PAEDIATRIC FORMULATIONS
Pharmaceutical Development and Clinical Evaluation
Annette van der Vossen
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PAEDIATRIC FORMULATIONS
Pharmaceutical Development and Clinical Evaluation

KINDERFORMULERINGEN
Farmaceutische Ontwikkeling en Klinische Evaluatie

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PART I

Unfulfilled needs and poor practices relating to pharmaceutical products applied in paediatrics in daily clinical practice
Introduction
The development of medicines for children has long been a neglected area. Until late into the 20th century, the general view was that, for ethical reasons, children should not be subjected to clinical research. Nowadays, the consensus is that children are entitled to medicines that have been appropriately evaluated for their use, but other barriers still remain. As the paediatric population from premature neonate to adolescent is very heterogeneous, it cannot be approached as a uniform group. This brings not only practical issues in study design, but the smaller populations also mean a lower returns on investment for companies. As a result, a paucity exists in medicines designed and studied for use in children. On a European level, at the end of 2006, of the 317 centrally authorised medicines, 43% had a potential paediatric use, but were not authorised in this manner (1).

European legislation and incentives for the development of paediatric medicines

Within the European Union, this paucity in paediatric medicines was acted upon by specific legislation in the form of the Paediatric Regulation (EC No 1902/2006), following the example of the US Best Pharmaceuticals for Children Act. When this regulation came into effect in 2007, one of the first measures that were taken was the establishment of the Paediatric Committee, with its main role of scientific assessments and agreement of paediatric investigation plans (PIP). Since then, all applications for marketing authorisation for new medicines have to include the results of studies as described in an agreed PIP, unless the medicine is exempt because of a deferral or waiver. This has resulted in 949 agreed PIPs by the end of 2016, of which 131 had been completed (2). Between the adoption of the Paediatric Regulation in 2007 and the end of 2016, 101 of 399 (26%) centrally authorised new medicines received a paediatric indication. The Paediatric Regulation is therefore seen as successful, but the above applies mainly to innovative medicines, and does not include the development of off-patent medicines.

To stimulate the development of off-patent medicines for paediatric patients, several measures were taken. Firstly, the Paediatric Use Marketing Authorisation (PUMA) was established by Article 30 of the Paediatric Regulation. It is an incentive for off-patent medicinal product development for paediatric use, which offers 10 years of data and marketing protection. Secondly, specific European funding for research into off-patent medicinal products was made available, for instance through the EU Framework Programmes for Research and Technological Development. Thirdly, an inventory of paediatric needs was made, which is published on the EMA website (3), and is meant to help developers identify opportunities. It consists of lists of medicines by therapeutic class, which identify needs with respect to clinical data and age appropriate formulations. From these lists, it is evident that there is a great lack of age-appropriate formulations for off-patent medicines. Unfortunately, up to 2018, only four PUMAs have been granted (4), and it seems that the data and marketing protection is not an effective incentive.

The role of pharmacists in supplying paediatric patients with age-appropriate formulations

Even though the development of new medicines has improved greatly since the introduction of the Paediatric Regulation, there are many therapeutic areas in which there is still a need for paediatric formulations of older medicines. When age-appropriate licensed formulations are not available, pharmacists have several options in providing paediatric patients with suitable preparations. The most preferred option would be to seek a licensed therapeutic alternative. Examples of drug classes where substitution is common
are proton pump inhibitors and NSAIDs. Importation of products that are authorised in another EU country is a second option, but this can be time consuming and costly, and is often subject to strict regulations, which are country-specific. In the Netherlands, reimbursement is also difficult for non-licensed imported products. A third option is the compounding of medicines within the pharmacy, defined as the preparation of an unlicensed medicine to meet the specific needs of a patient. This can either be using raw materials, or the authorised dosage form. These three options are much preferred above the alternative; the manipulation of licensed dosage forms, such as splitting or crushing of tablets, or mixing with fluids or food, by parents and caregivers. With this option, the risk of quality issues is probable, and bioavailability may be substantially altered. When crushing Kaletra (lopinavir/ritonavir) tablets for example, lopinavir and ritonavir exposure in children reduced by 45% and 47%, respectively (5).

Officially, two types of pharmacy preparations are recognised in Directive 2001/83/EC, known as magistral formulae (any medicinal product prepared in a pharmacy in accordance with a medical prescription for an individual patient) and officinal formulae (any medicinal product which is prepared in a pharmacy in accordance with the prescriptions of a pharmacopoeia and is intended to be supplied directly to the patients served by the pharmacy in question). In The Netherlands, as in several other European Member States, an alternative practice is common, where centralised, GMP-certified pharmacies manufacture unlicensed medicines and supply them to local pharmacies. Although in conflict with Directive 2001/83/EC, it is officially allowed by the Health and Youth Care Inspectorate because of the obvious improvement in pharmaceutical quality it provides, but it is tightly regulated.

Practices concerning compounding/manufacturing of unlicensed paediatric formulations and the facilities and equipment available to pharmacists are highly variable across the European Union. In an effort to standardise quality and availability throughout the EU, initiatives are currently undertaken towards the compilation of a pan-European Paediatric Formulary, consisting of monographs for extemporaneous formulations, based on national or regional information. Led by the European Committee on Pharmaceuticals and Pharmaceutical Care (CD-P-PH) and the European Pharmacopoeia Commission, a working party of European experts is currently working on the selection and elaboration of the formulations to be included (6). It is expected that the Formulary of Dutch Pharmacists (FNA) will contribute largely to this Paediatric Formulary.

**Paediatric product development**

Most of the unlicensed products dispensed to paediatric inpatients are manufactured at GMP-pharmacies, and are thus based on pharmaceutical quality data and extensive product dossiers. This also applies to the two drug products presented in this thesis, which were designed at the pharmacy of the Erasmus MC and studied in association with the Laboratory of Dutch Pharmacists (LNA). The LNA is a department of the Royal Dutch Pharmacists Association and supports pharmacist in the compounding of essential medicines of good quality, when licensed products are not available.

The starting point of product development for new paediatric products is always the clinical need. Generally, therapeutic rationale has been established, but the available dosage forms fall short. The EMA has offered some guidance for the selection of dosage forms in relation to the acceptability by paediatric patients, summarised in a reflection
One of the main considerations is the ability to deliver the correct dose to the patient. Within the heterogeneous paediatric population, this means that dosing flexibility is required for a specific drug, and it reduces the options to low-dose solid dosage forms, liquids or parenteral formulations. In the inpatient setting, as a large proportion of the patients is below the age of two or is dependent on a feeding tube, liquid formulations are usually the first choice if non-parenteral administration is aimed for. In addition to the standard drug and formulation properties such as dosage strength, solubility, taste and stability, certain aspects of the formulation need specific attention when designing a product for paediatric patients, in particular the choice of excipients. The EMA guideline on pharmaceutical development of medicines for paediatric use (EMA/CHMP/QWP/805880/2012) offers useful guidance for the selection of excipients, and a hierarchized list of information sources to consult in order to assess the safety profile of each one. Another important property to consider is the palatability of the excipients and the drug product as a whole. Palatability, a combination of taste, after-taste, mouth-feel, fragrance and appearance, is one of the main elements determining the acceptability of paediatric medicinal products.

**In vitro evaluation of paediatric products**

Currently, most officinal formulae that are compounded or manufactured in the Netherlands and applied in pediatrics, have not been clinically evaluated. This has led to unexpected deviations in exposure to the drug in multiple occasions, an example being the reduced oral bioavailability of tacrolimus suspension, compared to tacrolimus capsules (9). Ideally, in the future, in vivo performance of oral dosage forms in children can be predicted with use of in vitro biopharmaceutical techniques. Unfortunately, the drug absorption processes in children have not yet been sufficiently elucidated to develop and validate accurate biopharmaceutical methods.

**In vivo studies**

When formulation development has been completed, sometimes it is necessary to evaluate the product in vivo. A general principle is that paediatric patients should be given medicines that have been appropriately evaluated for their use. Unnecessary clinical trials in (paediatric) patients should however be avoided. From a regulatory perspective, a new formulation that has not been tested in efficacy trials, requires a bioequivalence study, which should typically be performed in adults (10).

Bioequivalence studies are performed to make sure that two formulations have the same rate and extent of absorption (within predefined limits), to ensure comparable in vivo drug exposure. The parameters area under the curve (AUC), maximum plasma concentration (C\text{max}) and sometimes time to maximum plasma concentration (t\text{max}), are calculated from dense sampling schemes and compared between formulations. Bioequivalence studies may however be exempted, if in vitro data can be expected to adequately predict the in vivo performance. These so-called biowavers are based on the Biopharmaceutic Classification System (BCS, Figure 1). The BCS is a system to differentiate drugs on the basis of their solubility and permeability (11). A drug is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1 to 7.5. A drug is considered highly permeable when the extent of absorption in humans is determined to be 90% or more of the administered dose based on a mass-balance determination or in comparison to an intravenous dose. BCS class 1 (highly soluble and...
highly permeable) and sometimes class 3 drugs (highly soluble and low permeable) are eligible for biowaivers. Additional conditions for a biowaiver are rapid dissolution and similar excipients, if they might affect the bioavailability.

![Biopharmaceutical classification system](image)

**Figure 1.1** Biopharmaceutical classification system. The x-axis shows the volume (ml) required to dissolve the highest dose strength of the drug at the lowest solubility over the pH range 1–7.5. Permeability is defined by various *in vivo* or *in vitro* assays. A drug is considered highly permeable when the extent of oral absorption in humans is determined to be 90% or more of the administered dose based on a mass-balance determination or in comparison to an intravenous dose.

When it comes to paediatric formulations, there are some limitations to this approach. Both the parameters solubility and permeability may not be extrapolated to paediatric population. Consequently, BCS-based biowaivers, as well as adult bioequivalence studies, need to be regarded with caution in the pediatric setting.
Aims and outline of this thesis

This thesis we describe the work that was carried out towards a framework for the development of paediatric oral liquids and their evaluation in the target population.

In part one of this thesis we aimed to identify the unfulfilled needs and poor practices relating to pharmaceutical products applied in paediatrics in daily clinical practice. It has two main focus points: firstly, the availability and suitability of drug products for paediatric patients, and secondly, the practical issues regarding administration of drug products to paediatric patients. In chapter 2 we describe studies into the drug products that were dispensed from the pharmacy and assessed their suitability for the specific patient according to EMA guidelines. Furthermore, we identified liquid drug products that are unsuitable due to the presence of potentially harmful excipients, based on the extent of exposure. In chapter 3 we surveyed the extent of manipulation of drug products required to adequately administer the drug to the patient. Both parents and nurses were involved in the study, using questionnaires (parents) and observation (nurses) as main methods.

The second part of this thesis contains the formulation development that was conducted in collaboration with the Laboratory of Dutch Pharmacists. For children, oral liquid formulations with acceptable palatability, good pharmaceutical quality and possibility of flexible dosing are still urgently needed. As a proof of concept, two drugs were selected, both frequently used in children; amlodipine representing a typical BCS class I drug, and lorazepam as an example of a drug with poor aqueous solubility. In chapter 4 we describes the pharmaceutical development of an amlodipine 0.5 mg/ml oral liquid, and chapter 5 proposes a liquid formulation for poorly soluble compounds with lorazepam as a proof of concept. Chapter 6, which was a collaboration with the University of Bath, explores in vitro biopharmaceutical methods that could be used to predict formulation performance in paediatric patients.

