6: Spontaneous reports on local irritation; in both groups 52% of the patients did not report on any local irritation. The intensity of the local irritation was rated as mild (MD: 39%, UD 24%), moderate (MD: 9%, UD: 17%), and strong (MD: 0%, UD: 7%).**

### Monitoring

Two months after study start, difficulties in patient recruitment in two study centers (B, C) were detected. Patients often admitted their fear only at the beginning or even during MRI examination. To avoid delay of day's schedule, patients admitting their fear only during MRI examination, were not invited to enter the study and the routine anxiolytic treatment was offered.

To call attention for the possibility of treatment options for claustrophobia and general anxiety, we designed posters and attached them in the waiting room of Centre B and C.

## 7.4 Discussion

This multicenter trial was accomplished to compare two preparations for transmucosal nasal delivery of low-dose midazolam. This trial was implemented at three radiologic departments and one private radiologic institution. The indication of interest was procedural anxiety during MRI examinations. The purpose was to investigate therapeutic equivalence of the two preparations.

Midazolam (oral, i.v., or nasal) is commonly used for procedural anxiety during MRI examinations. In several radiologic departments (e.g., Institute of Diagnostic Radiology, University Hospital Zurich Institute of Radiology, Kantonsspital Winterthur) midazolam nasal spray, prepared according to the monograph of NRF [NRF 2002], is routinely offered to anxious and/or claustrophobic patients for procedural anxiety.

The presented study was designed to compare two preparations (today's routine nasal spray MD and new UD nasal spray) and not for the quantitative assessment of efficacy in prevention of claustrophobia during MRI examinations. The design of a clinical trial to prove efficacy of nasal delivered low-dose midazolam in prevention of procedural anxiety and/or claustrophobia, is supposed to be randomized, placebo controlled, and preferably double blinded. For such a placebo-controlled study it would be essential, that the administration of the placebo nasal spray is identical concerning sensation of application and local irritation.

For the present trial, blinding was not feasible, due to different shapes of the two nasal sprays (MD and UD nasal spray). Furthermore, the application characteristics were integral part of the comparison of the two nasal sprays. The only option for blinding would have been a double-dummy study design, and consequently simultaneous nasal administration with both nasal sprays: in treatment group midazolam by multidose nasal spray and placebo by unit dose nasal spray and in comparator group vice versa, placebo by multidose nasal spray and midazolam by unit dose nasal spray. But because of the limited volumetric capacity of the nose, double-dummy design is not reasonable for nasal administration of liquid preparations. Hence, doubled application volume (placebo and treatment or placebo and comparator) would certainly result in swallowing of the midazolam preparation diluted by placebo.

Patients, self-assessing to be anxious and/or claustrophobic, were invited for participation in this trial with nasal midazolam. No standardized diagnostic tool was applied to identify claustrophobic patients. Intensity of anxiety and/or claustrophobia was rated by visual analog scale. McIsaac et al. assumed one single item to detect claustrophobia to be insufficient for predicting intense anxiety reactions during MRI examinations [Mc Isaac et al., 1998].

An elaborate claustrophobia questionnaire (with 29 items) for diagnosis of claustrophobia was proposed by Radosky et al. [Radosky et al., 2001], but in day-to-day operations at radiologic departments interviewing patients by this extensive questionnaire is too time-consuming. To help anxious patients to complete MRI examinations successfully it is not necessary to conclusively diagnose their claustrophobia. Therefore, Mc Isaac et al. reduced the extensive claustrophobia questionnaire to 6 items and suggested this screening tool for the identification of patients who are

most likely to experience claustrophobic fear or panic during MRI examinations. But this reduced questionnaire has not been validated yet and therefore reliability is still unsettled.

Because day's schedule in radiologic departments is tight, the accomplishment of a clinical trial should not affect work flow. Therefore, to successfully run a clinical trial in clinical everyday life it is crucial to deliver well structured self-explanatory study material. The documentation should be as less time-consuming as possible.

Schweizer et al. demonstrated in a pilot study the anxiolytic effect of low-dose midazolam (0.5-1 mg) in prevention of panic reactions [Schweizer, et al. 1992]. In addition, nasal delivery of low-dose midazolam proved to be efficient and safe for conscious sedation during MRI examinations [Tschirch, et al. 2006]. Nasally administered doses of 1 mg to 2 mg midazolam provided anxiolysis without sedation (i.e., conscious sedation) and therefore the patients were able to follow the instructions given during MRI examinations. Based on these findings for the present study the dose of 1 mg nasal midazolam (initial dose) was set with the possibility of delivering another 1 mg nasal midazolam if anxiolysis was not provided.

The composition and the multidose delivery device of the test preparation (Midazolam MD Nasal Spray 5 mg/ml) refer to the commonly used midazolam nasal spray, prepared by the local hospital pharmacies. Complying with monograph for nasal preparations of Ph.Eur., this aqueous nasal preparation, supplied in multidose delivery systems, is preserved. The multidose nasal spray used in this trial, delivers 0.1 ml per pump. But for correct dosing at least 1 ml preparation has to be filled into the delivery device. As for the administration of 1 mg to 2 mg midazolam only 0.2 ml to 0.4 ml are delivered, the major portion of the preparation is wasted. Furthermore, to get the multidose nasal spray ready for exact dosing, the pump needs to be prepared by pressing the pump several times previous to nasal administration. Preparing the pump inhalation of the atomized midazolam preparation is to be prevented. Since the upright position of the multidose nasal spray is essential for exact dosing, administration to lying patients is not possible. As used multidose nasal sprays do not look different from unused multidose nasal sprays, there is a certain risk for unhygienic multiple application to different patients.

The comparator (Midazolam UD Nasal Spray 1 mg) is supplied in a single-dose delivery device and is preservative-free. The dose of 1 mg midazolam is delivered within 0.1 ml by one-sided administration. For the administration of an additional dose, 0.1 ml is sprayed into the opposite nostril by applying a second unit dose nasal spray. The unit dose nasal spray system contains 0.125 ml nasal preparation, thereof 0.1 ml is delivered independent of the position. Therefore, the unit dose system allows nasal administration of the anxiolytic treatment directly to patients lying on MRI table, ready to be introduced into the MRI tunnel. As often patients declare their anxiety only at the moment they are introduced into the MRI tunnel, administration independent of position is advantageous.

Many patients presenting to radiologists for MRI examination admit anxiety and/or claustrophobia, but only few of them are willing to participate in a clinical trial. Finally, 110 patients gave consent to enter the trial with nasal midazolam. One patient dropped out because of withdrawal of consent.

Due to his excellent experiences with i.v. anxiety during a former MRI examination, after administration of the initial nasal dose of midazolam he requested i.v. application of an anxiolytic treatment.

Generally, if anxiety of the initial treatment with 1 mg midazolam (MD or UD) was not sufficient to perform MRI examination, one additional dose of 1 mg midazolam was administered.

All patients, refusing or interrupting the MRI examinations (therapy failures, n=3) were treated with 2 mg midazolam. Probably in these cases, diffuse anxiety and/or claustrophobia were too intense to be cured with low-doses of midazolam (1-2 mg). But due to small number of patients requiring 2 mg midazolam, statistical analysis of the intensity of anxiety is not reasonable.

Acquired data of one patient had to be excluded from final analysis, because of protocol deviation. This patient of the comparator group was initially treated with UD nasal spray but the additional dose was administered by MD nasal spray.

As anxious patients are generally agitated they tend to move during MRI examination impairing image quality by generating motion artifacts. In a prospective double-blind placebo controlled randomized trial Hollenhorst et al. demonstrated significant reduction of MRI related anxiety in patients receiving nasal midazolam, and the reported anxiety reduction correlated with improved image quality. In the present multicenter study with nasal midazolam, overall 91 patients (MD n=42 and UD n=49) were treated 1 mg nasal midazolam (initial dose). All of them successfully completed MRI examination and the generated MRI images were of good or even excellent quality.

No patient fell asleep and therefore all patients were able to appropriately respond to instructions given during MRI examination. All MRI examinations were rated as normally feasible by radiologic technician. Beside local irritation for both tested midazolam nasal spray no adverse drug reaction was reported.

The parameters to assess the benefit of nasal anxiety for anxious patients undergoing MRI examination were: anxiety score before medication and after completing of the MRI examination, tolerance of nasal administration, local irritation, and willingness to repeat the MRI examination with the analog medication receiving in the study.

Regarding subjective anxiety reduction, willingness to repeat the procedure, and diagnostic image quality, the tested nasal sprays (MD and UD) were equivalent. Concerning convenience of administration (i.e., delivery independent of position) and hygienic security UD nasal spray was rated as superior to MD nasal spray.

## 7.5 Conclusions

Nasal delivery of low-dose midazolam is a safe therapy to provide procedural anxiolysis in patients undergoing MRI examinations.

The two compared midazolam preparations for transmucosal nasal delivery of low-dose midazolam showed therapeutic equivalence. Hence, anxious and/or claustrophobic patients equally benefit from procedural anxiolysis during MRI examinations provided by administration of low-dose midazolam with MD nasal spray or UD nasal spray.

Concerning convenient handling, administration, and hygienic aspects the new midazolam nasal spray (UD) is superior to the commonly used midazolam multidose nasal spray (MD).





## 8 Overall discussion

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The objectives of this thesis were to provide preparations for transmucosal nasal midazolam delivery (Project I), to assess the pharmacokinetic characteristics of nasally applied midazolam (Project II), and to compare the benefit of two nasal midazolam preparations for procedural anxiolysis in anxious patients undergoing MRI examinations (Project III).

Pharmacokinetic parameters of nasally delivered drugs depend on many-sided interactions of different preparation and administration related parameters. If the nasally delivered volumes exceed the recommended volume of about 0.2 ml per nostril, little viscous preparations tend to flow in the nasopharynx and are swallowed. To compare the outcome of different pharmacokinetic trials on nasal drug delivery, the nasally administered volumes need to be regarded. In foregoing research on nasal midazolam delivery the commercially available midazolam preparation for i.v. use (Dormicum® 5 mg/ml) has been instilled into the nostrils, and often the doses (i.e., the administered volumes) exceeded the volumetric capacity of the nose (see Appendix 10.1.1). Therefore, published pharmacokinetic characteristics of nasal midazolam delivery often refer to a combination of transmucosal nasal and gastrointestinal drug absorption [Burstein, et al. 1997].

To study exclusive transmucosal nasal midazolam delivery, not falsified by gastrointestinal absorption of the swallowed portion, nasal midazolam preparations adapted to the limited nasal capacity were developed (Project I). Addition of RM $\beta$ CD facilitated compounding of aqueous preparations with high midazolam concentrations (up to 30 mg/ml midazolam base). This allowed administration of therapeutic doses (1 mg to 6 mg midazolam) by nasal application of a minimized volume (0.1 ml per nostril). Since systemic bioavailability of nasally applied midazolam was 86% to 93%, nasal administration of 6 mg midazolam corresponds to about 5 mg i.v. administered midazolam. Hence, the range of midazolam doses (1 mg to 6 mg midazolam), delivered by the presented nasal midazolam preparations (Preparation 1 to 5), facilitates nasal midazolam administration to provide different dose related effects (e.g., anxiolysis, sedation, or anticonvulsion).

High midazolam concentrations and thus reduction of nasal administration volumes successfully prevented swallowing of nasally administered preparations (Project II). The profile of midazolam metabolites ( $\alpha$ -hydroxymidazolam and 4-hydroxymidazolam) demonstrated effective by-passing of first-pass effect and hence exclusive transmucosal nasal absorption of nasally applied midazolam. Consequently, transmucosal nasal midazolam delivery (bioavailability 86% to 93%) proved higher systemic bioavailability than oral midazolam delivery (bioavailability 30% to 50% [Documed 2006]). Pharmacokinetic parameters assessed for transmucosal nasal midazolam delivery proved comparable characteristics as pharmacokinetics following i.v. administration of midazolam. Hence, transmucosal nasal delivered midazolam demonstrated suitable administration and

pharmacokinetic characteristics to provide midazolam therapy in clinical situations where common drug delivery methods (e.g., i.v. or i.m. administration) are inappropriate but fast onset of therapeutic effect is required (e.g., anticonvulsive treatment of status epilepticus).

In particular, the tested chitosan hydrochloride containing nasal midazolam preparation (Preparation 5) demonstrated fast increasing of midazolam serum concentration providing maximal midazolam serum concentrations within  $7.1 \pm 0.6$  minutes (range  $t_{\max}$  1.9 min). These findings are in accordance with the data published by Illum demonstrating chitosan to promote nasal absorption of morphine [Illum, et al. 2002]. Furthermore, nasal morphine preparations containing chitosan provided fast onset of therapeutic effect in the treatment of breakthrough pain in cancer patients [Pavis, et al. 2002]. As well as therapy of breakthrough pain, treatment of seizures (epileptic or febrile) is also a clinical situation where drug administration is difficult and fast onset of therapeutic effect is urgently needed.

Chitosan containing nasal midazolam preparation (Preparation 5) showed most promising pharmacokinetic characteristics for the treatment of seizures. Providing anticonvulsive therapy by nasal administration of midazolam the local irritation is of secondary importance compared to the enormous benefit of this needle-free drug delivery option featuring rapid onset of therapeutic effect. However, to elongate shelf life and to reduce local irritation further optimization of chitosan containing nasal midazolam preparations is required.

The performed pharmacodynamic testing during Project II (VAS and CRTT) assessed volunteer's attention and their ability to respond. These tests aimed predicting the time needed to restore fitness to drive after procedural anxiolysis with nasally applied midazolam. However, for the quantification of the anticonvulsive effects of nasally delivered midazolam more specific pharmacodynamic characterization needs to be performed (e.g., electroencephalogram).

To assess the anxiolytic effects of benzodiazepines in healthy volunteers, different testing methods have been described in literature [Visser et al., 2003]. But no testing method is specific enough to differentiate sedating from anxiolytic effects. Anxiety and panic during MRI examinations is impossible to be simulated, since these phenomena involve different components (e.g., fear of enclosed places, pain, the unknown situation, as well as apprehension about what the test might reveal) [Katz, et al. 1994]. Consequently, evaluation of therapeutic equivalence of two nasal midazolam preparations was accomplished with anxious and/or claustrophobic patients undergoing MRI examinations and not with healthy volunteers (multicenter trial, Project III).

Therapeutic equivalence of multidose nasal spray (MD, Preparation 1) and unit dose nasal spray (UD, Preparation 3) demonstrated in Project III is in accordance with the congruent serum concentrations time profiles attesting bioequivalence for these two preparations.

However, less required additional doses (total administration of 2 mg midazolam) in the comparator group (UD, Preparation 3) cannot be explained by serum concentration time profiles. Drug administration with the unit dose system (UD, Preparation 3) is mostly a new experience for the patients, while nasal drug administration with the multidose nasal spray (MD, Preparation 1) resembles ordinary nasal delivery of common decongestants for the treatment of rhinitis. The

unusual application experience may have influenced the subjective assessment of therapeutic effect.

As the impact of cyclodextrin derivatives on drug release and absorption kinetics (*in vivo*) is controversially discussed in literature, midazolam was nasally delivered without RM $\beta$ CD (Preparation 1) and with equimolar RM $\beta$ CD (Preparation 2). In aqueous solutions maximal 5 mg/ml midazolam (base) can be solubilized by dissolving midazolam hydrochloride. Therefore, the impact of RM $\beta$ CD on pharmacokinetics of nasally applied midazolam was studied at the concentration of 5 mg/ml midazolam base (Preparation 1 and Preparation 2). This midazolam concentration is equal to Dormicum<sup>®</sup> 5 mg/ml and only adequate for transmucosal delivery of low-dose midazolam.

RM $\beta$ CD, added in equimolar amounts to midazolam, demonstrated neither penetration enhancing nor penetration inhibiting effects on transmucosal nasal delivered midazolam. RM $\beta$ CD proved to be an adequate solubilizer in preparations for transmucosal nasal midazolam delivery.

Bioequivalence was confirmed for all tested nasal administration modalities for nasal delivery of 1 mg midazolam (Project II). According to Fick's first law, absorption of 1 mg nasal delivered midazolam following nasal administration of half concentration (5 mg/ml) on doubled application area (two-sided administration) was equivalent with one-sided administration of doubled midazolam concentration (10 mg/ml, Preparation 3). Hence, transmucosal nasal midazolam absorption demonstrated direct proportionality to (total) midazolam concentration and to the application area.

Unlike *in vivo* midazolam absorption (Project II), *in vitro* midazolam release was reduced by equimolar RM $\beta$ CD (Project I): doubled midazolam concentration (solubilized with equimolar amount of RM $\beta$ CD) did not result in doubled midazolam release from the donor compartment into the receptor compartment (*in vitro*). Apparently, not the total midazolam but the free midazolam concentration determined midazolam release *in vitro*.

In the nasal cavities (*in vivo*) there are a lot of molecules (components of the mucus layer or of the cell surfaces) potentially interfering with the cyclodextrin-drug complexes. Therefore, midazolam release *in vivo* is probably facilitated by competitive displacement of complexed midazolam molecules [Stella, et al. 1999].

The *in vitro* drug release studies showed different midazolam release for midazolam preparations with RM $\beta$ CD (equimolar) and without RM $\beta$ CD. However, overall transmucosal nasal midazolam uptake, resulting of midazolam release, penetration, and permeation processes, did not reflect the differences of midazolam release observed *in vitro* (Project I). Apparently, *in vitro* drug release studies revealed differences in midazolam release, that *in vivo* were of inferior importance. Thus, drug release studies were not reliable models to predict absorption kinetics of nasally applied midazolam preparations.

Osmolality of all proposed preparations for transmucosal nasal midazolam delivery was adjusted to physiologic conditions. For preparation proposed by Roelofse et al., containing 20% of  $\beta$ CD to solubilize 10 mg/ml midazolam, pH was 5.8. But as this preparation showed reduced systemic midazolam bioavailability following nasal administration, adding of excess cyclodextrin was

considered as unfavorable. Consequently, by applying only equimolar amounts of RM $\beta$ CD (2%, 4%, and 12% to solubilize 5 mg/ml, 10 mg/ml, and 30 mg/ml midazolam) the pH of the nasal preparations could not be adapted to physiologic pH due to limited midazolam solubility. Despite low pH of the tested preparations (pH 3 to 4), no volunteer did rate the nasal administration as painful and nasal irritation was reversible within 30 minutes.

As the proposed preparations are not supposed for long term therapies but for sporadic application (e.g., treatment of status epilepticus and procedural anxiolysis or sedation) some reversible local irritation may be tolerable.

For transmucosal nasal drug delivery there is no recommendation whether the dose is preferably administered one-sided or two-sided. Commercially available nasal preparations for systemic treatment (e.g., Zomig<sup>®</sup> nasal, AstraZeneca, Zug, Switzerland) are supplied in unit dose systems for one-sided administration. The blood vessels inside the lining of the nose become engorged in a cyclic fashion, which causes congestion and then decongestion of the linings of each side of the nose. This so-called 'nasal cycle' is detectable in about 80% of the population [Hasegawa and Kern 1977]. The impact of this nasal cycle on transmucosal nasal drug delivery is unknown. Against this background two-sided nasal drug delivery may be preferable even though one-sided and two-sided nasal administration of a given midazolam dose (1 mg) resulted in identical serum concentration time profiles.

## 9 Final conclusions

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The developed midazolam preparations presented in this thesis facilitated pharmacokinetic characterization of nasally applied midazolam. Reduction of nasal administration volumes successfully prevented swallowing of nasally applied preparations. Consequently, the assessed pharmacokinetic parameters described exclusive transmucosal nasal midazolam absorption not biased by gastrointestinal midazolam uptake. Midazolam metabolites serum concentration profiles following nasal midazolam delivery, demonstrated efficient avoidance of first-pass effect.

Pharmacokinetics of nasally applied midazolam was not adversely affected by equimolar amounts of RM $\beta$ CD to solubilize midazolam. Chitosan hydrochloride promoted transmucosal nasal absorption of nasally applied midazolam. Transmucosal nasal midazolam delivery is a convenient needle-free drug administration option and demonstrated pharmacokinetic characteristics to provide fast onset of therapeutic effects. Pharmacokinetic characteristics predestine chitosan containing midazolam preparations for transmucosal nasal midazolam delivery to provide fast onset of therapeutic effect.

High systemic bioavailability of nasally applied midazolam demonstrated the veritable potential of transmucosal nasal drug delivery as alternative to invasive drug administration.



## Appendix





## 10.1.1 Pharmacokinetic data of nasally applied midazolam (previous studies)

Reference	Number of subjects	Dose	Treatment and comparator	Volume	Assessed Parameters	F	T <sub>max</sub>	C <sub>max</sub>	General remarks
[Lofsson et al., 2001]	12	0.06mg/kg	Dormicum® i.v. 2 mg (dose not adjusted to body weight) Comparator: midazolam (17mg/ml) 12.33% Sulfobutylether-β-CD, 1% HPMcellulose, preservatives	0.2-0.3ml	Midazolam plasma concentration (t: 5, 10, 15, 20, 30, 60, 120, 180, 240, and 360min)	73% 41-95%	15 min (10-20)	54.3ng/ml (40-72ng/ml)	Solubilizer (Sulfobutylether-β-CD) arises pH and improves nasal tolerability
[Roelofse et al., 2000]	12	7.5mg	Dormicum® 5mg/ml Comparator: Midazolam (10mg/ml) 20% RMβCD	n.a.: 1.5ml n.a.: 0.75ml	Midazolam plasma concentration (0, 2.5, 5, 10, 20, 30, 60, 120, 240 min), visual examination of nasal mucosa assessment of anoxylsis by VAS	77%	33.33 min ±20.15	Dormicum® : 63.21 ng/ml ± 20.29ng/ml Comparator: 35.68 ng/ml ± 12.39ng/ml	Study design: Randomized, crossover (12 subjects with wisdom tooth extraction)
[Knoester et al., 2002]	6	5mg	Midazolam i.v. or nasal spray Nasal preparation based on propyleneglycol (27.8mg/ml midazolam)		Midazolam plasma concentration (2, 5, 10, 15, 20, 30 and 60 min, 2, 3, 4, 5, 6, 7 and 8 h), EEG	79% ±30%	14 min ± 5	71ng/ml ± 25ng/ml	Quantification of α-hydroxymidazolam (active metabolite)
[Malinovsky et al., 1993]	45 <sup>1</sup>	0.2mg/kg			Midazolam plasma concentration (before and until 360 min after midazolam administration)	--	6 min	100 ng/ml	
[Burststein et al., 1997]	8	0.25mg/kg	Dormicum® 5mg/ml: i.v.: 2 mg nasal: Dormicum® for i.v. administration was instilled into the nostrils with common syringe.	up to 5ml	Midazolam plasma concentration (5, 10, 20, 30, 45, 60, 90, 120, 180, 240, and 360 min), Digit Symbol Substitution Test (cognitive function testing)	50% ±13%	25 min (10-48)	147ng/ml (91.3-224.3)	Randomized, double-blind, crossover Midazolam peak after breakfast indicates gastrointestinal midazolam uptake due to swallowing of extended nasal administered volumes (up to 5ml)
[Gudmundsdottir et al., 2001]	18	0.06mg/kg	Dormicum® i.v.: 2mg Comparator: Midazolam (17mg/ml) 12.33% Sulfobutylether-β-CD		Midazolam plasma concentration (5, 10, 15, 20, 30, 60, 120, 180, 240, 360 min), Assessment of local irritation by interview, Sedation was rated according to a 8 point scale	64% (±19)	15.5 ±7.9 (5-30)	42.1ng/ml ± 11.2 (29-72)	Comparison of AUC of i.v. and n.a. midazolam → onset of action 5-10 min
[Bjorkman et al., 1997]	14	0.15mg/kg	Dormicum® i.v.: 0.15mg/kg (nasal administration by nasal spray)		Midazolam plasma concentration (2, 4, 6, 9, 12, 15, 20, und 30min und 1, 1.5, 2, 3, 4, 6, 8 und 10 h)	83% (±15)	14 min ± 2	192µg/L ± 48	→ α-hydroxymidazolam time profile is identical to the profile metabolite profile after i.v. administration.

<sup>1</sup>Children (age 2-9 years, weight 10-30 kg); n.a.: nasal administration

## 10.2 Project I

### 10.2.1 Ratio of cyclodextrin-midazolam complexes

	HPbCD-Midazolam		HPgCD-Midazolam		RMbCD-Midazolam	
	HPbCD	Midazolam	HPgCD	Midazolam	RMbCD	Midazolam
Molecular weight (g/mol)	1400	326	1576	326	1310	326
Concentration %	10		10		10	
Concentration (mg/ml)		26.2		40.8		33.1
Concentration (g/L)	100.0	26.2	100.0	40.8	100.0	33.1
Concentration (mol/L)	0.07	0.08	0.06	0.13	0.08	0.10
Ratio	1.00	1.13	1.00	1.97	1.00	1.33

### 10.2.2 Accelerated stability testing: Preparation 3 (10 mg/ml)

day	room temperature		40°C		50°C		60°C	
	concentration	content	concentration	content	concentration	content	concentration	content
	[mg/ml]	[%]	[mg/ml]	[%]	[mg/ml]	[%]	[mg/ml]	[%]
0	10.2	100	10.2	100	10.2	100	10.2	100
3	10.4	102	10.8	105	10.2	101	10.1	105
6	11.1	109	10.2	100	9.8	96	9.9	100
9	11.8	116	11.7	115	11.8	115	9.9	115
12	10.0	98	10.0	98	10.2	100	10.0	98
15	10.3	101	10.0	98	9.7	95	10.0	98
18	9.8	96	10.1	99	10.3	101	10.2	99
21	9.8	96	10.2	100	10.2	100	10.1	100
24	9.7	96	10.0	98	10.2	100	10.3	98
27	9.8	96	10.2	100	10.0	98	10.1	100
30	9.9	97	10.1	99	10.3	101	10.1	99

Tage	room temperature			40°C			50°C			60°C		
	pH	color	precipitation	pH	color	precipitation	pH	color	precipitation	pH	color	precipitation
0	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no
3	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no
6	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no
9	3.9	colorless	no	4.0	colorless	no	3.9	colorless	no	4.0	colorless	no
12	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no
15	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no
18	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no
21	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no
24	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no
27	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no
30	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no	4.0	colorless	no

### 10.2.3 Accelerated stability testing: Preparation 4 (30 mg/ml)

day	room temperature		40°C		50°C		60°C	
	concentration [mg/ml]	content [%]	concentration [mg/ml]	content [%]	concentration [mg/ml]	content [%]	concentration [mg/ml]	content [%]
0	30.0	100	30.0	100	30.0	100	30.0	100
3	30.0	100	29.6	99	30.6	102	30.6	102
6	29.3	98	27.7	92	29.7	99	29.5	98
9	30.6	102	30.5	102	30.0	100	30.1	100
12	29.8	99	29.7	99	30.8	103	30.1	100
15	29.7	99	30.5	102	29.5	98	29.5	98
18	30.1	100	30.7	102	30.1	100	30.3	101
21	30.7	102	30.0	100	30.3	101	29.9	100
24	30.5	102	30.3	101	30.2	101	30.4	101
27	30.2	101	30.6	102	30.3	101	29.8	99
30	30.2	101	30.2	101	30.8	103	30.4	101

Tage	room temperature			40°C			50°C			60°C		
	pH	color	precipitation	pH	color	precipitation	pH	color	precipitation	pH	color	precipitation
0	3.7	colorless	no	3.7	colorless	no	3.7	colorless	no	3.7	colorless	no
3	3.7	colorless	no	3.7	colorless	no	3.7	colorless	no	3.7	colorless	no
6	3.7	colorless	no	3.7	colorless	no	3.7	colorless	no	3.7	colorless	no
9	3.7	colorless	no	3.7	colorless	no	3.7	yellowish	no	3.7	yellowish	no
12	3.7	colorless	no	3.7	colorless	no	3.7	yellowish	no	3.7	yellowish	no
15	3.7	colorless	no	3.7	colorless	no	3.7	yellowish	no	3.7	yellowish	no
18	3.7	colorless	no	3.7	colorless	no	3.7	yellowish	no	3.7	yellowish	no
21	3.7	colorless	no	3.7	colorless	no	3.7	yellowish	no	3.7	yellowish	no
24	3.7	colorless	no	3.7	colorless	no	3.7	yellowish	no	3.7	yellowish	no
27	3.7	colorless	no	3.7	colorless	no	3.7	yellowish	no	3.6	yellowish	no
30	3.7	colorless	no	3.7	colorless	no	3.7	yellowish	no	3.7	yellowish	no

10.2.4 Specification: Midazolam UD Nasal Spray 0.5mg (Preparation 1a)

Datum: 04.05.06  
Seite: 1 von 2

Gültig ab: 04.05.06  
Autorisierte Kopie Nr.: 04.05.06  
Dokumentnummer: SPO871-V03.doc

**Universitätsspital  
Basel  
Spital-Pharmazie**

**Spezifikation**

Midazolam UD Nasenspray 0,5mg  
Pharmakokinetik-Studie

Autorisierte Kopie - Nur gültig mit rotem Aufdruck

---

Datum: 04.05.06  
Seite: 2 von 2

Gültig ab: 04.05.06  
Autorisierte Kopie Nr.: 04.05.06  
Dokumentnummer: SPO871-V03.doc

**Universitätsspital  
Basel  
Spital-Pharmazie**

**Spezifikation**

Midazolam UD Nasenspray 0,5mg  
Pharmakokinetik-Studie

Autorisierte Kopie - Nur gültig mit rotem Aufdruck

---

**Geprüft und Genehmigt:**

Name:	K. Suter	Datum:	9.5.06	Unterschrift:	<i>K. Suter</i>
Autor:	Dr. R. Wiemer		9.5.06		<i>R. Wiemer</i>
Leiter Herstellung:	V. Figueiredo		8.5.06		<i>V. Figueiredo</i>
Leiter Qualitätskontrolle:	Dr. R. Leu Marseller		10.5.06		<i>R. Leu Marseller</i>

**1. Identifikation:** Midazolam UD Nasenspray 0,5mg für Pharmakokinetik-Studie  
entfällt  
Artikel-ID: entfällt

**Bezeichnung:** Midazolam UD Nasenspray 0,5mg für Pharmakokinetik-Studie  
entfällt

**SAP-Name:** Eigenprodukt - Studienware

**Kategorie:** Studienware

**Lieferant(en):** Herstellung steril

**2. Beschreibung:**

**Bezeichnung Inhaltsstoffe:** Herstellvorschrift: HV0871  
5,55mg  
Midazolamhydrochlorid DAC  
q.s.  
Säure 0,1M 500ml  
SP0106  
Natriumchlorid-Lösung 0,9%, Ovalla  
Fresenius

**Summe:** 1,00ml  
(=1,01g)

**Spezifikation:** Bezeichnung Verpackung:  
SP0605 Primär: Unitidose Nasenspray  
SP0015 Etikette Primärverpackung: Etikette 25x50mm weiss  
SP0326 Etikette Sekundärverpackung: Etikette 70x70mm doppelt abziehbar  
SP0597 Sekundär: Minigrip Beutel 75x90x0,05mm

**3. Anforderungen:** Prüfvorschrift: IP-Kontrolle  
Eigenschaft: klare farblose Lösung entspricht  
Identität: Midazolam entspricht  
Natriumchlorid-Lösung 0,9% Visum Pharmazeut  
Midazolam-Basis 4,50-5,50mg/ml  
Gehalt: 270-330µmol/kg  
pH-Wert: 3,0-3,5  
Osmolalität: 270-330mosmol/kg

**4. Hinweise:** Raumbedingung  
6 Monate, zum einmaligen Gebrauch.  
Der Midazolam UD Nasenspray 0,5mg wird für die Bestimmung der Pharmakokinetik von nasal appliziertem Midazolam im Rahmen einer klinischen Studie eingesetzt.  
Gemäss Studienprotokoll. Die nasale Applikation von einem Hub entspricht der Dosis von 0,5mg Midazolam (berechnet als Base).  
Die Wirkung gleichzeitig eingenommener sedierender Medikamente kann verstärkt werden.  
Das System gibt einen Hub mit dem Volumen von 100µl ab und kann nicht weiter verwendet werden. Der Unitidose Nasenspray ist gebrauchsfertig und kann nach dem Entfernen der Schutzkappe unmittelbar appliziert werden. Das Hubvolumen von 100µl wird lageunabhängig abgegeben, der Spray kann auch dem liegenden Patienten verabreicht werden. Die Dichte beträgt 1,0063g/ml.

---

**Bemerkung**

Die Formulierung entspricht jener des "Midazolam Nasenspray 5mg/ml für multizentrische Analyse-Studie" (SP/HV/PV0806), ist jedoch nicht konserviert.

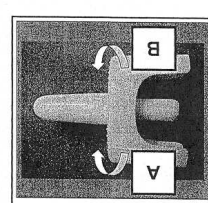
**5. Etikette(n):**

Die Primäretikette wird halbiert und wie auf untenstehender Abbildung dargestellt auf den Unitidose Nasenspray geklebt.

Autorisierte Kopie - Nur gültig mit rotem Aufdruck

---

**Primäretikette (Teil A und B)**



Für die klinischen Versuche  
Pharmakokinetik-Studie  
Midazolam UD Nasenspray 0,5mg  
PK-Studie / Formulierung 1

ANWENDUNG GEMÄSS STUDIENPROTOKOLL  
1 Hub enthält 0,5mg Midazolam  
(berechnet als Base)  
Prüfart: Dr. med. M. Haschke  
Tel.: 061 266 88 60  
Ch. Nr. 000000500 EXP. 000000  
**UNIVERSITÄTSSPITAL BASEL**

Autorisierte Kopie - Nur gültig mit rotem Aufdruck

10.2.5 Specification: Midazolam MD Nasal Spray 5mg/ml (Preparation 1b)

Datum: 21.09.06  
Seite: 2 von 2  
Gültig ab: Siehe Deckblatt  
Autorisierte Kopie Nr.: Siehe Deckblatt  
Dokumentnummer: SP0806-V02.doc

**Spezifikation**

Midazolam Nasenspray 5mg/ml  
Für multizentrische Anxiolyse-Studie

**Interaktion:** Die Wirkung von gleichzeitig eingenommenen sedierend wirkenden Medikamenten kann verstärkt werden.  
**Besondere Hinweise:** Von der Applikation muss die Zerstäuberpumpe 5mal betätigt werden (das Einatmen des Sprühnebels soll vermieden werden). Der Midazolam Nasenspray 5mg/ml ist während der Applikation adreht zu halten, damit der gesamte Inhalt des Inhalators in das Nasenspray gelangt.  
Die Dosis des Midazolam Nasenspray 5mg/ml beträgt 1,0621g/ml.  
Die Rezeptur für den Midazolam Nasenspray 5mg/ml basiert auf der NRF<sup>1</sup> Rezeptur Midazolamhydrochlorid Nasenspray (Stand 6.11.2002).  
Die Zusammensetzung der vorliegenden Rezeptur ist so gewählt, dass sie pro ml 5,00mg Midazolam Base enthält. Um eine ausreichende Midazolam-Löslichkeit zu erreichen, ist die pH-Senkung durch Salzsäure notwendig.