In the third part of this thesis we present the clinical studies that were conducted following the pharmaceutical development of the two experimental formulations. Chapter 7 contains the results of a bioequivalence trial in adults of commercial amlodipine tablets and the oral liquid described in chapter 4. This liquid was subsequently studied in the target population using a population pharmacokinetic design. The retrospective study in chapter 9 evaluates the effects of an IV midazolam to oral lorazepam conversion on withdrawal and sedation levels on the paediatric intensive care unit. The subsequent clinical trial in which the bioavailability and pharmacokinetics of our lorazepam oral liquid is studied is described in chapter 10.

Finally, the results, conclusions and recommendation from the studies described in this thesis are discussed in a summarizing discussion.
REFERENCES


PART II

Pharmaceutical development and *in vitro* evaluation of the formulations
Design and stability study of an oral solution of amlodipine besylate for pediatric patients

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O.S.N.M. Smeets
D.J. Postma
A. Vermes
B.C.P. Koch
A.G. Vulto
L.M. Hanff

ABSTRACT

Introduction

Amlodipine is an antihypertensive agent recommended for the management of hypertension in children and adolescents. The commercially available tablets of 5 and 10 mg do not provide the necessary flexibility in dosing needed for treating children. Our goal was to develop a pediatric oral solution of amlodipine, using a robust manufacturing process suitable for ex-tempora and larger scale production.

Methods

The parameters API and preservative content, related substances, appearance and pH were studied under four different storage conditions. Samples were analyzed up to 12 months. Microbiological quality was studied in an 18-week in-use test based on a two-times daily dosing schedule.

Results

The stability of the formulation was influenced by storage conditions and composition. A formulation containing amlodipine besylate, sucrose syrup and methyl paraben remained physically stable for 12 months at 4°C with no loss of amlodipine content. Related substances increased during the study but remained below 0.5%. In-use stability was proven up to 18 weeks.

Discussion

Storage under refrigerated conditions was necessary to prevent precipitation and to obtain an acceptable shelf-life. In conclusion, we have developed and validated an amlodipine oral solution, suitable for the pediatric population. This liquid formulation is preferred over manipulated commercial dosage forms or non-standardized extemporaneously compounded formulations.
INTRODUCTION

Amlodipine (3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate) is a long-acting dihydropyridine L-type calcium channel blocker widely used in both adults and children. It selectively inhibits calcium ion influx in vascular smooth muscle and cardiac muscle, thereby inhibiting the contractile processes of these tissues. The resulting peripheral arterial vasodilation and reduction in peripheral vascular resistance reduces the arterial blood pressure (1). Currently, amlodipine is one of the antihypertensive agents recommended by the European Society of Hypertension for the management of hypertension in children and adolescents (2). Within the group of calcium channel blocking agents, amlodipine is considered first choice treatment for chronic hypertension in children, because of its pharmacological characteristics and it being the most extensively studied drug within this class (2, 3). Calcium channel blockers are also specifically recommended as the preferred drug class in pediatric posttransplantation hypertension (2).

Amlodipine is prescribed off-label to children from the age of 1 month in a dose of 0.06-0.3 mg/kg per day (2). Using the commercially available tablets of 5 and 10 mg, these dosages cannot be administered accurately in young children. Amlodipine has therefor been added to the ‘Inventory of paediatric therapeutic needs’ published by the European Medicines Agency (EMA), as there is no age-appropriate formulation available (4).

According to the EMA reflection paper on pediatric formulations, an oral liquid dosage form would generally be the form of choice and best applicable to administer systemic medication to infants and toddlers (1m-2y) and young children (2-5y). If the physical and chemical characteristics as well as the taste of the drug substance are appropriate, solutions are preferred over suspensions due to better oral acceptance. In addition, solutions are less susceptible to dosing errors resulting from insufficient re-dispersion and are easier to administer through an enteral feeding tube. Further properties that need to be taken into account when designing a pediatric oral liquid dosage form are dose volume (preferably ≤ 5mL for children under 5 years) and use of child-friendly excipients (5).

Since amlodipine is slightly soluble in water (6, 7) and not prone to chemical degradation when protected from light (8), an oral solution might be a feasible dosage form. Its pharmacokinetic characteristics make a once-daily dosing schedule possible, without the need for a controlled release formulation (9). This aids in compliance and acceptability by pediatric patients (10).

For amlodipine only extemporaneously compounded suspensions have been formulated, using commercially available generic suspension bases (Ora-Plus®/Ora-Sweet® 1:1 and Syrspend® SF) and crushed tablets or amlodipine besylate raw material, resulting in a limited stability of 3 months (8, 11, 12). In The Netherlands, as well as in other EU countries, centralized officinal production of unlicensed medicines by GMP-certified pharmacies is common practice. These products are supplied to other pharmacies, after which they are dispensed to the patient. Simultaneously, a part of the community pharmacies still has compounding facilities to provide ex-tempora formulations to their own patients. This situation requires a formulation design that provides an acceptable shelf-life for batch-production, but at the same time allows for individual ex-tempora compounding.

Our goal was to develop and validate a pediatric oral solution of amlodipine, using a
robust manufacturing process (suitable for extemporaneous compounding). To maximize affordability of and accessibility to the formulation, we chose to make use of manufacturing methods suitable for individual and larger scale production.

MATERIAL AND METHODS

Initially an attempt was made to develop a solution of 1 mg/mL amlodipine besylate, preserved with methyl paraben 0.15% and buffered with citric acid. The aqueous solubility of amlodipine was so low that it required heat to dissolve and precipitated shortly after preparation. Lowering the amount of methyl paraben or citric acid buffer did not improve the stability. The concentration of amlodipine was then lowered to 0.5 mg/mL (equal to 0.69 mg/mL amlodipine besylate), which is an acceptable concentration for application in clinical practice.

Composition

The starting point for the comprehensive development of our formulation was an aqueous solution containing amlodipine besylate. Methyl paraben was maintained as a preservative, since it is considered suitable for use in pediatric formulations (13). Because we aimed for a shelf-life of at least six months, preservative-free formulations were not considered. To enhance the taste of the formulation, sucrose syrup was added until an acceptable taste achieved, according to our experienced formulation developers (Composition A). Additional artificial flavors were omitted to preclude a negative influence on the physical stability. Because of the limited aqueous solubility of amlodipine, a second formulation containing propylene glycol as a co-solvent was studied (composition B). Thirdly, a formulation containing amlodipine maleate (Composition C) was studied, to examine if the aqueous solubility of the maleate form would be better. All formulations were manufactured in batches of 2500 mL (A and B) or 5000 mL (C) and put into 100 mL, amber-colored polyethylene terephthalate (PET) (A, B and C) or glass containers (C). Composition A was prepared with active pharmaceutical ingredient (API) from two suppliers (Duchefa and Wyeth), so four batches of amlodipine solution were manufactured, of which the compositions can be found in Table 4.1. Amlodipine besylate, maleate and all other excipients were European Pharmacopoeia grade.

Long-term stability studies

The influence of temperature, packaging material and amlodipine salt form on long term stability were investigated. Samples were stored in climate cabinets at 4±2°C (Elbanton type 5KV-2-50), 25±2°C (Elbanton type LC 500) and 40±2°C (Elbanton type LTKB-ST650). In each cabinet the temperature was registered hourly. Samples of composition A and C were additionally stored at ambient temperature and indirect daylight. Influence of packaging material was studied on composition C, samples were stored in PET and glass containers under each storing condition. API and preservative content were examined over time. Initially, we aimed for a shelf life of 6 months. Samples were analyzed at 0, 1, 2, 3 and 6 months. With an extension of the stability studies, samples stored at 4°C were subsequently also analyzed at 9 ant 12 months.
Table 4.1 Compositions of the studied formulations, which were manufactured in batches of 2500 mL (A and B) or 5000 mL (C) and put into 100 mL, amber-colored polyethylene terephthalate (PET) (A, B and C) or glass containers (C).

<table>
<thead>
<tr>
<th>Composition A</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine besylate</td>
<td>69 mg</td>
</tr>
<tr>
<td>Methyl paraben solution 15% m/v*</td>
<td>287 mg</td>
</tr>
<tr>
<td>Sucrose syrup^</td>
<td>32 g</td>
</tr>
<tr>
<td>Purified water</td>
<td>75,137 g</td>
</tr>
<tr>
<td></td>
<td>107,51 g (=100 mL)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Composition B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine besylate</td>
<td>69 mg</td>
</tr>
<tr>
<td>Methyl paraben solution 15% m/v*</td>
<td>432 mg</td>
</tr>
<tr>
<td>Sucrose syrup^</td>
<td>10 g</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>3,796 g</td>
</tr>
<tr>
<td>Purified water</td>
<td>88,133 g</td>
</tr>
<tr>
<td></td>
<td>102,43 g (=100 mL)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Composition C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine maleate</td>
<td>64 mg</td>
</tr>
<tr>
<td>Methyl paraben solution 15% m/v*</td>
<td>304 mg</td>
</tr>
<tr>
<td>Sucrose syrup^</td>
<td>32 g</td>
</tr>
<tr>
<td>Purified water</td>
<td>75,442 g</td>
</tr>
<tr>
<td></td>
<td>107,81 g (=100 mL)</td>
</tr>
</tbody>
</table>

*Methyl paraben is processed as a 15% m/v solution in propylene glycol.

^ Sucrose syrup contains 63% m/v sucrose and 0.1% m/v methyl paraben.

The stability indicating HPLC-UV method for determination of API, related substances and preservative content was modified from the Ph. Eur. method of amlodipine besylate drug substance by introduction of a gradient in the mobile phase. Analytical specifications can be found in Table 4.2. Release and end-of-shelf-life specifications are displayed in Table 4.3.
Table 4.2 Analytical specifications of the stability indicating HPLC-UV assay of amlodipine oral solution, derived from the Ph. Eur. monograph of amlodipine besylate.

<table>
<thead>
<tr>
<th>Column</th>
<th>Spherisorb ODS1, 5 µm, 250 x 4.0 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test solution</td>
<td>20 µL of 2 ml amlodipine besilate oral liquid in 18 ml water R</td>
</tr>
<tr>
<td>Reference solution</td>
<td>20 µL of 0.05 mg/ml amlodipine as besilate in 1:9 methanol R and water R</td>
</tr>
<tr>
<td>Wavelength</td>
<td>237 nm</td>
</tr>
<tr>
<td>Flow</td>
<td>1.5 ml/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>A: 2.3 g/L ammonium acetate R in water R</td>
</tr>
<tr>
<td></td>
<td>B: Methanol R</td>
</tr>
<tr>
<td>Gradient</td>
<td></td>
</tr>
<tr>
<td>Time (min.)</td>
<td>Solution A (%)</td>
</tr>
<tr>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>36</td>
<td>50</td>
</tr>
<tr>
<td>45</td>
<td>50</td>
</tr>
</tbody>
</table>

In-use stability

An in-use test was performed on Composition A based on a two-times daily dosing schedule. Based on the results from the stability studies, the containers were stored at 4°C and twice-daily removed from the climate chamber to be exposed to air, light and ambient temperature for 30 minutes at every dosing simulation. Samples of 0.4 mL were withdrawn until a quantity of 25 mL remained after which the dosing simulation continued without taking samples. After 18 weeks the samples were analyzed in accordance with the specifications in Table 4.3.

Manufacturing procedure

The amlodipine drug substance was added to ca. 60% of the total volume of distilled water. Using a magnetic stirrer and heating up to 50°C, amlodipine dissolved completely. Methyl paraben solution 15% m/v was added and the mixture was stirred vigorously using the magnetic stirrer. The mixture was cooled to ambient temperature and the sucrose syrup was added. Finally distilled water was added to the solution to reach the desired volume.
**Table 4.3** Release and end-of-shelf-life specifications amlopidine besylate solution 0.5 mg/mL. Microbiological tests of the formulation were performed in two samples from the finished in-use stability study.

<table>
<thead>
<tr>
<th>Test Item</th>
<th>Method</th>
<th>Reference</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification</td>
<td>According to assay</td>
<td>Ph. Eur. Amlodipine Monograph</td>
<td>Spectra should be identical to reference</td>
</tr>
<tr>
<td>Assay (API and preservative)</td>
<td>HPLC-UV</td>
<td>Modified Ph. Eur. method</td>
<td>90% ≤ content ≤ 110%</td>
</tr>
<tr>
<td>Related substances</td>
<td>HPLC-UV</td>
<td>Modified Ph. Eur. method</td>
<td>Total related substances ≤ 1.5%</td>
</tr>
<tr>
<td>Appearance</td>
<td>Visual observation</td>
<td>Ph. Eur. 2.2.1</td>
<td>Clarity ≤ O1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ph. Eur. 2.2.2</td>
<td>Coloration &lt; GY6</td>
</tr>
<tr>
<td>pH</td>
<td>pH meter</td>
<td>Ph. Eur. 2.2.3</td>
<td>Range 5.0 – 6.5</td>
</tr>
<tr>
<td>Microbiological quality</td>
<td>Milliflex Plus</td>
<td>In-house procedure</td>
<td>TAMC ≤ 10^2 CFU/mL</td>
</tr>
<tr>
<td></td>
<td>0.45 μm funnel 100 mL</td>
<td>TSA Casette</td>
<td></td>
</tr>
</tbody>
</table>

Ph. Eur. = European Pharmacopoeia; TAMC = Total aerobic microbial count
RESULTS

Stability studies

The physical and chemical stability of the amlodipine solutions were influenced by the storage conditions. At 25°C and 40°C, resulting from both precipitation and chemical degradation, amlodipine content declined over time as displayed in Figure 4.1. Results after six months for Composition A and B are displayed in Table 4.4. A gradual increase in related substances was seen in all samples, but was notably higher with increasing temperatures.

Table 4.4 Results from the stability studies at 6 months.

<table>
<thead>
<tr>
<th>Storage condition and composition</th>
<th>Appearance</th>
<th>pH</th>
<th>Amlodipine content (%)</th>
<th>Preservative content (%)</th>
<th>Related substances (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T=0 A</td>
<td>≤ O1</td>
<td>5.9</td>
<td>100.0</td>
<td>95.6</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>T=6 4°C A</td>
<td>≤ O1</td>
<td>5.7</td>
<td>98.8</td>
<td>95.3</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>25°C A</td>
<td>≤ O1</td>
<td>5.6</td>
<td>92.0</td>
<td>95.6</td>
<td>2.1</td>
</tr>
<tr>
<td>40°C A</td>
<td>&lt; O3</td>
<td>4.6</td>
<td>55.3</td>
<td>93.7</td>
<td>4.0</td>
</tr>
<tr>
<td>Ambient conditions A</td>
<td>NA</td>
<td>NA</td>
<td>94.1</td>
<td>95.4</td>
<td>1.5</td>
</tr>
</tbody>
</table>

1 Refer to Ph. Eur. 2.2.1
2 Refer to Ph. Eur. 2.2.2.
NA; not available

Based upon the stability results at six months, the stability studies were continued with composition A, since the addition of propylene glycol to Composition B did not provide any advantages. During the extended stability studies, Composition A remained physically stable for 12 months at 4°C with an amlodipine content of 98.7% (see Figure 4.1) and total related substances of 0.5% (not shown). At ambient temperature, the formulation was physically stable for at least two months.

In Composition C particles were first seen after two weeks at 40°C. Crystal depositions were visible in both PET and glass containers. After 3 weeks particles were also seen in Composition C stored at 25°C. The stability studies of Composition C were at this point discontinued.

No changes in color and clarity were observed in any of the samples before precipitation occurred. The methyl parabens content did not decrease in any of the formulations during
the stability studies.

**Figure 4.1** Amlodipine besylate content over time for Composition A and B under different storing conditions. After six months stability studies were continued with the preferred composition A.

**In-use stability**

The samples of Composition A remained physically stable during the in-use study, no crystal depositions of amlodipine were formed. Both the content of amlodipine and methyl paraben increased with 6-8% as a result of evaporation of water. The related substances reached a maximum of 0.2%. The total bacteria count was less than 100 cfu/mL at week 18 of the in-use study in all samples.

**DISCUSSION**

In this study, we developed an oral solution of amlodipine besylate with adequate physical and chemical stability, a shelf-life of 12 months and excipients suitable for pediatric patients.

The biggest challenge in the development of the formulation was the poor aqueous solubility of amlodipine. The Ph. Eur. describes amlodipine besylate as ‘slightly soluble in water’, which would mean that it has a solubility of 1 to 10 mg/mL. This is consistent with the solubilities submitted by Pfizer in the US patent (14). In our pre-studies, a stable aqueous solution of 1 mg/mL or higher appeared not to be feasible, therefore we reduced the concentration to 0.5 mg/mL. Storage under refrigerated conditions was necessary to prevent precipitation and to obtain an acceptable shelf-life. With precipitation occurring faster at higher temperatures, it appears to be an endothermal process.

The addition of propylene glycol (Composition B) to enhance the solubility did not provide any advantages in the physical stability of the product. Since propylene glycol can
be a harmful excipient for pediatric patients (15), we decided to discontinue the stability studies with Composition B after 6 months. The substitution of amlodipine maleate for amlodipine besylate did not improve the stability of the formulation.

Due to the unpleasant taste of amlodipine besylate, we had to increase the amount of sucrose syrup above the recommended range of 10-20% required for acceptable palatability (16). Although the use of cariogenic sweeteners, such as sucrose, should be restricted for chronic use in pediatric formulations, acceptance of the formulation will highly depend on how it tastes (14). The sucrose syrup concentration was therefore considered to be acceptable. The palatability of our formulation was later surveyed in a bioequivalence study in healthy adult volunteers, and on average rated between “not good, not bad” and “good” (17).

In conclusion, we have developed a well-validated amlodipine oral solution, suitable for the pediatric population and able to provide the required dosing flexibility. This formulation is preferable to manipulated commercial dosage forms and non-standardized extemporaneously compounded formulations. It is suitable for large-scale production as well as extemporaneous compounding, which is in many situations necessary for the pediatric population. Our formulation has already proven to be bioequivalent to 5 mg tablets (17) and is now being studied in the pediatric population in order to construct a population pharmacokinetic model.

ACKNOWLEDGEMENTS

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REFERENCES


Formulating a poorly water soluble drug into an oral solution suitable for paediatric patients; lorazepam as a model drug

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ABSTRACT

Introduction

Many drugs are unavailable in suitable oral paediatric dosage forms, and pharmacists often have to compound drugs to provide paediatric patients with an acceptable formulation in the right dose. Liquid formulations offer the advantage of dosing flexibility and ease of administration to young patients, but drug substances often show poor aqueous solubility. The objective of this work was to study different solvents and matrices to design a liquid formulation for poorly water soluble drugs, using lorazepam as model drug.

Methods

Three different formulation strategies were explored to improve the solubility. Firstly, water-soluble organic solvents were used to improve the aqueous solubility directly, secondly, ionic surfactants were used to solubilise the model drug, and thirdly, complexation of lorazepam with cyclodextrin was studied. Specific attention was paid to excipients, adequate taste correction and palatability. For the final formulation, physical and chemical stability and microbiological quality were assessed for 12 months.

Results

An organic solvent based formulation, containing a mixture of polyethylene glycol and glycerol 85%, with a minimum amount of propylene glycol, proved to be physically and chemically stable. Development of the non-ionic surfactants formulation was discontinued due to taste problems. The cyclodextrin formulations were physically stable, but lorazepam content declined to 90% within five months. The final formulation contained in volume concentration (%v/v) 87% glycerol, 10% polyethylene glycol 400 and 3% propylene glycol. Orange essence was the preferred taste corrector. The formulation remained stable for 12 months at 4°C, with lorazepam content remaining > 95%. Related substances increased during the study period but remained below 2%. In-use stability was proven up to 4 weeks.

Conclusion

An organic solvent based oral formulation was shown to be superior to a non-ionic surfactant based formulation or a cyclodextrin formulation. These results may help to formulate paediatric formulations of other poorly water soluble drugs, to aid pharmacy compounding.
INTRODUCTION

Many drugs are unavailable in suitable oral paediatric dosage forms (1), therefore, pharmacists often have to compound drugs to provide paediatric patients with an acceptable formulation in the right dose. In the reflection paper released by the paediatric working party of the European Medicines Agency (EMA) on formulations of choice for the paediatric population, solutions/drops and effervescent dosage forms are considered to have the highest applicability in a population of young patients (2). Capsules can be compounded extemporaneously in the dosage needed, but they need to be dissolved before administration and are difficult to administer through feeding tubes. Another disadvantage of extemporaneously compounded capsules is the difficulty in obtaining adequate content uniformity at low dosages.

Liquid formulations have the advantage of dosing flexibility and a reduced risk of choking. They can also be applied in other populations, such as geriatric patients with swallowing difficulties, or in a palliative setting. Possible disadvantages of liquid formulations are issues with stability and palatability, parameters that need to be considered in the design. As an alternative for liquid formulations, the development of mini-tablets has been given a lot of attention in the past years (3). They provide dosing flexibility and ease of administration, and generally solid formulations are more stable than liquid formulations. However, for most compounding pharmacies, tableting is not an available technique. Liquid formulations are therefore still commonly applied by pharmacist that need to compound for paediatric patients, both on individual and batch scale.

Drug substances sometimes show poor aqueous solubility. The use of solubilizing excipients can improve this, but especially in the paediatric population, the use of excipients needs to be considered carefully, with respect to safety and palatability. The objective of this study was to explore different formulation strategies for a poorly water soluble drug substance, lorazepam was chosen as a model drug.

Lorazepam (7-chloro-5-(2-chlorophenyl)-3-hydroxy-2,3-dihydro-1H-1,4-benzodiazepin-2-one) is a benzodiazepine indicated for the treatment of generalized anxiety disorder and pre-surgical anxiety in patients from the age of twelve years (4). Off-label, it is applied in a wide range of indications and patient categories, because of its sedative and anticonvulsive activity and absence of active metabolites. Within paediatrics, it is administered to children from the age of one month for acute anxiety, sedation, chemotherapy induced- or associated nausea, status epilepticus or for weaning purposes (5).

Currently, no liquid dosage form of lorazepam is available in the EU. An extemporaneous suspension of 1 mg/mL, prepared from 2 mg tablets, distilled water, Ora-Plus® and Ora-Sweet®, has been proven to be chemically stable for up to three months when stored at 4°C (6). However, a subsequent study using this suspension proved that dosage measurement by paediatric intensive care nurses led to significant deviations from the intended dose (7). These inaccurate dosage measurements are less likely to occur in the case of an oral solution, but the physical and chemical characteristics of lorazepam make this a challenge.