Autorisierte Kopie - Nur gültig mit rotem Aufdruck

**Primäretikette**

**Extern** Nur für den klinischen Versuch  
Multizentrische Anxiolyse-Studie  
**Midazolam Nasenspray 5mg/ml**  
Anwendung gemäss Studienprotokoll  
1-Hub-Box (Glas-Inhalator) (unsteril, ab 6 Jahre)  
Dosierung gemäss Studienprotokoll  
Cespar - Benzolimidazol, Natriumchlorid  
Ch.Nr.: 000000000 EXP: TMMMJJ  
**UNIVERSITÄTSSPITAL BASEL**

**Sekundäretikette**

**Extern** Nur für den klinischen Versuch  
Multizentrische Anxiolyse-Studie  
**Midazolam Nasenspray 5mg/ml**  
Anwendung gemäss Studienprotokoll  
1-Hub-Box (Glas-Inhalator) (unsteril, ab 6 Jahre)  
Dosierung gemäss Studienprotokoll  
Cespar - Benzolimidazol, Natriumchlorid  
Ch.Nr.: 000000000 EXP: TMMMJJ  
**UNIVERSITÄTSSPITAL BASEL**

Datum: 21.09.06  
Seite: 1 von 2  
Gültig ab: 09.09.06  
Autorisierte Kopie Nr.: 01.09.06  
Dokumentnummer: SP0806-V02.doc

**Spezifikation**

Midazolam Nasenspray 5mg/ml  
Für multizentrische Anxiolyse-Studie

**Gepflicht und Genehmigt:** Name: K. Suter  
Datum: 22.3.06  
Unterschrift: K. Suter

**Autor:** Dr. R. Werner  
Datum: 22.9.06  
Unterschrift: R. Werner

**Leiter Herstellung:** V. Figueredo  
Datum: 22.9.06  
Unterschrift: V. Figueredo

**Leiter Qualitätskontrolle:** Dr. R. Leu Marseller  
Datum: 22.9.06  
Unterschrift: R. Leu Marseller

**Leiter Qualitätssicherung:** Dr. R. Leu Marseller  
Datum: 22.9.06  
Unterschrift: R. Leu Marseller

**1. Identifikation:** Artikel-ID: entfällt

**Bezeichnung:** Midazolam Nasenspray 5mg/ml für multizentrische Anxiolyse-Studie

**SAP-Name:** entfällt

**Kategorie:** Eigenprodukt - Studienware

**Lieferant(en):** Herstellung nicht steril

**2. Beschreibung:**

Bezeichnung	Inhaltsstoffe	Menge / Einheit
SP0038	Midazolamhydrochlorid DAC	5,55mg
SP0030	Benzalkoniumchlorid-Lösung	197µg
SP0027	Natriumdecal PhEur	1,00mg
SP0004	Wasser für Injektionszwecke PhEur	98,8mg
SP0106	Salzsäure 0,1M 500ml	q.s.
B. Braun	Natriumchlorid-Lösung 0,9%, Spüllösung (Artikel-ID: 4002718)	ad 1,00ml
	<b>Summe:</b>	1,00ml
		1,01g

**Spezifikation:** **Bezeichnung Verpackung:**

SP0323 Primär: Pipettenglas 10ml, braun  
SP0808 Primär: Nasenspray-Zerstäuberpumpe  
SP0136 Etikette Primärverpackung: Etikette 32x50mm weiss  
SP0328 Etikette Sekundärverpackung: Etikette 70x70mm doppelt abziehbar (sekundär)  
SP0705 Zusatzzeitkette: Etikette 10x34mm weiss  
SP0524 Sekundär: Milingrip Beutel 100x220x0,05mm

**3. Anforderungen:**

Menge / Einheit	Füllmenge	Prüfvorschrift:
>2g	klare, farblose Lösung	PV0808
	Midazolam	entspricht
	Benzalkoniumchlorid	entspricht
	Visum Pharmazeut	Visum Pharmazeut
	EDITA	Visum Pharmazeut
	Midazolam-Base	4,50-5,50mg/ml
		3,0-3,5
	Osmolalität:	270-330mosm/kg

**4. Hinweise:**

**Lagerungsbedingungen:** Raumbedingung  
**Verwendungsdauer:** 6 Monate  
**Indikationen:** Der Midazolam Nasenspray 5mg/ml wird zur Anxiolyse vor MRI-Untersuchungen eingesetzt.  
**Dosierung/Applikation:** Vor der Untersuchung je ein Hub pro Nasenloch applizieren (0,1ml enthält 0,5mg Midazolam Base). Bei Bedarf kann die Applikation von 1 Hub pro Nasenloch wiederholt werden. Anwendung gemäss Studienprotokoll.

Autorisierte Kopie - Nur gültig mit rotem Aufdruck

**Reserve**

**Rückstellmuster**

**Patienten Nr.:** 0000

**Rückstellmuster:** Rückstellmuster

**Zusatzetiketten:** Um den Verwendungszweck des klinischen Prüfmusters anzugeben, werden Zusatzetiketten auf die unbedruckte Stelle der Sekundäretikette geklebt.

**Patientennummer:** Reserve: Rückstellmuster:

<sup>1</sup> NRF: Neues Rezeptur-Formularium, herausgegeben von der Bundesvereinigung deutscher Apothekerverbände.

10.2.6 Specification: Cyclodex-Midazolam UD Nasal Spray 0.5mg (Preparation 2)

**Universitätsspital Basel**  
Spital-Pharmazie

Cyclodex-Midazolam UD Nasenspray 0,5mg für Pharmakokinetik-Studie

Datum: 04.05.08  
Seite: 2 von 2  
Gültig ab: Siehe Deckblatt  
Autorisierte Kopie Nr.: Siehe Deckblatt  
Dokumentnummer: SP0894-V03.doc

**Spezifikation**

**Bemerkung** Die Formulierung des „Midazolam UD Nasenspray 0,5mg“ basiert auf jener des „Midazolam UD Nasenspray 1mg – für multizentrische Anxiolyse-Studie“ (SP/HV/PV0803).

**5. Etikette(n):**  
Die Primäretikette wird halbiert und wie auf untenstehender Abbildung dargestellt auf den Unitdose Nasenspray geklebt.

**Primäretikette (Teil A und B)**

**Sekundäretikette**

**EXTERN** Nur für den klinischen Versuch  
Pharmakokinetik-Studie  
Cyclodex-Midazolam UD Nasenspray 0, 5mg  
PK-Studie / Formulierung 2

Anwendung gemäss Studienprotokoll  
1 Hub enthält 0, 5mg Midazolam  
(berechnet als Base)

Prüfart: Dr. med. M. Haschke  
Tel.: 061 266 88 60  
Ch. Nr. 000000S00 EXP. 000000  
UNIVERSITÄTSSPITAL BASEL

Autorisierte Kopie - Nur gültig mit rotem Aufdruck

**Universitätsspital Basel**  
Spital-Pharmazie

Cyclodex-Midazolam UD Nasenspray 0,5mg für Pharmakokinetik-Studie

Datum: 04.05.08  
Seite: 1 von 2  
Gültig ab: 10.11.06  
Autorisierte Kopie Nr.: 01.11.06  
Dokumentnummer: SP0894-V03.doc

**Spezifikation**

Geprüft und Genehmigt:	Name:	Datum:	Unterschrift:
Autor	K. Suter	3.5.06	K. Suter
Leiter Herstellung	Dr. R. Wiener	3.5.06	R. Wiener
Leiter Qualitätskontrolle	V. Figueiredo	8.5.06	V. Figueiredo
Leiter Qualitätssicherung	Dr. R. Leu Marseller	10.11.06	R. Leu Marseller

**1. Identifikation:** **Artikel-ID:** entfällt

**Bezeichnung:** Cyclodex-Midazolam UD Nasenspray 0,5mg für Pharmakokinetik-Studie

**SAP-Name:** entfällt

**Kategorie:** Eigenprodukt - Studienware

**Lieferant(en):** Herstellung sterl

**2. Beschreibung:** **Bezeichnung Inaktivstoffe:** Menge / Einheit: **Herstellervorschrift:** **HV0864**

SP0098 Midazolamhydrochlorid DAC 5,55mg

SP0804 randomisiert methyliertes b-Cyclodextrin 20,1mg

SP0006 Natriumchlorid für Parenterals PHEur 7,85mg

SP0004 Wasser für Injektionszwecke PHEur ad 1,00ml

**Summe:** 1,00ml (=1,0g)

**Spezifikation:** **Bezeichnung Verpackung:**

SP0805 Primär: Unitdose Nasenspray

SP0015 Etikette Primärverpackung: Etikette 25x50mm weiss

SP0326 Etikette Sekundärverpackung: Etikette 70x70mm doppelt abziehbar

SP0597 Sekundär: Minigrip Beutel 75x90x0,05mm

**3. Anforderungen:** **Prüfvorschrift:** **IP-Kontrolle**

**Eigenschaft:** Klare farblose Lösung entspricht

**Identität:** Midazolam randomisiert methyliertes b-Cyclodextrin entspricht

Visum Pharmazeut

**Gehalt:** Midazolam-Base 4,50-5,50mg/ml

**pH-Wert:** 3,5-4,5

**Osmolalität:** 270-330mosmol/kg

**4. Hinweise:**

**Lagerungsbedingungen:** Raumbedingung

**Verwendungsdauer:** 6 Monate, zum einmaligen Gebrauch.

**Indikationen/Kontraind.:** Der Cyclodex-Midazolam UD Nasenspray 0,5mg wird für die Bestimmung der Pharmakokinetik von nasal appliziertem Midazolam im Rahmen einer klinischen Studie eingesetzt.

**Dosierung/Applikation:** Gemäss Studienprotokoll. Die nasale Applikation von einem Hub entspricht der Dosis von 0,5mg Midazolam (berechnet als Base).

**Interaktion:** Die Wirkung gleichzeitig eingenommener sedierender Medikamente kann verstärkt werden.

**Besondere Hinweise:** Das System gibt einen Hub mit dem Volumen von 100µl ab und kann nicht weiter verwendet werden. Der Unitdose Nasenspray ist gebrauchsfertig und kann nach dem Entfernen der Schutzkappe unmittelbar appliziert werden. Das Hubvolumen von 100µl wird lageunabhängig abgeben, der Spray kann auch dem liegenden Patienten verabreicht werden. Die Dichte beträgt 1,01945g/ml.

Autorisierte Kopie - Nur gültig mit rotem Aufdruck

10.2.7 Specification: Cyclodex-Midazolam UD Nasal Spray 1mg (Preparation 3)

**Universitätsspital Basel**  
Spital-Pharmazie

**Cyclodex-Midazolam UD Nasenspray 1mg**  
für Pharmakokinetik-Studie

Datum: 04.05.06  
Seite: 1 von 2  
Gültig ab: 10.11.06  
Autorisierte Kopie Nr.: 01.11.05.06  
Dokumentnummer: SP0883-V03.doc

**Spezifikation**

**Geprüft und Genehmigt:** Name: K. Suter, Datum: 3.5.06, Unterschrift: *K. Suter*  
 Leiter Herstellung: Dr. R. Werner, Datum: 3.5.06, Unterschrift: *R. Werner*  
 Leiter Qualitätskontrolle: V. Figueiredo, Datum: 3.5.06, Unterschrift: *V. Figueiredo*  
 Leiter Qualitätssicherung: Dr. R. Leu Marseller, Datum: 10.11.06, Unterschrift: *R. Leu Marseller*

**1. Identifikation:** Bezeichnung: Cyclodex-Midazolam UD Nasenspray 1mg für Pharmakokinetik-Studie, Artikel-ID: entfällt  
 SAP-Name: entfällt  
 Kategorie: Eigenprodukt - Studienware  
 Lieferant(en): Herstellung steril

**2. Beschreibung:** Bezeichnung Inbalsstoffe: Hersteller/Vorschrift: HV0863  
 SP0088: Midazolamhydrochlorid DAC, Menge/Einheit: 11, 1mg  
 SP0804: randomisiertes B-Cyclodextrin, 40,2mg  
 SP0006: Natriumchlorid für Paracetamol, PNEUR, 6,70mg  
 SP0004: Wasser für Injektionszwecke PNEUR, ad 1,00ml  
 Summe: 1,00ml (=1,02g)

**Spezifikation:** Bezeichnung Verpackung:  
 SP0805 Primär: Unitöse Nasenspray  
 SP0015 Etiketle Primärverpackung: Etiketle 25x50mm weiss  
 SP0326 Etiketle Sekundärverpackung: Etiketle 70x70mm doppelt abziehbar  
 SP0597 Sekundär: MiniGrüP Beutel 75x80x0,05mm

**3. Anforderungen:** Anfordernungen: klare farblose Lösung, IP-Kontrolle entspricht  
 Eigenschaften: Midazolam, randomisiertes methylertes b- Cyclohexan, entspricht  
 Identität: Midazolam-Basis, Visum Pharmazeut

Gehalt: 9,00-11,0mg/ml  
 pH-Wert: 3,5-4,5  
 Osmolalität: 270-330mosmol/kg

**4. Hinweise:** Lagerungsbedingungen: Raumbedingung  
 Verwendungsduer: 6 Monate, zum einmaligen Gebrauch.  
 Indikationen/Kontraind.: Der Cyclodex-Midazolam UD Nasenspray 1mg wird für die Bestimmung der Pharmakokinetik von nasal appliziertem Midazolam im Rahmen einer klinischen Studie eingesetzt.  
 Gemäss Studienprotokoll. Die nasale Applikation von einem Hub entspricht der Dosis von 1mg Midazolam (berechnet als Base). Die Wirkung gleichzeitig eingenommener sedierender Medikamente kann verstärkt werden.  
 Dosierung/Applikation: Das System gibt einen Hub mit dem Volumen von 100µl ab und kann nicht weiter verwendet werden. Der Unitöse Nasenspray ist gebrauchsfertig und kann nach dem Entfernen der Schutzkappe unmittelbar appliziert werden. Das Hubvolumen von 100µl wird lageunabhängig abgegeben, der Spray kann auch dem liegenden Patienten verabreicht werden. Die Dichte beträgt 1,01705g/ml.  
 Interaktion:  
 Besondere Hinweise:

**Universitätsspital Basel**  
Spital-Pharmazie

**Cyclodex-Midazolam UD Nasenspray 1mg**  
für Pharmakokinetik-Studie

Datum: 04.05.06  
Seite: 2 von 2  
Gültig ab: Siehe Deckblatt  
Autorisierte Kopie Nr.: Siehe Deckblatt  
Dokumentnummer: SP0883-V03.doc

**Spezifikation**

**Bemerkung:** Die Zusammensetzung der Formulierung entspricht jener des Midazolam UD Nasenspray 1mg – für multizentrische Anxiolyse-Studien\* (SPHV/PV0803)

**5. Etikette(n):** Die Primäretikette wird halbiert und wie zur untenstehenden Abbildung dargestellt auf den Unitöse Nasenspray geklebt.

**Primäretikette (Teil A und B)**

**Sekundäretikette**

**EXTERN** Nur für den klinischen Versuch  
 Pharmakokinetik-Studie  
 Cyclodex-Midazolam UD Nasenspray 1mg  
 PK-Studie / Formulierung 3

Anwendung gemäss Studienprotokoll  
 1 Hub enthält 1mg Midazolam  
 (berechnet als Base)  
 Prüfarzt: Dr. med. M. Haschke  
 Tel.: 061 265 88 60  
 Ch. Nr. 000000500 EXP. 000000  
**UNIVERSITÄTSSPITAL BASEL**

Datum: 21.09.06  
 Seite: 1 von 2  
 Gültig ab: 22.09.06  
 Autorisierte Kopie Nr.: 01/02/03/06  
 Dokumentennummer: SP0803-V02.006

Universitätsspital  
 Basel  
 Spital-Pharmazie

Midazolam UD Nasenspray 1mg für multizentrische Anxiolyse-Studie

Spezifikation

Geprüft und Genehmigt:	Name:	Datum:	Unterschrift:
Autor	K. Suter	22.9.06	<i>K. Suter</i>
Leiter Herstellung	Dr. R. Werner	22.9.06	<i>R. Werner</i>
Leiter Qualitätskontrolle	V. Figueiredo	21.9.06	<i>V. Figueiredo</i>
Leiter Qualitätssicherung	Dr. R. Leu Marseller	22.9.06	<i>R. Leu Marseller</i>

**1. Identifikation:** Artikel-ID: entfällt

**Bezeichnung:** Midazolam UD Nasenspray 1mg für multizentrische Anxiolyse-Studie

**SAP-Name:** entfällt

**Kategorie:** Eigenprodukt - Studienware

**Lieferant(en):** Herstellung steril

**2. Beschreibung:** **Herstellung:** steril

**Herstellervorschrift:** HV0803

**Spezifikation:** **Bezeichnung Inhaltsstoffe:**

	Menge / Einheit:
SP0089	11,1mg
SP0804	randomisiert methyliertes b-Cyclodextrin
SP0006	Natriumchlorid für Parenterala PhEur
SP0004	Wasser für Injektionszwecke PhEur
	96,0mg
	Summe:
	1,02g (=1,00ml)

**Spezifikation:** **Bezeichnung Verpackung:**

SP0805 Primar: Unitdose Nasenspray

SP0015 Etikette Primärverpackung: Etikette 25x50mm weiss

SP0326 Etikette Sekundärverpackung: Etikette 70x70mm doppelt abziehbar

SP0705 Zusatzetiketten: Etikette 18x34mm weiss

SP0597 Sekundär: Minigrip Beutel 75x90x0,05mm

**3. Anforderungen:** Prüfvorschrift: PV0803

**Eigenschaft:** klare farblose Lösung

**Identität:** Midazolam entspricht  
 Natrium Visum Pharmazeut entspricht  
 Midazolam-Base 9,00-11,0mg/ml entspricht

**Gehalt:** 9,00-11,0mg/ml

**pH-Wert:** 3,5-4,5

**Osmolalität:** 270-330mosmol/kg

**Sterilität:** steril

**4. Hinweise:**

**Lagerungsbedingungen:** Raumbedingung

**Verwendungsdauer:** 6 Monate, zum einmaligen Gebrauch.

**Indikationen/Kontraind.:** Der Midazolam UD Nasenspray 1mg wird zur Anxiolyse vor MRI-Untersuchungen eingesetzt.

**Dosierung/Application:** Die nasale Applikation von einem Hub entspricht der Dosis von 1mg Midazolam (berechnet als Base), bei Bedarf kann 1mg nachdosiert werden.

**Interaktion:** Die Wirkung gleichzeitig eingenommener sedierender Medikamente kann verstärkt werden.

Autorisierte Kopie - Nur gültig mit rotem Aufdruck

Datum: 21.09.06  
 Seite: 2 von 2  
 Gültig ab: Siehe Deckblatt  
 Autorisierte Kopie Nr.: Siehe Deckblatt  
 Dokumentennummer: SP0803-V02.006

Universitätsspital  
 Basel  
 Spital-Pharmazie

Midazolam UD Nasenspray 1mg für multizentrische Anxiolyse-Studie

Spezifikation

**Besondere Hinweise:** Der Midazolam UD Nasenspray ist eine Monodose und wird daher einmalig eingesetzt. Das System gibt einen Hub mit dem Volumen von 100µl ab und kann nicht weiter verwendet werden. Der Midazolam UD Nasenspray ist gebrauchsfertig und kann nach dem Entfernen der Schutzkappe unmittelbar appliziert werden. Das Hubvolumen von 100µl wird lagunabhängig abgegeben, der Spray kann zusätzlich noch durch die Lippen des Patienten verabreicht werden. Die Dichte beträgt 1,0170 g/ml.

**5. Etikette(n):**

Die Primäretikette wird halbiert und wie auf untenstehender Abbildung dargestellt auf den Unitdosen Nasenspray geklebt.

A

B

A

B

**Sekundäretikette**  
 Nur für den klinischen Versuch  
**EXTERN**  
 Multizentrische Anxiolyse-Studie  
**Midazolam UD Nasenspray 1mg**  
 Anwendung gemäss Studienprotokoll

**Primäretikette (Teil A und B)**

Midazolam UD Nasenspray 1mg

UNIVERSITÄTSSPITAL BASEL

**Zusatzetiketten:** Um den Verwendungszweck des klinischen Prüfmoders anzugeben, werden Zusatzetiketten auf die unbedruckte Stelle der Sekundäretikette geklebt.

Nachdosierung: Rückstellmuster:  
 Patientennummer: 0000 Rückstellmuster  
 Patientennr. 0000

**UNIVERSITÄTSSPITAL BASEL**  
 EXP.: 17MMJJ  
 Ch. Nr.: 000000600

**UNIVERSITÄTSSPITAL BASEL**  
 Prof. Dr. Peter Dominik Weissenhof  
 Universitätsklinik für Psychiatrie  
 Kantonsspital Winterthur (092.896.46.19)  
 Radiologie Winterthur (092.106.142.42)  
 Orthopädische Universitätsklinik Balgrist (044.895.18.13)

Autorisierte Kopie - Nur gültig mit rotem Aufdruck



10.2.8 Specification: Cyclodex-Midazolam UD Nasal Spray 3mg (Preparation 4)

**Universitätsspital Basel**  
Spital-Pharmazie

Cyclodex-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie

Datum: 04.05.06  
Seite: 2 von 2  
Gültig ab: 10.11.06  
Autorisierte Kopie Nr.: 04.05.06  
Dokumentnummer: SP0865-V03.doc

**Spezifikation**

**Geprüft und Genehmigt:** Name: K. Suter, Datum: 3.1.06, Unterschrift: *K. Suter*  
 Autor: Dr. R. Werner, Datum: 9.5.06, Unterschrift: *R. Werner*  
 Leiter-Herstellung: V. Figueiredo, Datum: 9.5.06, Unterschrift: *V. Figueiredo*  
 Leiter-Qualitätskontrolle: Dr. R. Leu Marseller, Datum: 11.11.06, Unterschrift: *R. L. Marseller*

**1. Identifikation:** Bezeichnung: Cyclodex-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie, Artikel-ID: entfällt  
 SAP-Name: entfällt  
 Kategorie: Eigenprodukt - Studienware  
 Lieferant(en): Herstellung steril  
**2. Beschreibung:** Bezeichnung Inhaltsstoffe: HV0865  
 SP0088 Midazolamhydrochlorid DAC, Menge / Einheit: 33,3mg  
 SP0804 randomisiert methylertes b-Cyclodextrin, Menge / Einheit: 1211mg  
 SP0006 Natriumchlorid für Parenteralia PhEur, Menge / Einheit: 1,801mg  
 SP0004 Wasser für Injektionszwecke PhEur, Menge / Einheit: ad 1,00ml  
 Summe: 1,00ml (=1,04g)

**Spezifikation:** Bezeichnung Verpackung: SP0805 Primär: Unitidose Nasenspray, Etlkette 25x50mm weiss  
 SP0015 Etlkette Primärverpackung: Etlkette 70x70mm doppelt abziehbar  
 SP0326 Sekundär: Minigrip Beutel 75x90x0,05mm  
 SP0557

**3. Anforderungen:** Prüfverfahren: klare farblose Lösung, IP-Kontrolle entspricht  
 Identität: Midazolam, Osmolalität: entspricht  
 pH-Wert: randomisiert methylertes b-Cyclodextrin, Osmolalität: Visum Pharmazeut  
 Gehalt: Midazolam-Basis, Osmolalität: 27,0-33,0mg/ml, 3,0-4,5  
 Osmolalität: 270-330mosmol/kg

**4. Hinweise:** Lagerungsbedingungen: Raumbedingung  
 Verwendungsduer: 6 Monate, zum einmaligen Gebrauch  
 Indikationen/Kontraind.: Der Cyclodex-Midazolam UD Nasenspray 3mg wird für die Bestimmung der Pharmakokinetik von nasal appliziertem Midazolam im Rahmen einer klinischen Studie eingesetzt.  
 Dosierung/Applikation: Gemäss Studienprotokoll. Die nasale Applikation von einem Hub entspricht der Dosis von 3mg Midazolam (berechnet als Base). Die Wirkung gleichzeitig eingenommener sedierender Medikamente kann verstärkt werden.  
 Interaktion: Das System gibt einen Hub mit dem Volumen von 100µl ab und kann nicht weiter verwendet werden. Der Unitidose Nasenspray ist gebrauchsfertig und kann nach dem Entleeren der Schutzkappe unmittelbar appliziert werden. Das Hubvolumen von 100µl wird lageunabhängig abgegeben, der Spray kann auch dem liegenden Patienten verabreicht werden. Die Dichte beträgt 1,04284g/ml.  
 Besondere Hinweise:

Autorisierte Kopie - Nur gültig mit rotem Aufdruck

**Primäretikette (Teil A und B)**

EXTERN  
Nur für den klinischen Versuch  
Pharmakokinetik-Studie  
Cyclodex-Midazolam UD Nasenspray 3mg  
PK-Studie / Formulierung 4

Anwendung gemäss Studienprotokoll  
1 Hub enthält 3 mg Midazolam (berechnet als Base)  
Prüfart: Dr. med. M. Haschke  
Tel.: 061 265 88 60  
Ch. Nr. 000000S00 EXP. 000000  
UNIVERSITÄTSSPITAL BASEL

**Sekundäretikette**

EXTERN  
Nur für den klinischen Versuch  
Pharmakokinetik-Studie  
Cyclodex-Midazolam UD Nasenspray 3mg  
PK-Studie / Formulierung 4

Anwendung gemäss Studienprotokoll  
1 Hub enthält 3 mg Midazolam (berechnet als Base)  
Prüfart: Dr. med. M. Haschke  
Tel.: 061 265 88 60  
Ch. Nr. 000000S00 EXP. 000000  
UNIVERSITÄTSSPITAL BASEL

10.2.9 Specification: Chitosan-CD-Midazolam UD Nasal Spray 3mg (Preparation 5)

**Universitätsspital Basel**  
Spital-Pharmazie

**Chitosan-CD-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie**

**Spezifikation**

**Geprüft und Genehmigt:**

Name:	Unterschrift:
K. Suter	<i>K. Suter</i>
Leiter Herstellung	<i>28.7.06</i>
Leiter Qualitätskontrolle	<i>V. Figueredo</i>
Leiter Qualitätssicherung	<i>Dr. R. Leu Marseller</i>

**Datum:** 28.07.06  
**Seite:** 1 von 2  
**Gültig ab:** *18.07.06*  
**Autorsierte Kopie Nr.:** *01.18.07.06*  
**Dokumentnummer:** SP086-V04.doc

**Universitätsspital Basel**  
Spital-Pharmazie

**Chitosan-CD-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie**

**Spezifikation**

unmittelbar appliziert werden. Das Hubvolumen von 100µl wird abgemessen abgegeben. Der Spray wird auch dem liegenden Patienten verabreicht werden. Die Dosis beträgt 104205µl/ml.

Die Zusammensetzung der Formulierung basiert auf jener des 'Midazolam-CD-Nasenspray 1mg für klinische Anxiolyse-Studie' (SPHVPV0803), der allerdings kein Chitosan enthält.

**5. Etikette(n):**  
Die Primäretikette wird halbiert und wie auf untenstehender Abbildung dargestellt auf den Unitdose Nasenspray geklebt.

**Primäretikette (Teil A und B)**

**Sekundäretikette**

**EXTERNA** Nur für den klinischen Versuch  
Pharmakokinetik-Studie  
Chitosan-CD-Midazolam UD Nasenspray 3mg  
PK-Studie / Formulierung 5

Anwendung gemäss Studienprotokoll  
1 Hub enthält 3mg Midazolam  
(berechnet als Base)  
Prüfart: Dr. med. M. Haschke  
Tel.: 061 265 88 60  
Ch. Nr. 000000300 EXP. 000000  
UNIVERSITÄTSSPITAL BASEL

**EXTERNA** Nur für klinischen Versuch  
PK-Studie  
Formulierung 5

Midazolam 3mg  
Chitosan-CD-Nasenspray 1mg  
Chitosan-CD-Nasenspray 1mg

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**Universitätsspital Basel**  
Spital-Pharmazie

**Chitosan-CD-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie**

**Spezifikation**

**Geprüft und Genehmigt:**

Name:	Unterschrift:
K. Suter	<i>K. Suter</i>
Leiter Herstellung	<i>28.7.06</i>
Leiter Qualitätskontrolle	<i>V. Figueredo</i>
Leiter Qualitätssicherung	<i>Dr. R. Leu Marseller</i>

**Datum:** 28.07.06  
**Seite:** 1 von 2  
**Gültig ab:** *18.07.06*  
**Autorsierte Kopie Nr.:** *01.18.07.06*  
**Dokumentnummer:** SP086-V04.doc

**1. Identifikation:**

Bezeichnung:	Chitosan-CD-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie	entfällt
SAP-Name:	Eigenprodukt - Studienware	
Kategorie:	Herstellung steril	
Lieferant(en):	Herstellung	

**2. Beschreibung:**

Spezifikation:	Bezeichnung Inhaltsstoffe:	Menge / Einheit:	HV0866
SP0038	Midazolamhydrochlorid DAC	33,3mg	
SP0804	randomisiertes methylertes b-Cyclodextrin	121mg	
SP0862	Chitosanhydrochlorid für Pharmakokinetik-Studie	5,00mg	
SP0564	Salzsäure 1M 500ml	q.s.	
SP0006	Natriumchlorid für Parenteralia PhEur	1,24mg	
SP0004	Wasser für Injektionszwecke PhEur	ad 1,00ml	
<b>Summe:</b>			1,00ml (=1,04g)

**3. Anfordertungen:**

Primär:	Unitdose Nasenspray	
Etikette Primärverpackung:	Etikette 25x50mm weiss	
Etikette Sekundärverpackung:	Etikette 70x70mm doppelt abziehbar	
Sekundär:	Minigrip Beutel 7,9x9,0x0,05mm	

**4. Hinweise:**

Spezifikation:	Bezeichnung Verpackung:	
SP0805	Primär: Unitdose Nasenspray	
SP0019	Etikette Primärverpackung: Etikette 25x50mm weiss	
SP0326	Etikette Sekundärverpackung: Etikette 70x70mm doppelt abziehbar	
SP0597	Sekundär: Minigrip Beutel 7,9x9,0x0,05mm	

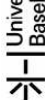
**5. Anfordertungen:**

Klare farblose Lösung	IP-Kontrolle	entspricht
Midazolam	entspricht	
randomisiertes methylertes b-Cyclodextrin	Visum Pharmazeut	
Chitosanhydrochlorid	Visum Pharmazeut	
Natriumchlorid für Parenteralia PhEur	Visum Pharmazeut	
Midazolam-Base	27,0-33,0mg/ml	
	3,0-3,5	
	270-330mosmol/kg	

**6. Hinweise:**

Raumbedingung:	
Lagerungsbedingungen:	6 Monate, zum einmaligen Gebrauch.
Verwendungsdauer:	Der Chitosan-CD-Midazolam UD Nasenspray 3mg wird für die Bestimmung der Pharmakokinetik von nasal appliziertem Midazolam im Rahmen einer klinischen Studie eingesetzt.
Indikation/Kontraindication:	Gemäss Studienprotokoll. Die nasale Applikation von einem Hub entspricht der Dosis von 3mg Midazolam (berechnet als Base).
Dosierung/Applikation:	Die Wirkung gleichzeitig eingenommener sedierender Medikamente kann verstärkt werden.
Interaktion:	Das System gibt einen Hub mit dem Volumen von 100µl ab und kann nicht weiter verwendet werden. Der Unitdose Nasenspray ist gebrauchsfertig und kann nach dem Entfernen der Schutzkappe
Besondere Hinweise:	

## 10.2.10 Stability report 10 months: Midazolam MD Nasal Spray 5mg/ml (Preparation 1b)

 <b>Universitätsklinik Basel</b> Spital-Pharmazie	Datum: 09.03.07
	Seite: 2 von 4
	Dokument: StabMdzMD-
<b>Stabilitätsbericht</b>	
Midazolam Nasenspray 5mg/ml für multizentrische Anxiolyse-Studie	

### 1 Festgelegtes Verfalldatum

In der zu Beginn der Stabilitätsuntersuchung gültigen Herstellvorschrift wurde eine Haltbarkeit von 6 Monaten festgelegt. Eine Stabilitätsprüfung wurde durchgeführt um die festgelegte Haltbarkeit zu bestätigen. Von der Charge 060321N01 liegen momentan Stabilitätsdaten über 6 Monate und 10 Monate vor.

Die Ergebnisse der Stabilitätsprüfungen belegen, dass eine Haltbarkeit von 6 Monaten gegeben werden kann. Obwohl die gemessene pH Erhöhung nach 10 Monaten Lagerung bei Raumtemperatur nicht zu einer Ausfällung geführt hat, wird von einer Verädgerung der Haltbarkeit abgeraten.

### 2 Spezifikation

Die Spezifikation 0806 liegt mittlerweile in Version 02 vor.

#### Zusammensetzung:

Spezifikation:	Bezeichnung	Inhaltsstoffe:	Menge / Einheit:
SP0098	Midazolamhydrochlorid DAC		5,58mg
SP0030	Benzalkoniumchlorid-Lösung PhEur		197µg
SP0027	Natriumacetat PhEur		1,00mg
SP0004	Wasser für Injektionszwecke PhEur		98,8mg
SP0106	Salzsäure 0,1M 500ml		g.s.
B. Braun	Natriumchlorid-Lösung 0,9%, Spüllösung (Artikel-ID: 4002718)		ad 1,00ml
SP0806	<b>Summe:</b>		1,00ml 1,01g

#### Verpackung:

Spezifikation:	Bezeichnung	Verpackung:
SP0323	Primär: Pipettenglas 10ml, braun	
SP0808	Primär: Nasenspray-Zerstäuberpumpe	
SP0136	Etikette Primärverpackung: Etikette 32x50mm weiss	
SP0326	Etikette Sekundärverpackung: Etikette 70x70mm doppelt abziehbar (sekundär)	
SP0705	Zusatzetiketten: Etikette 18x34mm weiss	
SP0524	Sekundär: Minigrip Beutel 160x220x0,05mm	

#### Anforderungen:

Eigenschaft:	Prüfvorschrift:	PV0808
Identität:	klare, farblose Lösung	entspricht
Gehalt:	Midazolam	entspricht
pH-Wert:	Midazolam-Base	4,50-5,50mg/ml
Osmolalität:		3,0-3,5
		270-330mosm/kg

#### Lagerung und Haltbarkeit:


Lagerungsbedingungen:	Raumbedingung
Verwendungsdauer:	6 Monate, zum einmaligen Gebrauch.