There are different strategies to formulate a poorly water soluble drug substance into an oral solution. pH Adjustment can be used to ionize a compound, which generally will result in increased aqueous solubility. In the case of lorazepam (aqueous solubility 0.08 mg/mL) (8), with pKas of 1.3 and 11.5 (9), pH adjustment is not a feasible method to increase the solubility. It is also sensitive to hydrolysis in both acidic and basic environments (10).
and shows temperature-dependent degradation (11). Organic solvents can be used as an alternative to water, but specific attention has to be paid to safety in paediatric patients. A distinction can be made between water-soluble and water-insoluble organic solvents. Water-soluble co-solvents, like ethanol (lorazepam solubility 14 mg/ml) and propylene glycol (lorazepam solubility 16 mg/ml) (8), create a mixed aqueous/organic solution. These excipients are readily available and easy to process, but they can convey a risk of toxicity to children (2). A combination of water-insoluble organic solvents, such as medium-chain and long-chain triglycerides and oleic acid, can be used to disperse lipophilic drugs. Alternatively, a poor water-soluble drug can be solubilized using surfactants, like polysorbate 20 and 80 (Tween) or polyoxyl hydrogenated castor oil (Cremophor), to obtain micelles in an aqueous environment. Similarly, surfactants can be used to obtain a microemulsion, when combined with a polar solvent, an oil, and a cosurfactant. Lastly, complexation of poorly soluble drugs with cyclodextrins has been a strategy to increase the aqueous solubility and bioavailability of compounds, while at the same time masking the taste (12), an important aspect in the design of paediatric formulations.

The objective of this study was to explore different formulation strategies to process a poorly soluble drug substance into a clear oral solution, using lorazepam as a model drug. The formulation needed to be suitable for paediatric patients from the age of one month, and have adequate stability to allow for individual and batch production within the pharmacy.

**MATERIAL AND METHODS**

**Materials**

Lorazepam drug substance was bought from Fagron BV (Capelle a/d IJssel, The Netherlands) and Duchefa Farma BV (Haarlem, The Netherlands). Lorazepam related compound B and hydroxypropyl-β-cyclodextrin (HP-β-CD, substitution degree 0.6) were bought from Sigma-Aldrich Chemie BV (Zwijndrecht, The Netherlands). Lorazepam related compounds C and D were bought from USP Switzerland (Basel, Switzerland). Colour Reference Solutions Y were bought from Merck Millipore (Amsterdam, The Netherlands). Lorazepam drug substance and all other excipients were European Pharmacopoeia grade.

**Formulation development**

The dosage strength was chosen based on the target population of children from the age of one month to 18 years old, receiving a maximum dose of 0.6 mg/kg/day (5). To limit the volume needed and excipients administered, we aimed for a strength of 1 mg/ml. Three different formulation strategies were explored to improve the solubility. Firstly, water-soluble organic solvents were used to improve the aqueous solubility directly, secondly, non-ionic surfactants were used to solubilise the model drug, and thirdly, complexation of lorazepam with cyclodextrin was studied. Parameters that were studied were; physical stability (by visual inspection), chemical stability, using the analytical assay described in section 2.5, and palatability (see 2.3). Physical instability was defined as the presence of visible precipitation. The visual inspection of the samples was performed according to Ph. Eur. 2.2.1., with use of commercial reference solutions. The physical and chemical stability were initially studied for 5 months.

**Organic solvents**

For the organic solvents-based formulation, we experimented with different ratios of
propylene glycol (PG), poly ethylene glycol 400 (PEG400) and glycerol 85%. Efforts were directed towards a glycerol/PEG400 based mixture containing minimal amounts of propylene glycol (Figure 5.1).

**Figure 5.1** Lorazepam 1 mg/ml test formulations containing water-soluble organic solvents.

**Non-ionic surfactants**

The second strategy that was explored was the use of non-ionic surfactants to create a micellar solution. Polysorbate 80 and sorbitan monooleate were mixed in a ratio to obtain a hydrophilic/lipophilic balance (HLB) of 11.5. The total surfactant content in the test formulations ranged from 1-5%. PEG400 was used to dissolve lorazepam, after which the micellar solution was slowly added to the PEG400. The volume per test formulation was 50 mL, the composition of the excipients is displayed in Figure 5.2.

**Figure 5.2** Lorazepam 1 mg/ml test formulations containing non-ionic surfactants.
Cyclodextrin

For the cyclodextrin formulation, HP-β-CD was chosen as the complexing agent, because of its high water solubility, lower cost compared to other cyclodextrins, low toxicity (12), and based on previous work investigating different cyclodextrins for inclusion complexation of lorazepam (13). A phase solubility diagram was made to measure the solubility of lorazepam as a function of the HP-β-CD concentration. This revealed that a minimum of 54 mg/mL HP-β-CD was required to obtain a 1 mg/ml lorazepam solution after 4 hours of ultrasonification. However, a HP-β-CD solution of 60 mg/mL (formulation C1) proved not sufficient to maintain a stable product after one week, therefore the HP-β-CD concentration was increased to 100 mg/ml (formulation C2). Glycerol 85% was added as a preservative in an amount of 35% m/v.

Palatability

The palatability of the test formulations was assessed by three adults, experienced in taste assessment. Characteristics that were evaluated were smell, taste, aftertaste and mouthfeel, and they were independently and qualitatively described by the taste panel. Taste correction possibilities were assessed with formulation C2, O6 and O7, using lemon, banana, raspberry and orange essence. Raspberry and banana were chosen as they are regularly applied in paediatric formulations. Lemon and orange flavours are good taste maskers for bitter drug substances.

Long-term stability studies

After the preliminary formulation studies, a decision was made to continue the development with formulation O7 (Table 3). To this end, two batches of 3000 ml each were compounded, to investigate the influence of temperature and packaging material on long term stability. The test formulations were prepared with active pharmaceutical ingredient (API) from two different suppliers (Fabbrica Italiana Sintetici S.p.A and Cambrex Profarmaco Milano S.r.l.). Samples were stored in climate cabinets at 4 °C (VTL650K, range 2-8 °C) and 25°C 60% relative humidity (Elbanon type LC 500, range 23-27 °C, 55-65% RH) in amber-coloured polyethylene terephthalate (PET) and glass containers. In each cabinet the temperature was registered hourly. Because of the known temperature dependent degradation of lorazepam, stability studies at 40°C were omitted. Samples were tested against the release or end-of-shelf life specifications, based on the United States Pharmacopeia (USP) monograph for lorazepam oral concentrate and the general Ph. Eur. monograph for microbiological quality of non-sterile pharmaceutical preparations, shown in Table 5.1. Samples stored at 25°C were analysed at 0, 1, 2, and 3 months. Samples stored at 4°C were also analysed at 6, 9 and 12 months.
Table 5.1 Release and end-of-shelf life specifications.

<table>
<thead>
<tr>
<th>Test Item</th>
<th>Method</th>
<th>Reference</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification</td>
<td>According to assay</td>
<td>Ph. Eur. Lorazepam Monograph</td>
<td>Spectra should be identical to reference</td>
</tr>
<tr>
<td>Appearance</td>
<td>Visual Observation</td>
<td>Ph. Eur. 2.2.1</td>
<td>Clarity ≤ Susp. I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ph. Eur. 2.2.2</td>
<td>Coloration ≤ Y5</td>
</tr>
<tr>
<td>Assay</td>
<td>HPLC-UV</td>
<td>Modified Ph. Eur. method</td>
<td>Lorazepam 90-110%</td>
</tr>
<tr>
<td>Microbiological</td>
<td>Bioburden filtration</td>
<td>Ph. Eur. 2.6.1</td>
<td>E. Coli Absent</td>
</tr>
<tr>
<td>quality</td>
<td></td>
<td></td>
<td>TAMC (CFU/mL) &lt; 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TYMC (CFU/mL) &lt; 10</td>
</tr>
</tbody>
</table>

CFU = Colony-forming unit; TAMC = Total aerobic microbial count; TYMC = Total combined yeasts/moulds count

Analytical assay

For the quantitative analysis of lorazepam and lorazepam related compounds (USP) B, C and D [2-amino-2,5'-dichlorobenzophenone, 6-chloro-4-(o-chlorophenyl)-2-quinazolinecarboxaldehyde and 6-chloro-4-(o-chlorophenyl)-2-quinazolinecarboxylic acid, respectively] a high performance liquid chromatography combined with UV (HPLC-UV) detection method was used. The components were separated using a Shimadzu LC20 system, on a C18 analytical column (Inertsil ODS-3.5 µm 150x4.6 mm) with a mixture of acetonitrile, methanol and ammonium acetate solution (100 mM, pH 6.0 ± 0.04 adjusted with 1 M acetic acid) in the ratio 1:1:1 (v/v/v) as mobile phase, at a flow rate of 1.0 mL/min. Column temperature was kept at 30 ± 0.1°C and UV detection for quantification was performed at 230 nm using a Shimadzu M20A diode array detector, while the wavelength range of 200-400 nm was continuously monitored for unidentified peaks. The injection volume was 20 µl. The method was validated for the quantification of lorazepam in the cyclodextrin and PG/PEG 400/glycerol sample matrices and in the presence of related compounds B, C and D, for the parameters shown in Table 5.2. The response factors of related compounds B, C and D were determined to allow for accurate quantification of these compounds on lorazepam calibration curves.
Table 5.2 Validation parameters of the developed HPLC-UV analytical assay.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test</th>
<th>n</th>
<th>Specification</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy (12.5 - 37.5 µg/mL)</td>
<td>Recovery (%)</td>
<td>12</td>
<td>98.0 - 102.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Coefficient of variation (%)</td>
<td>12</td>
<td>&lt; 1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Linearity (0 - 1.25 µg/mL)</td>
<td>F-value (12;1 p=0.05)</td>
<td>14</td>
<td>&lt; 4.747</td>
<td>1.508</td>
</tr>
<tr>
<td></td>
<td>Correlation coefficient</td>
<td>14</td>
<td>&gt; 0.9950</td>
<td>0.9978</td>
</tr>
<tr>
<td>Linearity (12.5 - 37.5 µg/mL)</td>
<td>F-value (10;1 p=0.05)</td>
<td>12</td>
<td>&lt; 4.965</td>
<td>2.050</td>
</tr>
<tr>
<td></td>
<td>Correlation coefficient</td>
<td>12</td>
<td>&gt; 0.9950</td>
<td>0.9997</td>
</tr>
<tr>
<td>Limits</td>
<td>LLOQ (µg/mL)</td>
<td>26</td>
<td>-</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>LOD (µg/mL)</td>
<td>26</td>
<td>-</td>
<td>0.018</td>
</tr>
<tr>
<td>Intra-assay precision (0.25 µg/mL)</td>
<td>Coefficient of variation (%)</td>
<td>6</td>
<td>&lt; 1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Intra-assay precision (25 µg/mL)</td>
<td>Coefficient of variation (%)</td>
<td>6</td>
<td>&lt; 1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Inter-assay precision (25 µg/mL)</td>
<td>Coefficient of variation (%)</td>
<td>6</td>
<td>&lt; 2.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Response factors</td>
<td>Related compound B</td>
<td>4</td>
<td>-</td>
<td>0.707</td>
</tr>
<tr>
<td></td>
<td>Related compound C</td>
<td>4</td>
<td>-</td>
<td>1.085</td>
</tr>
<tr>
<td></td>
<td>Related compound D</td>
<td>4</td>
<td>-</td>
<td>0.999</td>
</tr>
<tr>
<td>Specificity</td>
<td>Lorazepam (%)</td>
<td>2</td>
<td>&gt; 99.5</td>
<td>99.7</td>
</tr>
<tr>
<td></td>
<td>Related compound B (%)</td>
<td>2</td>
<td>&gt; 99.5</td>
<td>99.9</td>
</tr>
<tr>
<td></td>
<td>Related compound C (%)</td>
<td>2</td>
<td>&gt; 99.5</td>
<td>99.8</td>
</tr>
<tr>
<td></td>
<td>Related compound D (%)</td>
<td>2</td>
<td>&gt; 99.5</td>
<td>99.6</td>
</tr>
</tbody>
</table>

LLOQ lower limit of quantification, LOD limit of detection

**Calibration and sample analysis**

Samples were diluted 40 times to 25 µg/mL with mobile phase and quantified on a calibration curve (20–30 µg/mL) of freshly prepared standard solutions of lorazepam RS in mobile phase using the validated HPLC method. All duplicate sample analyses were preceded by a system suitability test consisting of replicate (n=5) injections of an equal
mixture of lorazepam RS 25 µg/mL in mobile phase and lorazepam related compound D, 25 µg/mL RS in mobile phase. Specifications for the relative standard deviation in the lorazepam peak areas and the resolution between the lorazepam and lorazepam related compound D peaks were ≤0.5% and 3.8-4.6, respectively. If unavailable, lorazepam related compound D can be created in situ by diluting a lorazepam RS 1000 µg/mL solution in methanol 40 times with 1 M sodium hydroxide and exposing it to a temperature of 70°C for two hours, then neutralized by mixing with an equal volume of 1 M hydrochloric acid.