 <b>Universitätsklinik Basel</b> Spital-Pharmazie	Datum: 09.03.07
	Seite: 1 von 4
	Dokument: StabMdzMD-
<b>Stabilitätsbericht</b>	
Midazolam Nasenspray 5mg/ml für multizentrische Anxiolyse-Studie	

## Stabilitätsbericht (10 Monate Lagerung bei Raumtemperatur)

**Produkt:**  
**Midazolam Nasenspray 5mg/ml**  
**für multizentrische Anxiolyse-Studie**  
**SP/HV/PV0806**

Name:	Unterschrift:
K. Suter	<i>K. Suter</i>
Datum:	9.3.07
Autor:	

 Universitätsspital Basel Spital-Pharmazie	Datum:	06.03.07
	Seite:	3 von 4
	Dokument:	StabMz.MD-
<b>Stabilitätsbericht</b>		
Midazolam Nasenspray 5mg/ml für multizentrische Anxiolyse-Studie		

 Universitätsspital Basel Spital-Pharmazie	Datum:	06.03.07
	Seite:	4 von 4
	Dokument:	StabMz.MD-
<b>Stabilitätsbericht</b>		
Midazolam Nasenspray 5mg/ml für multizentrische Anxiolyse-Studie		

### 3 Literatur

- Lichtschutz nötig
  - Midazolam hydrochlorid ist im pH-Bereich zwischen 3 bis 3,6 chemisch relativ stabil. Bei der Erhöhung des pH über 4 fällt das schwerlösliche Midazolam aus.
  - [1] in Ethanol gelöst
  - [2] in Wasser gelöst
  - [3] in Wasser gelöst. Tiefe pH's sind vorzuziehen
- [1] R. Selkamaa, S. Tammielto, Photochemical decomposition of midazolam. I. Isolation and identification of products. *Int. J. Pharm.* 49 (1987) 83.
- [2] R. Andersin, J. Ovaskainen, S. Kaitia, Photochemical decomposition of midazolam. III. Isolation and identification of products in aqueous solutions. *Journal of Pharmaceutical and Biomedical Analysis* 12 (1994) 165.
- [3] R. Andersin, S. Tammielto, Photochemical decomposition of midazolam. IV. Study of pH-dependent stability by high-performance liquid chromatography. *International Journal of Pharmaceutics* 123 (1995) 229.
- [4] Kommentar zur PhEur und Auszug aus Hagers Handbuch

### 4 Durchgeführte Stabilitätsprüfungen

Lagerungsbedingungen:	Raumtemperatur
Prüfdaten:	6 Monate (165 Tage), 10 Monate (300 Tage)
Anzahl geprüfte Chargen:	1 (060321N01)
Analysenparameter:	gemäss PV0803
Datum der Herstellung:	27.4.2006

Charge 060321N01, Verfalldatum 27.10.2006

	Probenr.	Anz. Tage seit Herst.	Eigenschaft	Identität	Gehalt mg/ml	pH-Wert	Osmolalität mOsm/kg
Freigabe-Analyse 9.5.2006	060508-03	12	entspricht	entspricht	4.83	3.47/3.46	290/289
9.10.2006	061006-06	165	entspricht	entspricht	4.84	3.49/3.49	293/296
21.2.2007	070221-07	300	entspricht	entspricht	4.78	3.71 <sup>1</sup>	291/293

<sup>1</sup> Der gemessene pH Wert liegt ausserhalb der Limiten, welche in der Spezifikation gesetzt wurden.


### 5 Resultate und deren Bewertung


Die geprüfte Charge erfüllt nach 6 Monaten die geprüften Anforderungen der Spezifikation (Eigenschaften, Identität, Gehalt, pH, Osmolalität).  
 Nach 10 Monaten Lagerung bei Raumtemperatur liegt der pH-Wert der geprüften Charge ausserhalb der in der Spezifikation gesetzten Limiten. Die weiteren gemessenen Parameter (Eigenschaften, Identität, Gehalt, Osmolalität) liegen innerhalb der in der Spezifikation 0806-V02 gesetzten Toleranzen.

### 6 Schlussfolgerungen

Die Ergebnisse der Stabilitätsuntersuchungen bestätigen eine Haltbarkeit von 6 Monaten bei Lagerung unter Raumbedingungen.  
 Bei einer Lagerung von 10 Monaten werden die in der Spezifikation 0806-V02 gesetzten Toleranzen bezüglich pH nicht eingehalten. Von einer Verlängerung der Haltbarkeit wird abgeraten, da unter steigendem pH das Risiko für Midazolam-Ausfällungen steigt.

10.2.11 Stability report 10 months: Cyclodex-Midazolam UD Nasal Spray 1mg (Preparation 3)

 <b>Universitätsspital Basel</b> Spital-Pharmazie	Datum: 09.03.07
	Seite: 1 von 4
	Dokument: StabMdzUD1mg_10Mo.
<b>Stabilitätsbericht</b>	
Midazolam UD Nasenspray 1mg für multizentrische Anxiolyse-Studie	

 <b>Universitätsspital Basel</b> Spital-Pharmazie	Datum: 09.03.07
	Seite: 2 von 4
	Dokument: StabMdzUD1mg_10Mo.
<b>Stabilitätsbericht</b>	
Midazolam UD Nasenspray 1mg für multizentrische Anxiolyse-Studie	

**1 Festgelegtes Verfalldatum**

In der zu Beginn der Stabilitätsuntersuchung gültigen Herstellvorschrift wurde eine Haltbarkeit von 6 Monaten festgelegt. Eine Stabilitätsprüfung wurde durchgeführt um die festgelegte Haltbarkeit zu bestätigen. Von der Charge 060321S01 liegen momentan Stabilitätsdaten über 6 Monate und 10 Monate vor.

Die Ergebnisse der Stabilitätsprüfungen belegen die chemische Stabilität über 10 Monate. Eine Verlängerung der Haltbarkeit (momentan 6 Monate) kann in Erwägung gezogen werden.

**2 Spezifikation**

Die Spezifikation 0803 liegt mittlerweile in Version 02 vor.

Zusammensetzung:

Spezifikation:	Bezeichnung	Inhaltsstoffe:	Menge / Einheit:
SP0098	Midazolamhydrochlorid DAC		11,1mg
SP0804	randomisiertes methylieres b-Cyclodextrin		40,2mg
SP0006	Natriumchlorid für Parenterala PhEur		6,70mg
SP0004	Wasser für Injektionszwecke PhEur		960mg
SP0803	<b>Summe:</b>		1,02g (=1,00ml)

Verpackung:

<b>Spezifikation:</b>	<b>Bezeichnung</b>	<b>Verpackung:</b>
SP0805	Primär: Unitdose Nasenspray	
SP0015	Etikette Primärverpackung: Etikette 25x50mm weiss	
SP0326	Etikette Sekundärverpackung: Etikette 70x70mm doppelt abziehbar	
SP0705	Zusatzetiketten: Etikette 18x34mm weiss	
SP0597	Sekundär: Minigrip Beutel 75x90x0,05mm	

Anforderung:

<b>Eigenschaft:</b>	klare farblose Lösung	<b>Prüfvorschrift:</b>	<b>PV0803</b>
<b>Identität:</b>	Midazolam		entspricht
<b>Gehalt:</b>	Midazolam-Basis		entspricht
<b>pH-Wert:</b>			9,00-11,0mg/ml
<b>Osmolalität:</b>			3,5-4,5
<b>Sterilität:</b>			270-330mosmol/kg
			steril

Lagerung und Haltbarkeit:

<b>Lagerungsbedingungen:</b>	Raumbedingung
<b>Verwendungsdauer:</b>	6 Monate, zum einmaligen Gebrauch.

**Stabilitätsbericht**  
(10 Monate Lagerung bei Raumtemperatur)

**Produkt:**

**Midazolam UD Nasenspray 1mg für multizentrische Anxiolyse-Studie**  
**SP/HV/PV0803**

→ die Formulierung SP/HV/PV0803 ist identisch mit der Formulierung von Cyclodex-Midazolam UD Nasenspray 1mg für Pharmakokinetik-Studie SP/HV/PV0863.

<b>Name:</b>	<b>Datum:</b>	<b>Unterschrift:</b>
K. Suter	9.3.07	<i>K. Suter</i>
<b>Autor</b>		

**Universitätsspital Basel**  
Spital-Pharmazie

Datum: 09.03.07  
Seite: 3 von 4  
Dokument: StabMdzUD:1mg\_10Mo.

**Stabilitätsbericht**

Midazolam UD Nasenspray 1mg  
für multizentrische Anxiolyse-Studie

**3 Literatur**

- Lichtschutz nötig
  - Midazolam hydrochlorid ist im pH-Bereich zwischen 3 bis 3.6 chemisch relativ stabil. Bei der Erhöhung des pH über 4 fällt das schwerlösliche Midazolam aus.
    - [1] in Ethanol gelöst
    - [2] in Wasser gelöst
    - [3] in Wasser gelöst. Tiefe pHs sind vorzuziehen
- [1] R. Seikamaa, S. Tammliehto, Photochemical decomposition of midazolam. I. Isolation and identification of products. Int. J. Pharm. 49 (1987) 83.
  - [2] R. Andersin, J. Ovasikainen, S. Kallia, Photochemical decomposition of midazolam. III. Isolation and identification of products in aqueous solutions. Journal of Pharmaceutical and Biomedical Analysis 12 (1994) 165.
  - [3] R. Andersin, S. Tammliehto, Photochemical decomposition of midazolam. IV. Study of pH-dependent stability by high-performance liquid chromatography. International Journal of Pharmaceutics 123 (1995) 229.
  - [4] Kommentar zur PhEur und Auszug aus Hagers Handbuch

Bemerkung:  
Das schwerlösliche Midazolam wird auf Grund des beigefügten randomisiert methylertes b-Cyclodextrin nicht ausfallen obwohl der pH-Wert der Formulierung von Midazolam UD Nasenspray 1mg bei 4 liegt.

**4 Durchgeführte Stabilitätsprüfungen**

Lagerungsbedingungen:	Raumtemperatur
Prüfintervall:	6 Monate (172 Tage)
Anzahl geprüfte Chargen:	1 (060321S01)
Analyseparameter:	gemäss PV0803 (ohne Prüfung auf Sterilität)
Datum der Herstellung:	20.4.2006

Charge 060321S01, Verfalldatum 20.10.2006

	Proben-Nummer	Anz. Tage seit Herst.	Eigenschaft	Identität	Gehalt mg/ml	pH-Wert	Osmolalität Osm/kg	Sterilitest
Freigabe-Analyse 25.4.2006	060425-01	5	entspricht	entspricht	9.74	3.94/3.93	296/297	entspricht
9.10.2006	061006-03	172	entspricht	entspricht	9.87	3.90/3.90	304/302	nicht überprüft
21.2.2007	070221-05	307	entspricht	entspricht	9.61	4.01	306/304	nicht überprüft

**Universitätsspital Basel**  
Spital-Pharmazie

Datum: 09.03.07  
Seite: 4 von 4  
Dokument: StabMdzUD:1mg\_10Mo.

**Stabilitätsbericht**

Midazolam UD Nasenspray 1mg  
für multizentrische Anxiolyse-Studie


**5 Resultate und deren Bewertung**


Die geprüfte Charge erfüllt nach 10 Monaten die geprüften Anforderungen der Spezifikation (Eigenschaften, Identität, Gehalt, pH, Osmolalität). Die Sterilität wurde nicht überprüft.

**6 Schlussfolgerungen**

Die Ergebnisse bestätigen somit eine Haltbarkeit von 6 Monaten bei Lagerung unter Raumbedingungen. Die in der Spezifikation 0603-V02 gesetzten Toleranzen können auch nach 10 Monaten Lagerung unter Raumbedingungen eingehalten werden.  
Für eine Verlängerung der Haltbarkeit wird empfohlen ebenfalls die mikrobielle Stabilität zu überprüfen.  
Da die Formulierungen und die Verpackungen der beiden Produkte (Spezifikation 0603 und 0663) identisch sind, gelten die vorliegenden Stabilitätsdaten ebenfalls für den Cyclohex-Midazolam UD Nasenspray 1mg (für Pharmakokinetik-Studie) SP/HV/PV0863. Die beiden genannten Nasensprays unterscheiden sich lediglich in der Beschriftung der Etiketten (Prüfmuster für zwei unterschiedliche klinische Studien).

10.2.12 Stability report 6 months: Midazolam UD Nasal Spray 0.5mg (Preparation 1)

		Datum: 03.09.07
Universitätsspital Basel		Seite: 1 von 4
Spital-Pharmazie		Dokument: StabMz_UD_0.5mg_6
<b>Stabilitätsbericht</b>		
Midazolam UD Nasenspray 0.5mg für Pharmakokinetik-Studie		

		Datum: 09.03.07
Universitätsspital Basel		Seite: 2 von 4
Spital-Pharmazie		Dokument: StabMz_UD_0.5mg_6
<b>Stabilitätsbericht</b>		
Midazolam UD Nasenspray 0.5mg für Pharmakokinetik-Studie		

**Stabilitätsbericht**

(6 Monate Lagerung bei Raumtemperatur)

**Produkt:**

**Midazolam UD Nasenspray 0.5mg für Pharmakokinetik-Studie SP/HV/PV0871**

**1 Festgelegtes Verfalldatum**

In der zu Beginn der Stabilitätsuntersuchung gültigen Herstellvorschrift wurde eine Haltbarkeit von 6 Monaten festgelegt. Eine Stabilitätsprüfung wurde durchgeführt um die festgelegte Haltbarkeit zu bestätigen. Von der Charge 060814.S01 liegen momentan Stabilitätsdaten über 6 Monate vor. Die Ergebnisse der Stabilitätsprüfungen belegen, dass eine Haltbarkeit von 6 Monaten gegeben werden kann.

**2 Spezifikation**

Die Spezifikation 0871 liegt mittlerweile in Version 03 vor.

**Zusammensetzung:**

Spezifikation:	Bezeichnung Inhaltsstoffe:	Menge / Einheit:
SP0098	Midazolamhydrochlorid DAC	5,55mg
SP0106	Salzsäure 0.1M 500ml	q. s.
Fresenius	Natriumchlorid-Lösung 0.9%, Ovalla	ad 1,00ml
	<b>Summe:</b>	<b>1,00ml (=1,01g)</b>

**Verpackung:**


Spezifikation:	Bezeichnung Verpackung:
SP0805	Primär: Unidose Nasenspray
SP0015	Etikette Primärverpackung: Etikette 25x50mm weiss
SP0326	Etikette Sekundärverpackung: Etikette 70x70mm doppelt abziehbar
SP0597	Sekundär: Minigrip Beutel 75x90x0,05mm


**Anforderungen:**


Anforderungen:	Prüfvorschrift:	IP-Kontrolle
Eigenschaft:	klare farblose Lösung	entspricht
Identität:	Midazolam	entspricht
Gehalt:	Natriumchlorid-Lösung 0.9%	Visum Pharmazeut
pH-Wert:	Midazolam-Base	4,50-5,50mg/ml
Osmolalität:		3,0-3,5
		270-330mosmol/kg

**Lagerung und Haltbarkeit:**

Lagerungsbedingungen:	Raumbedingung
Verwendungsdauer:	6 Monate, zum einmaligen Gebrauch.

Autor	Name: K. Suter	Datum: 9.3.07	Unterschrift: 
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 <b>Universitätsspital Basel</b> Spital-Pharmazie		Datum:	30.03.07
		Seite:	3 von 4
		Dokument:	StabMdz_UD_0.5mg_6
Midazolam UD Nasenspray 0.5mg für Pharmakokinetik-Studie		<b>Stabilitätsbericht</b>	

 <b>Universitätsspital Basel</b> Spital-Pharmazie		Datum:	30.03.07
		Seite:	4 von 4
		Dokument:	StabMdz_UD_0.5mg_6
Midazolam UD Nasenspray 0.5mg für Pharmakokinetik-Studie		<b>Stabilitätsbericht</b>	

### 3 Literatur

- Lichtschutz nötig
  - Midazolam hydrochlorid ist im pH-Bereich zwischen 3 bis 3.6 chemisch relativ stabil. Bei der Erhöhung des pH über 4 fällt das schwerlösliche Midazolam aus.
  - [1] in Ethanol gelöst
  - [2] in Wasser gelöst
  - [3] in Wasser gelöst. Tiefe pH's sind vorzuziehen
- [1] R. Selkamaa, S. Tammilehto. Photochemical decomposition of midazolam. I. Isolation and identification of products. *Int. J. Pharm.* 49 (1987) 83.
- [2] R. Andersin, J. Ovaskainen, S. Kallia. Photochemical decomposition of midazolam. III. Isolation and identification of products in aqueous solutions. *Journal of Pharmaceutical and Biomedical Analysis* 12 (1994) 165.
- [3] R. Andersin, S. Tammilehto. Photochemical decomposition of midazolam. IV. Study of pH-dependent stability by high-performance liquid chromatography. *International Journal of Pharmaceutics* 123 (1995) 229.
- [4] Kommentar zur PhEur und Auszug aus Hagers Handbuch

### 4 Durchgeführte Stabilitätsprüfungen

Lagerungsbedingungen:	Raumtemperatur
Prüfintervall:	6 Monate (191 Tage)
Anzahl geprüfte Chargen:	1 (060814S01)
Analysenparameter:	gemäss PV0871
Datum der Herstellung:	14.8.2006

Charge 060814S01, Verfalldatum 14.2.2007

	Proben-nummer	Anz. Tage seit Herst.	Eigenschaft	Identität	Gehalt mg/ml	pH-Wert	Osmolalität mOsm/kg
Freigabe-Analyse 14.8.2006	060814-03	0	entspricht	entspricht	4.93	3.13	303
21.2.2007	070221-06	191	entspricht	entspricht	4.75	3.38	309/311

### 5 Resultate und deren Bewertung


Die geprüfte Charge erfüllt nach 6 Monaten die geprüften Anforderungen der Spezifikation (Eigenschaften, Identität, Gehalt, pH, Osmolalität).

### 6 Schlussfolgerungen

Die Ergebnisse bestätigen somit eine Haltbarkeit von 6 Monaten bei Lagerung unter Raumbedingungen. Die in der Spezifikation 0871-V03 gesetzten Toleranzen können bestätigt werden.



**10.2.13 Stability report 6 months: Cyclodex-Midazolam UD Nasal Spray 0.5mg (Preparation 2)**

 <b>Universitätsspital Basel</b> Spital-Pharmazie	Datum: 09.03.07
	Seite: 2 von 4
	Dokument: StabMdz_UD_CD_0.5m
<b>Stabilitätsbericht</b>	
Cyclodex-Midazolam UD Nasenspray 0.5mg für Pharmakokinetik-Studie	

**1 Festgelegtes Verfalldatum**

In der zu Beginn der Stabilitätsuntersuchung gültigen Herstellvorschrift wurde eine Haltbarkeit von 6 Monaten festgelegt. Eine Stabilitätsprüfung wurde durchgeführt um die festgelegte Haltbarkeit zu bestätigen. Von der Charge 060717.S01 liegen momentan Stabilitätsdaten über 6 Monate vor. Die Ergebnisse der Stabilitätsprüfungen belegen, dass eine Haltbarkeit von 6 Monaten gegeben werden kann.

**2 Spezifikation**

Die Spezifikation 0864 liegt mittlerweile in Version 03 vor.

Zusammensetzung:

Spezifikation:	Bezeichnung	Inhaltsstoffe:	Menge / Einheit:
SP0098	Midazolamhydrochlorid DAC		5,55mg
SP0804	randomisiert methyliertes b-Cyclodextrin		20,1mg
SP0006	Natriumchlorid für Parenteralia PhEur		7,85mg
SP0004	Wasser für Injektionszwecke PhEur		ad 1,00ml
	<b>Summe:</b>		<b>1,00ml (=1,01g)</b>

Verpackung:


Spezifikation:	Bezeichnung	Verpackung:
SP0805	Primär: Unitdose Nasenspray	
SP0015	Etikette Primärverpackung: Etikette 25x50mm weiss	
SP0326	Etikette Sekundärverpackung: Etikette 70x70mm doppelt abziehbar	
SP0597	Sekundär: Mimgrip Beutel 75x90x0,05mm	

Anforderungen:

Eigenschaft:	Prüfvorschrift:	IP-Kontrolle
Identität:	klare farblose Lösung	entspricht
Gehalt:	Midazolam	entspricht
pH-Wert:	randomisiert methyliertes b-Cyclodextrin	Visum Pharmazeut
Osmolalität:	Midazolam-Base	4,50-5,50mg/ml
		3,5-4,5
		270-330mosmol/kg

Lagerung und Haltbarkeit:

Lagerungsbedingungen:	Raumbedingung
Verwendungsdauer:	6 Monate, zum einmaligen Gebrauch.

 <b>Universitätsspital Basel</b> Spital-Pharmazie	Datum: 09.03.07
	Seite: 1 von 4
	Dokument: StabMdz_UD_CD_0.5m
<b>Stabilitätsbericht</b>	
Cyclodex-Midazolam UD Nasenspray 0.5mg für Pharmakokinetik-Studie	

**Stabilitätsbericht**

**(6 Monate Lagerung bei Raumtemperatur)**

**Produkt:**

**Cyclodex-Midazolam UD Nasenspray 0.5mg  
für Pharmakokinetik-Studie  
SP/HV/IPV0864**

Autor	Name:	K. Suter	Datum:	9.3.07	Unterschrift:	
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**Universitätsspital Basel**  
Spital-Pharmazie

Cyclohex-Midazolam UD Nasenspray 0.5mg  
für Pharmakokinetik-Studie

Datum: 09.03.07  
Seite: 3 von 4  
Dokument: StabMdz\_UD\_CD\_0.5m

**Stabilitätsbericht**

**Universitätsspital Basel**  
Spital-Pharmazie

Cyclohex-Midazolam UD Nasenspray 0.5mg  
für Pharmakokinetik-Studie

Datum: 09.03.07  
Seite: 4 von 4  
Dokument: StabMdz\_UD\_CD\_0.5m

**Stabilitätsbericht**

**3 Literatur**

- Lichtschutz nötig
  - Midazolam hydrochlorid ist im pH-Bereich zwischen 3 bis 3.6 chemisch relativ stabil. Bei der Erhöhung des pH über 4 fällt das schwerlösliche Midazolam aus.
  - [1] in Ethanol gelöst
  - [2] in Wasser gelöst
  - [3] in Wasser gelöst. Tiefe pH's sind vorzuziehen
- [1] R. Seikamaa, S. Tammielto, Photochemical decomposition of midazolam. I. Isolation and identification of products. Int. J. Pharm. 49 (1987) 83.
  - [2] R. Andersin, J. Ovaskainen, S. Kalita. Photochemical decomposition of midazolam. III. Isolation and identification of products in aqueous solutions. Journal of Pharmaceutical and Biomedical Analysis 12 (1994) 165.
  - [3] R. Andersin, S. Tammielto. Photochemical decomposition of midazolam. IV. Study of pH-dependent stability by high-performance liquid chromatography. International Journal of Pharmaceutics 123 (1995) 229.
  - [4] Kommentar zur PhEur und Auszug aus Hagers Handbuch

**4 Durchgeführte Stabilitätsprüfungen**

Bemerkung:  
Das schwerlösliche Midazolam wird auf Grund des beigefügten randomisiert methylertes b-Cyclodextrin nicht ausfallen obwohl der pH-Wert der Formulierung von Midazolam UD Nasenspray 1mg bei 4 liegt.

Lagerungsbedingungen:	Raumtemperatur
Prüfintervall:	6 Monate (218Tage)
Anzahl geprüfte Chargen:	1 (060717S01)
Analyseparameter:	gemäss PV0664
Datum der Herstellung	18.7.2006

Charge 060717S01, Verfalldatum 18.1.2007

	Probennummer	Anz. Tage seit Herst.	Eigenschaft	Identität	Gehalt mg/ml	pH-Wert	Osmolalität mOsm/kg
Freigabe-Analyse 7.8.2006	060719-05	20	entspricht	entspricht	5.03	4.06	274
21.2.2007	070221-07	218	entspricht	entspricht	4.82	4.24	281/278


**5 Resultate und deren Bewertung**

Die geprüfte Charge erfüllt nach 6 Monaten die geprüften Anforderungen der Spezifikation (Eigenschaften, Identität, Gehalt, pH, Osmolalität).

**6 Schlussfolgerungen**

Die Ergebnisse bestätigen somit eine Haltbarkeit von 6 Monaten bei Lagerung unter Raumbedingungen. Die in der Spezifikation 0864 V03 gesetzten Toleranzen können bestätigt werden.

10.2.14 Stability report 6 months: Cyclodex-Midazolam UD Nasal Spray 3mg (Preparation 4)

 Universitätsspital Basel Spital-Pharmazie	Datum:	09.03.07
	Seite:	2 von 4
	Dokument:	StablMdz_UD_CD_3mg
<b>Stabilitätsbericht</b>		
Cyclodex-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie		

1 Festgelegtes Verfalldatum

In der zu Beginn der Stabilitätsuntersuchung gültigen Herstellvorschrift wurde eine Haltbarkeit von 6 Monaten festgelegt. Eine Stabilitätsprüfung wurde durchgeführt um die festgelegte Haltbarkeit zu bestätigen. Von der Charge 060502.S03 liegen momentanen Stabilitätsdaten über 6 Monate vor. Die Ergebnisse der Stabilitätsprüfungen belegen, dass eine Haltbarkeit von 6 Monaten gegeben werden kann.

2 Spezifikation

Die Spezifikation 0865 liegt mittlerweile in Version 03 vor.

Zusammensetzung:

Spezifikation:	Bezeichnung Inhaltsstoffe:	Menge / Einheit:
SP0088	Midazolamhydrochlorid DAC	33,3mg
SP0804	randomisiertes methylertes b-Cyclodextrin	12 mg
SP0006	Natriumchlorid für Parenteralia PhEur	1,80mg
SP0004	Wasser für Injektionszwecke PhEur	ad 1,00ml
	<b>Summe:</b>	<b>1,00ml (=1,04g)</b>

Verpackung:


Spezifikation:	Bezeichnung Verpackung:
SP0805	Primär: Unitidose Nasenspray
SP0015	Etikette Primärverpackung: Etikette 25x50mm weiss
SP0326	Etikette Sekundärverpackung: Etikette 70x70mm doppelt abziehbar
SP0597	Sekundär: Minigrip Beutel 75x90x0,05mm

Anforderung:

Eigenschaft:	Prüfvorschrift:	IP-Kontrolle
Identität:	Klare farblose Lösung	entspricht
Gehalt:	Midazolam	entspricht
pH-Wert:	randomisiertes methylertes b-Cyclodextrin	Visum Pharmazeut
Osmolalität:	Midazolam-Base	27,0-33,0mg/ml
		3,0-4,5
		270-330mosmol/kg


Lagerung und Haltbarkeit:


Lagerungsbedingungen:	Raumbedingung
Verwendungsdauer:	6 Monate, zum einmaligen Gebrauch.

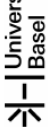
 Universitätsspital Basel Spital-Pharmazie	Datum:	09.03.07
	Seite:	1 von 4
	Dokument:	StablMdz_UD_CD_3mg
<b>Stabilitätsbericht</b>		
Cyclodex-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie		

Stabilitätsbericht  
(6 Monate Lagerung bei Raumtemperatur)

Produkt:  
Cyclodex-Midazolam UD Nasenspray 3mg  
für Pharmakokinetik-Studie  
SP/HV/IPV0865

Autor	Name: K. Suter	Datum: 9.3.07	Unterschrift: 
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 <b>Universitätsspital Basel</b> Spital-Pharmazie	Datum:	09.03.07
	Seite:	3 von 4
	Dokument:	StabMdz_UD_CD_3mg
Cyclodex-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie		Stabilitätsbericht

 <b>Universitätsspital Basel</b> Spital-Pharmazie	Datum:	09.03.07
	Seite:	3 von 4
	Dokument:	StabMdz_UD_CD_3mg
Cyclodex-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie		Stabilitätsbericht

### 3 Literatur

- Lichtschutz nötig
- Midazolam hydrochlorid ist im pH-Bereich zwischen 3 bis 3,6 chemisch relativ stabil. Bei der Erhöhung des pH über 4 fällt das schwerlösliche Midazolam aus.
- [1] in Ethanol gelöst
- [2] in Wasser gelöst
- [3] in Wasser gelöst. Tiefe pH's sind vorzuziehen

- [1] R. Selkamaa, S. Tammilehto, Photochemical decomposition of midazolam. I. Isolation and identification of products. *Int. J. Pharm.* 49 (1987) 83.
- [2] R. Andersin, J. Ovaskainen, S. Kallia, Photochemical decomposition of midazolam. III. Isolation and identification of products in aqueous solutions, *Journal of Pharmaceutical and Biomedical Analysis* 12 (1994) 165.
- [3] R. Andersin, S. Tammilehto, Photochemical decomposition of midazolam. IV. Study of pH-dependent stability by high-performance liquid chromatography. *International Journal of Pharmaceutics* 123 (1995) 229.
- [4] Kommentar zur PhEur und Auszug aus Hagers Handbuch

#### Bemerkung:

Das schwerlösliche Midazolam wird auf Grund des beigefügten randomisiert methylertes b-Cyclodextrin nicht ausfallen obwohl der pH-Wert der Formulierung von Midazolam UD Nasenspray 1mg bei 4 liegt.

### 4 Durchgeführte Stabilitätsprüfungen

Lagerungsbedingungen:	Raumtemperatur
Prüfintervall:	6 Monate (222Tage)
Anzahl geprüfte Chargen:	1 (060502S03)
Analysenparameter:	gemäss PV0865
Datum der Herstellung	14.7.2006

#### Charge 060502S03, Verfalldatum 14.1.2007

Freigabe-Analyse	Probennummer	Anz. Tage seit Herst.	Eigenschaft	Identität	Gehalt mg/ml	pH-Wert	Osmolalität mOsm/kg
7.8.2006	060719-06	24	entspricht	entspricht	29.8	3.68	308
21.2.2007	070221-09	222	entspricht	entspricht	29.0	3.78	315/315


### 5 Resultate und deren Bewertung


Die geprüfte Charge erfüllt nach 6 Monaten die geprüften Anforderungen der Spezifikation (Eigenschaften, Identität, Gehalt, pH, Osmolalität).

### 6 Schlussfolgerungen

Die Ergebnisse bestätigen somit eine Haltbarkeit von 6 Monaten bei Lagerung unter Raumbedingungen. Die in der Spezifikation 0865 V03 gesetzten Toleranzen können bestätigt werden.

**10.2.15 Stability report 6 months: Chitosan-CD-Midazolam UD Nasal Spray 3mg (Preparation 5)**

 <b>Universitätsspital Basel</b> Spital-Pharmazie	Datum: 09.03.07
	Seite: 2 von 4
	Dokument: StabMdz_UD_CDChl_3
<b>Stabilitätsbericht</b>	
Chitosan-CD-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie	

 <b>Universitätsspital Basel</b> Spital-Pharmazie	Datum: 09.03.07
	Seite: 1 von 4
	Dokument: StabMdz_UD_CDChl_3
<b>Stabilitätsbericht</b>	
Chitosan-CD-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie	

**1 Festgelegtes Verfalldatum**

In der zu Beginn der Stabilitätsuntersuchung gültigen Herstellvorschrift wurde eine Haltbarkeit von 6 Monaten festgelegt. Eine Stabilitätsprüfung wurde durchgeführt um die festgelegte Haltbarkeit zu bestätigen. Von der Charge 060717 S02 liegen momentan Stabilitätsdaten über 6 Monate vor. Die Ergebnisse der Stabilitätsprüfungen belegen, dass eine Haltbarkeit von 6 Monaten gegeben werden kann.

**2 Spezifikation**

Die Spezifikation 0866 liegt mittlerweile in Version 04 vor.

**Zusammensetzung:**

Spezifikation:	Bezeichnung Inhaltsstoffe:	Menge / Einheit:
SP0098	Midazolamhydrochlorid DAC	33,3mg
SP0804	randomisiert methyliertes b-Cyclodextrin	121mg
SP0862	Chitosanhydrochlorid für Pharmakokinetik-Studie	5,00mg
SP0554	Salzsäure 1M/500ml	q. s.
SP0006	Natriumchlorid für Parenteralia PhEur	1,24mg
SP0004	Wasser für Injektionszwecke PhEur	ad 1,00ml
	<b>Summe:</b>	<b>1,00ml (=1,04g)</b>

**Verpackung:**

Spezifikation:	Bezeichnung Verpackung:
SP0805	Primär: Unitidose Nasenspray
SP0015	Etikette Primärverpackung: Etikette 25x50mm weiss
SP0326	Etikette Sekundärverpackung: Etikette 70x70mm doppelt abziehbar
SP0597	Sekundär: Mningrip Beutel 75x90x0,05mm

**Anforderung:**

Eigenschaft:	Prüfvorschrift:	IP-Kontrolle
Identität:	Klare, farblose Lösung	entspricht
	Midazolam	entspricht
	randomisiert methyliertes b-Cyclodextrin	Visum Pharmazeut
	Chitosanhydrochlorid	Visum Pharmazeut
Gehalt:	Natriumchlorid für Parenteralia PhEur	Visum Pharmazeut
pH-Wert:	Midazolam-Basis	27,0-33,0mg/ml
Osmolalität:		3,0-3,5
		270-330mosmol/kg


**Lagerung und Haltbarkeit:**


Lagerungsbedingungen:	Raumbedingung
Verwendungsdauer:	6 Monate, zum einmaligen Gebrauch.