In-use stability

An in-use test was performed on the final formulation (O7) based on a four-times daily dosing schedule. The containers were stored at 4°C (range 2-8°C) and based on the application in our PICU, four-times daily removed from the climate chamber to be exposed to air, light and ambient temperature for 15 minutes at every dosing simulation. Samples of 0.25 mL were withdrawn. After 28 days the samples were analysed in accordance with the specifications in Table 5.1. Microbiological quality was tested in accordance with the bioburden filtration method of Ph. Eur. 2.6.1.

Manufacturing procedure

The manufacturing procedure was developed with the intention to be suitable for individual and batch compounding. The lorazepam drug substance was levigated in a mortar with the solvent mixture. The remaining solvent was added by geometric dilution. Orange essence was added and the solution was magnetically stirred for one hour to achieve complete solution of the lorazepam.

RESULTS

Formulation development

The organic solvents-based formulations O1-O7 all resulted in physically stable products for at least 5 months. In formulation O1-O4, the lorazepam content declined to around 80-90% after 5 months at 4°C. Formulations O5-O7 were also chemically stable, with lorazepam content remaining around 100% after five months at 4°C. For this reason, we chose formulation O7, with the lowest propylene glycol content, to take into further development (Table 5.3).

Table 5.3 Composition of the lorazepam formulation studied for long-term and in-use stability.

<table>
<thead>
<tr>
<th>Lorazepam</th>
<th>100 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly Ethylene Glycol 400</td>
<td>10 g</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>3 g</td>
</tr>
<tr>
<td>Orange Essence</td>
<td>100 mg</td>
</tr>
<tr>
<td>Glycerol 85% ad 108.1 g (=100 ml)</td>
<td></td>
</tr>
</tbody>
</table>

The surfactant-based formulations gave variable results. Formulations S1-S3 precipitated within a few days (S1) to two months (S3). Formulations S4-S6 remained physically stable during the study period. The content of S4 declined towards the end-of-shelf life limit of 90% within 3 months at 4°C. S5 and S6 remained chemically stable, but development of
these formulations was discontinued due to the bad soapy taste of the liquid.

The cyclodextrin formulation C2 containing 100 mg/ml HP-β-CD remained physically stable during the 5 month study period. The lorazepam content declined to around 90% after 5 months at 4°C with formation of related substance C up to 2.9%.

**Palatability**

The taste assessment results within the panel were consistent. Both cyclodextrin formulations had a neutral scent, slightly sweet taste, and a faint bitter taste caused by the lorazepam. There was no obvious aftertaste, but a prickly sensation on the tongue was sometimes observed. The lemon essence was the preferred taste corrector for formulation C2. Formulations S4 and S4 both had an overpowering soapy smell and taste, which was the reason for discontinuing the development of the surfactant-based formulations. All organic solvent-based formulations had a neutral scent, a sweet taste and a bitter aftertaste. Formulations with 20% PEG400 had a stronger bitter taste than formulations with 10% PEG400. Orange essence was the preferred taste corrector for formulation O6 and O7.

**Long-term stability**

The long-term chemical stability studies of formulation O7 showed that lorazepam content declined over time as displayed in Figure 5.3. A gradual increase in related compounds, mainly related compound C, was seen in all samples, but was notably higher at 25°C. Therefore, stability studies at 25°C were stopped after 3 months. At 12 months, related compound B was first measured in the 4°C samples and also an unknown impurity was found. Related compound C remained below 2.0%. The packaging material did not influence the chemical degradation of lorazepam. No changes in colour and clarity were observed in any of the samples.

![Figure 5.3](image)

**Figure 5.3** Average lorazepam content (left graph) with SD (n=4) and related compound C content (right graph) with SD (n=4) of formulation O7 at 4 and 25 °C.

**In-use stability**

The samples of formulation O7 remained stable during the in-use study, no visual changes were observed. The content of lorazepam did not decrease during the in-use study. Related substance C reached a maximum of 0.5% and the remaining related substances
DISCUSSION

In this study, we explored different formulation strategies to compound a poorly water-soluble drug into a clear oral liquid formulation, using lorazepam as a model drug. With the intended application in paediatric patients, specific attention was paid to child-friendly excipients and adequate palatability. We developed an oral solution of lorazepam at a concentration of 1 mg/ml with adequate physical and chemical stability, and a shelf-life of at least 12 months. This clear solution can be expected to provide good dosing accuracy.

In our final, organic solvent based formulation, a small volume (3% m/v) propylene glycol was still needed to ensure adequate stability. Recently the European Medicines Agency has published a new assessment report concerning the safety of propylene glycol in paediatric formulations (14). In this report, new safety limits were set, expressed in terms of maximum daily doses that are considered to be safe whatever the duration and the route of administration. For neonates up to 28 days, this limit is set at 1 mg/kg, for children 1 month to 4 years old it is set at 50 mg/kg, and for children aged five years and up it is set at 500 mg/kg. Even in the rare occasion that the maximum dose of 0.6 mg/kg/day is required, the intake limits for patients above 28 days old will not be reached with our formulation. If administration to neonates is required, the propylene glycol limit of 1 mg/kg/day may be exceeded, and therefore its use is not recommended for neonates.

In the last decades, an increasing amount of research has been performed into cyclodextrins as a pharmaceutical excipient. The best known example of cyclodextrin in a commercial formulation, is itraconazole (Trisporal®) 10 mg/ml oral solution, containing 40% HP-β-CD and 2.5% propylene glycol, which is used off-label in children. HP-β-CD seems to be a promising option for a lorazepam solution. However, our results showed a restricted stability of maximum of 5 months, most likely due to hydrolysis of lorazepam. The compounding method, needing 4 hours of ultrasonification, proved impractical for individual preparations. The high amount of HP-β-CD required in this composition also makes it expensive. A possible solution that is currently being studied is the spray-drying of lorazepam-cyclodextrin 1:1 complexes, to provide a dry, and thus stable, semi-finished product, which can be compounded by pharmacist for individual patients.

Besides the technical challenges, there are also uncertainties around the safety of cyclodextrins in children below the age of 2 years. The oral bioavailability of HP-β-CD very low, and high doses could cause reversible diarrhoea. For children below the age of 2 years, the currently suggested permitted daily exposure of HP-β-CD is set at 16 mg/kg/day for oral ingestion (12). This is set at one tenth of the adult value, as there are insufficient data in this age group. It corresponds with a maximum allowable lorazepam intake of 0.16 mg/kg/day, which may be surpassed in clinical practice. In summary, a cyclodextrin formulation is a feasible option, but would require considerable additional research.

Our efforts to create a micellar solution of lorazepam resulted in a physically and chemically stable product, and the high amounts of surfactants required to obtain a stable solution would not exceed the Acceptable Daily Intake (ADI) limits for food additives set by the WHO (15, 16). However, the taste of the formulation made it unacceptable for use in children. The development of this formulation was therefore discontinued.
With regard to the palatability assessment by healthy volunteers, it is known that children experience different taste sensations than adults (17). In this stage of development we considered a first screening by an adult tasting panel acceptable. A palatability assessment is included in the clinical trial that is currently performed with our formulation in paediatric ICU patients.

In conclusion, we have studied different options for an oral solution of a poorly water soluble drug, using lorazepam a model drug. The organic solvent based formulation showed adequate stability, taste and dosing flexibility, rendering it suitable for the paediatric population above the age of one month. Our final, organic solvent-based formulation is currently used in a paediatric clinical trial to study the oral pharmacokinetics of lorazepam in PICU patients from the age of 1 month to 12 years old. This formulation is preferable to manipulation of commercial dosage forms and non-standardized extemporaneously compounded formulations, and may serve as an example for the development of comparable drug substances into oral liquid formulations.

ACKNOWLEDGEMENTS

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REFERENCES


PART III

Clinical evaluation of the formulations
Bioequivalence study of an extemporaneously prepared oral solution of amlodipine suitable for use in pediatric patients compared to commercial tablets

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ABSTRACT

Objective

Amlodipine, a long-acting dihydropyridine calcium channel blocker, is frequently prescribed to pediatric patients. To date, no suitable pediatric formulation has been available. In this study, an amlodipine oral solution was developed and tested for bioequivalence to tablets in healthy adult volunteers.

Methods

This study was designed as an open-label, single-dose, two-sequence, two-period, crossover trial to assess the bioequivalence of a newly developed amlodipine besylate oral solution 0.5 mg/mL compared to Norvasc® 5 mg tablets. Thirteen adult subjects (mean [standard deviation] age of 23.2 [3.6] years, weight 71.5 [7.7] kg) were included and blood samples were collected for 72 hours. Amlodipine plasma levels were determined using a validated UPLC-MS/MS assay. Non-compartmental pharmacokinetic parameters were compared between the formulations according to European Medicines Agency (EMA) bioequivalence guidelines.

Results

The 90% confidence intervals of the test/reference ratios of the geometric means for the primary pharmacokinetic parameters $\text{AUC}_{0-72}$ (88.24 - 104.37%) and $\text{C}_{\text{max}}$ (99.00 - 121.40%) were within the acceptance range of 80.00-125.00% for bioequivalence. Mean (SD) $\text{AUC}_{0-72}$ was 102.7 (26.8) µg*h/L for the solution and 108.2 (30.6) µg*h/L for the tablet. Mean (SD) $\text{C}_{\text{max}}$ of the solution was 3.11(1.06) µg/L with a median (IQR) $T_{\text{max}}$ of 4.0 (2.6-7.5) hours. Mean (SD) $\text{C}_{\text{max}}$ of the tablet was 2.91 (0.84) µg/L with a median (IQR) $T_{\text{max}}$ of 6.0 (4.0-14.0) hours. Intrasubject coefficients of variation were 10.2% ($\text{AUC}_{0-72}$) and 12.4% ($\text{C}_{\text{max}}$).

Conclusions

The formulations are bioequivalent according to EMA guidelines. This warrants further study of our novel amlodipine oral solution in pediatric patients.
INTRODUCTION

Amlodipine is a long-acting dihydropyridine calcium channel blocker widely used in both adults and children. Currently, it is one of the antihypertensive agents recommended by the European Society of Hypertension for the management of hypertension in children and adolescents (1). Within the group of calcium channel blocking agents, amlodipine is considered first choice treatment for chronic hypertension in children, based upon its pharmacological characteristics and as the most extensively studied drug within this class (2). Its main advantage is its long half-life, enabling once or twice daily administration.