**Stabilitätsbericht  
(6 Monate Lagerung bei Raumtemperatur)**

**Produkt:**  
**Chitosan-CD-Midazolam UD Nasenspray 3mg**  
**für Pharmakokinetik-Studie**  
**SP/HV/PV0866**

Name:	Unterschrift:
K. Suter	<i>K. Suter</i>
Datum:	9.3.07

 <b>Universitätsspital Basel</b> Spital-Pharmazie	Datum:	09.03.07
	Seite:	4 von 4
	Dokument:	StabMdz_UD_CDChI_3
Chitosan-CD-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie		<b>Stabilitätsbericht</b>

 <b>Universitätsspital Basel</b> Spital-Pharmazie	Datum:	09.03.07
	Seite:	3 von 4
	Dokument:	StabMdz_UD_CDChI_3
Chitosan-CD-Midazolam UD Nasenspray 3mg für Pharmakokinetik-Studie		<b>Stabilitätsbericht</b>

### 3 Literatur

- Lichtschutz nötig
- Midazolam hydrochlorid ist im pH-Bereich zwischen 3 bis 3,6 chemisch relativ stabil. Bei der Erhöhung des pH über 4 fällt das schwerlösliche Midazolam aus.
- [1] in Ethanol gelöst
- [2] in Wasser gelöst
- [3] in Wasser gelöst. Tiefe pH's sind vorzuziehen

- [1] R. Selkamaa, S. Tammielto, Photochemical decomposition of midazolam. I. Isolation and identification of products. *Int. J. Pharm.* 49 (1987) 83.
- [2] R. Andersin, J. Ovasainen, S. Kallia, Photochemical decomposition of midazolam. III. Isolation and identification of products in aqueous solutions. *Journal of Pharmaceutical and Biomedical Analysis* 12 (1994) 165.
- [3] R. Andersin, S. Tammielto, Photochemical decomposition of midazolam. IV. Study of pH-dependent stability by high-performance liquid chromatography. *International Journal of Pharmaceutics* 123 (1995) 229.
- [4] Kommentar zur PhEur und Auszug aus Hagers Handbuch

#### Bemerkung:

Das schwerlösliche Midazolam wird auf Grund des beigefügten randomisiert methyliertes b-Cyclodextrin nicht ausfallen obwohl der pH-Wert der Formulierung von Midazolam UD Nasenspray 1mg bei 4 liegt.

### 4 Durchgeführte Stabilitätsprüfungen

Lagerungsbedingungen:	Raumtemperatur
Prüfintervall:	6 Monate (201Tage)
Anzahl geprüfte Chargen:	1 (060717S02)
Analysenparameter:	gemäss PV0866
Datum der Herstellung	4.8.2006

Charge 060717S02, Verfalldatum 4.2.2007

Freigabe-Analyse	Probennummer	Anz. Tage seit Herst.	Eigenschaft	Identität	Gehalt mg/ml	pH-Wert	Osmolalität Osm/kg
7.8.2006	060804-05	3	entspricht	entspricht	29,5	3,15	301
21.2.2007	070221-10	201	entspricht	entspricht	25,1 <sup>1</sup>	3,30	312/314

<sup>1</sup> Der Gehalt liegt außerhalb der von der Ph.Eur vorgeschriebenen Toleranzen!

### 5 Resultate und deren Bewertung

Da der Gehalt der geprüften Charge außerhalb der Toleranzen liegt muss bei einer neuen Produktion die Haltbarkeit verkürzt werden.

### 6 Schlussfolgerungen

Die gegebene Haltbarkeit von 6 Monaten kann nicht bestätigt werden. Die in der Spezifikation 0866 V04 muss vor der Produktion weiterer Chargen angepasst werden.

## Project II

## 10.3.1 Study protocol (without appendix)

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Version 01 – 6.3.2006

Seite II

# Pharmakokinetik von nasal appliziertem Midazolam

## Studienprotokoll mit Anhang

**1. Studiensynopsis**

<b>STUDIENTITEL:</b>	Pharmakokinetik und Pharmakodynamik von nasal appliziertem Midazolam
<b>PRINCIPAL INVESTIGATOR:</b>	Dr. med. Manuel Haschke Klinische Pharmakologie & Toxikologie Universitätsklinik Basel Hebelstrasse 2, 4031 Basel Tel: 061 265 88 60 Fax: 061 265 88 64 MHaschke@uhbs.ch
<b>CO-INVESTIGATOR:</b>	Prof. Dr. Christian Surber Fachtechnischer Leiter Spital-Pharmazie Universitätsklinik Basel Spitalstrasse 26, 4031 Basel Tel.: 061 265 29 05 Fax: 061 265 88 75 Christian.Surber@unibas.ch
<b>CO-INVESTIGATOR:</b>	Katja Zimmermann, dipl. pharm. Doktorandin Spital-Pharmazie Universitätsklinik Basel Spitalstrasse 26, 4031 Basel Tel.: 061 265 85 33 Fax: 061 265 88 75 Katja.Zimmermann@unibas.ch
<b>STUDIENORT:</b>	Universitätsklinik Basel, Clinical Research Center (CRC)
<b>SPONSOR:</b>	Spital-Pharmazie, Universitätsklinik Basel
<b>DESIGN:</b>	nicht verblindete sequentielle Pilotstudie
<b>ENTWICKLUNGSPHASE:</b>	Phase I
<b>PRIMÄRES STUDIENZIEL:</b>	Pharmakokinetik unterschiedlicher Formulierungen von nasal appliziertem Midazolam
<b>SEKUNDÄRE STUDIENZIELE:</b>	- Verträglichkeitsprüfung verschiedener nasaler Midazolam-Formulierungen - Pharmakodynamik: Evaluation sedierender Effekte mittels standardisierter Reaktionszeiterfassung im CRTT (Choice Reaction Time Task) -Test
<b>PROBANDEN:</b>	8 gesunde Freiwillige
<b>EINSCHLUSSKRITERIEN:</b>	- Männer im Alter zwischen 18 und 45 Jahren - Nichtraucher, BMI 18-30 kg/m <sup>2</sup> - Freiwillige Unterzeichnung der Einverständniserklärung
<b>AUSSCHLUSSKRITERIEN:</b>	- bekannte oder vermutete Überempfindlichkeit gegenüber Midazolam (bzw. vorhergehende paradoxe Reaktion nach Benzodiazepingabe) oder gegenüber einem der eingesetzten Hilfsstoffe (randomisiert-methylierte-β-Cyclodextrin, Chitosan-HCl)

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**2. Einleitung**

**2.1 Nasale Applikation von systemischen Wirkstoffen**

Das Interesse an der nasalen Applikation von Wirkstoffen mit systemischer Wirkung ist in den letzten Jahren stark gestiegen. Dank einer hoher Vaszkularisierung und relativ durchlässiger Membranen ist die nasale Schleimhaut ein geeigneter Ort für die Applikation von Wirkstoffen mit systemischer Wirkung. Für Wirkstoffe mit geringer oraler Bioverfügbarkeit bedingt durch einen hohen First-Pass-Effekt (z.B. Midazolam, Hormone etc.) oder den Abbau im Gastrointestinaltrakt (z.B. Peptide) kann die nasale Applikation eine interessante Alternative zur parenteralen Verabreichung sein. Im Weiteren kann die nasale Arzneimittelgabe in bestimmten Situationen (Pädiatrie, kranke Patienten, Anfallstherapie etc.) den herkömmlichen Verabreichungsarten (i.v. i.m. rektal, oral) überlegen sein.

**2.2 Nasale Applikation von Midazolam**

Aus der ausgeprägt sedierenden und schlafanstressenden Wirkung, sowie dem anxiolytischen, antikonvulsiven und muskelrelaxierenden Effekt, ergeben sich unterschiedliche Indikationen für Midazolam. Dank raschem Wirkungseintritt und kurzer Wirkdauer ist Midazolam besonders geeignet für die Prämedikation vor der Narkoseeinleitung sowie zur Basisanasthetik vor unangenehmen medizinischen oder chirurgischen Prozeduren zu diagnostischen oder therapeutischen Zwecken. Midazolam steht auf dem Arzneimittelmarkt lediglich als Tabletten und als Ampullen zur i.v., i.m. und rektalen (Pädiatrie) Verabreichung zur Verfügung.

Die orale Applikation von Midazolam als Tablette hat den Nachteil eines verzögerten Wirkungseintritts, der zudem von der Nahrungsaufnahme abhängig ist. Die Wirkung tritt 15 bis 30 Minuten nach der oralen Verabreichung ein, das Wirkungsmaximum wird erst rund eine Stunde nach der Applikation erreicht. Die Latenzzeit bis zum Wirkungseintritt ist insbesondere beim Einsatz von Midazolam zur Anxiolyse vor kurzen diagnostischen und chirurgischen Eingriffen problematisch, da der richtige Medikationszeitpunkt schwer abgeschätzt werden kann.

Da die erwähnten Applikationsformen manchen Anforderungen an die Medikation in verschiedenen Anwendungsbereichen nicht gerecht werden, besteht das Bedürfnis nach alternativen Verabreichungsmöglichkeiten. Obwohl keine nasale Midazolam-Formulierung im Handel erhältlich ist existieren zahlreiche Berichte über die erfolgreiche nasale Applikation von Midazolam. Dazu wird Dormicum® entweder mittels Spritze (ohne Nadel) oder Nasen-Spray in die Naseroffnung verabreicht. In der Tabelle 2-1 sind verschiedene Anwendungsbereiche aufgeführt für welche die nasale Midazolam-Applikation dokumentiert ist. In den meisten beschriebenen Fällen und publizierten Studien wurde Midazolam üblicherweise in einer Dosierung von 4 bis 7mg appliziert. In der vorliegenden Studie soll Pharmakokinetik, Pharmakodynamik und Verträglichkeit verschiedener Formulierungen von nasal appliziertem low-dose Midazolam systematisch untersucht werden.

	<ul style="list-style-type: none"> <li>- Bestehende Kontraindikation für Midazolam</li> <li>- Bekannte Allergie gegen Krustentiere<sup>1</sup></li> <li>- Einnahme von CYP3A4-Induktoren oder CYP3A4-Inhibitoren</li> <li>- Medikamenten-, Drogen- oder Alkoholmissbrauch</li> <li>- Teilnahme an einer anderen klinischen Studie innerhalb der vergangenen 30 Tage</li> <li>- chronisch behinderte Nasenatmung</li> <li>- symptomatische Nasenpolypen oder Nasenpolyphen in der Anamnese</li> </ul>
<b>PRÜFPRÄPARATE:</b>	<ul style="list-style-type: none"> <li>- Midazolam UD Nasenspray 0.5mg (Formulierung 1)</li> <li>- Cyclodex-Midazolam UD Nasenspray 0.5mg (Formulierung 2)</li> <li>- Cyclodex-Midazolam UD Nasenspray 1mg (Formulierung 3)</li> <li>- Cyclodex-Midazolam UD Nasenspray 3mg (Formulierung 4)</li> <li>- Chitosan-CD-Midazolam UD Nasenspray 3mg (Formulierung 5)</li> </ul>
<b>BEHANDLUNG:</b>	<ul style="list-style-type: none"> <li>- Midazolam 1 mg intravenös</li> <li>- Midazolam nasal, 3 zeitlich getrennte Applikationen mit je 1mg (Formulierungen 1 bis 3) und 2 weitere Applikationen mit je 3 mg (Formulierungen 4 und 5)</li> </ul>
<b>STUDIENPARAMETER:</b>	<p>Pharmakokinetik:</p> <ul style="list-style-type: none"> <li>- Midazolam-Serumkonzentration zu den Zeitpunkten 1, 2, 5, 10, 15, 20, 30, 45, 60, 120, 180, 240, 360 Minuten nach Applikation der Wirkstoffe</li> <li>- Analyse der Konzentrations-Zeit-Daten mittels nicht-kompartimenteller Standardmethode mit Ableitung der pharmakokinetischen Parameter <math>C_{max}</math>, <math>t_{max}</math>, <math>AUC_{0-360}</math>, <math>AUC_{0-inf}</math> (extrapol.)-<math>t_{1/2}</math>, Cl, Vd, F.</li> </ul> <p>Pharmakodynamik:</p> <ul style="list-style-type: none"> <li>- standardisierte, computergestützte Reaktionszeitmessung (Choice Reaction Time Task, CRTT)</li> </ul>
<b>STATISTIK:</b>	<p>Standardisierte dosisnormalisierte Bioäquivalenzprüfung von <math>AUC_{0-360}</math>, <math>AUC_{0-inf}</math> (extrapol.)-<math>C_{max}</math> der einzelnen Formulierungen</p> <p>Testung der Effektparameter mittels repeated measures ANOVA (oder analoges nicht parametrisches Testverfahren bei nicht normal verteilten Daten)</p>
<b>DATEN:</b>	<p>Protokoll-Einreichung EKBB: 06.04.2006</p> <p>Geplanter Studienstart: 01.06.2006</p> <p>Geplanter Rekrutierungsstopp: 31.09.2006</p> <p>Abschlussbericht: 31.12.2006</p>

<sup>1</sup> Chitosan wird aus den Schalen von Krustentieren gewonnen

Tabelle 2-1: Zusammenstellung von Literatur über die nasale Applikation von Midazolam

Anwendung	Quelle
Prämedikation vor der Narkoseeinleitung	<b>Kinder:</b> (Davis et al., 1995; Geldner et al., 1997; Griffin et al., Jaennet 1999; Kogan et al., 2002; Lejus et al., 1997; Ljung and Andreasson 1996; Rey et al., 1991; Rose et al., 1990) <b>Erwachsene:</b> (J.Haas 1998)
Notfallsituationen	<b>Kinder:</b> (Goepfert 1996) <b>Erwachsene:</b> (Wolfe and Bernstone 2004)
Behandlung des Status Epilepticus	<b>Kinder:</b> (Fisgin et al., 2000; Fisgin et al., 2002; Jaennet 1999; Kuttu et al., 2000; Lahat et al., 1998) <b>Erwachsene:</b> (Gilat et al., 2003; Kendall et al., 1997; Lahat et al., 1992; Scheepers et al., 2000; Scheepers et al., 1998; Scott et al., 1999; Scott et al., 1997)
Behandlung von kindlichen Fieberkrämpfen	(Johnson 2001; Koren 2000; Lahat et al., 2000)
<b>Sedation vor medizinischen Untersuchungen oder Eingriffen:</b> Kleinere chirurgische Eingriffe	<b>Kinder:</b> (Ljungman 2000; Malinovsky et al., 1993; Saint-Maurice et al., 1990)
MRI Untersuchung	<b>Erwachsene:</b> (Moss et al., 1993; Munte Sinikka 2002; Schweizer et al., 1992)
Endoskopie des oberen Gastrointestinaltraktes	<b>Erwachsene:</b> (Uygur-Bayramcili et al., 2002)
Zahnärztlichen Behandlung	<b>Kinder:</b> (Abrams et al., 1993; al-Rakaf et al., 2001; Fuks et al., 1994; Hargraves and Prinosch 1994; Kaufman et al., 1994; Kupietzky et al., 1996; Lloyd et al., 2000) <b>Erwachsene:</b> (Burstein et al., 1996; Fukuta et al., 1993; Fukuta et al., 1994; Fukuta et al., 1997; Kaufman et al., 1994; Roelfse et al., 2000)

### 2.3 Pharmakokinetik von nasalem Midazolam

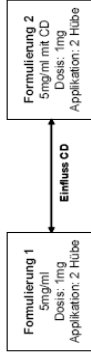
In unterschiedlichen Studien wurde die Pharmakokinetik von nasal appliziertem Midazolam untersucht. Im Unterschied zu der geplanten Studie mit low-dose Midazolam wurden die für die orale Applikation übliche Dosierungen von 0.05 mg/kg bis 0.25 mg/kg Körpergewicht bzw. eine Dosis von 5 mg bis 7.5 mg appliziert. Da üblicherweise die Dormicum® Injektionslösung eingesetzt wurde, wurden Volumina von 0.2 ml bis zu 5 ml nasal appliziert. Bei der nasalen Applikation von 5 ml Formulierungen zeigte die beobachtete Pharmakokinetik die Charakteristika einer Pharmakokinetik nach oraler Applikation. Diese orale Pharmakokinetik lässt sich mit dem Herunterfließen der Formulierung in den Nasenrachenraum gefolgt vom Schlucken der Formulierung und einer anschließenden gastrointestinalen Wirkstoffaufnahme erklären. Da sich die bisher untersuchten Formulierungen hinsichtlich der verwendeten Hilfsstoffe sowie der Midazolam-Konzentration unterscheiden und zudem

höhere Midazolam Dosen oder Volumina appliziert wurden, können die gemessenen Parameter lediglich als Anhaltspunkte für die geplante Studie dienen.

### 2.4 Low-Dose-Midazolam

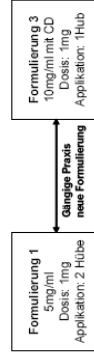
Midazolam zeigt ein Wirkspektrum das sich von der Anxiolyse bis zur tiefen Sedation erstreckt. Verschiedene Publikationen berichten über den erfolgreichen Einsatz von tiefen Midazolam Dosen zur Anxiolyse vor diagnostischen Eingriffen (Steinlauf et al., 1999), (Aeschbacher et al., 1998) bzw. zur Behandlung von Angststörungen (Schweizer, et al. 1992). Zurzeit läuft eine Multizenterstudie, welche die Wirksamkeit von low-dose Midazolam in der Prävention von Klaustrophobie bei der MRT-Untersuchung evaluiert (Studiennummer EKBB: 23/06: Wirksamkeit von nasalem low-dose Midazolam in der Prävention von Klaustrophobie bei MRT-Untersuchungen - Vergleich zweier Formulierungen und Applikationssysteme). Bisher wurde jedoch keine systematische Untersuchung von Pharmakokinetik und Pharmakodynamik von nasal appliziertem low-dose-Midazolam durchgeführt.

(5 mg/ml) durchgeführt werden, da bei einer Midazolam-Konzentration von  $\geq 5$  mg/ml das Cyclohexim als Lösungsvermittler unabhängig ist.



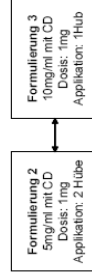
**3. Vergleich der gängigen Praxis der nasalen Midazolam Applikation mit einer neuen Formulierung in einem neuen Applikationssystem**

In dieser Versuchsanordnung wird die Pharmakokinetik der in der Multizenterstudie (Studiennummer EKBB: 23/06) auf deren Effektivität in der Prävention von Klausurophobie bei MRT-Untersuchungen getesteten Studienmedikation untersucht. Diese Studienmedikation entspricht in der Zusammensetzung den hier gezeigten Formulierungen 1 und 3. Es wird eine neue Formulierung mit doppelter Konzentration und dem Lösungsvermittler CD appliziert in einem Hub (Unitdose, Formulierung 3) mit der heute gängigen Praxis (Dormicum® im Multidose-Nasenspray, Formulierung 1) verglichen.



**4. Einseitige oder beidseitige Applikation**

Dieser Vergleich soll aufzeigen in welchem Ausmass der Applikations-Modus, insbesondere die Applikationsfläche, die pharmakokinetischen Messparameter von 1 mg nasal appliziertem Midazolam beeinflusst. Verglichen werden dabei die Parameter  $AUC_{0-360}$ ,  $C_{max}$ ,  $t_{max}$ .

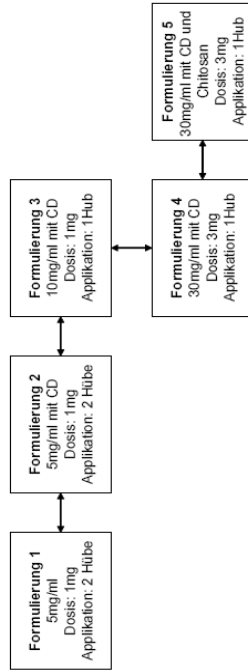


**3. Studienziele**

**3.1 Primäres Studienziel**

Primäres Ziel dieser Studie ist die Untersuchung des Effektes verschiedener Formulierungen und Applikationsarten (nasal einseitig vs. zweiseitig) auf die Pharmakokinetik und insbesondere die Bioverfügbarkeit von nasal appliziertem Midazolam.

Im folgenden Schema sind die Formulierungen, welche nasal appliziert werden aufgeführt. Zur Bestimmung der Bioverfügbarkeit der nasalen Formulierungen wird den Probanden an einem separaten Studientag 1 mg Midazolam i.v. (Dormicum® Amp.) appliziert. Bei den Formulierungen 1-3 wird jeweils eine Dosis von 1 mg, bei den Formulierungen 4 und 5 eine Dosis von 3 mg nasal appliziert.



CD: Cyclohexim

Nachfolgend sind die einzelnen Formulierungen und die damit verbundenen Fragestellungen beschrieben:

**1. Bioverfügbarkeit von nasal appliziertem Midazolam**

Zur Bestimmung der Bioverfügbarkeiten der nasal applizierten Formulierungen (1- 5) wird neben den nasal applizierten Formulierungen auch 1 mg Midazolam i.v. (Dormicum® Amp.) verabreicht

**2. Einfluss des Lösungsvermittlers**

In der Literatur wird der Effekt der Cyclohexime (CD) kontrovers diskutiert. In manchen Publikationen werden sie als permeationsfördernd (Merkus 1959) in anderen als permeationsverzögernd (Roelofse, et al. 2000) bezeichnet. Zu den Cycloheximen und deren Eigenschaften siehe auch Kapitel 4.3.3. Der Vergleich von Formulierung 1 und Formulierung 2 soll zeigen, ob und in welchem Ausmass die Resorption von Midazolam durch die Anwesenheit des als Lösungsvermittlers eingesetzten Cyclohexims beeinflusst wird. Dieser Versuch kann nur mit der tieferen Midazolam-Konzentration

4. Versuchsplanung

4.1 Ablauf der Studie (Rekrutierung bis Nachuntersuchung)

Die Abbildung 4-1 zeigt den Ablauf der einzelnen Studientage. Die Probanden werden über Aushänge in den Instituten der Universität Basel und auf der Homepage der Universität Basel gesucht (Inseratext im Anhang 10.1). Jeder Proband wird vor Aufnahme in die Studie über Wissen, Zielsetzung, Ablauf, Bedeutung sowie Tragweite der Studie aufgeklärt und eingehend über die Wirkungen, Nebenwirkungen und die Risiken der Studienteilnahme in Kenntnis gesetzt (siehe Probandeninformation im Anhang 10.2). Die Probandeninformation setzt den Probanden darüber in Kenntnis, dass die Teilnahme an der Studie freiwillig und die Einwilligung jederzeit und ohne Angabe von Gründen zurückgezogen werden kann.

Bei der Visite 1 (Eintrittsuntersuchung) werden noch offene Fragen durch den Prüfarzt geklärt. Nach dem Unterzeichnen der Einverständniserklärung (Anhang 10.3) wird der Proband medizinisch untersucht (CRF Visite 1 im Anhang 10.4). In den Visiten 2 bis Visite 7 wird dem Probanden die Studienmedikation Formulierung 1 bis 5 sowie Midazolam 1 mg i.v. (siehe 4.3.6) verabreicht. Die Überwachung der Probanden und die Dokumentation der Studien-Parameter erfolgt gemäss dem Case Report Form (CRF, Anhang 10.5). Zwischen den einzelnen Studientagen liegen mindestens 2 Tage.

Eine Woche bis maximal 14 Tage nach der letzten Verabreichung der Studienmedikation (oder nach Abbruch/Ausschluss) wird der Proband abschliessend medizinisch untersucht (CRF Visite 8 im Anhang 10.6).

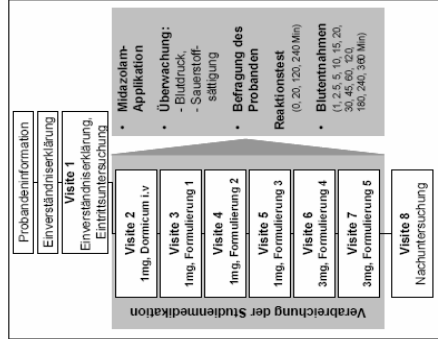


Abbildung 4-1: Studienablauf zur Untersuchung der Pharmakokinetik von nasal appliziertem Midazolam

5. Einfluss von Chitosan-HCl

In der Literatur wird Chitosan zur Verbesserung der Permeation von Wirkstoffen durch unverhornte Epithelien eingesetzt (Illum 1998; Illum et al., 2002; Martinac et al., 2005; Yu et al., 2004). Im Weiteren konnte gezeigt werden, dass die Variabilität der Bioverfügbarkeiten (bzw. weiterer pharmakokinetischer Parameter wie AUC, C<sub>max</sub>, t<sub>max</sub>) durch die Zugabe von Chitosan vermindert werden kann (Hinchcliffe et al., 2005). Zu Chitosan-HCl und dessen Eigenschaften siehe auch Kapitel 4.3.4.

Mit dem Vergleich der **Formulierungen 4 und 5**, soll untersucht werden, ob durch die Chitosan-Zugabe die nasale Resorption von optimiert werden kann. Gemäss den Untersuchungen von (Hinchcliffe, et al 2005) wird eine Verminderung der Variabilität der pharmakokinetischen Parameter (AUC, C<sub>max</sub>, t<sub>max</sub>) erwartet.



3.2 Sekundäre Studienziele

3.2.1 Verträglichkeit

Sekundäres Studienziel ist die Erfassung der nasalen Verträglichkeit der verschiedenen Formulierungen. Daneben wird auch die Ansprechbarkeit der Probanden überwacht und dokumentiert (siehe Case Report Form (CRF) im Anhang 10.5).

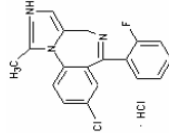
3.2.2 Pharmakodynamik: Reaktionszeitmessung mittels Choice Reaction Time Task (CRTT)

Mögliche sedierende Effekte werden mittels Messung der Reaktionszeit erfasst. Computergestützt wird das Zeitintervall zwischen zwei Stimuli (verschiedenfarbene Punkte auf Computerbildschirm) als Arbeitsgeschwindigkeit und die Latenz zwischen Stimulus und Reaktion der Testperson (Drücken der entsprechenden Fabtaste auf Tastatur) als Reaktionszeit erfasst. Dabei wird die Arbeitsgeschwindigkeit laufend so an die Performance der Versuchsperson angepasst, dass gemittelt über total 500 Stimuli eine konstante Fehlerrate von 50% erreicht wird. Das Testverfahren ist standardisiert und zeigt eine hohe Test-Re-Test-Zuverlässigkeit (Langewitz 1987).

Der Test dauert insgesamt zwischen 5 und 8 Minuten. Vor der ersten Messung wird ein Testlauf durchgeführt, um die Versuchspersonen mit dem Test vertraut zu machen. Die Reaktionszeitmessungen werden vor Applikation von Midazolam sowie 20 Minuten (maximaler Benzodiazepin-Effekt) und 240 Minuten nach Midazolam Applikation durchgeführt.

### 4.3 Prüfpräparate

#### 4.3.1 Midazolam-Hydrochlorid



**Wirkung:** Midazolam gehört zu den kurzwirksamen Tranquillantien der Imidazo-Benzodiazepine und wirkt beruhigend, entspannend, anxiolytisch, hypnotisch, antikonvulsiv und muskellaxierend. Der Hauptwirkungsort ist das limbische System. Die Anzahl der Nervenimpulse in diesem Gebiet wird verringert.

Der sedierende Effekt tritt nach ca. 4 Minuten (i.v.), 15 Minuten (i.m.) bzw. 0,5-1,5 Stunden (peroral) ein. Für den Wirkungseintritt nach nasaler Applikation werden in der Literatur 5 bis 10 Minuten angegeben (Malinovsky et al., 1995), (Goeplert 1996).

Midazolam wird zur Sedation in der Prämedikation vor diagnostischen oder operativen Eingriffen, zur Narkoseeinleitung und –aufrechterhaltung und zur Notfalltherapie des Status epilepticus eingesetzt. Die Bioverfügbarkeit nach oraler Applikation beträgt wegen dem ausgeprägten First-pass Metabolismus lediglich 50%. In verschiedenen Publikationen wird die Bioverfügbarkeit nach nasaler Verabreichung höher als jene nach oraler Applikation angegeben, begründet wird dies durch die Umgehung des First-pass-Effektes bei der nasalen Verabreichung.

Midazolam wird durch Hydroxylierung mit anschließender Glucuronidierung hepatisch eliminiert. Der Hauptmetabolit,  $\alpha$ -Hydroxymidazolam besitzt ca. 10% der pharmakologischen Aktivität von Midazolam. Die Plasmahalbwertszeit von Midazolam beträgt 1,5 bis 2,5h (die Metaboliten haben eine kürzere Halbwertszeit als die Muttersubstanz).

**Dosierung:** Für die Prämedikation vor einer Operation werden im schweizerischen Arzneimittelkompendium 2006 folgende Dosen vorgeschlagen:

Indikation	Kinder	Erwachsene	Ältere und geschwächte Patienten
Intramuskuläre Verabreichung	0,15-0,20mg/kg	0,07-0,1mg/kg übliche Dosis: 5mg	0,025-0,05mg/kg
Rectale Verabreichung	0,35-0,45mg/kg (BV:50%)	---	---

Dornicum® Tabletten sind in den Dosierungsstärken 7,5 mg und 15 mg erhältlich.

### 4.2 Studienpopulation

#### 4.2.1 Stichprobenumfang

Es werden 8 Probanden rekrutiert. Bei vorzeitigem Ausscheiden eines Probanden wird ein Ersatzproband rekrutiert, so dass nach Beendigung der Studie 8 vollständige Datensätze ausgewertet werden können.

#### 4.2.2 Einschlusskriterien

- Männliche Probanden im Alter zwischen 18 und 45 Jahren
- Nichtraucher
- BMI mindestens 18 kg/m<sup>2</sup>, höchstens 30 kg/m<sup>2</sup>
- freiwillige Unterzeichnung der Einverständniserklärung

#### 4.2.3 Ausschlusskriterien

Es werden nur Probanden zur Studien-Teilnahme zugelassen, für welche keines der Ausschlusskriterien zutrifft. Entwickelt sich im Laufe der Studien-Teilnahme eines der Ausschlusskriterien wird der Proband von der weiteren Studien-Teilnahme ausgeschlossen.

- Probanden, welche nicht teilnehmen wollen, ihre Einwilligung zurückziehen oder die Einverständniserklärung nicht schriftlich abgeben können.
- bekannte oder vermutete Überempfindlichkeit gegenüber Midazolam (bzw. vorhergehende paradoxe Reaktion nach Benzodiazepingabe)
- Bestehende Kontraindikation für Midazolam (Überempfindlichkeit gegenüber Midazolam oder andere Benzodiazepine, Myasthenia gravis, anamnestische oder aktuelle Schlafmittel-, und / oder Psychopharmakaaabhängigkeit, schwere Leberfunktionsstörungen, akutes Engwinkelglaukom und akute Ateminsuffizienz)
- bekannte oder vermutete Überempfindlichkeit gegenüber einem der eingesetzten Hilfsstoffe (randomisiert-methylertes- $\beta$ -Cyclodextrin, Chitosan)
- bekannte Allergie gegen Krustentiere<sup>2</sup>
- Einnahme von **CYP3A4-Induktoren** (z.B. Phenobarbital, Carbamacepin, Rifampizin, Phenytoin, Hypericin bzw. Johanniskrautpräparate) oder **CYP3A4-Inhibitoren** (z.B. Amiodaron, Amprenavir, Atorvastatin, Cimetidin, Ciprofloxacin, Clarithromycin, Clotrimazol, Diazepam, Diltiazem, Erythromycin, Fluconazol, Grapefruitsaft, Indinavir, Isomiazid, Itraconazol, Ketoconazol, Metronidazol, Nelfinavir, Norfloxacin, Ritonavir, Saquinavir, Simvastatin, Verapamil)
- Medikamenten-, Drogen- oder Alkoholmissbrauch
- Probanden, die zur Zeit oder in den vergangenen 30 Tagen an einer anderen klinischen Studie teilgenommen haben
- chronisch behinderte Nasenatmung
- symptomatische Nasenpolypen oder Nasenpolypen in der Anamnese

<sup>2</sup> Chitosan wird aus den Schalen von Krustentieren gewonnen

**Therapeutische Midazolam-Blutkonzentrationen:** Im Konzentrationsbereich von 50 bis 100 µg/l ist mit einer Sedation zu rechnen, ab 100 µg/l kann eine Amnesie auftreten und ab 400 bis 500 µg/l tritt Bewusstlosigkeit ein (Informationen der Ratiopharm).

**Unerwünschte Wirkungen:** Die unerwünschten Wirkungen von Midazolam entsprechen jenen von anderen Vertretern der Benzodiazepine: Müdigkeit, Schläfrigkeit während des Tages, reduzierte Vigilanz, Verwirrtheit, Kopfschmerzen, Schwindel, Muskelschwäche, Ataxie.

Paradoxe Reaktionen wie Ruhelosigkeit, Erregbarkeit, Erregungszustände, Aggressivität, Halluzinationen, Verhaltensstörungen sind im Zusammenhang mit Benzodiazepin-Einnahmen beschreiben. Sie treten häufiger bei älteren Patienten auf und erfordern ein Absetzen der Therapie und eine adäquate Überwachung und Unterstützung des Patienten. Sehr selten können allergische Reaktionen (z.B. Hautausschläge) auftreten.

**Anterograde Amnesie:** Patienten, die am Einschlafen gehindert oder in den ersten Stunden nach der Einnahme von Dormicum geweckt werden, können in seltenen Fällen amnestische Episoden über alltägliche Handlungen, die in den Wachphasen ausgeführt werden, durchmachen. Es ist bekannt, dass solche Episoden häufiger bei Patienten unter aussergewöhnlichen psychischen Stressbedingungen auftreten. Bei schneller parenteraler Anwendung von Midazolam kann eine Blutdrucksenkung bzw. eine Beeinträchtigung der Atmung auftreten.

**Schwangerschaftstillzeit:** Midazolam passiert die Placentastranke und wird in die Muttermilch sezerniert. (Für diese Studie werden keine Frauen rekrutiert). Es wurden keine embryotoxischen, teratogenen und mutagene Wirkungen festgestellt.

**Akute Toxizität und chronische Toxizität:** Midazolam hat sich hinsichtlich der akuten, subakuten und chronischen Toxizität als eine sehr gut verträgliche Substanz mit einer grossen therapeutischen Breite erwiesen. Bei der parenteralen Anwendung (i.m und i.v) zeigte sich auch eine gute lokale Verträglichkeit.

**Kontraindikationen:** Überempfindlichkeit gegenüber Midazolam oder anderen Benzodiazepinen, Myasthenia gravis, Abhängigkeitsanamnese, akute Alkohol-, Schlafmittel-, sowie Psychopharmakavergiftung, Säuglinge bis zum 4. Lebensmonat, erstes Trimenon der Schwangerschaft und Zeitpunkt der Geburt, Stillzeit, schwere Leberfunktionsstörungen, akutes Engwinkelglaukom und akute Ateminsuffizienz.

#### 4.2 Hilfsstoffe für nasale Midazolam Zubereitung

Für lipophile Wirkstoffe wie Midazolam ist die nasale Verabreichung möglich wenn sie in der nasalen Zubereitung gelöst werden können. Midazolam ist bei einem pH-Wert von 2,8 bis zu einer Konzentration von 22 mg/ml, bei einem pH-Wert von 5,1 lediglich bis 1,1 mg/ml löslich. Um eine für die nasale Applikation geeignete Formulierung bereit zu stellen, können verschiedene Hilfsstoffe eingesetzt werden. Neben den Hilfsstoffen zur Löslichkeitsverbesserung können auch Hilfsstoffe zur Verbesserung der Bioverfügbarkeit eingesetzt werden.

Das geringe nasal applizierbare Volumen (0,1 bis maximal 0,4 ml pro Nasenöffnung) (Penker 2001) erfordert eine Midazolam-Konzentration, welche die Löslichkeit von Midazolam in Wasser übersteigt. Folglich wird die Verwendung eines geeigneten Lösungsvermittlers notwendig. Da das applizierte Volumen möglichst gering gehalten werden soll, ist es unerlässlich den Wirkstoff in einer ausreichenden Konzentration in Lösung zu bringen. Um beispielsweise die Dosis von 3 mg in einem Volumen von 0,1 ml verabreichen zu können, ist eine Wirkstoffkonzentration von 30 mg Midazolam pro ml erforderlich. Diese Konzentration an Midazolam kann nur durch die Zugabe eines geeigneten Lösungsvermittlers erreicht werden.

#### 4.3 Randomisiert-methyliertes- $\beta$ -Cyclodextrin (RM $\beta$ CD)

Das randomisiert-methylierte- $\beta$ -Cyclodextrin (RM $\beta$ CD) erwies sich bei in vitro Freisetzungsversuchen durch künstliche Membranen, im Rahmen der Formulierungsentwicklung als der am besten geeignete Lösungsvermittler für die Zubereitung einer physiologischen Midazolam-Formulierung zur nasalen Applikation.

#### Mechanismus der Löslichkeitsverbesserung/Peneetrationsförderung

Cyclodextrine führen einerseits durch erhöhte Löslichkeit und andererseits durch Verbesserung der nasalen Absorption zu einer gesamthaft höheren nasalen Bioverfügbarkeit (Merkus 1999).

Die Lösungsvermittlung der Cyclodextrine beruht auf einer Einschlusskomplexbildung. Verschiedene Untersuchungen zeigen, dass die Freisetzung der komplexierten Wirkstoffe üblicherweise rasch und vollständig ist (Stella et al., 1999).

Das bevorzugte Komplex-Verhältnis von CD und eingeschlossenem Molekül ist neben den physikochemischen Eigenschaften auch von der räumlichen Ausdehnung/Gestalt der Moleküle abhängig. Im Rahmen der Formulierungsentwicklung durchgeführte Untersuchungen zeigen, dass sich bei der Löslichkeitsvermittlung von Midazolam mittels RM $\beta$ CD ein 1:1 Einschlusskomplex bildet. Aufgrund dieser Erkenntnisse erscheint es sinnvoll, eine äquimolare Menge des Lösungsvermittlers (gleiche Anzahl CD-Moleküle wie Midazolam-Moleküle) einzusetzen. Daraus resultiert, dass die Formulierungen entsprechend ihrer Midazolam-Konzentration eine entsprechende Menge an RM $\beta$ CD enthalten: bei 5 mg Midazolam pro ml: 2% RM $\beta$ CD; bei 10 mg Midazolam pro ml: 4% RM $\beta$ CD; bei 30 mg Midazolam pro ml: 12% RM $\beta$ CD (MW von Midazolam-HCL: 362,3 g/mol, MW von RM $\beta$ CD: 1310 g/mol)

**Beurteilung der Sicherheit**

In den zitierten Toxikologie-Studien (Ratten) wurden relativ hohe RM $\beta$ CD-Konzentrationen (bis 20%) untersucht und während einer langen Expositionsdauer von bis zu 60 Minuten in Kontakt mit der nasalen Schleimhaut gebracht ohne eine histologisch sichtbare Schädigungen zu verursachen. Die Formulierungen welche im Rahmen der Pharmakokinetik-Studie den freiwilligen Probanden appliziert werden, enthalten eine RM $\beta$ CD-Konzentrationen von maximal 12%. Dabei wird lediglich ein Volumen von 0.1 ml pro Nasenöffnung appliziert (Dosies RM $\beta$ CD: max. 12 mg, weniger als 0.2mg/kg Körpergewicht, bei der oralen Applikation soll die Dosis von 1 mg/kg KG nicht überschritten werden). Im Weiteren ist zu berücksichtigen, dass bei der Applikation die verabreichte Formulierung sogleich durch den nasalen Mucus verdünnt wird. Zudem wird die Kontaktzeit einer nasal applizierten Formulierung durch die nasale Clearance<sup>4</sup> begrenzt. Auf Grund dieser begrenzten Exposition der Nasenschleimhaut mit RM $\beta$ CD ist keine Beeinträchtigung der nasalen Schleimhaut zu erwarten. Das RM $\beta$ CD ist in einem auf dem Markt erhältlichen Nasenspray zur Estrogentherapie<sup>5</sup> enthalten und wurde somit als Hilfsstoff einer Zubereitung für die nasale Langzeittherapie von der Swissmedic sowie anderen europäischen Arzneimittelbehörden genehmigt.

**4.3.4 Chitosanhydrochlorid Ph.Eur.**

Chitosan wird aus Chitin gewonnen, einem Polysaccharid, das in den Schalen von Garnelen, Krabben und anderen Krustentieren vorkommt. Chitosan-HCl ist ein Chitosanderivat (Salz) mit verbesserter Wasserlöslichkeit. Chitosan-HCl ist in der Ph.Eur. monographiert. Chitosan und dessen besser wasserlösliche Salze werden in nasalen und pulmonalen Formulierungen zur Verbesserung der Bioverfügbarkeit eingesetzt (Illum 1998; Illum, et al. 2002; Martinac, et al. 2005; Yu, et al. 2004). Die Erhöhung der Bioverfügbarkeit basiert einerseits auf der längeren Schleimhaftung der Formulierung (Mucoadhäsion). (Soane et al., 1999; Soane et al., 2001), andererseits wird die Zellschicht durch eine transiente reversible Öffnung der tight junctions permeabler. Die transiente Öffnung der tight junctions ist vollständig reversibel, gemäss Dodane et al. (Dodane et al., 1999) wird die nasale Schleimhaut nicht beeinträchtigt. Die Mucoadhäsion kommt durch die Interaktion zwischen dem linearen kationischen Polysaccharid Chitosan und der negativ geladenen nasalen Mucosa zustande. Dadurch wird die nasale Clearance von chitosamhaltigen Formulierungen verzögert (Aspdien et al., 1997). Die verlängerte Kontaktzeit von Formulierung und nasaler Schleimhaut begünstigt die Absorption von Wirkstoffen (Pavis et al., 2002). Chitosan wird nicht absorbiert, ist biokompatibel, nicht toxisch, weder irritierend noch allergisierend (Dodane and Vilivalam 1998). Der umfassende Review-Artikel von Dodane (68 Referenzen) bestätigt Chitosan als sicheren und effektiven Permeationsverbesserer für die nasale Applikation von systemischen Wirkstoffen (Dodane and Vilivalam 1998).

<sup>4</sup> unter den **nasalen Clearance** versteht man den Reinigungsprozess der nasalen Schleimhaut, welcher durch die Zilienbewegung des respiratorischen Epithels in Richtung Rachen zustande kommt.

<sup>5</sup> Aerodio<sup>®</sup> (Estradiol Hemihydrat), Servier S. A.

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Aufgrund ihrer Grösse und der Hydrathülle zeigen Cyclodextrine keine Membrangängigkeit. Die komplexierten Wirkstoffmoleküle diffundieren als geladener Komplex zur lipophilen Membran, wo sie (ohne hydratisiert zu werden) direkt in die Membran übergehen können, das Cyclodextrin bleibt in der wässrigen Phase ausserhalb der Membran. Die Abbildung 4-2 zeigt die schematische Darstellung des Gleichgewichts zwischen Komplexbildung und Wirkstofffreisetzung in einem wässrigen System an einer biologischen Membran (Gröger M., 2001).

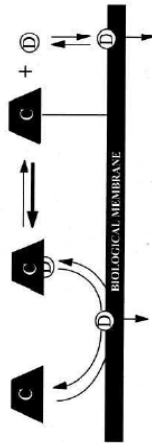


Abbildung 4-2 : Drug-Release aus dem Cyclodextrin-Wirkstoff-Komplex; C: Cyclodextrin, D: Wirkstoff

Die permeationsverbessernde Wirkung der Cyclodextrine wird jedoch kontrovers diskutiert. Gemäss (Illum 2002) wirken die Cyclodextrine nicht direkt als Penetrations- sondern lediglich als Löslichkeitsverbesserer. Die aus der verbesserten Löslichkeit resultierenden höheren Wirkstoffkonzentrationen führen zu höheren Konzentrationsgradienten, was zu einer rascheren Penetration führt.

Als Beispiel für den Einsatz von Cyclodextrinen (RM $\beta$ -CD) als Lösungsvermittler kann der Östrogen-Nasenspray von Servier (Aerodio<sup>®</sup>) genannt werden, welcher in verschiedenen europäischen Ländern im Handel ist.

**Toxikologie**

Eine Studie am Tierrmodell (Ratte) untersuchte den Effekt unterschiedlicher Cyclodextrin-Typen (CD-Typen) auf die Integrität der Nasenschleimhaut. Nach einer in vivo Exposition der Nasenschleimhaut während 60 Minuten mit einer 10% CD-Lösung (RM $\beta$ CD bzw. HP $\beta$ CD<sup>3</sup>) konnte in der anschliessenden lichtmikroskopischen Untersuchungen keine sichtbare Schädigung erkannt werden. Eine wiederholte Applikation von RM $\beta$ CD (20%) zeigte keinerlei toxische Effekte. Die Resultate zeigen, dass die Applikation der untersuchten CD-Typen (RM $\beta$ CD bzw. HP $\beta$ CD) keinen schädigenden Einfluss auf die Integrität der nasalen Mucosa haben (Asai et al., 2002).

Die wiederholte nasale und okkulare Applikation (2 mal täglich 7.5% RM $\beta$ CD, während 28 Tagen) verursachte keine histologischen Veränderungen. Untersucht wurden Hunde, Kaninchen, und Meerschweinchen (Angaben von Servier, Hersteller des Aerodio<sup>®</sup> Nasensprays) Aufgrund der Erfahrungen des Herstellers von RM $\beta$ CD (Wacker) sollte bei der oralen Applikation die Dosis von 1 mg RM $\beta$ CD pro kg Körpergewicht nicht überschritten werden.

<sup>3</sup> Hydroxypropyl- $\beta$ -Cyclodextrin

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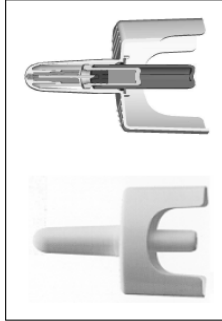


Abbildung 4-3: Untidose Nasenspray (Pfleifer)

#### 4.3.6 Zusammensetzung der Prüfpräparate

In Tabelle 4-1 sind die Bestandteile und die verabreichte Dosis der eingesetzten Formulierungen aufgeführt. Alle Formulierungen sind wässrige Lösungen und sind durch die Zugabe von NaCl isotoniert. Die Formulierungen 1 und 3 entsprechen den Formulierungen die in der multizentrischen Studie zur Untersuchung der Wirksamkeit von nasalem low-dose Midazolam in der Prävention von Klausurphobie bei MRT-Untersuchungen (Studiennummer EKBB: 23/06) eingesetzt werden. Das RM<sub>BCD</sub> bildet einen 1:1 Einschlusskomplex mit Midazolam. Folglich ist die Menge des Lösungsvermittlers der Midazolam-Menge angepasst, sodass die beiden Komponenten in einem äquimolaren Verhältnis vorliegen (5 mg Midazolam pro ml 2% RM<sub>BCD</sub>; bei 10 mg Midazolam pro ml: 4% RM<sub>BCD</sub>; bei 30 mg Midazolam pro ml: 12% RM<sub>BCD</sub>).

Tabelle 4-1: Zusammensetzung und Bezeichnung der Formulierungen und entsprechendes Applikationsvolumen

	Formulierung	Midazolam	RMBCD <sup>1</sup>	Chitosan-HCl	Applikation	Dosis
1	Midazolam UD Nasenspray 0.5mg	5mg/ml	---	---	nasal, 2x0.1ml	1mg
2	Cyclodex-Midazolam UD Nasenspray 0.5mg	5mg/ml	2%	---	nasal, 2x0.1ml	1mg
3	Cyclodex-Midazolam UD Nasenspray 1mg	10mg/ml	4%	---	nasal, 0.1ml	1mg
4	Cyclodex-Midazolam UD Nasenspray 3mg	30mg/ml	12%	---	nasal, 0.1ml	3mg
5	Chitosan-CD-Midazolam UD Nasenspray 3mg	30mg/ml	12%	0.5%	nasal, 0.1ml	3mg

<sup>1</sup> Randomisiert-methylertes-β-Cyclodextrin

Für die Konzentration von 0.5% Chitosan-HCl konnte gezeigt werden, dass die Permeation unterschiedlicher Wirkstoffe durch die nasale Schleimhaut verbessert werden kann, ohne dass eine Schädigung der Zellen auftritt. Im Weiteren konnte gezeigt werden, dass Chitosan weder bei nasaler noch bei i.v. Applikation eine humorale Immunantwort hervorruft (Illium 1998). In einer Studie mit 14 Patienten wurde ein chitosanhaltiger Morphin-Nasenspray zur Therapie von Durchbruchschmerzen bei Krebspatienten untersucht. Die beobachteten unerwünschten Effekte (bitterer Geschmack, Müdigkeit) stehen im Zusammenhang mit dem applizierten Wirkstoff (Pavis, et al. 2002).

Aspden et al. untersuchten den Effekt einer Chitosanlösung (0.25%) auf die nasale Schleimhaut in vitro sowie in vivo an 10 gesunden Probanden. Nach 7 Tagen Behandlung mit 0.25% Chitosanlösung konnte weder bei der Endoskopischen Untersuchung der Nasenschleimhaut (in vivo), noch bei der lichtmikroskopischen Untersuchung (in vitro) Entzündungszeichen festgestellt werden. Die Applikation der chitosanhaltigen Lösung wurde von allen 10 Probanden gut toleriert (Aspden, et al. 1997). Da das Chitosan Fett adsorbierende Eigenschaften aufweist ist es in unterschiedlichen Nahrungsergänzungsmitteln enthalten. Das Xitofarm Geränpulver der Phytopharma SV (Grandvillard) enthält 2 g Chitosan in 100 g Pulvermischung. Bei der Einnahme der empfohlenen Tagesdosis von 3 mal 8 g Xitofarm werden insgesamt 480 mg Chitosan eingenommen. In den USA zählt Chitosan zu den Nahrungsergänzungsmitteln mit GRAS-Status („Generally Recognized as Safe“) (Shahidi and Abuzaytoon 2005).

#### Beurteilung der Sicherheit

Bei der nasalen Applikation der chitosanhaltigen Formulierung (Formulierung 5) werden einmalig 0.5 mg Chitosan-HCl appliziert. Diese Dosis liegt weit unter der Chitosan Menge, die z.B. bei der Einnahme von Xitofarm, einem chitosanhaltigen Nahrungsergänzungsmittel eingenommen wird (480mg/d). Aufgrund der vorliegenden Daten zu der Sicherheit von nasal appliziertem Chitosan, wird das Risiko bei der nasale Applikation einer 0.5% Chitosan-Lösung als sehr gering eingestuft.

#### 4.3.5 Das Applikationssystem: Untidose Nasenspray (Pfleifer)

Die klinischen Prüfmuster zur nasalen Applikation werden in Untidose Nasenspray von Pfeifer abgefüllt (Abbildung 4-3). Der befüllte Untidose Nasenspray ist gebrauchsfertig und gibt ein Hubvolumen von 0.1 ml ab. Das Hubvolumen von 0.1 ml wird lageunabhängig abgegeben, die Applikation kann folglich auch bei liegenden Patienten/Probanden vorgenommen werden. Der Untidose Nasenspray ist zum einmaligen Gebrauch (Monodose). Für die Applikation von 2 mal 0.1 ml werden pro Proband 2 Untidose Nasensprays eingesetzt. Der Zornig® nasal (Nasenspray) von AstraZeneca entspricht, abgesehen vom Design der Umhüllung, dem hier vorgestellten Untidose Nasenspray.



## 4.3.7 Herstellung der Prüfpräparate

Alle Formulierungen werden in der Spital-Pharmazie des Universitätsspitals Basel gemäss den GMP-Richtlinien hergestellt (Swissmedic Bewilligungsnummer 1008282). Die Formulierungen zur nasalen Applikation werden in Unitidose Nasenspray (Pfeiffer) zum einmaligen Gebrauch bereitgestellt (vgl. Kapitel 4.3.5).

Von jeder Formulierung werden 2 Rückstellmuster gemäss der GMP-Arbeitsanweisung der Spital-Pharmazie des Universitätsspitals Basel (AA0016) aufbewahrt.

## 5. Studienparameter und Analytik

## 5.1 Messpunkte

Um zu überprüfen, ob mit den festgelegten Messzeitpunkten (1, 2, 5, 5, 10, 15, 20, 30, 60, 120 Minuten) die maximale Midazolam-Konzentration erfasst wird, wurde in einem Pilotversuch (Eigenversuch) Midazolam 1 mg und 3 mg nasal appliziert. Wie die Abbildung 5-1 zeigt wird die Serumkonzentrations-Zeitkurve gut durch die gewählten Messzeitpunkte beschrieben. Um eine bessere Extrapolation der terminalen Elimination und der davon abhängigen pharmakokinetischen Parameter (u.a.  $AUC_{0-\infty}$ ) zu ermöglichen werden nach 45, 180, 240 und 360 Minuten weitere Blutproben entnommen. Insgesamt werden also zu folgenden Zeitpunkten 5 ml Vollblut entnommen: 1, 2, 5, 10, 15, 20, 30, 45, 60, 120, 180, 240 und 360 Minuten. Dies ergibt ein totales Volumen von ca. 390 ml Blut, welches verteilt über die ganze Studiendauer pro Testperson entnommen wird.

Für die Bestimmung der Pharmakokinetik-Parameter nach der i.v. Applikation von 1 mg Midazolam werden zu denselben Zeitpunkten Blutproben entnommen wie nach der nasalen Applikation.

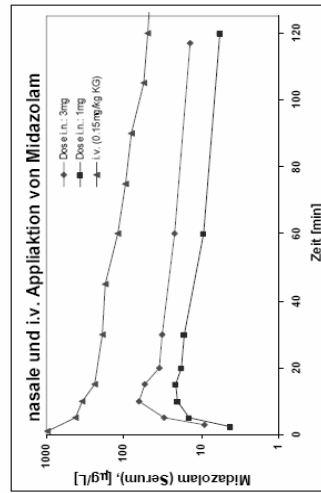


Abbildung 5-1: Pharmakokinetik von nasalem Midazolam 3 mg, 1 mg (Daten aus Pilotversuch) und 0.15mg/kg Körpergewicht i.v. Amrein, 1981)

## 5.2 Analytische Methode (Midazolam, a-Hydroxy- und 4-Hydroxy-Midazolam)

Das abzentrifugierte Serum wird tiefgekühlt ( $-20^{\circ}\text{C}$ ) und anschliessend für die Quantifizierung von Midazolam, a-Hydroxy- und 4-Hydroxy-Midazolam an das Institut für Rechtsmedizin<sup>6</sup> weitergeleitet.

<sup>6</sup> Dr. phil. Thomas Briellmann, Institut für Rechtsmedizin, Pestalozzistrasse 22, 4056 Basel, Tel.: 061 267 38 73, Fax: 061 267 39 07, Thomas.Briellmann@bs.ch

Die Quantifizierung von Midazolam und den Metaboliten  $\alpha$ -Hydroxy- und 4-Hydroxymidazolam im Serum erfolgt mittels LC-MS (Dussy et al., 2005). Der Probe wird ein interner Standard (Brotizolam) zugesetzt, anschliessend wird mit 1-Chlorbutan extrahiert und der eingeeingte Extrakt analysiert.

### 5.3 Auswertung

#### 5.3.1 Berechnung der Pharmakokinetik-Parameter

Die Serumkonzentrations-Zeit Daten werden mittels nicht-kompartimenteller Standardmethoden mit der Software WinNonlin (Version 3.1, Pharsight Corporation, Mountain View, CA, USA) analysiert.  $C_{max}$  und  $t_{max}$  werden direkt aus den Konzentrations-Zeit Profilen bestimmt. Die terminale Eliminationskonstante  $\lambda_z$  wird durch log-lineare Regression aus dem terminalen Anteil der Konzentrations-Zeit-Profilen bestimmt. Die Halbwertszeit  $t_{1/2}$  wird aus  $\ln 2 / \lambda_z$  berechnet. Die  $AUC_{0-360}$  wird mittels Trapezoid-Regel bestimmt. Die  $AUC_{0,inf}$  wird aus  $AUC_{0-360}$  mittels  $\lambda_z$  extrapoliert. Sekundäre pharmakokinetische Parameter (Cl, Vd, F) werden mittels Standardberechnungen aus den oben bestimmten Parametern abgeleitet.

#### 5.3.2 Statistik

Für die einzelnen Formulierungen wird die Bioäquivalenz ( $AUC_{0-360}$ ,  $AUC_{0,inf}$  (extrapol.),  $C_{max}$ ) dosisnormalisiert geprüft. Bioäquivalenz wird angenommen, wenn Mittelwert und 90%-Konfidenzintervalle für die log-transformierten individuellen Quotienten (Testformulierung vs. Referenz) für die einzelnen Parameter komplett innerhalb des Intervalls von 0.8 – 1.25 liegen. Nicht normal verteilte Parameter wie  $t_{max}$ , Cl, Vd, F, werden mit Hilfe von nicht parametrischen Methoden (Wilcoxon signed rank test, Friedman) getestet. Effektparameter der verschiedenen Behandlungsphasen werden formal auf Normalverteilung getestet. Normal verteilte Parameter werden mittels repeated measures ANOVA, nicht normalverteilte Parameter durch einen entsprechenden nicht parametrischen Test (zB Friedman) geprüft. Alle statistischen Tests werden mit der Software SPSS, Version 13.0 für Windows durchgeführt. Als Signifikanzniveau wird ein  $p=0.05$  festgelegt.

## 6. Probandensicherheit

### 6.1 Blutentnahme und Applikation der Studienmedikation

Das Legen des Venenkatheters (Venflon) in eine Vorderarmvene für die Blutentnahmen wird von ärztlichem Studienpersonal vorgenommen. Die i.v. Applikation von 1 mg Midazolam und die anschliessende Überwachung werden vom Principal Investigator vorgenommen. Die Blutentnahmen werden durch geschultes Studienpersonal durchgeführt. Die Probanden stehen während der gesamten Versuchsdurchführung unter ärztlicher Aufsicht.

### 6.2 Erwartete Effekte von low-dose Midazolam

Auf Grund der erreichten Serumkonzentrationen (maximal ca. 25 µg/l, siehe Abbildung 5-1) wird erwartet, dass nach nasaler Applikation von 1 mg Midazolam der beobachtete Effekt von Entspannung bis zur leichten Schläfrigkeit reicht. Bei nasaler Applikation von 3 mg Midazolam (maximale Serumkonzentration ca. 70 µg/l, siehe Abbildung 5-1) kann die Wirkung von leichter Schläfrigkeit bis zum Einschlafen des Probanden führen, ohne dass eine Beeinträchtigung der Aufweckbarkeit erwartet wird.

In einer randomisiert doppelblinden placebokontrollierten Studie mit 200 Patienten, in welcher i.v. low-dose Midazolam zur Anxiolyse/Sedierung bei transoesophagalen Echokardiographien in der Dosierung von 2 mg bis 3 mg i.v. eingesetzt wurde, wurden keine emalen unerwünschten Effekte beobachtet (Aeschbacher, et al. 1998). Gestützt auf die Studiendaten von (Aeschbacher, et al. 1998), wird bei der nasalen Applikation von 1 mg bis 3 mg keine Beeinträchtigung der vitalen Funktionen erwartet.

### 6.3 Mögliche unerwünschte Effekte von low-dose Midazolam

Neben einer vorübergehenden unangenehmen Empfindung bei der nasalen Applikation der Nasensprays wird lediglich mit einer vorübergehenden Beeinträchtigung der Aufmerksamkeit gerechnet. Die Probanden werden darüber informiert, dass die aktive Teilnahme am Strassenverkehr am Tag der Studienteilnahme zu unterlassen ist.

Die Entwicklung einer paradoxen Reaktion, die bei der Applikation von Benzodiazepinen gelegentlich auftritt, kann trotz der Applikation einer geringen Dosis nicht mit Sicherheit ausgeschlossen werden. Kinder, Patienten ab 65 Jahren (Weinbroum et al., 2001), Patienten mit einer anamnestischen oder aktuellen psychischen Erkrankung, sowie Alkoholiker zeigen ein erhöhtes Risiko für die Entwicklung einer paradoxen Reaktion nach der Verabreichung von Benzodiazepinen (Mancuso et al., 2004). Bei der gewählten Studienpopulation besteht kein erhöhtes Risiko eine paradoxen Reaktion zu entwickeln. Im Weiteren werden paradoxen Reaktionen mit der Applikation von hohen Benzodiazepindosen in Verbindung gebracht (Litchfield 1981). Da in der geplanten Studie die applizierte Dosis von 1 mg bis maximal 3 mg Midazolam viel tiefer ist als für die Sedation zur Ruhigstellung bei diagnostischen oder chirurgischen Eingriffen üblich, wird das Risiko für das Auftreten von Nebenwirkungen im Allgemeinen als gering eingeschätzt. Die Dosisabhängigkeit wird auch für die Entwicklung einer paradoxen

Reaktion angenommen, da gemäss (Litchfield 1981) vor allem bei einer hohen Benzodiazepin Dosis paradoxe Effekte zu beobachten sind.  
Die in der Literatur beschriebenen Fälle von paradoxer Reaktion traten im Zeitraum von „soon after application“ bis 210 Minuten nach der ersten i.v. Applikation auf (dabei ist zu beachten, dass in den meisten Studien Midazolam auftitriert wurde bis die gewünschte Sedationstiefe erreicht war). Dabei wurden deutlich höhere Midazolam-Dosen appliziert. Mit dem Beobachten der Patienten während 6 h nach der nasalen Applikation von maximal 3 mg Midazolam wird, auf Grund der oben aufgeführten Argumentation, davon ausgegangen, dass eine allfällige paradoxe Reaktion vom Studienpersonal bemerkt wird und entsprechend reagiert werden kann.

## 6.4 Überwachung und Intervention

In den ersten 30 Minuten nach der Verabreichung der Studienmedikation, während der Anflutung bis zur maximalen Serumkonzentration, werden der Blutdruck und die Sauerstoffsättigung (Pulsoximetrie) überwacht.

In Tabelle 6-1 sind die zur Überwachung der Probanden gemessenen Parameter sowie die Interventionsgrenzen und die zu treffenden Massnahmen aufgeführt.

**Tabelle 6-1:** zur Überwachung der Probanden gemessene Parameter, Interventionsgrenzen und Interventionen

Parameter	Interventionsgrenze	Intervention
<b>Sauerstoffsättigung</b>	S <sub>O</sub> <sub>2</sub> (transcutan) ≤ 90%	→ 2L Sauerstoff nasal
<b>Sedation</b>	- schwere Sedation (d.h. Proband nicht weckbar)	Anexate® (Flumazenil) → 0.2mg i.v. innerhalb 15 sec. → falls nach 60 sec. nach der ersten Verabreichung von Flumazenil der Proband das Bewusstsein nicht erlangt, wird eine zweite Dosis von 0.1mg injiziert. Bei Bedarf kann alle 60 sec. eine Dosis von 0.1mg Flumazenil nachdosiert werden. Die maximale Dosis von 1mg sollte nicht überschritten werden. → Probanden welche unter der Studienmedikation einen relevanten Abfall der Vitalparameter zeigten, werden von der weiteren Studien-Teilnahme ausgeschlossen.
<b>Vitalparameter</b>	- relevanter Abfall der Vitalparameter (BD <sub>95%</sub> < 85 mmHg und/oder Herzfrequenz < 45/Min)	Volumengabe (NaCl 0.9%, 500-1000 ml i.v. falls nötig Atropin 0.5 mg i.v. (bis max. 2 mg), ggf. Intensivüberwachung
<b>Unverträglichkeitsreaktion</b>	- mögliche allergische Reaktion	→ Proband überwachen, vitale Parameter sicherstellen, der Prüfarzt verordnet die notwendigen therapeutischen Massnahmen (Clemastin 2 mg i.v., falls nötig Volumengabe, Methylprednisolon 250 mg i.v.) → Der Proband wird von der weiteren Studien-Teilnahme ausgeschlossen
<b>Paradoxe Reaktion</b>	- Selbstgefährdung der Testperson	Es liegt im Ermessen des Studienarztes bei ausgeprägtem paradoxem Verhalten des Probanden Flumazenil zu applizieren.

**7. Ethik**

**7.1 Verpflichtungen der Studienverantwortlichen**

Die Studie wird gemäss der Verordnung über klinische Prüfungen des Schweizerischen Heilmittelinstituts, den Richtlinien der Guten Klinischen Praxis (GCP) sowie den Prinzipien der Deklaration von Helsinki des Weltärztebundes durchgeführt, welche auch die Verpflichtungen und Verantwortungen der Studienverantwortlichen beschreiben.

**7.2 Datenschutz**

Bei der ersten Visite (Eintrittsuntersuchung) wird den Probanden eine Probandennummer zugeordnet. Die Liste über die Zuordnung der Probandennummern wird vom Principal Investigator gemäss den Bestimmungen des Datenschutzes verwaltet. Sämtliche für die Auswertung der Studie benötigte Dokumente (Einschlussuntersuchung, CRF, Nachuntersuchung, Probenbeschriftung) werden anonymisiert und über die Probandennummer identifiziert.

**7.3 Aufwandsentschädigung**

Für die Teilnahme an der Studie werden die Probanden mit CHF 1000.- entschädigt. Falls ein Proband die Teilnahme frühzeitig beendet oder beenden muss, wird er seinem Aufwand entsprechend (pro rata temporis) entschädigt. Anfallende Spesen werden den Probanden nicht zusätzlich vergütet.

**7.4 Probandenversicherung**

Das Universitätsspital Basel ersetzt allfällige Schäden, welche die Probanden im Rahmen des klinischen Versuches erleiden sollten. Zu diesem Zweck hat das Universitätsspital Basel bei der Zurich AG Versicherung eine Versicherung abgeschlossen (vgl. Nachweis des Versicherungsschutzes im Anhang 10.7). Der Principal Investigator ist verpflichtet im Falle eines Schadenfalles die notwendigen Schritte einzuleiten, damit die Probanden für die allfällig erlittenen Schäden entschädigt werden.

**6.5 Schwerwiegende Unerwünschte Ereignisse (SAE, Serious Adverse Event)**

Jedes SAE, welches sich während der Studienteilnahme oder während der darauf folgenden 30 Tage ereignet, muss so schnell wie möglich dem Principal Investigator (Tel. 061 / 265.88.60) gemeldet werden. Er klassifiziert das Ereignis als unerwartet, unerwartet lange oder häufig und informiert die EKBB gemäss den gültigen Richtlinien bezüglich SAE-Meldungen and die EKBB (Oktober 2002).

**Tabelle 6-2:** Definition und zu ergreifende Massnahmen beim Auftreten von schwerwiegenden Unerwünschten Ereignissen (Serious Adverse Event, SAE) als SAE gelten:

	<b>zu ergreifende Massnahme:</b>
<ul style="list-style-type: none"> <li>• Tod</li> <li>• Lebensbedrohliche Ereignisse,</li> <li>• Hospitalisation oder Verlängerung bestehender Hospitalisationen,</li> <li>• Ereignisse, die zu einer bleibenden Behinderungen oder einer kongenitalen Anomalie oder einem Geburtsfehler führen,</li> <li>• Ereignisse, welche per se nicht zu Tod etc. führen, aber welche Interventionen nötig machen, damit solche Ereignisse nicht auftreten.</li> </ul>	<p>→ Klassifizierung des SAE durch den Prinzipal Investigator als: unerwartet, unerwartet lange oder häufig bzw. erwarteter SAE</p> <p>→ Meldung an die EKBB gemäss Richtlinien</p>


<sup>7</sup> Definition der ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Genf)

## 9 Unterschriften

Wir haben das vorliegende Studienprotokoll und die Angaben gelesen und bestätigen, dass alle darin zur Durchführung der Studie notwendigen Informationen enthalten sind. Die Unterzeichnenden werden die Studie gemässe Studienprotokoll durchführen. Sie verpflichten sich der Geheimhaltung aller Probandendaten im Sinne des Datenschutzgesetzes.

### Unterschriften der Studienverantwortlichen

Dr. med. Manuel Haschke (Principal Investigator)

.....  
  
 Unterschrift  
 Dr. M. Haschke  
 Klin. Pharmakologie  
 & Toxikologie

Ort, Datum

Prof. Dr. Christian Surber (Co-Investigator)

.....  
  
 Unterschrift  
 Prof. Dr. Christian Surber  
 Institut für Spital-Pharmazie  
 Bereich Medizinische Oeschäftsleistungen

Ort, Datum

Katja Zimmermann (Co-Investigator)

.....  
  
 Unterschrift

Ort, Datum

## 8. Studien Management

### 8.1 Datenerhebung

Beim Ausfüllen der Versuchsprotokolle ist Kugelschreiber/Tinte zu verwenden. Korrekturen müssen mit Namen und Datum versehen werden und der korrigierte Eintrag muss lesefähig bleiben.

### 8.2 Änderung des Studienprotokolls

Erforderliche Änderungen des Studienprotokolls, die sich während des Studienablaufs ergeben, werden als Amendment formeller Bestandteil des Studienprotokolls. Amendments sind vom Studienleiter und allen beteiligten Verantwortlichen zu unterzeichnen. Änderungen, welche die Sicherheit der Probanden in der Studie betreffen, müssen der EKBB vor Inkrafttreten vorgelegt und von dieser genehmigt werden.

### 8.3 Aufbewahrung der Studiendokumentation

Nach Abschluss der Studie werden die Studienunterlagen während 15 Jahren im Archiv der Spital-Pharmazie des Universitätsspitals Basel archiviert.

### 8.4 Kriterien für den Abbruch der klinischen Studie

Der Abbruch der Studie aus medizinischen Gründen (unerwünschte Ereignisse, Unverträglichkeiten der Formulierungen; Verschlechterung der Nutzen-Risiko-Abwägung) kann nur nach gemeinsamer Absprache des Studienleiters und der Co-Investigatoren vorgenommen werden, und muss der Ethikkommission mitgeteilt werden.