Amlodipine is officially licensed for treatment of children from the age of six, but also prescribed off-label to children from the age of 1 month in a dose of 0.06-0.3mg/kg per day (1). However, the commercial available formulations are limited to tablets of 5 or 10 mg. These tablets are not suitable for the youngest age group, in which lower dosages and higher dose flexibility are generally needed. A liquid formulation would therefore be a more appropriate dosage form for young patients. Some liquid formulations of amlodipine have been proposed, but have been composed as suspensions (3-5). For a drug that can be highly toxic when overdosed, especially in children (6), a suspension is not preferred. Suspensions can become inhomogeneous, leading to accidental administration of wrong dosages. For this reason we developed a solution of amlodipine, for oral pediatric use. We validated its stability and the formulation has shown to be stable for at least one year (7).

Amlodipine immediate release, solid dosage forms of ≤5 milligrams are classified in Biopharmaceutics Classification System class I by the WHO (8). This implies a high gastrointestinal solubility and permeability of amlodipine. Given these characteristics, we expect our oral solution to be bioequivalent to amlodipine tablets according to European Medicines Agency (EMA) guidelines (9). However, with the test product being a solution, a shift in $T_{\text{max}}$ might occur. To be able to safely apply the oral solution in the pediatric population, we chose to first elucidate the pharmacokinetic parameters of the oral liquid in adult volunteers.

In adults, amlodipine is slowly and completely absorbed after oral ingestion with peak plasma concentrations between 3 and 12 hours (10-12). As a result of its first-pass effect, tablets show high, but variable, bioavailability (50-90%), which is not influenced by food (10, 12, 13). Amlodipine is extensively metabolized in the liver, mainly by CYP3A4 (12). Initial metabolism involves the oxidation of the dihydropyridine ring to the pyridine analogue, complemented by side-chain oxidation and hydrolysis of one or both side-chain ester groups. Around 60% of amlodipine is excreted in the urine, with up to 5% in unchanged form (14). The half-life ranges between 30 and 50 hours, and seems to increase with age (10, 14).

In this study we investigate the bioequivalence of 10 mL amlodipine besylate 0.5 mg/mL in comparison with the innovator 5 mg Norvasc® (amlodipine besylate) tablet after a single oral dose in healthy volunteers, in a crossover design.
METHODS

Drug formulations

The quantitative composition of the amlodipine oral solution is shown in Table 7.1. Long-term stability studies have proven that the solution is stable for at least one year when stored at 4°C, with the contents of amlodipine besylate not dropping below 95% and related substances remaining below 0.4%. The reference treatment consisted of Norvasc® (Pfizer BV, Capelle a/d Ijssel, The Netherlands) 5 mg tablets, containing the excipients sodium starch glycollate (type A), calcium hydrogen phosphate, anhydrous, cellulose, microcrystalline and magnesium stearate.

Table 7.1 Composition of the test formulation amlodipine besylate oral liquid 0.5 mg/mL.

<table>
<thead>
<tr>
<th>Composition of amlodipine oral liquid</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine besylate</td>
<td>69.0 mg</td>
</tr>
<tr>
<td>Methyl paraben solution* 15% m/v FNA</td>
<td>304 mg</td>
</tr>
<tr>
<td>Sucrose syrup^</td>
<td>8.53 g</td>
</tr>
<tr>
<td>Purified water</td>
<td>Ad 100 mL</td>
</tr>
</tbody>
</table>

FNA Formulary of Dutch Pharmacists; * solution of methyl paraben in propylene glycol^solution of 630 mg sucrose and 1 mg methyl parahydroxybenzoate in 1 g of water

Study population and Recruitment

From March to May 2013, we recruited healthy male and female volunteers in Rotterdam, The Netherlands. Subjects were eligible for inclusion if they met the following criteria: age between 18 and 55 years, Caucasian and body mass index from 19 to 25. All subjects were considered healthy on the basis of a physical examination and recording of medical history performed by a physician. Exclusion criteria for participation were: sitting blood pressure lower than 120 mmHg systolic and 80 mmHg diastolic in resting conditions, use of any other medication excluding contraceptives, smoking, pregnancy, history of alcohol or drug abuse, known hypersensitivity to dihydropyridine derivatives or any other contraindication for amlodipine use.

The study was conducted in line with Good Clinical Practice and the Declaration of Helsinki and approved by the Medical Ethics Committee of the Erasmus Medical Centre and by the Dutch competent authority. Written informed consent was obtained from each subject. All subjects had the right to withdraw from the study at any time without any consequences. The trial was registered in the Dutch Trial Register.

Study design

The study was conducted in a single-center, randomized, open-label, two-sequence, two-period, crossover design at the Erasmus Medical Centre, Rotterdam, The Netherlands. We evaluated two single-dose treatments of amlodipine. Two sequences (test-reference and reference-test) were randomly allocated to subjects using the Trial Online Process (TOP) program of the HOVON data center, Rotterdam, The Netherlands. Administration of the study drug was not blinded, because of the difference of appearance of tablets and liquid
and because it was not deemed necessary for the purpose of the study.

A wash-out period of at least 14 days was maintained between test and reference treatment. An intravenous catheter was placed on the day of study drug administration to draw blood samples. Samples were taken at baseline and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14, 24, 48 and 72 hours post dose. The samples at 24, 48 and 72 were drawn by venipuncture. The test product, 10 mL of amlodipine oral liquid 0,5 mg/mL, was dispensed in an oral syringe and ingested without additional fluid, to enable a taste assessment. The reference product (tablet) was ingested with tap water. The subjects underwent an overnight fast for at least eight hours. Water and tea were allowed before and during the study period. Standardized meals were provided at 1, 5 and 11 hours after administration of the study drug. Consumption of grapefruit juice and smoking was not allowed during the study.

For safety reasons, we monitored sitting blood pressure and heart rate of all subjects at baseline and 1, 3, 6, 8, 10, 12, 14, 24 48 and 72 hours post dose for both study drugs using an automated oscillometric device. Subjects were excluded if their systolic blood pressure dropped below 70 mmHg or if their diastolic blood pressure dropped below 40 mmHg. After administration of the test product, the subjects had to fill out a taste assessment form based on a five-point hedonic scale. We surveyed the subjects for adverse events during the study period and one week after.

**Amlodipine analysis**

Plasma concentrations of amlodipine were determined using a validated ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method, that was developed for the purpose of this clinical study.

Plasma samples were stored at -80°C until analysis. After thawing at room temperature, protein was precipitated by adding 600µl methanol, containing the internal standard (amlodipine-d4, Art Molecule, Poitiers, France), to 200µl plasma sample. After vortexing for ten seconds, samples were centrifuged at 15973 g for five minutes. After centrifugation 600µl supernatant was diluted with 150µl of methanol: water (50:50 v/v) and vortexed for 5 seconds. Samples were kept at room temperature until analysis. A 5-µl sample was injected onto the UPLC-MS/MS system (Thermo Scientific Dionex Ultimate 3000) consisting of an Ultimate 3000 RS quaternary UHPLC-pump, an Ultimate 3000 RS auto sampler and an Ultimate 3000 RS column compartment in combination with a Thermo Scientific TSQ vantage MS/MS for mass spectrometric detection. The auto sampler was kept at 15°C. Isocratic elution was achieved with a mixture of 65% of 2mM Ammoniumacetate + 0,1% formic acid in water (mobile phase A) and 35% of 2mM Ammoniumacetate + 0,1% formic acid in methanol (mobile phase B) at a flow rate of 0,4 ml/min. The column (Waters Acquity UPLC HSS T3, 2.1x100 mm, 1.8 µm) temperature was set at 40°C. The total runtime was 4 minutes. Compounds were detected using Electron Spray Ionization (ESI) in positive mode. The SRM transitions of Amlodipine and Amlodipine-d4 were 409 > 238 and 413 > 238 [M+H]+ respectively. Optimal MS settings were as follows: Spray Voltage 3000V, Capillary Temp 200°C, Vaporizer Temp. 400°C, collision gas pressure 1,5 mTorr, Sheat gas 50, Ion Sweep gas 5, Aux gas 20, Collision Energy 10V and S-lens RF amplitude 64. Data processing was performed with LcQuan 2.7 software (Thermo Scientific).

Satisfying results of intraday precision, interday precision and accuracy were conclusively
demonstrated during the period of method validation. Six replicates of three levels of quality control (QC) samples (0.493, 7.398 and 16.77 µg/L) were used to determine accuracy and intraday precision with the maximum coefficient of variation (%CV) set at 15%. For interday precision three levels of QC samples (0.493, 7.398 and 16.77 µg/L) were analyzed in duplicate on six consecutive days. The maximum %CV was also set at 15%. The linear calibration curves were obtained in the concentration range of 0.2 to 20 µg/L using a weighing factor of 1/x. The correlation coefficient was 0.9965. The lower limit of quantification was 0.1 µg/L.

Pharmacokinetic and statistical evaluation

The intra-subject coefficient of variation (CV) for pharmacokinetic parameters was assumed to be 16% (4) and the geometric mean ratio (test/reference) of the pharmacokinetic parameters was assumed to be 1.05. We expected the minimum sample size of 12 evaluable subjects to be sufficient to reject the null hypothesis “lack of bioequivalence between test and reference treatment” with α = 0.05 and a power of at least 80% (15). We performed the statistical analysis according to recommendations of the European Medicines Agency on the investigation of bioequivalence (9). The assessment of bioequivalence was based upon 90% confidence intervals for the ratio of the population geometric means of the test and reference formulation for the parameters $\text{AUC}_{0-72}$ and $\text{C}_{\text{max}}$. Equivalent to two one-sided tests with the null hypothesis of bioinequivalence at the 5% significance level. $\text{AUC}_{0-72}$ was considered adequate for comparison of extent of exposure of the two immediate release formulations, as the absorption phase of amlodipine has been covered. We calculated the $\text{AUC}_{0-72}$ using the linear trapezoidal rule. Extrapolation to infinity ($\text{AUC}_{0-\infty}$) was performed by dividing the last measurable serum concentration by the elimination rate constant ($\lambda_z$). ANOVA was carried out using the respective log-transformed data. The mean square error of ANOVA was used as a variance estimate to calculate the 90% CI. The predefined acceptance range was 80.00% to 125.00% for $\text{AUC}_{0-72}$ and $\text{C}_{\text{max}}$. The elimination half-life was determined from $0.693/\lambda_z$. We estimated $\text{C}_{\text{max}}$ values and $T_{\text{max}}$ directly from the observed plasma concentration–time data. The software used for all calculations was Microsoft Excel 2010 (Microsoft Corporation, Seattle, Washington), IBM SPSS Statistics 21.0.0.1 (IBM Corporation, Armonk, New York) and WinNonlin 6.01 (Pharsight Corporation, Palo Alto, California).

RESULTS

A total of 13 subjects were enrolled in the study (4 male, 9 female, mean [SD] age of 23.2 [3.6] years; weight 71.5 [7.7] kg; height 177.5 [8.5] cm). One subject dropped out during study period 2, due to displacement of the intravenous catheter. After $t=1.5$ no evaluable blood samples were obtained and subsequently this subject was excluded from the pharmacokinetic analysis.