Bei mangelnder Rekrutierung oder bei anhaltenden oder wiederkehrenden schweren Verstössen gegen den Prüfplan kann der Studienleiter die Studie vorzeitig beenden.

Tritt im Rahmen dieser Studie ein SAE auf muss eine neue Sicherheitsbeurteilung vorgenommen werden, der Entscheid über die Weiterführung bzw. den Abbruch der Studie erfolgt in Absprache mit der EKBB.

### 10.3.2 Case report form: Visit 1 (screening)

CRF / Visite 1	Probandennummer:  __ _ _   __ _ _   __ _ _   __ _ _	
<b>VISITE 1</b> Datum  __ _ _   __ _ _		
<b>A. Proband</b>		
Geschlecht: männlich <input type="checkbox"/>	weiblich <input type="checkbox"/> (Ausschluss)	
Geburtsdatum:  __ _ _ _   __ _	Alter:  __ _ _  (18-45 Jahre)	
<b>B. Anamnese</b>		
1. Persönliche Anamnese		
Hospitalisationen? _____		
Operationen? _____		
Noxen	Nikotin: Nein <input type="checkbox"/> Ja <input type="checkbox"/>	
	Koffein: Nein <input type="checkbox"/> Ja <input type="checkbox"/>	
	Alkohol: Nein <input type="checkbox"/> Ja <input type="checkbox"/>	
	Drogen: Nein <input type="checkbox"/> Ja <input type="checkbox"/>	
Allergien: _____		
Medikamente: _____		
	CYP3A4-Induktor: Nein <input type="checkbox"/> Ja <input type="checkbox"/> (Ausschluss)	
	CYP3A4-Inhibitor: Nein <input type="checkbox"/> Ja <input type="checkbox"/> (Ausschluss)	
2. Familienanamnese		
_____		
_____		
<b>C. Status</b>		
Gewicht  __ _ _ _   __ _ _   __ _ _   __ _ _  kg	Grösse:  __ _ _   __ _ _   __ _ _  cm	BMI:  __ _ _   __ _ _   __ _ _  kg/m <sup>2</sup> (18 - 30)
Blutdruck:  __ _ _   __ _ _  /  __ _ _   __ _ _  mmHg	Herzfrequenz:  __ _ _  /min	Temp:  __ _ _   __ _ _  °C

CRF- Visite 1	Probandennummer:  __ _ _   __ _ _   __ _ _   __ _ _
<b>Pharmakokinetik von nasal appliziertem Midazolam</b>  <b>CRF / Visite 1</b>  <b>Eintrittsuntersuchung</b>	
Pharmakokinetik von nasal appliziertem Midazolam	
CRF / Visite 1 – Version 01; 6.4.2006	



CRF / Visite 1 Probandennummer: | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ | \_ |

**E. Ein- / Ausschlusskriterien**

Geschlecht	männlich <input type="checkbox"/>		weiblich <input type="checkbox"/> (Ausschluss)	
	Ja <input type="checkbox"/>	<input type="checkbox"/>	Nein <input type="checkbox"/>	(Ausschluss)
Alter zwischen 18 und 45 Jahren?	Ja <input type="checkbox"/>		Nein <input type="checkbox"/> (Ausschluss)	
BMI zwischen 18 und 30 kg/m <sup>2</sup> ?	Ja <input type="checkbox"/>		Nein <input type="checkbox"/> (Ausschluss)	
Liegt eine schriftliche Einwilligung vor?	Ja <input type="checkbox"/>		Nein <input type="checkbox"/> (Ausschluss)	
Bekannte Überempfindlichkeit gegen Midazolam, Chitosan, randomisiert-methyliertes $\beta$ -Cyclodextrin?	Ja <input type="checkbox"/> (Ausschluss)		Nein <input type="checkbox"/>	
Vorhergehende paradoxe Reaktion nach der Verabreichung von Midazolam oder eines anderen Benzodiazepins?	Ja <input type="checkbox"/> (Ausschluss)		Nein <input type="checkbox"/>	
Liegt eine Kontraindikation für Midazolam vor?	Ja <input type="checkbox"/> (Ausschluss)		Nein <input type="checkbox"/>	
Allergie auf Krustentiere?	Ja <input type="checkbox"/> (Ausschluss)		Nein <input type="checkbox"/>	
Einnahme von CYP-3A4 Inhibitoren?	Ja <input type="checkbox"/> (Ausschluss)		Nein <input type="checkbox"/>	
Einnahme von CYP-3A4 Induktoren?	Ja <input type="checkbox"/> (Ausschluss)		Nein <input type="checkbox"/>	
Medikamenten-/Drogen-/Alkoholabusus?	Ja <input type="checkbox"/> (Ausschluss)		Nein <input type="checkbox"/>	
Chronisch behinderte Nasenatmung?	Ja <input type="checkbox"/> (Ausschluss)		Nein <input type="checkbox"/>	
Symptomatische Nasenpolypen oder Nasenpolypen in der Anamnese?	Ja <input type="checkbox"/> (Ausschluss)		Nein <input type="checkbox"/>	
Zurzeit (oder in den vergangenen 30 Tagen) Teilnahme an anderer klinischen Studie?	Ja <input type="checkbox"/> (Ausschluss)		Nein <input type="checkbox"/>	

Aufnahme in die Studie Ja  Nein

Datum: \_\_\_\_\_ Unterschrift Prüferarzt: \_\_\_\_\_





<p style="text-align: center;">CRF- Visite <span style="float: right;">Probandennummer: _____</span></p> <hr/> <p><b>Haben Sie seit der letzten Visite Medikamente (auch selbst gekaufte) eingenommen?</b></p> <p><input type="checkbox"/> Nein  <input type="checkbox"/> Ja, nämlich: .....</p> <p>→ Handelt es sich bei den eingenommenen Wirkstoffen um CYP3A4-Inhibitoren/-Induktoren?</p> <p><input type="checkbox"/> Nein  <input type="checkbox"/> Ja. → Massnahmen: <input type="checkbox"/> Ausschluss des Probanden, da Langzeittherapie  <input type="checkbox"/> Studienfortsetzung zu einem späteren Zeitpunkt  <input type="checkbox"/> die Studienmedikation kann trotzdem verabreicht werden          → Begründung: .....</p> <hr/> <p><b>liegt zur Zeit eine behinderte Nasenatmung vor?</b></p> <p><input type="checkbox"/> Nein  <input type="checkbox"/> Ja (Studienfortsetzung zu einem späteren Zeitpunkt)</p> <hr/> <p><b>Bemerkungen:</b></p> <p>_____</p> <p>_____</p> <hr/> <p><b>Die Studienteilnahme kann heute stattfinden</b></p> <p><input type="checkbox"/> Ja  <input type="checkbox"/> Nein <input type="checkbox"/> Ausschluss des Probanden → Termin für die Nachkontrolle vereinbaren  <input type="checkbox"/> Studienfortsetzung zu einem späteren Zeitpunkt → neuer Termin vereinbaren</p> <p>Datum: _____ Unterschrift Prüfarzt: _____</p> <hr/> <p><b>Vorbereitung</b></p> <ul style="list-style-type: none"> <li>• Legen des Venenkatheters (<b>ACHTUNG: Reaktionstest! Nicht an führender Hand!</b>)  <input type="checkbox"/> rechter Vorderarm <input type="checkbox"/> linker Vorderarm</li> <li>• NaCl 0.9% Infusion anbringen</li> <li>• Kontrolle der bereitgestellten Studienmedikation (Applikations-Reihenfolge)</li> </ul> <hr/> <p style="font-size: small;">Pharmakometrik von nasal appliziertem Midazolam CRF / Visite 2 bis 7 – Version 03.25.07.2006</p> <p style="text-align: right; font-size: x-small;">Seite 3 von 5</p>	<p style="text-align: center;">CRF- Visite <span style="float: right;">Probandennummer: _____</span></p> <hr/> <p><b>iv. Applikation der Studienmedikation (gemäss Plan)</b></p> <p><input type="checkbox"/> Dormicum® 1 mg/ml → <b>Applikation: 1ml</b>  <input type="checkbox"/> Midazolam UD Nasenspray 0.5mg (Formulierung 1) → <b>Applikation von 2 Hüben</b>  <input type="checkbox"/> Cyclodex-Midazolam UD Nasenspray 0.5mg (Formulierung 2) → <b>Applikation von 2 Hüben</b>  <input type="checkbox"/> Cyclodex-Midazolam UD Nasenspray 1mg (Formulierung 3) → <b>Applikation von 1 Hub</b>  <input type="checkbox"/> Cyclodex-Midazolam UD Nasenspray 3mg (Formulierung 4) → <b>Applikation von 1 Hub</b>  <input type="checkbox"/> Chitosan-CD<sub>4</sub>-Midazolam UD Nasenspray 3mg (Formulierung 5) → <b>Applikation von 1 Hub</b></p> <p style="text-align: center; font-size: small;">Bitte hier doppelabziehbare Etikette des Prüfpräparates aufkleben</p> <hr/> <p><b>Nasale Applikation</b>          iv. Applikation von 1mg Dormicum®          in welches Nasenloch erfolgte die Applikation?          → bei der Applikation von 2 Hüben 1x rechts und 1x links applizieren</p> <p><input type="checkbox"/> rechtes Nasenloch <input type="checkbox"/> linkes Nasenloch  <input type="checkbox"/> rechter Arm <input type="checkbox"/> linker Arm</p> <p>→ Die Applikation muss auf der Gegenseite des Venenkatheters erfolgen!</p> <hr/> <p style="font-size: small;">Pharmakometrik von nasal appliziertem Midazolam CRF / Visite 2 bis 7 – Version 03.25.07.2006</p> <p style="text-align: right; font-size: x-small;">Seite 4 von 5</p>
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CRF- Visite   ___										Probandennummer:   ___NI   ___NZ   ___V   ___   ___   ___									
t	Min	Zeit (geplant)	Zeit (effektiv)	Blut-Entnahme	BD	HF	SaO2	Reaktions-test	Bemerkungen										
t <sub>0</sub>									Bei Visite 2: Übungsdurchgang des Reaktionstests → o.k. <input type="checkbox"/> Reaktionstest t <sub>0</sub>										
t <sub>1</sub>	1								Beschreibung der Applikation: <input type="checkbox"/> neutral <input type="checkbox"/> erträglich <input type="checkbox"/> unangenehm <input type="checkbox"/> schmerzhaft Empfindung: <input type="checkbox"/> Brennen <input type="checkbox"/> Jucken <input type="checkbox"/> bitterer Geschmack <input type="checkbox"/> Intensität der Empfindung: <input type="checkbox"/> sehr schwach <input type="checkbox"/> schwach <input type="checkbox"/> mittel <input type="checkbox"/> stark <input type="checkbox"/> sehr stark										
t <sub>2</sub>	2.5																		
t <sub>3</sub>	5								unangenehme Empfindung noch: <input type="checkbox"/> sehr schwach <input type="checkbox"/> schwach <input type="checkbox"/> mittel <input type="checkbox"/> stark <input type="checkbox"/> sehr stark										
t <sub>4</sub>	10																		
t <sub>5</sub>	15								unangenehme Empfindung noch: <input type="checkbox"/> sehr schwach <input type="checkbox"/> schwach <input type="checkbox"/> mittel <input type="checkbox"/> stark <input type="checkbox"/> sehr stark										
t <sub>6</sub>	20																		
t <sub>7</sub>	30								unangenehme Empfindung noch: <input type="checkbox"/> sehr schwach <input type="checkbox"/> schwach <input type="checkbox"/> mittel <input type="checkbox"/> stark <input type="checkbox"/> sehr stark										
t <sub>8</sub>	45																		
t <sub>9</sub>	60																		
t <sub>10</sub>	120																		
t <sub>11</sub>	180																		
t <sub>12</sub>	240								unangenehme Empfindung noch: <input type="checkbox"/> sehr schwach <input type="checkbox"/> schwach <input type="checkbox"/> mittel <input type="checkbox"/> stark <input type="checkbox"/> sehr stark										
t <sub>13</sub>	360																		

### 10.3.4 Case report form: Visit 8 (follow-up)

CRF / Visite 8	Probandnummer:
----------------	----------------

**VISITE 8** Datum | | | | | | | |

Unerwünschte Ereignisse (unerwünschte Arzneimittelwirkungen, UAW) seit dem letzten Studientag?

Nein  (weiter auf Seite 2) Ja  (→ Blatt ausfüllen)

**1. Beschreibung der UAW**

---



---

2. UAW-Typ  Typ A  Typ B

3. UAW-Dauer: UAW-Beginn: UAW-Ende:

4. Schweregrad:  leichte Reaktion  
 mittlere bis schwere Reaktion  
 lebensbedrohliche Reaktion

**5. Therapeutische Massnahmen:**

Datum	Medikament	Dosis	Beginn	Ende	Visum

**6. Zusammenhang mit verabreichter Studienmedikation**

- sicher  
 wahrscheinlich  
 möglich  
 unwahrscheinlich  
 nicht klassierbar

Datum: \_\_\_\_\_ Unterschrift Prüfarzt: \_\_\_\_\_

CRF - Visite 8	Probandnummer:
----------------	----------------

**Pharmakokinetik von nasal appliziertem Midazolam**

CRF / Visite 8

Nachuntersuchung



10.3.5 Intensity of local irritation after nasal administration of 1 mg

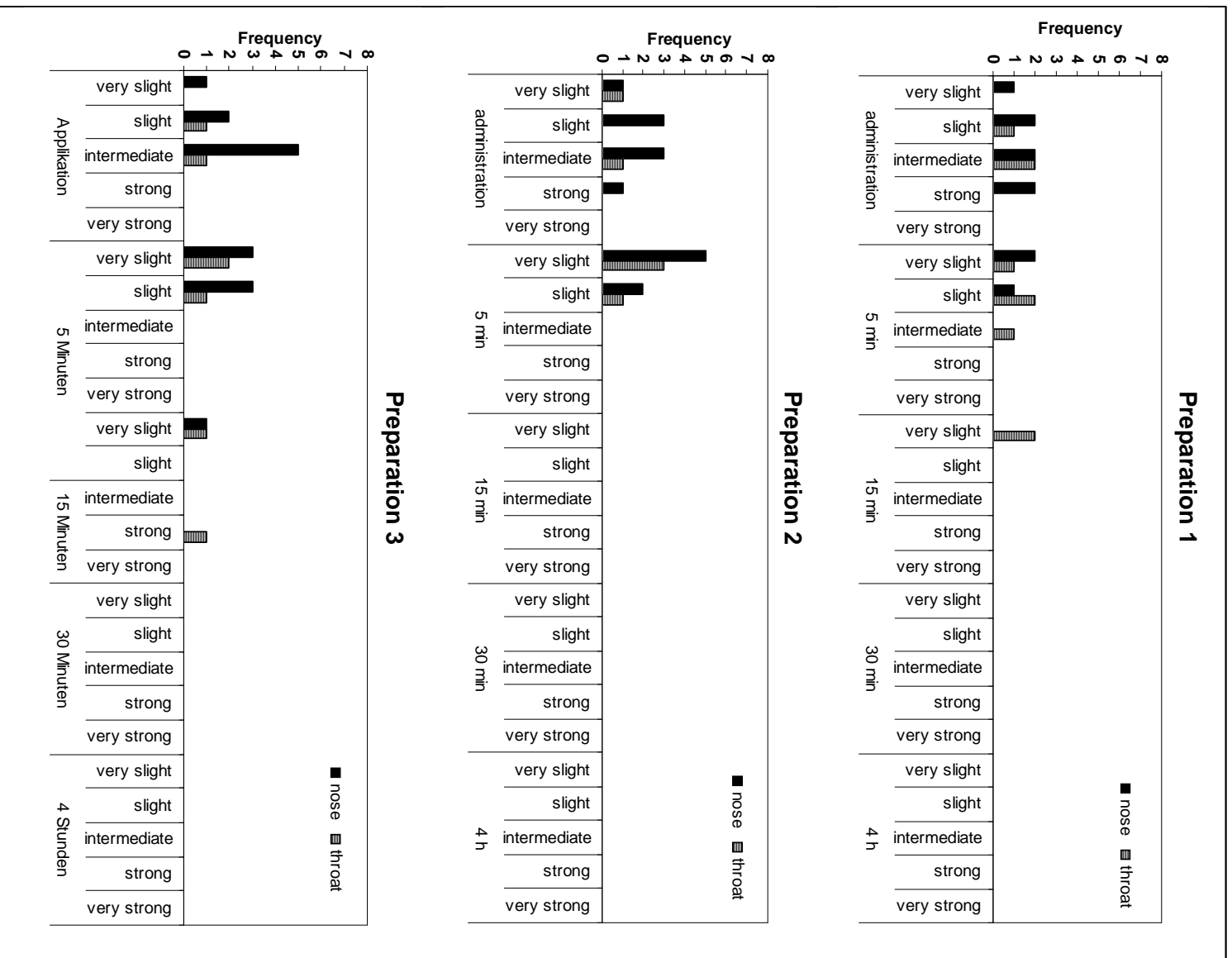


Figure 10-1: Intensity of local irritation (nose and throat area) after nasal administration of Preparation 1, 2 and 3 (1 mg midazolam), immediately after administration, 5 min, 15 min, 30 min, and 4 h after nasal administration

10.3.6 Intensity of local irritation after nasal administration of 3 mg

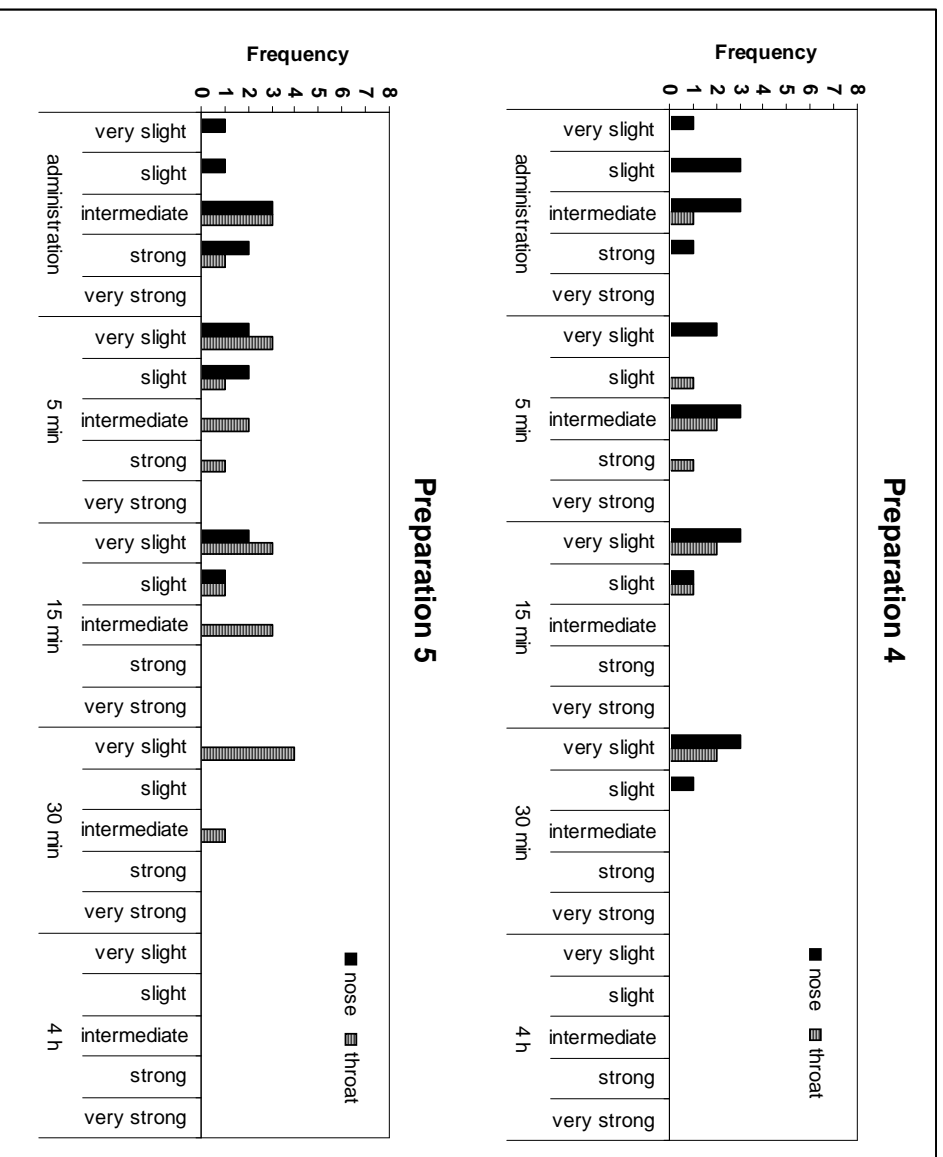


Figure 10-2: Intensity of local irritation (nose and throat area) after nasal administration of Preparation 4 and 5 (3 mg midazolam), immediately after administration, 5 min, 15 min, 30 min, and 4 h after nasal administration

### 10.3.7 Serumconcentration of midazolam and main metabolites (original data)

#### Subject 1: midazolam and metabolites serum concentrations

##### Dormicum i.v. (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
1-V2-t0	nn	nn	nn
1-V2-t1	107.4	nn	nn
1-V2-t2	46.1	nn	nn
1-V2-t3	25.2	<0.2	nn
1-V2-t4	16.5	0.5	nn
1-V2-t5	15.8	0.4	nn
1-V2-t6	15.5	0.5	nn
1-V2-t7	13.2	0.9	<0.2
1-V2-t8	11.0	1.0	<0.2
1-V2-t9	8.0	0.9	nn
1-V2-t10	5.0	0.6	<0.2
1-V2-t11	3.0	0.6	<0.2
1-V2-t12	2.0	0.3	nn
1-V2-t13	0.9	0.2	nn

##### Preparation 1 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
1-V3-t0	nn	nn	nn
1-V3-t1	nn	nn	nn
1-V3-t2	3.6	nn	nn
1-V3-t3	14.8	nn	nn
1-V3-t4	18.8	<0.2	nn
1-V3-t5	13.8	0.3	nn
1-V3-t6	10.7	0.6	nn
1-V3-t7	8.6	0.9	nn
1-V3-t8	7.4	1.2	<0.2
1-V3-t9	6.9	0.8	nn
1-V3-t10	4.0	0.7	<0.2
1-V3-t11	3.0	0.6	nn
1-V3-t12	2.0	0.5	nn
1-V3-t13	0.9	0.2	nn

##### Preparation 2 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
1-V4-t0	nn	nn	nn
1-V4-t1	nn	nn	nn
1-V4-t2	9.2	nn	nn
1-V4-t3	19.8	nn	nn
1-V4-t4	23.0	nn	nn
1-V4-t5	17.7	0.4	nn
1-V4-t6	14.9	0.9	<0.2
1-V4-t7	10.2	1.2	<0.2
1-V4-t8	7.4	0.9	<0.2
1-V4-t9	6.7	1	<0.2
1-V4-t10	3.7	0.8	<0.2
1-V4-t11	2.2	0.5	nn
1-V4-t12	1.6	0.4	nn
1-V4-t13	0.6	0.3	nn

##### Preparation 3 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
1-V5-t0	nn	nn	nn
1-V5-t1	nn	nn	nn
1-V5-t2	6.6	nn	nn
1-V5-t3	15.5	nn	nn
1-V5-t4	18.8	<0.2	nn
1-V5-t5	16.3	0.3	nn
1-V5-t6	12.5	0.6	nn
1-V5-t7	10.1	0.9	<0.2
1-V5-t8	8.6	1.0	nn
1-V5-t9	7.1	1.1	<0.2
1-V5-t10	4.1	0.8	<0.2
1-V5-t11	2.3	0.4	<0.2
1-V5-t12	1.9	0.3	nn
1-V5-t13	0.8	0.2	nn

##### Preparation 4 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
1-V6-t0	nn	nn	nn
1-V6-t1	nn	nn	nn
1-V6-t2	4.3	nn	nn
1-V6-t3	12.8	nn	nn
1-V6-t4	38.9	<0.2	nn
1-V6-t5	39.9	0.6	nn
1-V6-t6	29.8	1.5	<0.2
1-V6-t7	41.7	2.4	0.2
1-V6-t8	27.1	3.5	0.4
1-V6-t9	29.2	3.5	0.4
1-V6-t10	19.4	3.2	0.4
1-V6-t11	10.8	2.3	0.4
1-V6-t12	8.0	1.7	0.3
1-V6-t13	2.5	0.7	<0.2

##### Preparation 5 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
1-V7-t0	nn	nn	nn
1-V7-t1	nn	nn	nn
1-V7-t2	31.7	nn	nn
1-V7-t3	61.0	nn	nn
1-V7-t4	65.7	0.5	nn
1-V7-t5	47.5	1.5	<0.2
1-V7-t6	35.1	2.6	0.3
1-V7-t7	27	3.2	0.4
1-V7-t8	23.8	3.0	0.4
1-V7-t9	18.0	3.0	0.4
1-V7-t10	10.5	2.2	0.3
1-V7-t11	7.3	1.5	0.3
1-V7-t12	5.6	1.3	0.3
1-V7-t13	2.2	0.6	<0.2



### Subject 3: midazolam and metabolites serum concentrations

#### Dormicum i.v. (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
3-V2-t0	nn	nn	nn
3-V2-t1	145.6	nn	nn
3-V2-t2	66.8	<0.2	nn
3-V2-t3	42.8	0.7	nn
3-V2-t4	33.8	3.3	<0.2
3-V2-t5	26	3.2	0.2
3-V2-t6	22.6	3	0.2
3-V2-t7	17	2.8	0.2
3-V2-t8	12.0	2.3	0.2
3-V2-t9	10.2	1.8	<0.2
3-V2-t10	5.8	0.9	<0.2
3-V2-t11	4.6	0.9	<0.2
3-V2-t12	2.7	0.4	nn
3-V2-t13	1.2	0.2	nn

#### Preparation 1 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
3-V3-t0	nn	nn	nn
3-V3-t1	1.1	nn	nn
3-V3-t2	6.4	nn	nn
3-V3-t3	25.7	nn	nn
3-V3-t4	37.5	0.2	nn
3-V3-t5	39.9	1	nn
3-V3-t6	34.8	1.8	<0.2
3-V3-t7	25.3	2.2	0.2
3-V3-t8	16.2	2	<0.2
3-V3-t9	13.5	1.6	<0.2
3-V3-t10	8.2	1.1	<0.2
3-V3-t11	4.9	0.8	<0.2
3-V3-t12	3.4	0.6	<0.2
3-V3-t13	1.9	0.3	nn

#### Preparation 2 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
3-V4-t0	nn	nn	nn
3-V4-t1	nn	nn	nn
3-V4-t2	7.7	nn	nn
3-V4-t3	22.9	nn	nn
3-V4-t4	29.1	0.3	nn
3-V4-t5	33.5	1.1	nn
3-V4-t6	28.6	1.9	<0.2
3-V4-t7	28.1	2.8	<0.2
3-V4-t8	16.2	2.7	<0.2
3-V4-t9	13.2	2.4	0.2
3-V4-t10	7.9	1.7	<0.2
3-V4-t11	4.6	1	<0.2
3-V4-t12	3.6	0.8	<0.2
3-V4-t13	2	0.5	nn

#### Preparation 3 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
3-V5-t0	nn	nn	nn
3-V5-t1	nn	nn	nn
3-V5-t2	2.5	nn	nn
3-V5-t3	11.9	nn	nn
3-V5-t4	24.1	nn	nn
3-V5-t5	24.9	0.3	nn
3-V5-t6	27.1	0.7	nn
3-V5-t7	25.5	1.4	<0.2
3-V5-t8	19.1	1.8	<0.2
3-V5-t9	12.6	1.7	<0.2
3-V5-t10	7.8	1.2	<0.2
3-V5-t11	5.6	0.8	<0.2
3-V5-t12	3.6	0.5	<0.2
3-V5-t13	1.7	0.5	nn

#### Preparation 4 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
3-V6-t0	nn	nn	nn
3-V6-t1	<0.5	nn	nn
3-V6-t2	9.4	nn	nn
3-V6-t3	41	nn	nn
3-V6-t4	88.5	0.6	nn
3-V6-t5	89.8	2.2	0.2
3-V6-t6	80.1	4.2	0.4
3-V6-t7	57.7	6.5	0.6
3-V6-t8	44.6	6.1	0.6
3-V6-t9	35.2	6	0.6
3-V6-t10	21.6	4.4	0.6
3-V6-t11	14.3	2.5	0.4
3-V6-t12	10.8	1.9	0.3
3-V6-t13	5.9	1.3	0.2

#### Preparation 5 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
3-V7-t0	nn	nn	nn
3-V7-t1	nn	nn	nn
3-V7-t2	16.8	nn	nn
3-V7-t3	91.1	nn	nn
3-V7-t4	102.8	0.8	nn
3-V7-t5	71.9	2.5	<0.2
3-V7-t6	66.4	4	0.3
3-V7-t7	49.8	4	0.3
3-V7-t8	36.3	4.5	0.4
3-V7-t9	28.3	4.3	0.4
3-V7-t10	20.2	3	0.3
3-V7-t11	12.2	2.1	0.3
3-V7-t12	8.3	1.4	0.2
3-V7-t13	5.9	0.8	<0.2

## Subject 4: midazolam and metabolites serum concentrations

### Dormicum i.v. (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
4-V2-t0	nn	nn	nn
4-V2-t1	198.8	nn	nn
4-V2-t2	54.3	nn	nn
4-V2-t3	29.2	0.5	nn
4-V2-t4	24.9	1.9	<0.2
4-V2-t5	17.9	2.2	<0.2
4-V2-t6	19	1.9	<0.2
4-V2-t7	14.8	1.7	<0.2
4-V2-t8	11.8	1.5	<0.2
4-V2-t9	9.3	1.3	<0.2
4-V2-t10	4.8	0.8	<0.2
4-V2-t11	3.4	0.5	<0.2
4-V2-t12	2.4	0.4	nn
4-V2-t13	1.3	<0.2	nn

### Preparation 1 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
4-V3-t0	nn	nn	nn
4-V3-t1	nn	nn	nn
4-V3-t2	5.4	nn	nn
4-V3-t3	15.8	nn	nn
4-V3-t4	23.1	<0.2	nn
4-V3-t5	18.7	0.5	nn
4-V3-t6	18.1	0.8	nn
4-V3-t7	12.7	1.2	<0.2
4-V3-t8	9.9	1.3	<0.2
4-V3-t9	6.6	1.5	<0.2
4-V3-t10	4.3	0.8	<0.2
4-V3-t11	3.5	0.6	<0.2
4-V3-t12	2.3	0.4	nn
4-V3-t13	1.5	0.4	nn

### Preparation 2 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
4-V4-t0	nn	nn	nn
4-V4-t1	nn	nn	nn
4-V4-t2	8.6	nn	nn
4-V4-t3	21	nn	nn
4-V4-t4	29.2	<0.2	nn
4-V4-t5	25.6	0.5	nn
4-V4-t6	25.2	0.7	nn
4-V4-t7	20.8	1.1	<0.2
4-V4-t8	15.5	1.2	<0.2
4-V4-t9	11.7	1	<0.2
4-V4-t10	7.4	0.8	<0.2
4-V4-t11	4.7	0.5	<0.2
4-V4-t12	3.2	0.5	<0.2
4-V4-t13	1.8	0.3	nn

### Preparation 3 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
4-V5-t0	nn	nn	nn
4-V5-t1	nn	nn	nn
4-V5-t2	6.4	nn	nn
4-V5-t3	15.8	nn	nn
4-V5-t4	30.7	0.2	nn
4-V5-t5	30.1	0.7	nn
4-V5-t6	24.7	1.3	<0.2
4-V5-t7	20	2.5	<0.2
4-V5-t8	13.4	2.1	0.2
4-V5-t9	10	1.5	0.2
4-V5-t10	5.4	0.8	<0.2
4-V5-t11	3.1	0.6	<0.2
4-V5-t12	2.6	0.4	<0.2
4-V5-t13	1	0.2	<0.2

### Preparation 4 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
4-V6-t0	nn	nn	nn
4-V6-t1	nn	nn	nn
4-V6-t2	10.6	nn	nn
4-V6-t3	43	nn	nn
4-V6-t4	56.2	0.3	nn
4-V6-t5	55.3	1.2	<0.2
4-V6-t6	61.8	2.3	0.3
4-V6-t7	49.5	3.4	0.4
4-V6-t8	35.5	3.5	0.4
4-V6-t9	31.1	3.7	0.5
4-V6-t10	16.1	2.4	0.4
4-V6-t11	11.4	1.8	0.4
4-V6-t12	8.8	1.2	0.3
4-V6-t13	3.8	0.6	<0.2

### Preparation 5 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
4-V7-t0	nn	nn	nn
4-V7-t1	nn	nn	nn
4-V7-t2	1.9	nn	nn
4-V7-t3	72.1	nn	nn
4-V7-t4	66.4	1	nn
4-V7-t5	65.6	4	0.3
4-V7-t6	62.1	6.8	0.5
4-V7-t7	37.2	6.4	0.6
4-V7-t8	23	4.4	0.4
4-V7-t9	22.1	4.6	0.5
4-V7-t10	13.4	3	0.4
4-V7-t11	9	1.9	0.3
4-V7-t12	5.7	1.3	0.3
4-V7-t13	3.1	0.7	<0.2

## Subject 6: midazolam and metabolites serum concentrations

### Dormicum i.v. (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
6-V2-t0	nn	nn	nn
6-V2-t1	15.4	nn	nn
6-V2-t2	61	nn	nn
6-V2-t3	32.5	nn	nn
6-V2-t4	23.4	0.7	nn
6-V2-t5	20.1	1.5	nn
6-V2-t6	17.2	1.5	<0.2
6-V2-t7	15.9	1.5	<0.2
6-V2-t8	12.1	1.5	<0.2
6-V2-t9	10.7	1.4	<0.2
6-V2-t10	6.5	1	<0.2
6-V2-t11	4.0	0.7	<0.2
6-V2-t12	2.7	0.5	nn
6-V2-t13	1.2	0.2	nn

### Preparation 1 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
6-V3-t0	nn	nn	nn
6-V3-t1	nn	nn	nn
6-V3-t2	9.8	nn	nn
6-V3-t3	21.9	nn	nn
6-V3-t4	27.1	0.2	nn
6-V3-t5	20.7	0.7	nn
6-V3-t6	18.2	1.3	<0.2
6-V3-t7	13.5	1.5	<0.2
6-V3-t8	9.5	1.5	<0.2
6-V3-t9	8.3	1.5	<0.2
6-V3-t10	5.3	1.1	<0.2
6-V3-t11	3.0	0.7	nn
6-V3-t12	2.1	0.5	nn
6-V3-t13	0.8	0.2	nn