No serious adverse events occurred during the study period. The blood pressures remained above the threshold for exclusion in all subjects. One subject complained of headache in the week after the second treatment period, but this was not attributed to the study medication. Another subject suffered from the flu between treatment periods but was considered healthy at the start of the second study period.
Pharmacokinetics

The liquid test and the solid reference preparation showed similar pharmacokinetic properties. Figure 7.1 shows the mean amlodipine concentration versus time plots. Individual amlodipine concentration versus time plots are shown in Figure 7.2. No relevant pre-dose amlodipine concentrations were observed. An overview of the pharmacokinetic parameters of the two amlodipine dosage forms is given in Table 7.2. Mean (SD) \( \text{AUC}_{0-72} \) was 102.7 (26.8) \( \mu \text{g*h/L} \) for the test product and 108.2 (30.6) \( \mu \text{g*h/L} \) for the reference product. Mean (SD) \( \text{AUC}_{0-\infty} \) was extrapolated to 141.3 (50.3) \( \mu \text{g*h/L} \) for the test product and 147.4 (49.6) \( \mu \text{g*h/L} \) for the reference product. Mean (SD) \( C_{\text{max}} \) of the test product was 3.11 (1.06) \( \mu \text{g/L} \) with a median (IQR) \( T_{\text{max}} \) of 4.0 (2.6-7.5) hours. Mean (SD) \( C_{\text{max}} \) of the reference product was 2.91 (0.84) \( \mu \text{g/L} \) with a median (IQR) \( T_{\text{max}} \) of 6.0 (4.0-14.0) hours. Intrasubject coefficients of variation (derived from the mean square error of the ANOVA) were 10.2\% (\( \text{AUC}_{0-72} \)) and 12.4\% (\( C_{\text{max}} \)). A non-parametric Wilcoxon signed rank test demonstrated a significant difference in \( T_{\text{max}} \) (\( p=0.007 \)).

Table 7.2 Pharmacokinetic parameters for amlodipine test (liquid) and reference (tablet) formulations after administration of a single 5-mg dose in 12 healthy adult volunteers.

<table>
<thead>
<tr>
<th></th>
<th>Test (liquid)</th>
<th>Reference (tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{\text{max}} ) (h)</td>
<td>Median (IQR)</td>
<td>4.0 (2.6-7.5)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>2.00 - 10.0</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (( \mu \text{g/L} ))</td>
<td>Mean (SD)</td>
<td>3.11 (1.06)</td>
</tr>
<tr>
<td></td>
<td>Geometric mean (Geometric CV)</td>
<td>2.97 (30.8%)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>2.07 - 5.83</td>
</tr>
<tr>
<td>( \text{AUC}_{0-72} ) (( \mu \text{g*h/L} ))</td>
<td>Mean (SD)</td>
<td>102.7 (26.8)</td>
</tr>
<tr>
<td></td>
<td>Geometric mean (Geometric CV)</td>
<td>99.4 (27.7%)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>57.83 - 141.44</td>
</tr>
<tr>
<td>( \text{AUC}_{0-\infty} ) (( \mu \text{g*h/L} ))</td>
<td>Mean (SD)</td>
<td>141.3 (50.3)</td>
</tr>
<tr>
<td></td>
<td>Geometric mean (Geometric CV)</td>
<td>133.7 (36.0%)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>66.49 - 260.42</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>Mean (SD)</td>
<td>36.2 (10.9)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>25.2 - 66.1</td>
</tr>
</tbody>
</table>

\( t_{\text{max}} \) = time to reach \( C_{\text{max}} \); \( C_{\text{max}} \) = maximum plasma concentration; \( \text{AUC}_{0-72} \) = area under the concentration-time curve from time zero to 72 hours; \( \text{AUC}_{0-\infty} \) = AUC from time zero to infinity; \( t_{1/2} \) = terminal elimination half-life.
Bioequivalence

The 90% confidence intervals of the test/reference ratios of the geometric means for the primary pharmacokinetic parameters $\text{AUC}_{0-72}$ (88.24 - 104.37%) and $\text{C}_{\text{max}}$ (99.00 - 121.40%) are within the acceptance range of 80.00-125.00% for bioequivalence, showing that the liquid is bioequivalent to the tablet.

![Figure 7.1](image1.png)

**Figure 7.1** Composite (mean±SD) plasma concentration versus time curves after administration of a single 5-mg dose the test (liquid) and reference (tablet) formulations in healthy adult volunteers.

Taste assessment

The average first impression of the taste of the oral solution was rated between ‘not good, not bad’ and ‘good’ (mean 2.75 on 5 point scale where 1= very good and 5= very bad), with two subjects rating it as ‘bad’. Two minutes after ingestion the taste of the oral solution was on average rated as ‘good’ (mean 2.17). Subjects described the taste as a combination

![Figure 7.2](image2.png)

**Figure 7.2** Individual amlodipine plasma concentration versus time plots of the liquid test formulation and the solid reference formulation.
DISCUSSION

In this study a novel liquid formulation of amlodipine besylate was tested for bioequivalence to the reference Norvasc® tablet. The 90% confidence intervals of the ratios of the geometric means for the primary endpoints $AUC_{0-72}$ and $C_{max}$ were well within the pre-defined bioequivalence acceptance range of 80.00-125.00%. This means that the two formulations are bioequivalent according to EMA guidelines.

PK parameters for both formulations were similar, except for the anticipated shorter $T_{max}$ observed for the liquid formulation. Since amlodipine is absorbed relatively slowly and no significant change in peak concentration occurs, this difference is deemed acceptable. The PK parameters were likewise comparable to previously published bioequivalence studies using Norvasc® 5 mg ($AUC_{0-\infty}$ mean 166.3, SD 76.7 µg*h/L (16) and $AUC_{0-\infty}$ mean 203.2, SD 52.1 µg*h/L (17)). The mean elimination half-life of 36 hours and its marked between subject variability found in this study are consistent with previous studies in young, healthy volunteers (10-12, 16, 18, 19). The individual half-lives in this study ranged between 25 and 66 hours, but the wash-out period between treatments was sufficiently large to achieve more than six half-lives for all subjects.

In both the mean concentration-time plots (Figure 7.1) and multiple individual concentration-time plots (Figure 7.2), a second peak was observed around $t=12$ hours. Other pharmacokinetic studies on amlodipine (10-13, 16, 18), did not show this profile, possibly due to more sparse blood sampling around that time point. However, in accordance with our results, a similar profile with a secondary peak was found in a study on the influence of gastrointestinal transit times on the AUC of several calcium antagonists, including amlodipine (20). It has been suggested that amlodipine undergoes enterohepatic circulation (21), which is supported by the excretion of metabolites in the feces (14). A possible explanation of the second peak could be re-entering of amlodipine in the intestinal tract with the excretion of bile during/after the evening meal.

Although the EMA guideline for the investigation of bioequivalence (9) recommends no intake of food for at least four hours post-dose, we limited the fasting period to one hour after the administration of the study drug, based upon several studies showing no direct influence of food on the absorption of amlodipine (22, 23). Likewise there was no restriction on water and tea intake as the dissolution of amlodipine from the dosage form is unlikely to effect the absorption (10, 21).

For small molecules, the EMA considers bioequivalence studies in healthy volunteers to be adequate to detect formulation differences and to allow for extrapolation of the results to populations for which the reference medicinal product is approved (9). It is generally accepted that gastro-intestinal permeability in children above the age of 2 years is equivalent to that observed in adults (24). For in vivo solubility however, there is debate as to whether results from bioequivalence studies can be directly extrapolated to pediatric patients (25), because of the relatively smaller volume of gastro-intestinal fluid in children. It is therefore desirable to further elucidate the pharmacokinetic performance of the amlodipine oral solution in the pediatric population. The results of this study will form the basis for a study protocol in children.
CONCLUSION

In conclusion, in this study we showed bioequivalence of the newly developed amlodipine oral solution compared to Norvasc® 5 mg tablets. With these results, the use of the liquid in the intended target population, children with chronic hypertension, can be safely explored in future studies.

ACKNOWLEDGEMENTS

This study was supported by ZonMW, The Hague, The Netherlands.
REFERENCES


17. Park JY, Kim KA, Park PW, Lee OJ, Kim JS, Lee GH, et al. Comparative pharmacokinetic and pharmacodynamic characteristics of amlodipine besylate and amlodipine nicotinate


Oral lorazepam can be substituted for intravenous midazolam when weaning paediatric intensive care patients off sedation.

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ABSTRACT

Aim

Intravenous sedatives used in the paediatric intensive care unit (PICU) need to be tapered after prolonged use to prevent iatrogenic withdrawal syndrome (IWS). We evaluated the occurrence of IWS and the levels of sedation before and after conversion from intravenous midazolam to oral lorazepam.

Methods

This was a retrospective, observational, single cohort study of children under the age of 18 admitted to the PICU of the Erasmus MC-Sophia Children's Hospital, Rotterdam, The Netherlands, between January 2013 and December 2014. The outcome parameters were the Sophia Observation withdrawal Symptoms (SOS) scale scores and COMFORT Behavior scale scores before and after conversion.

Results

Of the 79 patients who were weaned, 32 and 39 had before and after SOS scores and 77 had COMFORT scores. IWS was reported in 15/79 patients (19.0%) during the 48 hours before the start of lorazepam and 17/79 patients (21.5%) during the 48 hours after treatment started. Oversedation was seen in 16/79 patients (20.3%) during the 24 hours before substitution and in 30/79 patients (38.0%) during the 24 hours after substitution.

Conclusion

The weaning protocol was not able to prevent IWS in all patients, but converting from intravenous midazolam to oral lorazepam did not increase the incidence.
INTRODUCTION

Most children admitted to a paediatric intensive care unit (PICU) receive intravenous sedatives and analgesics to relieve anxiety, distress and pain and to tolerate mechanical ventilation and other PICU-related procedures. The most commonly used sedatives and analgesics in paediatrics are midazolam and opioids (1). Unfortunately, these drugs can cause iatrogenic withdrawal syndrome (IWS) after prolonged use (2).

To prevent IWS, a protocolled approach to taper the drugs and to regularly monitor withdrawal symptoms and sedation levels is recommended. Intravenously administered medication can be switched to oral dosage forms, to facilitate gradual weaning without the need for cardiorespiratory monitoring required for intravenous sedation, and to omit the need for intravenous access. Treatment can then be continued outside the PICU and removing intravenous access lowers the risk of infection. Oral lorazepam has a long half-life in children, with a median of 17 hours (range 8-53 hours), which prevents large fluctuations in plasma concentrations, and also has a lack of active metabolites. That is why it is often used off-label as a substitute for intravenous midazolam (2-4).

In our local weaning protocol, the calculation of the initial dose of oral lorazepam was based on a conversion factor proposed by Tobias et al (5), which assumed that lorazepam was twice as potent as midazolam and had a six-time longer half-life, based on adult data. This lorazepam starting dose is calculated irrespective of the potential impact of maturation of lorazepam and midazolam metabolism due to age or other factors influencing drug exposure, such as critical illness. In addition to this, the bioavailability of oral lorazepam in children is unknown and, therefore, no correction is possible for a potential incomplete bioavailability. In summary, this means that the current dosage of lorazepam for weaning of midazolam may not be optimal. At the time of our study, no clinical data on the conversion from midazolam to lorazepam in PICU settings was available in the literature.

Due to this limited information, the aim of this study was to evaluate the occurrence of IWS and the level of sedation before and after conversion from intravenous midazolam to oral lorazepam. We also wanted to assess the safety of our current midazolam to lorazepam conversion protocol.