### Preparation 2 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
6-V4-t0	nn	nn	nn
6-V4-t1	nn	nn	nn
6-V4-t2	8.7	nn	nn
6-V4-t3	28.6	nn	nn
6-V4-t4	37.9	<0.2	nn
6-V4-t5	28.2	0.5	nn
6-V4-t6	22.6	0.9	nn
6-V4-t7	17.7	1.3	<0.2
6-V4-t8	12.6	1.2	<0.2
6-V4-t9	9.8	0.9	<0.2
6-V4-t10	5.5	0.8	<0.2
6-V4-t11	4.6	0.7	<0.2
6-V4-t12	2.7	0.4	nn
6-V4-t13	1.2	0.2	nn

### Preparation 3 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
6-V5-t0	nn	nn	nn
6-V5-t1	2.6	nn	nn
6-V5-t2	10.4	nn	nn
6-V5-t3	16.2	nn	nn
6-V5-t4	17.1	nn	nn
6-V5-t5	26.5	0.2	nn
6-V5-t6	24.9	0.5	nn
6-V5-t7	15.3	0.9	nn
6-V5-t8	13.1	1.0	<0.2
6-V5-t9	10.8	1	<0.2
6-V5-t10	6.9	0.8	<0.2
6-V5-t11	3.9	0.6	<0.2
6-V5-t12	2.6	0.5	nn
6-V5-t13	1.1	0.4	nn

### Preparation 4 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
6-V6-t0	nn	nn	nn
6-V6-t1			
6-V6-t2			
6-V6-t3			
6-V6-t4	54.1	<0.2	nn
6-V6-t5	51.3	1.3	<0.2
6-V6-t6	57.7	2.4	<0.2
6-V6-t7	46.2	3.7	0.3
6-V6-t8	35.7	3.9	0.4
6-V6-t9	28.9	3.3	0.4
6-V6-t10	17.8	3.3	0.4
6-V6-t11	11.3	2.3	0.4
6-V6-t12	7.4	1.5	0.3
6-V6-t13	2.8	0.8	0.1

### Preparation 5 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
6-V7-t0	nn	nn	nn
6-V7-t1	<0.5	nn	nn
6-V7-t2	22.5	nn	nn
6-V7-t3	64.6	nn	nn
6-V7-t4	77.5	0.4	nn
6-V7-t5	58.3	0.9	<0.2
6-V7-t6	48.9	2.1	0.2
6-V7-t7	35.7	3.8	0.3
6-V7-t8	28.9	2.6	0.3
6-V7-t9	23.9	2.3	0.3
6-V7-t10	17	2.6	0.4
6-V7-t11	12.8	2.1	0.3
6-V7-t12	8.8	1.5	0.3
6-V7-t13	3.1	0.6	<0.2

## Subject 9: midazolam and metabolites serum concentrations

### Dormicum i.v. (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
9-V2-t0	nn	nn	nn
9-V2-t1	2.5	nn	nn
9-V2-t2	25.7	nn	nn
9-V2-t3	22.3	nn	nn
9-V2-t4	16.4	0.3	nn
9-V2-t5	15.1	0.6	nn
9-V2-t6	14.3	1	nn
9-V2-t7	12.7	1.2	<0.2
9-V2-t8	9.7	1.1	<0.2
9-V2-t9	8.1	1	<0.2
9-V2-t10	4.3	0.6	<0.2
9-V2-t11	2.9	0.4	nn
9-V2-t12	2.1	0.4	nn
9-V2-t13	1.3	0.2	nn

### Preparation 1 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
9-V3-t0	nn	nn	nn
9-V3-t1	nn	nn	nn
9-V3-t2	8.1	nn	nn
9-V3-t3	19.7	nn	nn
9-V3-t4	16.3	nn	nn
9-V3-t5	18.6	0.2	nn
9-V3-t6	16.7	0.5	nn
9-V3-t7	13	0.8	nn
9-V3-t8	9.7	0.9	nn
9-V3-t9	8.3	0.9	nn
9-V3-t10	5.0	0.7	nn
9-V3-t11	3.4	0.4	nn
9-V3-t12	2.6	0.3	nn
9-V3-t13	1.6	0.2	nn

### Preparation 2 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
9-V4-t0	nn	nn	nn
9-V4-t1	nn	nn	nn
9-V4-t2	3.9	nn	nn
9-V4-t3	16	nn	nn
9-V4-t4	19.5	<0.2	nn
9-V4-t5	16.1	0.3	nn
9-V4-t6	12.1	0.4	nn
9-V4-t7	11.1	0.7	<0.2
9-V4-t8	9.2	0.8	nn
9-V4-t9	6.8	0.6	nn
9-V4-t10	3.9	0.5	nn
9-V4-t11	2.3	0.3	nn
9-V4-t12	2.1	0.3	nn
9-V4-t13	1.2	<0.2	nn

### Preparation 3 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
9-V5-t0	nn	nn	nn
9-V5-t1	nn	nn	nn
9-V5-t2	6	nn	nn
9-V5-t3	18.3	nn	nn
9-V5-t4	26.5	nn	nn
9-V5-t5	23.4	0.5	nn
9-V5-t6	19	0.8	nn
9-V5-t7	12.8	1.1	nn
9-V5-t8	10.2	1.1	nn
9-V5-t9	8	1.1	<0.2
9-V5-t10	4.9	0.7	nn
9-V5-t11	2.8	0.5	nn
9-V5-t12	2.3	0.4	nn
9-V5-t13	1.1	0.3	nn

### Preparation 4 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
9-V6-t0	nn	nn	nn
9-V6-t1	nn	nn	nn
9-V6-t2	9.6	nn	nn
9-V6-t3	39.7	nn	nn
9-V6-t4	66.2	0.3	nn
9-V6-t5	44.4	0.5	nn
9-V6-t6	43.5	1.9	<0.2
9-V6-t7	35.4	2.7	0.2
9-V6-t8	23.7	2.5	0.2
9-V6-t9	20.4	2.4	0.2
9-V6-t10	10	2	<0.2
9-V6-t11	7.5	1.3	<0.2
9-V6-t12	5.4	1	<0.2
9-V6-t13	2.7	0.5	nn

### Preparation 5 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
9-V7-t0	nn	nn	nn
9-V7-t1	1	nn	nn
9-V7-t2	11.9	nn	nn
9-V7-t3	59.9	nn	nn
9-V7-t4	60.2	1.2	nn
9-V7-t5	43.1	2.8	<0.2
9-V7-t6	38.9	3.9	0.2
9-V7-t7	28	3.7	0.3
9-V7-t8	18.8	3.4	0.3
9-V7-t9	18.3	3.4	0.3
9-V7-t10	9.6	2.4	0.2
9-V7-t11	6.5	1.7	<0.2
9-V7-t12	5.2	1.2	<0.2
9-V7-t13	2.8	0.5	nn

## Subject 11: midazolam and metabolites serum concentrations

### Dormicum i.v. (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
11-V2-t0	nn	nn	nn
11-V2-t1	42.9	nn	nn
11-V2-t2	48.7	nn	nn
11-V2-t3	32	nn	nn
11-V2-t4	18.8	0.2	nn
11-V2-t5	17.3	0.7	nn
11-V2-t6	14.3	0.9	nn
11-V2-t7	16.1	1.1	<0.2
11-V2-t8	11.2	1.1	<0.2
11-V2-t9	11.0	1	<0.2
11-V2-t10	6.8	0.8	<0.2
11-V2-t11	4.0	0.6	<0.2
11-V2-t12	2.8	0.5	nn
11-V2-t13	0.9	0.2	nn

### Preparation 1 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
11-V3-t0	nn	nn	nn
11-V3-t1	nn	nn	nn
11-V3-t2	13.2	nn	nn
11-V3-t3	26.5	nn	nn
11-V3-t4	32.9	nn	nn
11-V3-t5	25.7	0.3	nn
11-V3-t6	20.7	0.7	nn
11-V3-t7	15.5	1	<0.2
11-V3-t8	11.9	1.1	<0.2
11-V3-t9	9.9	0.9	<0.2
11-V3-t10	6.5	0.8	<0.2
11-V3-t11	4.3	0.6	<0.2
11-V3-t12	3.0	0.5	<0.2
11-V3-t13	1.2	<0.2	nn

### Preparation 2 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
11-V4-t0	nn	nn	nn
11-V4-t1	0.7	nn	nn
11-V4-t2	17.2	nn	nn
11-V4-t3	30.2	nn	nn
11-V4-t4	25.8	<0.2	nn
11-V4-t5	22.2	0.5	nn
11-V4-t6	19.6	0.9	nn
11-V4-t7	15.8	1.2	nn
11-V4-t8	12.4	1.3	<0.2
11-V4-t9	11.2	1.3	<0.2
11-V4-t10	5.8	0.9	<0.2
11-V4-t11	3.9	0.6	<0.2
11-V4-t12	2.9	0.4	<0.2
11-V4-t13	1.1	0.2	nn

### Preparation 3 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
11-V5-t0	nn	nn	nn
11-V5-t1	nn	nn	nn
11-V5-t2	7.9	nn	nn
11-V5-t3	32.8	nn	nn
11-V5-t4	29.5	<0.2	nn
11-V5-t5	22.6	0.4	nn
11-V5-t6	24.2	0.7	nn
11-V5-t7	18.1	1.1	<0.2
11-V5-t8	14.1	1.3	<0.2
11-V5-t9	12.7	1.2	<0.2
11-V5-t10	7	1	<0.2
11-V5-t11	4.3	0.6	<0.2
11-V5-t12	3.4	0.5	<0.2
11-V5-t13	1.5	0.2	nn

### Preparation 4 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
11-V6-t0	nn	nn	nn
11-V6-t1	nn	nn	nn
11-V6-t2	23.7	nn	nn
11-V6-t3	91.9	nn	nn
11-V6-t4	98.3	<0.2	nn
11-V6-t5	77.2	0.8	nn
11-V6-t6	62.6	2.6	0.2
11-V6-t7	51.6	3	0.3
11-V6-t8	38.5	2.6	0.3
11-V6-t9	33.2	2.7	0.3
11-V6-t10	18.1	2	0.3
11-V6-t11	11.7	1.6	0.3
11-V6-t12	8.1	1.1	<0.2
11-V6-t13	3.5	0.4	<0.2

### Preparation 5 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
11-V7-t0	nn	nn	nn
11-V7-t1	nn	nn	nn
11-V7-t2	44.1	nn	nn
11-V7-t3	96.9	nn	nn
11-V7-t4	98.2	0.3	nn
11-V7-t5	79.2	1.8	<0.2
11-V7-t6	54.1	3.1	0.2
11-V7-t7	42.8	3.9	0.4
11-V7-t8	29.1	3.3	0.3
11-V7-t9	27.3	2.8	0.3
11-V7-t10	17.1	2.5	0.2
11-V7-t11	10.6	1.9	0.2
11-V7-t12	7.6	1.2	0.2
11-V7-t13	3.2	0.5	<0.2

## Subject 13: midazolam and metabolites serum concentrations

### Dormicum i.v. (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
13-V2-t0	nn	nn	nn
13-V2-t1	19.4	nn	nn
13-V2-t2	64	nn	nn
13-V2-t3	34.3	<0.2	nn
13-V2-t4	25.9	1	<0.2
13-V2-t5	22.7	1.4	<0.2
13-V2-t6	21.1	1.4	0.2
13-V2-t7	18.2	1.3	0.2
13-V2-t8	13.3	1.0	0.2
13-V2-t9	13.1	0.9	0.2
13-V2-t10	7.9	0.8	<0.2
13-V2-t11	6.0	0.6	<0.2
13-V2-t12	4.2	0.4	<0.2
13-V2-t13	1.9	<0.2	nn

### Preparation 1 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
13-V3-t0	nn	nn	nn
13-V3-t1	nn	nn	nn
13-V3-t2	10.8	nn	nn
13-V3-t3	41.8	nn	nn
13-V3-t4	34.2	0.2	nn
13-V3-t5	31.8	0.8	nn
13-V3-t6	22.6	1	<0.2
13-V3-t7	21.7	1.4	<0.2
13-V3-t8	13.7	1.2	<0.2
13-V3-t9	12.4	1.2	0.2
13-V3-t10	8.6	0.9	<0.2
13-V3-t11	5.6	0.6	<0.2
13-V3-t12	4.2	0.6	<0.2
13-V3-t13	1.5	0.2	nn

### Preparation 2 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
13-V4-t0	nn	nn	nn
13-V4-t1	nn	nn	nn
13-V4-t2	9.7	nn	nn
13-V4-t3	38.3	nn	nn
13-V4-t4	38.1	<0.2	nn
13-V4-t5	29.8	0.4	nn
13-V4-t6	24.1	0.9	<0.2
13-V4-t7	20	1.2	<0.2
13-V4-t8	15.5	0.8	<0.2
13-V4-t9	13.6	0.9	<0.2
13-V4-t10	8.3	0.8	<0.2
13-V4-t11	5.9	0.6	<0.2
13-V4-t12	4.5	0.4	<0.2
13-V4-t13	1.9	0.2	<0.2

### Preparation 3 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
13-V5-t0	nn	nn	nn
13-V5-t1	nn	nn	nn
13-V5-t2	1.3	nn	nn
13-V5-t3	6.1	nn	nn
13-V5-t4	32.1	nn	nn
13-V5-t5	27.9	0.2	nn
13-V5-t6	25.3	0.5	nn
13-V5-t7	16.5	0.5	nn
13-V5-t8	14.6	0.7	<0.2
13-V5-t9	12.4	0.7	<0.2
13-V5-t10	9	0.7	<0.2
13-V5-t11	5.8	0.5	<0.2
13-V5-t12	4.4	0.5	nn
13-V5-t13	1.9	0.2	nn

### Preparation 4 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
13-V6-t0	nn	nn	nn
13-V6-t1	nn	nn	nn
13-V6-t2	0.5	nn	nn
13-V6-t3	14.3	nn	nn
13-V6-t4	67.7	nn	nn
13-V6-t5	66.3	0.4	nn
13-V6-t6	63.5	1.1	<0.2
13-V6-t7	52.1	2.7	0.3
13-V6-t8	41.6	2.6	0.3
13-V6-t9	41.9	3.7	0.4
13-V6-t10	31.2	3.3	0.6
13-V6-t11	19.7	2.3	0.5
13-V6-t12	13.6	1.6	0.4
13-V6-t13	5.8	0.7	0.2

### Preparation 5 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
13-V7-t0	nn	nn	nn
13-V7-t1	nn	nn	nn
13-V7-t2	6.1	nn	nn
13-V7-t3	63.1	nn	nn
13-V7-t4	68.6	0.3	nn
13-V7-t5	56.2	1.4	<0.2
13-V7-t6	52.3	2.7	0.3
13-V7-t7	46.7	3.1	0.4
13-V7-t8	38.1	2.9	0.5
13-V7-t9	36.2	3.1	0.5
13-V7-t10	21.2	2.1	0.5
13-V7-t11	17	2	0.4
13-V7-t12	12	1.4	0.4
13-V7-t13	5.1	0.6	<0.2

## Subject 14: midazolam and metabolites serum concentrations

### Dormicum i.v. (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
14-V2-t0	nn	nn	nn
14-V2-t1	49.9	nn	nn
14-V2-t2	47.9	nn	nn
14-V2-t3	26.1	0.3	nn
14-V2-t4	25.6	1.3	<0.2
14-V2-t5	21.7	1.7	<0.2
14-V2-t6	18.3	1.8	<0.2
14-V2-t7	16.1	1.8	<0.2
14-V2-t8	12.8	1.8	<0.2
14-V2-t9	11.1	1.6	<0.2
14-V2-t10	6.8	1.2	<0.2
14-V2-t11	4.8	1.1	<0.2
14-V2-t12	3.9	0.8	<0.2
14-V2-t13	2.3	0.3	nn

### Preparation 1 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
14-V3-t0	nn	nn	nn
14-V3-t1	nn	nn	nn
14-V3-t2	2.7	nn	nn
14-V3-t3	12.4	nn	nn
14-V3-t4	16.4	nn	nn
14-V3-t5	20	0.4	nn
14-V3-t6	21.7	1.2	<0.2
14-V3-t7	16.7	1.4	<0.2
14-V3-t8	12.6	1.4	<0.2
14-V3-t9	11.3	1.4	<0.2
14-V3-t10	7.7	1.4	<0.2
14-V3-t11	4.9	0.9	<0.2
14-V3-t12	3.8	0.8	<0.2
14-V3-t13	2.5	0.4	nn

### Preparation 2 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
14-V4-t0	nn	nn	nn
14-V4-t1	nn	nn	nn
14-V4-t2	10.5	nn	nn
14-V4-t3	26.5	nn	nn
14-V4-t4	28.8	0.3	nn
14-V4-t5	26.4	1	nn
14-V4-t6	21.8	1.3	<0.2
14-V4-t7	16.9	1.4	nn
14-V4-t8	12.8	1.4	<0.2
14-V4-t9	10.2	1.4	<0.2
14-V4-t10	6.8	1.1	<0.2
14-V4-t11	5.4	1	<0.2
14-V4-t12	4	0.8	<0.2
14-V4-t13	2.3	0.5	nn

### Preparation 3 (1mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
14-V5-t0	nn	nn	nn
14-V5-t1	nn	nn	nn
14-V5-t2	2.6	nn	nn
14-V5-t3	19.6	nn	nn
14-V5-t4	36.7	<0.2	nn
14-V5-t5	30.7	1	nn
14-V5-t6	23.3	1.6	<0.2
14-V5-t7	16.8	2.2	<0.2
14-V5-t8	11.4	1.9	<0.2
14-V5-t9	9.4	1.8	<0.2
14-V5-t10	5.6	1.1	<0.2
14-V5-t11	4.6	0.9	<0.2
14-V5-t12	4	0.9	<0.2
14-V5-t13	2	0.4	nn

### Preparation 4 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
14-V6-t0	nn	nn	nn
14-V6-t1	nn	nn	nn
14-V6-t2	3.1	nn	nn
14-V6-t3	26.1	nn	nn
14-V6-t4	67.9	<0.2	nn
14-V6-t5	69.3	1	<0.2
14-V6-t6	57.7	2.2	0.2
14-V6-t7	46.5	3.7	0.4
14-V6-t8	23.7	2.2	0.3
14-V6-t9	25.7	2.9	0.3
14-V6-t10	20.6	3.2	0.5
14-V6-t11	14.6	2.5	0.4
14-V6-t12	11.7	2.2	0.4
14-V6-t13	6.8	1.1	0.3

### Preparation 5 (3mg midazolam)

Identification	midazolam [ug/L]	$\alpha$ -hy-midazolam [ug/L]	4-hy-midazolam [ug/L]
14-V7-t0	nn	nn	nn
14-V7-t1	nn	nn	nn
14-V7-t2	9.3	nn	nn
14-V7-t3	83.0	nn	nn
14-V7-t4	71.9	1	nn
14-V7-t5	53	2.8	0.3
14-V7-t6	42.9	3.9	0.3
14-V7-t7	34.3	4.6	0.4
14-V7-t8	27.7	4.5	0.4
14-V7-t9	24.6	5.7	0.5
14-V7-t10	15.9	3.5	0.4
14-V7-t11	12.9	2.8	0.3
14-V7-t12	9.9	2.2	0.3
14-V7-t13	4.7	0.8	0.2

## 10.3.8 Midazolam serum concentration time profiles (all subjects)

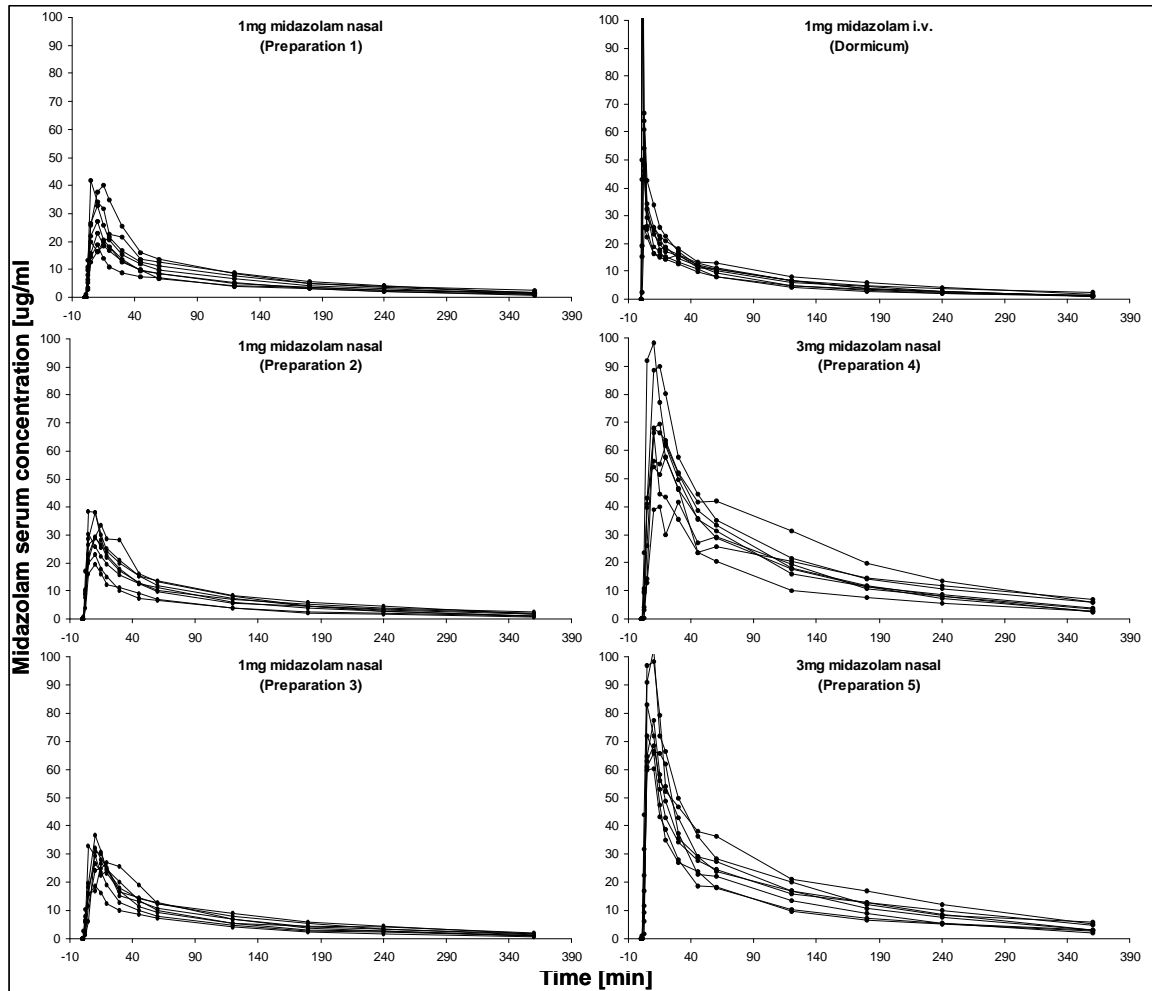


Figure 10-3: Overlaid midazolam serum concentration time profile after i.v. and nasal administration of 1 mg midazolam (Dormicum® and Preparation 1, 2, and 3), nasal administration of 3 mg (Preparation 4 and 5) to 8 healthy volunteers.



10.3.9 Nasal delivery of 1 mg midazolam (Preparation 1, 2, and 3)

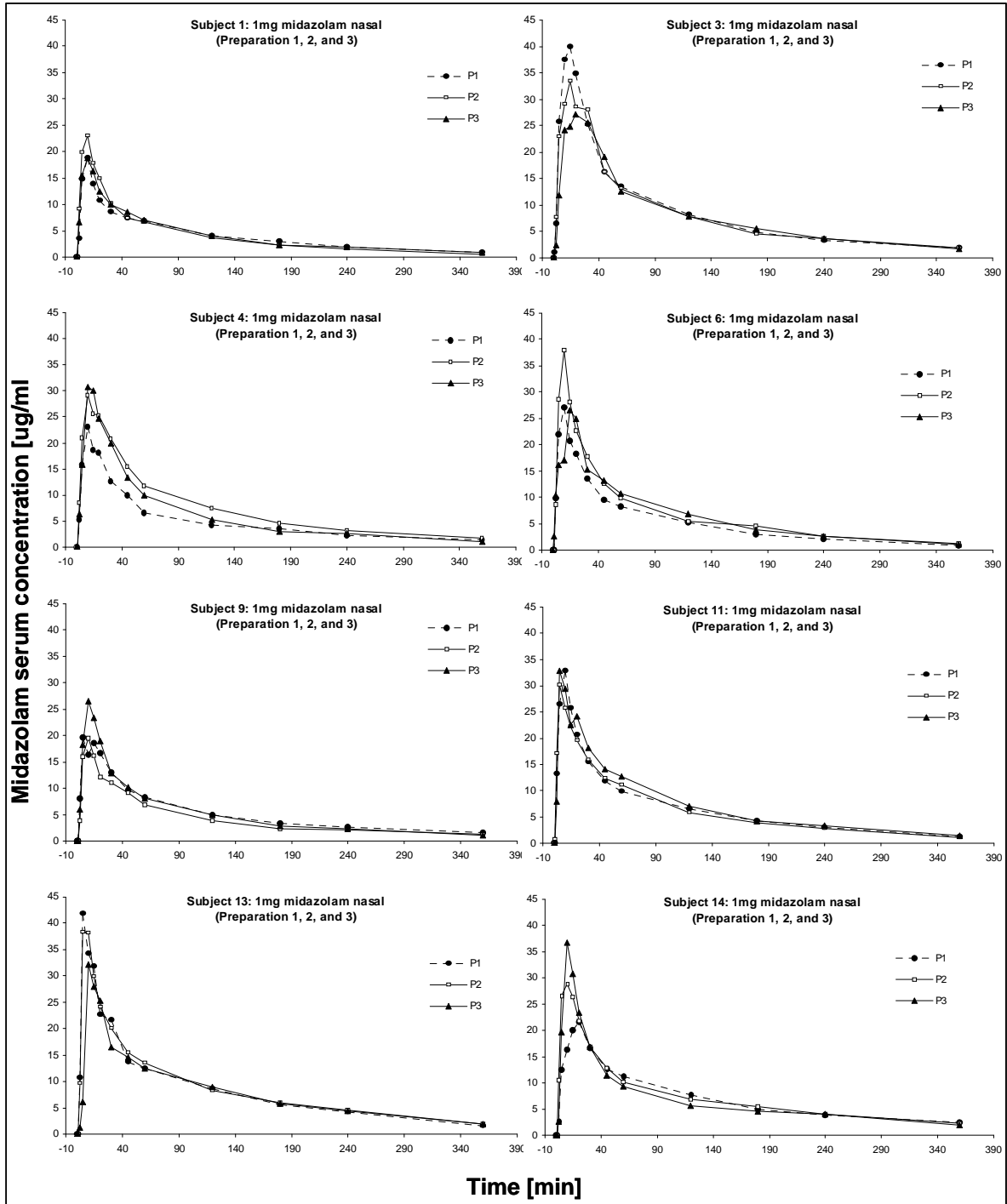
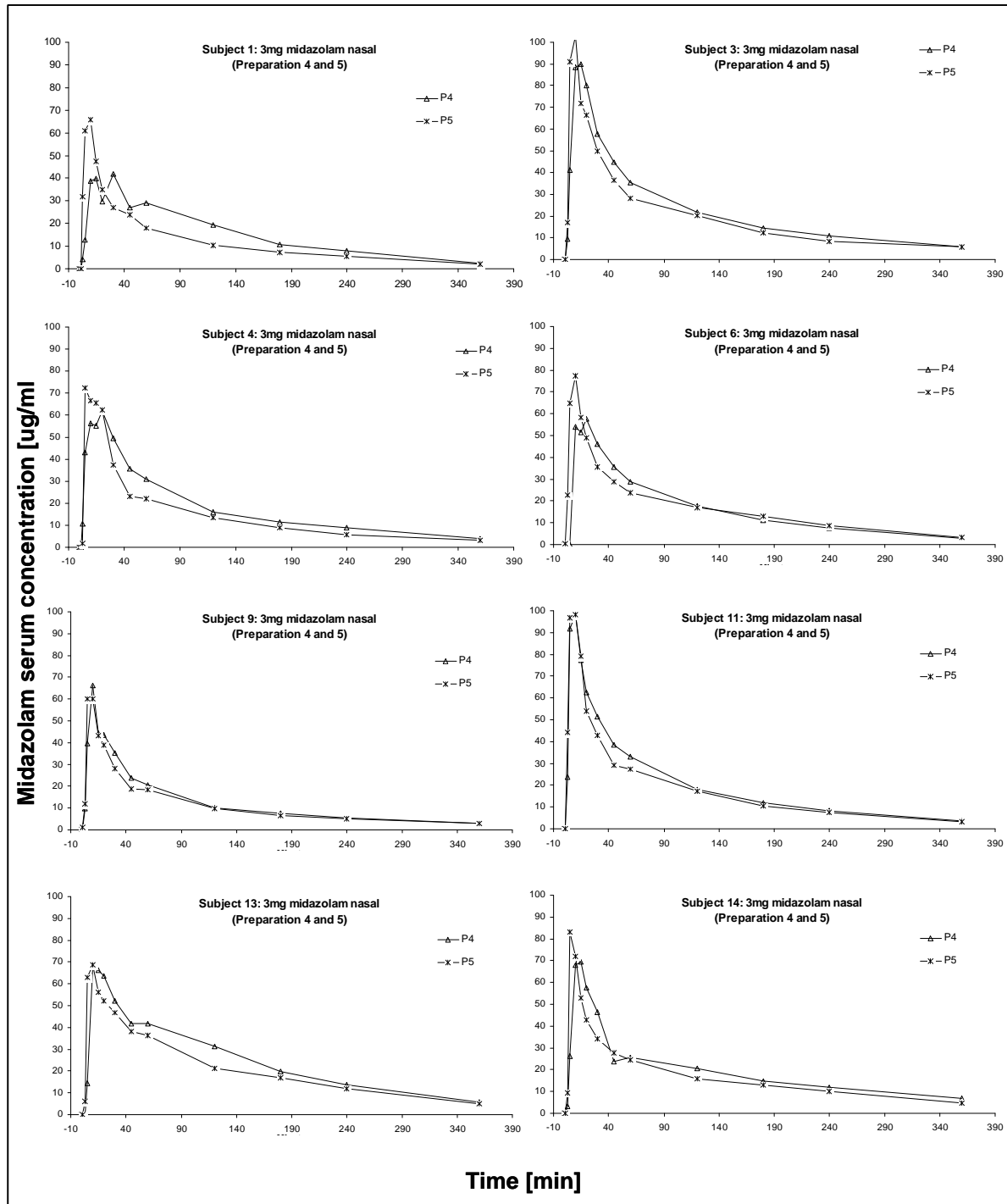


Figure 10-4: Nasal administration of 1 mg (Preparation 1, 2, and 3) to 8 healthy volunteers.

**10.3.10 Nasal delivery of 3 mg midazolam (Preparation 4 and 5)**



**Figure 10-5: Nasal administration of 3 mg (Preparation 4 and 5) to 8 healthy volunteers.**

## 10.4. Project III

### 10.4.1 Case report form (radiologist)

**6 Angaben zur MR-Untersuchung**

6.1 Untersuchte Körperregion/Untersuchungsprotokoll (Mehrfachnennungen möglich):

HWS  
 BWS  
 LWS  
 Schädeld  
 Hals  
 Thorax  
 Extremitäten/Gelenke, wo genau: \_\_\_\_\_  
 MR-Angiographie, wo genau: \_\_\_\_\_  
 andere: \_\_\_\_\_

Abdomen (Oberbauch: Leber, Niere, Nebennieren, Pankreas, etc.)  
 Abdomen (Unterbauch: gynäkologische Organe, Prostata, etc.)  
 Ossäres Becken/Sacrum/ISG  
 IV-Kontrastmittelgabe

6.2 **MR-Hauptdiagnose:** \_\_\_\_\_

6.3 **Relevante MRI-Nebendiagnosen:** \_\_\_\_\_

**7 Beurteilung der Bildqualität**

7.1 **Diagnostische Bildqualität für alle Sequenzen:**

optimal  leicht eingeschränkt  intermediär  deutlich vermindert  nicht diagnostisch

7.2 **Anzahl aller akquirierten Sequenzen:** \_\_\_\_\_

7.3 **Anzahl nicht diagnostischer Sequenzen:** \_\_\_\_\_

7.4 **Ursachen für eingeschränkte Bildqualität:**

Bewegungsartefakte  Spulenartefakte  
 andere: \_\_\_\_\_

**8 Anmerkungen**

Zuständiger Radiologe/In: \_\_\_\_\_

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**Multizenterstudie: Wirksamkeit von nasalen low-dose Midazolam in der Prävention von Klaustrophobie bei der MRT-Untersuchung – Vergleich zweier Formulierungen und Applikationssysteme**

**Ein-/Ausschluss von Patienten – Beurteilung der Untersuchung**  
vom untersuchenden Arzt auszufüllen

1 **Studienzentrum**  
 Universitätsklinik Zürich  Kantonsspital Winterthur  Imamed Radiologie Nordwest

2 **Angaben zum Patienten:** \_\_\_\_\_  
 Initialen: \_\_\_\_\_ Datum der Untersuchung: \_\_\_\_\_

3 **Einschlusskriterien:**  
 Zur Aufnahme in diese Studie müssen **alle Fragen mit Ja** beantwortet werden.  
 1. Patient/in gibt vor der MR-Untersuchung Angst bzw. Klaustrophobie an und wünscht nach ärztlichem Aufklärungsgespräch nach wie vor eine Medikation (Anxiolyse/Sedation)? **Ja** **Nein**    
 2. Ist der/die Patient/in mindestens 18 Jahre alt?    
 \*3. Der/die Patient/in wurde vom Prüfarzt aufgeklärt und hatte die Gelegenheit, Unklarheiten zu klären?    
 \*4. Die schriftliche Einwilligung wurde vom Patienten/von der Patientin unterzeichnet?

4 **Ausschlusskriterien:**  
 Zur Aufnahme in diese Studie müssen **alle Fragen mit Nein** beantwortet werden.  
 1. Liegen allgemeine MR-Kontraindikationen (Herzschrittmacher, Metallsplinter, Implantate/Prothesen, etc.) vor? **Ja** **Nein**    
 2. Ist die Probandin schwanger oder stillt sie? (Bei männlichen Probanden „Nein“ ankreuzen) Schwangerschaftstest durchgeführt:  Nein  Ja, Visum: \_\_\_\_\_  
 3. Bestehen allgemeine Kontraindikationen für Midazolam (Myasthenia gravis, paradoxe Reaktion, bekannte Überempfindlichkeitsreaktion auf Midazolam)?    
 \*4. Verordnet der Zuweiser eine andere Anxiolyse-/Sedationsmethode?  
 \*5. Wird eine MR-Untersuchung des Herzens mit Stressinduktion durchgeführt?  
 6. Liegt eine HNO-Erkrankung vor, welche die nasale Medikation ausschliesst (Rhinitis, Operation)?    
 \*7. Muss aufgrund mangelnder Sprachkenntnisse angenommen werden, dass der/die Patient/in Verständnisprobleme bei der Aufklärung hatte?    
 \*8. Nimmt der/die Patient/in gleichzeitig an einer anderen Studie teil?  
 \*9. Hat der/die Patient/in in den letzten 2 Tagen Beruhigungs- oder Schlafmittel eingenommen?  Nein  Ja, welche: \_\_\_\_\_ Dosierung: \_\_\_\_\_  
 Ist der/die Patient/in auf Grund dieser Medikation aus der Teilnahme an der Studie auszuschliessen?

5 **Beurteilung:**  
 Der/die Patient/in kann an der Multizenterstudie teilnehmen

Dem/der Patient/in wird folgende Nummer zugeordnet: PatientenNr.:

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### 10.4.2 Case report form (radiologic technician)

Multizenterstudie: Wirksamkeit von nasalem low-dose Midazolam in der Prävention von Klaustrophobie bei der MRT-Untersuchung – Vergleich zweier Formulierungen und Applikationssysteme

#### Versuchsprotokoll Fragebogen für MTRA und Patient

Die **grau** hinterlegten Fragen sind vom Patienten, die übrigen von der MTRA auszufüllen.

- Studienzentrum:**
 Universitätsklinik Zürich     Kantonsspital Winterthur     Imamed Radiologie Nordwest
- Angaben zum Patienten:**  
 Initialen: \_\_\_\_\_ Patient/Nr.: \_\_\_\_\_  
 Körpergewicht: \_\_\_\_\_ kg    Geburtsdatum: \_\_\_\_\_  
 männlich     ambulant     zu Fuss     mit Begleitperson während der Untersuchung  
 weiblich     stationär     im Rollstuhl     im Bett
- Untersuchung:**  
 Datum der Untersuchung: \_\_\_\_\_ Beginn: \_\_\_\_\_ Ende: \_\_\_\_\_  
 Eingesetzte Spule: \_\_\_\_\_  
 Rückenlage im MR     Bauchlage im MR     Kopf voraus     Füsse voraus
- Beurteilung der Angst vor der Medikation**  
 → Bitte kurz vor der Untersuchung die VAS-Angstskala vom Patienten ausfüllen lassen!  
 Geben Sie bitte mit Hilfe dieser Skala an, wie stark ihre Angst ist:  
 keine Angst | \_\_\_\_\_ | extreme Angst
- Applikation der Studien-Medikation**  
 5.1 Grund für die Anxiolyse/Sedation:  
 Platzangst     allgemeine Angst     Unruhe  
 sonstiges: \_\_\_\_\_

#### 5.2 Studien-Medikation

→ Bitte das Prüfpräparat mit der entsprechenden Patientennummer anwenden<sup>1</sup>

**Multidose-Nasenspray:**  
 Vor der Anwendung ist die Zerstäuberpumpe 5-mal zu betätigen (der Spürrübel soll nicht eingeatmet werden). Der Nasenspray ist bei der Anwendung aufrecht zu halten.  
**Dosierung: 1 Hub pro Nasenloch**  
**(die Applikation von 2 Hüben entspricht 1ml Midazolam-Base)**

**Unitidose Nasenspray:**  
 Midazolam UD Nasenspray 1mg  
 Der Unitidose-Nasenspray ist gebrauchsfertig und lageunabhängig einsetzbar.  
**Dosierung: 1 Hub**  
**(die Applikation von 1 Hub entspricht 1ml Midazolam-Base)**

Die Applikation des Unitidose Nasenspray erfolgte in das  
 rechte     linke Nasenloch des Patienten

Bitte die doppelabziehbare  
Etikette des Prüfpräparates hier aufkleben

#### 5.3 Nebenwirkungen

Der Patient meldet **keine** Nebenwirkungen → weiter bei Punkt 6

Nebenwirkungsmeldung  
 Uhrzeit: \_\_\_\_\_  
 Art der Nebenwirkung (z.B. brennen, jucken,...): \_\_\_\_\_  
 Intensität:  leicht     mittel     stark  
 Medikamentöse Behandlung:  nein     ja, wie: \_\_\_\_\_  
 Erholung des Patienten:  
 rasch (Minuten)     nach > 1h  
 Patient wurde stationär

<sup>1</sup> Die Prüfpräparate wurden gemäss einer Randomisierungsliste den Patienten zugeordnet. Die Prüfpräparate sind mit den fortlaufenden Patientennummern bezeichnet.  
 Versuchsprotokoll – revidierte Version: 05.07.2006

6. Nachdosierung → gleiche Medikation wie bei der Erstapplikation!

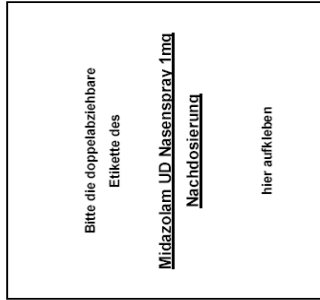
- es ist keine Nachdosierung erforderlich → weiter bei Punkt 7
- es wird eine Nachdosierung vorgenommen Uhrzeit: \_\_\_\_\_
- Multidose-Nasenspray: Zur Nachdosierung muss kein neues Nasenspray verwendet werden! (keine Etikette aufkleben)
- Dosierung: 1 Hub pro Nasenloch
- (die Applikation von 2 Hüben entspricht 1mg Midazolam-Base)

Anzahl applizierte Hübe: \_\_\_\_\_

- Unitdose Nasenspray: Für die Applikation einer Nachdosierung ist ein mit "Nachdosierung" gekennzeichnetes Midazolam UD Nasenspray 1mg zu verwenden.

Dosierung: 1 Hub  
(die Applikation von 1 Hub entspricht 1mg Midazolam-Base)

Die Nachdosierung mittels Unitdose erfolgte in das  
 rechte  linke Nasenloch des Patienten



6.1 Vom Patienten spontan gemeldete Nebenwirkungen nach der Nachdosierung

- Der Patient meldet keine Nebenwirkungen → weiter bei Punkt 7
- Nebenwirkungsmeldung Uhrzeit: \_\_\_\_\_
- Art der Nebenwirkung (z.B. bremsen, jucken,...): \_\_\_\_\_
- Intensität:  leicht  mittel  stark
- Medikamentöse Behandlung:  nein  ja, wie: \_\_\_\_\_
- Erhölung des Patienten:  rasch (Minuten)  nach > 1h
- Patient wurde stationär

7. Patientenbefragung nach der Untersuchung

7.1 VAS-Angstskala

→ Bitte kurz nach der Untersuchung vom Patienten ausfüllen lassen!  
 Geben Sie bitte mit Hilfe dieser Skala an, wie erträglich die Untersuchung für Sie bezüglich Ihrer Angst war.

problemlos | \_\_\_\_\_ | unerträglich

7.2 Gab es für Sie während der Untersuchung Probleme?

- Nein  Ja, welche:  Brennen in der Nase
- Schmerzen
- wiederkehrende Angst
- Klopfgeräusche
- Andere: \_\_\_\_\_

7.3 Würden Sie die Untersuchung wiederholen?

- Nein  Ja

Anmerkungen: \_\_\_\_\_

8. Beurteilung der Untersuchung durch zuständige MTRA

8.1 Durchführbarkeit

- MR konnte normal durchgeführt werden
- Abbruch während MR
- MR konnte gar nicht durchgeführt werden
- Gründe für Abbruch oder nicht durchgeführt:  wegen zu starker Angst
- wegen zu starken Nebenwirkungen
- Rücknahme der Einverständniserklärung
- andere: \_\_\_\_\_

8.2 Patienteneindruck/-kooperation während der Untersuchung

- ruhig, kooperativ  ein wenig ängstlich
- unruhig, kooperiert schlecht  sehr ängstlich
- Patient ist eingeschlafen, keine Kooperation  sonstiges: \_\_\_\_\_

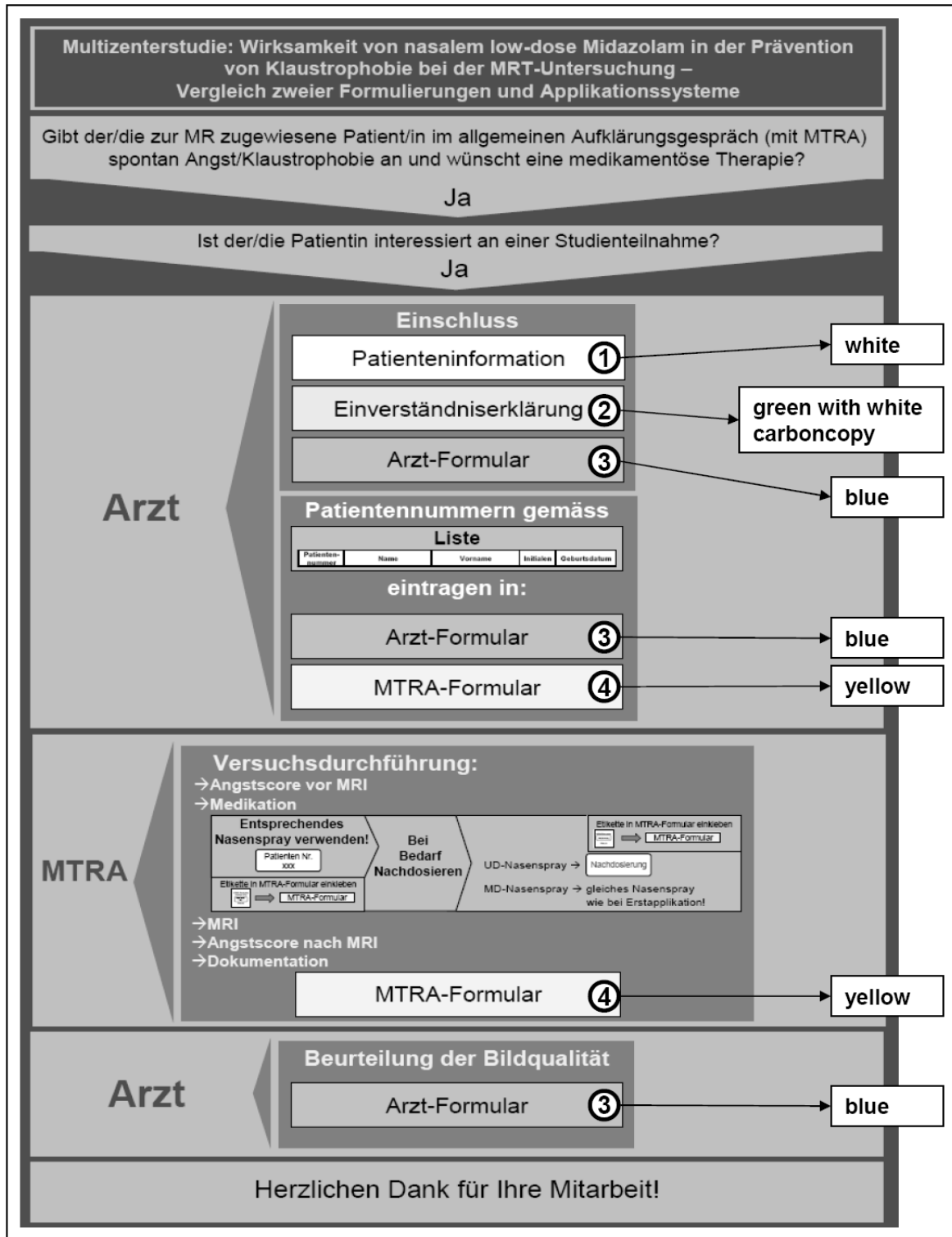
8.3 Wann traten Angstsymptomen/Kooperationsproblemen im Verlauf der Untersuchung auf

- am Anfang der Untersuchung  am Ende der Untersuchung
- mitten in der Untersuchung  keine Angstsymptome/Kooperationsprobleme

9. Abweichungen von der Methodik/Anmerkungen

\_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 Zuständiger/r MTRA: \_\_\_\_\_

10.4.3 Flowchart: multicenter trial



10.4.4 Instruction material

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## Rekrutierung von Patienten

**Rekrutierung** ↑

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## Multizenterstudie mit nasalem low-dose Midazololam

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## Patientenvorbereitung

- Vorauswahl der Patienten
- Formular nach Hause schicken
- In der Wartezone auflegen

**Ziel:**  
Die Patienten sollen sensibilisiert werden, Studie wird jedoch noch nicht erwähnt!

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### Einschluss von Patienten → Arzt

**Einschluss**

**Arzt**

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low-dose Midazolam

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### Einschluss für den Einschluss eines Patienten

**Arzt**

**Einschluss**

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low-dose Midazolam

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### Patienteninformation (2 Seiten, weiss)

**Patienteninformation zur Studie**  
**Vorbereitung der Fragebogen**

Hier werden Sie gebeten, ein paar Minuten Zeit zu investieren, um die Fragebogen auszufüllen. Die Angaben sind für die Studie wichtig und werden streng vertraulich gehalten. Ihre Angaben werden nicht an Dritte weitergegeben.

**Bitte durchlesen und allfällige Fragen stellen**

**→ Den Patienten mitgeben**

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low-dose Midazolam

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### Patienteninformation (2 Seiten, weiss)

**Patienteninformation zur Studie**  
**Vorbereitung der Fragebogen**

Hier werden Sie gebeten, ein paar Minuten Zeit zu investieren, um die Fragebogen auszufüllen. Die Angaben sind für die Studie wichtig und werden streng vertraulich gehalten. Ihre Angaben werden nicht an Dritte weitergegeben.

**Bitte durchlesen und allfällige Fragen stellen**

**→ Den Patienten mitgeben**

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low-dose Midazolam

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### Einverständniserklärung (grünes Formular mit Durchschlag)

**Einverständniserklärung** (grünes Formular mit Durchschlag)

**→ Unterzeichnen lassen**  
**→ Durchschlag dem Patienten mitgeben**

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low-dose Mizolamol

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### Einverständniserklärung (grünes Formular mit Durchschlag)

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low-dose Mizolamol

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### Arztfragebogen (2 Seiten, hellblau)

**→ Anhand Patientenvorbereitung ausfüllen**  
**→ bei erfolgreichem Einschluss Patientenummer vergeben**

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low-dose Mizolamol

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### Arztfragebogen (2 Seiten, hellblau)

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low-dose Mizolamol

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### Einschluss von Patienten - Patientennummern

**Arzt**

Patientennummern gemäss

eintragen in:

Arzt-Formular

MTRA-Formular

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low-dose Minoxidil

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### Vergabe der Patientennummer

Studie: Wirksamkeit von nasalem low-dose Minoxidil in der Prävention von Klausurprobleme... EK Nr. 009

Patientennummer	Name	Vorname	Initialen	Geburtsdatum
101	Meyer	Hans	MH	27.5.1956

Patientennummer übertragen in:

Arzt-Formular

MTRA-Formular

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low-dose Minoxidil

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### Versuchsdurchführung → MTRA

**Versuch**

Arzt

Einschluss

Patientennummer

Einschlussklärung

Arzt-Formular

MTRA-Formular

Versuchsdurchführung

Arzt

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low-dose Minoxidil

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### Versuchsdurchführung (MTRA)

**MTRA**

Versuchsdurchführung:

- Angstscore vor MRI
- Medikation

Einsprechendes Nasenspray verwenden!

Bei Entzündung/Nachschleim

→MRI

→Angstscore nach MRI

→Dokumentation

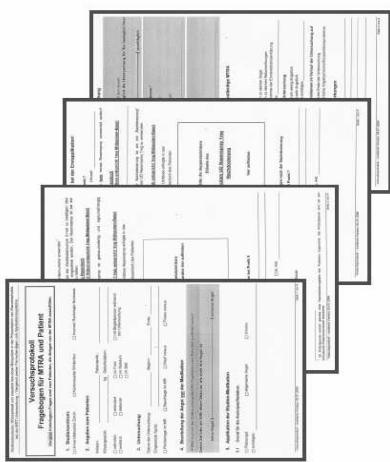
MTRA-Formular

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low-dose Minoxidil

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### MTRA-Fragebogen (Seite 1 bis 4)



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low-dose Midazolam

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### 1. Zentrum, 2. Patient, 3. Untersuchung (MTRA-Fragebogen)

**Versuchsprotokoll  
Fragebogen für MTRA und Patient**

Die grau hinterlegten Fragen sind vom Patienten, die übrigen von der MTRA auszufüllen.

**1. Studienzentrum:**  
 Universitätsklinik Zürich     Kantonsspital Winterthur     Imamed Radiologie Nordwest

**2. Angaben zum Patienten:**  
 Initialen: SMH    PatientenNr.: 101  
 Körpergewicht: 76 kg    Geburtsdatum: 27.5.1956  
 männlich     ambulant     zu Fuss     mit Begleitperson während der Untersuchung  
 weiblich     stationär     im Rollstuhl

**3. Untersuchung:**  
 Datum der Untersuchung: 8.5.2006    Beginn: 9:15    Ende: \_\_\_\_\_  
 Eingesetzte Spule: XYZ  
 Rückenlage im MR     Bauchlage im MR     Kopf voraus     Fusse voraus

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low-dose Midazolam

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### 4. Angstscore vor MRI (MTRA-Fragebogen)

**4. Beurteilung der Angst vor der Medikation**

→ Bitte kurz vor der Untersuchung die VAS-Angstskala vom Patienten ausfüllen lassen!  
 Geben Sie bitte mit Hilfe dieser Skala an, wie stark ihre Angst ist:

keine Angst |-----| extreme Angst

→ graue Felder vom Patienten ausfüllen lassen!

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low-dose Midazolam

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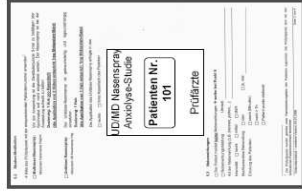


### 5.1 Grund für Anxiolyse/Sedation (MTRA-Fragebogen)

**5. Applikation der Studien-Medikation**

5.1 Grund für die Anxiolyse/Sedation:  
 Platzangst     allgemeine Angst     Unruhe  
 sonstiges: \_\_\_\_\_

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low-dose Midazolam

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<p><b>5.2 Studien-Medikation (MTRA-Fragebogen)</b></p> <p><b>5.2 Studien-Medikation</b></p> <p>→ Bitte das Prüfpräparat mit der entsprechenden Patientenummer anwenden!</p> <div style="display: flex; justify-content: space-around;"> <div style="border: 1px solid gray; padding: 5px; width: 45%;"> <p style="text-align: center;"><b>Entsprechendes Nasenspray verwenden!</b></p> <p style="text-align: center;">Patienten Nr. 101</p> </div> <div style="border: 1px solid gray; padding: 5px; width: 45%;"> <p style="text-align: center;"><b>Etikette in MTRA-Formular einkleben</b></p> <p style="text-align: center;">UDMD Nasenspray Analyse-Studie Patienten Nr. 101 Prüfärzte</p> </div> </div>  <p style="text-align: right;"><b>Seite 2</b></p> <p style="text-align: right;">USZ/SSW 17.5.2006</p> <p style="text-align: right; font-size: small;">Multizentrierte, Wirksamkeit von nasalem low-dose Midazolam</p> <p style="text-align: right;">21</p>	<p><b>5.2 Studien-Medikation (MTRA-Fragebogen)</b></p> <p>→ MD-Nasenspray</p> <p>Vor der Anwendung ist die Zerstäuberpumpe 5-mal zu betätigen (der Sprühnebel soll nicht eingeatmet werden. Der Nasenspray ist bei der Anwendung aufrecht zu halten)</p> <p><b>Dosierung: 1 Hub pro Nasenloch</b> <b>(die Applikation von 2 Hüben entspricht 1mg Midazolam-Base)</b></p>  <p style="text-align: right;">USZ/SSW 17.5.2006</p> <p style="text-align: right; font-size: small;">Multizentrierte, Wirksamkeit von nasalem low-dose Midazolam</p> <p style="text-align: right;">22</p>
<p><b>5.2 Studien-Medikation (MTRA-Fragebogen)</b></p> <p>→ UD-Nasenspray</p> <p>Der Unitidose-Nasenspray ist gebrauchsfertig und lageunabhängig einsetzbar.</p> <p><b>Dosierung: 1 Hub</b> <b>(die Applikation von 1 Hub entspricht 1mg Midazolam-Base)</b></p> <p>Die Applikation des Unitidose Nasenspray erfolgte in das</p> <p><input checked="" type="checkbox"/> rechte    <input type="checkbox"/> linke Nasenloch des Patienten</p>  <p style="text-align: right;">USZ/SSW 17.5.2006</p> <p style="text-align: right; font-size: small;">Multizentrierte, Wirksamkeit von nasalem low-dose Midazolam</p> <p style="text-align: right;">23</p>	<p><b>5.3 Nebenwirkungen (MTRA-Fragebogen)</b></p> <p><b>5.3 Nebenwirkungen</b></p> <p><input type="checkbox"/> Der Patient meldet keine Nebenwirkungen → weiter bei Punkt 6</p> <p><input type="checkbox"/> Nebenwirkungsmeldung</p> <p>Uhrzeit: _____</p> <p>Art der Nebenwirkung (z.B. brennen, jucken,...): _____</p> <p>Intensität:    <input type="checkbox"/> leicht    <input type="checkbox"/> mittel    <input type="checkbox"/> stark    <input type="checkbox"/> ja, wie: _____</p> <p>Medikamentöse Behandlung:    <input type="checkbox"/> nein    <input type="checkbox"/> ja, wie: _____</p> <p>Erholung des Patienten:    <input type="checkbox"/> rasch (Minuten)    <input type="checkbox"/> nach &gt; 1h</p> <p style="text-align: right;"><input type="checkbox"/> Patient wurde stationär</p> <p style="text-align: right;">USZ/SSW 17.5.2006</p> <p style="text-align: right; font-size: small;">Multizentrierte, Wirksamkeit von nasalem low-dose Midazolam</p> <p style="text-align: right;">24</p>

## 6. Nachdosierung (MTRA-Fragebogen)

6. Nachdosierung → gleiche Medikation wie bei der Erstapplikation!

- es ist **keine** Nachdosierung erforderlich → weiter bei Punkt 7
- es wird eine Nachdosierung vorgenommen. Uhrzeit: \_\_\_\_\_
- Multidose-Nasenspray:** Zur Nachdosierung muss **kein** neues Nasenspray verwendet werden! (keine Etikette aufkleben)
- Dosierung:** 1 Hub pro Nasenloch (die Applikation von 2 Hüben entspricht 1mg Midazolam-Base)
- Anzahl applizierte Hübe: \_\_\_\_\_
- Unitdose Nasenspray:** Für die Applikation einer Nachdosierung ist ein mit „Nachdosierung“ gekennzeichnetes Midazolam UD Nasenspray 1mg zu verwenden.
- Dosierung:** 1 Hub (die Applikation von 1 Hub entspricht 1mg Midazolam-Base)
- Die Nachdosierung mittels Unitdose erfolgte in das
  - rechte  linke Nasenloch des Patienten

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## 6. Nachdosierung (MTRA-Fragebogen)

**Seite 3**

**Bei Bedarf Nachdosieren**

UD-Nasenspray → neu:  
MD-Nasenspray → gleiches Nasenspray wie bei Erstapplikation!

**Etikette in MTRA-Formular einkleben**

UD-Nasenspray  
Analyse-Stufe  
Nachdosierung  
Pulverfarbe

↑

**Etikette in MTRA-Formular einkleben**

UD-Nasenspray  
Analyse-Stufe  
Patienten Nr.  
101  
Pulverfarbe

**Nachdosierung**

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## 6.1 Nebenwirkungen (MTRA-Fragebogen)

6.1 Vom Patienten spontan gemeldete Nebenwirkungen nach der Nachdosierung

- Der Patient meldet **keine** Nebenwirkungen → weiter bei Punkt 7
- Nebenwirkungsmeldung** Uhrzeit: \_\_\_\_\_
- Art der Nebenwirkung (z.B. brennen, jucken, ...): \_\_\_\_\_
- Intensität:  leicht  mittel  stark
- Medikamentöse Behandlung:  nein  ja, wie: \_\_\_\_\_
- Erholung des Patienten:  rasch (Minuten)  nach > 1h  Patient wurde stationär

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## 7. nach der Untersuchung (MTRA-Fragebogen)

7. Patientbefragung nach der Untersuchung

- 7.1 VAS-Angstskala  
→ Bitte kurz nach der Untersuchung vom Patienten ausfüllen lassen!  
Geben Sie bitte mit Hilfe dieser Skala an, wie erträglich die Untersuchung für Sie bezüglich Ihrer Angst war.
- problemlos \_\_\_\_\_ | \_\_\_\_\_ | unerträglich
- 7.2 Gab es für Sie während der Untersuchung Probleme?  
 Nein  Ja, welche:  Blenken in der Nase  
 Schmerzen  vorübergehende Angst  
 Kopfgalgen  Ängste: \_\_\_\_\_
- 7.3 Würden Sie die Untersuchung wiederholen?  
 Nein  Ja
- Anmerkungen: \_\_\_\_\_

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<h3 style="text-align: center;">8. Beurteilung (MTRA-Fragebogen)</h3> <p><b>8. Beurteilung der Untersuchung durch zuständige MTRA</b></p> <p><b>8.1 Durchführbarkeit</b></p> <p><input type="checkbox"/> MR konnte normal durchgeführt werden  <input type="checkbox"/> Abbruch während MR  <input type="checkbox"/> MR konnte gar nicht durchgeführt werden</p> <p><small>Günde für Abbruch oder nicht Durchführen:</small></p> <p><input type="checkbox"/> wegen zu starker Angst  <input type="checkbox"/> wegen zu starken Nebenwirkungen  <input type="checkbox"/> Rücknahme der Einverständniserklärung  <input type="checkbox"/> andere:</p> <p><b>8.2 Patienteneindruck/ -kooperation während der Untersuchung</b></p> <p><input type="checkbox"/> ruhig, kooperativ  <input type="checkbox"/> unruhig, kooperiert schlecht  <input type="checkbox"/> Patient ist eingeschlafen, keine Kooperation  <input type="checkbox"/> ein wenig ängstlich  <input type="checkbox"/> sehr ängstlich  <input type="checkbox"/> sonstiges:</p> <p><b>8.3 Wann traten Angstsymptome/Kooperationsproblemen im Verlauf der Untersuchung auf</b></p> <p><input type="checkbox"/> am Anfang der Untersuchung  <input type="checkbox"/> mitten in der Untersuchung  <input type="checkbox"/> am Ende der Untersuchung  <input type="checkbox"/> keine Angstsymptome/Kooperationsprobleme</p> <p style="text-align: right;">USZ/KSW 17.5.2006</p> <p style="text-align: right; font-size: small;">MUTZENTSTUDE: Wissensnetz von nasalem low-dose Midazolam</p> <p style="text-align: right;">29</p>	<h3 style="text-align: center;">9. Methodik (MTRA-Fragebogen)</h3> <p><b>9. Abweichungen von der Methodik/Anmerkungen</b></p> <hr/> <hr/> <hr/> <p>Zuständiger MTRA:</p> <div style="border: 2px solid black; padding: 10px; text-align: center; margin: 10px auto; width: 80%;"> <h2 style="margin: 0;">Ende des MTRA-Teils</h2> </div> <p style="text-align: right;">USZ/KSW 17.5.2006</p> <p style="text-align: right; font-size: small;">MUTZENTSTUDE: Wissensnetz von nasalem low-dose Midazolam</p> <p style="text-align: right;">30</p>
<h3 style="text-align: center;">8. Beurteilung der Bildqualität → Arzt</h3> <p style="text-align: right;">USZ/KSW 17.5.2006</p> <p style="text-align: right; font-size: small;">MUTZENTSTUDE: Wissensnetz von nasalem low-dose Midazolam</p> <p style="text-align: right;">31</p>	<h3 style="text-align: center;">Beurteilung der Bildqualität</h3> <p>• Punkt 6 bis 8 des Arzt-Fragebogen:</p> <p style="text-align: right;">USZ/KSW 17.5.2006</p> <p style="text-align: right; font-size: small;">MUTZENTSTUDE: Wissensnetz von nasalem low-dose Midazolam</p> <p style="text-align: right;">32</p>

## Beurteilung der Bildqualität

- Punkt 7: Bildqualität:

<input type="checkbox"/> optimal	→ Keine Bewegungsartefakte (Pulsation und Peristaltik ausgenommen).
<input type="checkbox"/> leicht eingeschränkt	→ Geringe Bewegungsartefakte, welche die diagnostische Auswertung nicht beeinflussen.
<input type="checkbox"/> intermediär	→ Bewegungsartefakte, welche die Bildqualität beeinflussen, eine Diagnose ist jedoch möglich.
<input type="checkbox"/> deutlich vermindert	→ Deutlich eingeschränkte Bildqualität, eine diagnostische Aussage ist nur sehr eingeschränkt möglich.
<input type="checkbox"/> nicht diagnostisch	→ Keine Diagnose möglich!

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## Monitoring / Besuche

Ziel:

- Gibt es Probleme
- Kann ein Ablauf vereinfacht werden
- Nachliefern von Formularen
- Werden neue Prüfmuster benötigt

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## Ersetzen von ausgetauschten Patienten

Die Patientennummer eines Ersatzpatienten lautet:  
**1-R- „Nummer des ersetzten Patienten“**

- Fällt der Patient mit der Nummer XYZ aus, so bekommt sein Ersatz die Nummer 1-R-XYZ

Patientennummer	Name	Vorname	Initialen	Geburtsdatum
1-R-101				
1-R-_____				
1-R-_____				
1-R-_____				
1-R-_____				

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## Bei Fragen

- Dr. med. F. Tschirch → Tel. 044 255 30 60
- Dr. J.M Fröhlich → Tel. 079 407 13 64
- K. Suter-Zimmermann → Tel. 061 265 85 33

→ Herzlichen Dank für Ihre Mitarbeit!

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## 12 Curriculum vitae

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### Personal Data

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Name	Katja Suter-Zimmermann
Date and place of birth	November 29, 1978; Aarau AG
Native place	Suhr AG, Oberbözing AG
Nationality	Swiss

### Education

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1985 - 1989	Primarschule, Leutwil AG
1989 - 1990	Mittelstufe, Dürrenäsch AG
1990 - 1994	Bezirksschule Seengen AG
1994 - 1998	Neue Kantonsschule Aarau AG (Matura Type D)
1998 - 2003	Studies in pharmaceutical sciences, University of Basel; Master's degree; Master's thesis "Midazolam in der Pädiatrie: Entwicklung einer oralen und nasalen flüssigen Midazolam-Formulierung für die Pädiatrie" (Hospital Pharmacy, University Hospital Basel), supervised by Prof. Dr. phil. C. Surber, Prof. Dr. phil. G. Imanidis, C. Pellanda
Since 2004	PhD at Hospital Pharmacy, University Hospital Basel supervised by Prof. Dr. phil. C. Surber: "Transmucosal Nasal Drug Delivery – Systemic Bioavailability of Nasally Applied Midazolam"
2004 - 2006	Graduate study in drug discovery and development, organized by the Pharmcenter Basel-Zurich in collaboration with Novartis and Roche
2005 - 2006	WIN-Project 05 (a project of the University of Basel and Novartis)

### Work Experience

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2000 - 2001	Practical training at Apotheke Göldlin Aarau AG (six months) and at the Hospital Pharmacy, Kantonsspital Aarau AG (six months)
2001 - 2007	regular stand-in at Apotheke Göldlin, Aarau AG
2002	stand-in at Pharmacia Nuova, Brissago TI (two weeks)
2004	stand-in at Dorf-Apotheke, Naters VS (one week)
2004	stand-in at Marty Apotheke, Brig VS (two months)
2005	Supervision of the Master's thesis of Robert Witschi "Entwicklung und in vitro Freisetzung von nasalen Midazolam Formulierungen"
2006	Planning and realization of a phase I pharmacokinetic trial (Pharmacokinetics of transmucosal nasal delivered midazolam). This project was carried out in collaboration with Dr. med. M. Haschke, Division of Clinical Pharmacology and Toxicology, University Hospital Basel
2006 - 2007	Clinical Research Assistance in a multicenter trial, phase III (Transmucosal nasal delivery of low-dose midazolam – evaluation of two preparations for procedural anxiety). This project was carried out in collaboration with PD Dr. med. D. Weishaupt and Dr. med. F.T.C. Tschirch, Institute of Diagnostic Radiology, University Hospital Zurich; Dr. med. B. Eckhardt and J. Beranek, Institute of Radiology, Kantonsspital Winterthur; Prof. Dr. med. A. Nidecker, Imamed Radiologie Nordwest, Basel; Prof. Dr. med. J. Hodler and Dr. med. U. Studler, Department of Radiology, Orthopedic University Hospital Balgrist, Zurich; and Dr. sc. nat. J.M. Fröhlich, Akroswiss AG, Zurich

### Award

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2003	1. Preis Junior für die Beste Arbeit, "Nasale Applikation in der Pädiatrie – Eine Alternative?" annual meeting 2003 of Swiss Society of Public Health Administration and Hospital Pharmacists
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During my academic education I attended lectures and practical courses given by following professors and lecturers:

Barras JP, Berger Büter K, Betz G, Bienz KA, Bölsterli U, Brunner E, Bruppacher R, Drewe J, Durrer H, Erb P, Ernst B, Fahr A, Folkers G, Günter TW, Gutmann H, Haag-Wackernagel D, Hädener A, Hauser PC, Hersberger K, Huber PR, Hunziker W, Iberg N, Imanidis G, Itin P, Kaden TA, Kessler M, Krähenbühl S, Kress A, Lampert ML, Landmann L, Leu Marseiler R, Leuenberger H, Meier B, Meyer CA, Meyer J, Moroni C, Mühlebach S, Müller HJ, Oelhafen PC, Plattner GR, Rätz-Bravo A, Regeniter A, Renggli V, Saxer A, Schaffner W, Scholer A, Schlienger R, Séquin U, Sigel H, Spornitz UM, Strazewski P, Surber C, Vedani A, Zaugg C, Zuberbühler AD