METHODS

Design and study population

A retrospective, single centre, cohort study was performed to evaluate the move from intravenous midazolam to oral lorazepam to keep patients comfortable and prevent IWS. Our study population was admitted to the level five PICU of the Erasmus MC - Sophia Children’s Hospital, Rotterdam, The Netherlands, between January 2013 and December 2014. Patients were selected from our Critical Care Suite electronic patient data management system (Picis Clinical Solutions SA, Barcelona, Spain), when they had received oral lorazepam following intravenous midazolam. The exclusion criteria were the use of midazolam and lorazepam for epilepsy or delirium, when the latter had been diagnosed by a trained psychiatrist, or for other reasons such as incidental sleep medication. The medical ethics committee of the hospital waived the need for institutional review board approval and informed consent according to the Dutch law on Medical Human Research.
IWS and sedation scores

To achieve optimal weaning, it is necessary to monitor symptoms of IWS from benzodiazepines and opioids and to monitor the level of sedation. These are assessed using the Sophia Observation withdrawal Symptoms (SOS) scale to determine IWS and the COMFORT behavior (COMFORT-B) scale to assess the level of sedation (6-8). The SOS scale consists of 15 items representing signs and symptoms of opioid and, or, benzodiazepine withdrawal, including changes in heart and respiratory rate and signs of discomfort. IWS scoring is initiated at start of weaning and performed at eight-hour intervals, when the occurrence of IWS is suspected, and to evaluate any interventions that were made to treat IWS. The COMFORT-B scale consists of six behavioural items and is applied in combination with the Nurses Interpretation of Sedation Score (NISS) (6) from the start of mechanical ventilation. It has been validated to assess the level of sedation in ventilated and non-ventilated children. Scoring is performed by the attending nurses at eight-hour intervals and if there are signs of distress or increasing discomfort. It continues until discharge from the PICU or until all sedative medication has been stopped.

Weaning protocol

Weaning of sedative and analgesic medication is initiated as soon as the patient’s underlying condition and pathology improves, their electrolytes are within normal range and they are cardiovascularly stable. The protocol for weaning of continuous opioids and sedatives implemented at our PICU starts with decreasing continuous infusion rates of the drugs and the intervals depend on the preceding length of treatment. Infusion rates are decreased, one drug at a time, by 10% of the initial rate. This occurs every 24 hours when the patient has received the drug for 6-9 days and every 48 hours when they have received the drug for 10 days or more. The intravenous medication is converted to an effect-equivalent dose of oral medication within the same therapeutic class when the patient is due to be discharged to the general ward without cardiorespiratory monitoring, when intravenous access is no longer required or available or when prolonged weaning is expected. The initial daily dose of oral lorazepam is calculated by dividing the daily dose of midazolam by 12. This conversion is based on the lorazepam and midazolam ratio for half-life (6:1) and its relative potency (2:1) in adults (5). This lorazepam dose is administered orally four times a day and the intravenous midazolam is tapered over 24 hours as shown in Figure 9.1. Lorazepam is subsequently tapered in steps of 10% of the initial dose every 24 or 48 hours. If there are withdrawal symptoms, indicated by an SOS score of four or more, a rescue dose of 0.1 mg/kg midazolam is administered or the oral lorazepam dose is increased to the previous strength. If applicable, opioids and other sedatives, such as morphine, fentanyl, clonidine and pentobarbital, are also converted to oral alternatives in a similar manner, for example methadone, clonidine per os and phenobarbital, preferably with a minimum of 48 hours between conversions. They are tapered according to the same principles.

Medication

Intravenous midazolam was administered using a Perfusor FM syringe pump (B Braun Medical, Oss, The Netherlands), in concentrations of 1 mg/ml or 5 mg/ml dissolved in 5% glucose, which were prepared by the pharmacy. Oral midazolam for rescue administrations was available as an extemporaneous liquid of 1 mg/ml. Oral lorazepam was administered as either commercial tablets, extemporaneous capsules of 0.1 mg or a 4 mg/ml commercial
injection fluid that was administered orally. Solid dosage forms were usually dispersed in water and administered through a feeding tube.

![Figure 9.1 Tapering of midazolam after substitution with oral lorazepam. The intravenous midazolam dose is halved after the second administration of lorazepam, again halved after the 3rd administration of lorazepam and ceased after the 4th administration of lorazepam (24h after switch). The first dose of lorazepam is calculated upon the last infusion rate of midazolam.](image)

**Data collection**

Data were extracted from the electronic medical records. The clinical and demographic parameters that were retrieved included age, sex, diagnosis, cumulative doses and duration of midazolam and lorazepam therapy, analgesic and sedative co-medication and the patient’s destination after their discharge from the PICU.

**Outcomes**

The SOS scores were retrieved to determine the incidence of withdrawal from 48 hours before substitution to 48 hours after substitution. A cut-off score of at least four was defined as withdrawal. The COMFORT-B scores and NISS scores were analysed from 48 hours before substitution to 48 hours after substitution to determine the level of sedation. COMFORT-B scores of ≥23 or 11-22 with a NISS of one were regarded as undersedation, COMFORT-B scores of 11-22 with a NISS of two were regarded as adequate sedation and COMFORT-B scores of ≤10 or 11-22 with a NISS of three were regarded as oversedation. Similarly, the number of rescue dosages of midazolam and other sedatives were compared from 48 hours before to 48 hours after substitution. The frequency and severity of apnoeas and the need for flumazenil during the 48 hours after start of lorazepam were used to assess the safety of the conversion. Apnoeas were registered manually in the patient data management system by the attending physician or nurse as part of standard care. The agreement of the actual midazolam to lorazepam conversion with the conversion protocol was assessed with respect to the dose calculation of lorazepam and the tapering of midazolam within 24 hours after conversion.

**Analysis**

Data were analysed using IBM SPSS statistics version 21.0 (IBM Corporation, New York,
USA). Demographic and clinical data were processed using descriptive statistics. The number of rescue administrations of midazolam and other sedatives before and after substitution were compared using a paired-sample t-test.

RESULTS

During the 24-month study period between January 2013 and December 2014, 111 cases met the inclusion criterion for oral lorazepam use after intravenous midazolam therapy. After excluding three patients who started lorazepam in 2012, 20 patients who received lorazepam for other purposes than weaning, and excluding multiple occasions within one subject (n=9), 79 cases were included for further analysis. The patient characteristics are listed in Table 9.1.

Table 9.1 Patient characteristics (n=79).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>37</td>
<td>46.8</td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
<td>53.2</td>
</tr>
<tr>
<td>Age median (months) (IQR)</td>
<td>5.3 (1.7-19.8)</td>
<td></td>
</tr>
<tr>
<td>Age:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-27 days</td>
<td>13</td>
<td>16.5</td>
</tr>
<tr>
<td>28 days -11 months</td>
<td>40</td>
<td>50.6</td>
</tr>
<tr>
<td>12-23 months</td>
<td>8</td>
<td>10.1</td>
</tr>
<tr>
<td>2-11 years</td>
<td>16</td>
<td>20.2</td>
</tr>
<tr>
<td>12-18 years</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Weight median (kg) (IQR)</td>
<td>5.5 (3.6-10.0)</td>
<td></td>
</tr>
<tr>
<td>Reason for PICU admission:</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Cardiac</td>
<td>30</td>
<td>28.0</td>
</tr>
<tr>
<td>Non-cardiac surgical</td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td>Neurological</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Infection/respiratory</td>
<td>19</td>
<td>24.1</td>
</tr>
<tr>
<td>Trauma</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Congenital</td>
<td>9</td>
<td>11.4</td>
</tr>
<tr>
<td>Other</td>
<td>14</td>
<td>17.7</td>
</tr>
<tr>
<td>Ventilation</td>
<td>79</td>
<td>100</td>
</tr>
<tr>
<td>ECMO therapy</td>
<td>7</td>
<td>8.9</td>
</tr>
<tr>
<td>Transfer after PICU:</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Home</td>
<td>7</td>
<td>8.9</td>
</tr>
<tr>
<td>Other hospital</td>
<td>18</td>
<td>22.8</td>
</tr>
<tr>
<td>Other department</td>
<td>45</td>
<td>57.0</td>
</tr>
<tr>
<td>Mortality</td>
<td>9</td>
<td>11.4</td>
</tr>
<tr>
<td>Median length of PICU stay</td>
<td>Days (range)</td>
<td>32 (4-183)</td>
</tr>
</tbody>
</table>

ECMO = extracorporeal membrane oxygenation; IQR = inter quartile range; PICU = paediatric intensive care unit
At the point of the midazolam to lorazepam switch, the median duration of midazolam infusion, from the day of admittance to the Sophia Children’s Hospital, was 12 days (range 1-69) and the median cumulative dose was 46.5 mg/kg (range 0.47-287). We also noted that 23 patients were still on invasive ventilation and 11 patients had received midazolam at infusion rates that were higher than 0.35 mg/kg/h during their admission. Further information on the patients’ sedative treatment during PICU admission is summarised in Table 9.2.

Table 9.2 Sedative treatment characteristics during PICU admission (n=79).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (range)</th>
<th>unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median dose per patient:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midazolama</td>
<td>130 (30-393)</td>
<td>mcg/kg/h</td>
</tr>
<tr>
<td>Lorazepamb</td>
<td>0.30 (0.08-2.76)</td>
<td>mg/kg/d</td>
</tr>
<tr>
<td>Cumulative dose:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midazolamc</td>
<td>46.5 (0.47-287)</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>1.42 (0.08-79.32)</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Maximum infusion rate before substitution:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midazolam</td>
<td>300 (12-1000)</td>
<td>mcg/kg/h</td>
</tr>
<tr>
<td>Duration of infusion until substitution:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midazolam</td>
<td>12 (1-69)</td>
<td>days</td>
</tr>
<tr>
<td>Duration of midazolam therapy until substitutiond</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>&lt; 5 days</td>
<td>3</td>
<td>3.8</td>
</tr>
<tr>
<td>5-10 days</td>
<td>16</td>
<td>20.3</td>
</tr>
<tr>
<td>&gt; 10 days</td>
<td>60</td>
<td>75.9</td>
</tr>
<tr>
<td>Duration of lorazepam taper:</td>
<td>Days</td>
<td>(range)</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>22 (3-97)</td>
<td>(n=45)</td>
</tr>
<tr>
<td>Fixed-interval and continuous sedative and analgesic co-medication:</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Alimemazine po</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Clonidine iv</td>
<td>41</td>
<td>52</td>
</tr>
<tr>
<td>Clonidine po</td>
<td>23</td>
<td>29</td>
</tr>
<tr>
<td>Esketamine iv</td>
<td>26</td>
<td>33</td>
</tr>
<tr>
<td>Fentanyl iv</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Methadone po</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Morphine iv</td>
<td>73</td>
<td>92</td>
</tr>
<tr>
<td>Pentobarbital iv</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Propofol iv</td>
<td>19</td>
<td>24</td>
</tr>
</tbody>
</table>

a Throughout PICU admission, b Starting dose at substitution, c Until substitution, d Midazolam therapy was calculated from the first administration to the last administration in the Sophia Children's hospital. The short administration of one day is due to the transfer from another hospital. e n=45. Total lorazepam duration, including use at home. Only the patients with complete post clinical duration were used to calculate the median.

po = orally; iv = intravenous; PICU = paediatric intensive care unit.
The SOS scores were available for 32/79 (40.5%) of the patients in the 48 hours before substitution and 39/79 (49.4%) of the patients in the 48 hours after substitution. The median score per patient before the start of lorazepam ranged between 0-9.0, with 15 patients (19.0%) having one or more SOS score of at least four, indicating IWS. After the start of lorazepam the median score per patient ranged between 0-5.0, with 17 patients (21.5%) having one or more SOS score of at least four. In eight of these 17 patients, the morphine infusion rates were decreased during the 96 hours around conversion. Figure 9.2 shows the range of the highest SOS score per patient within our study period. Seven patients experienced IWS both before and after substitution and 11 patients experienced both oversedation and IWS in the 48 hours after substitution.

Figure 9.2  Distribution of the highest SOS score per patient during the first 48 hours before substitution (grey bars) and 48 hours after substation (open bars) of iv midazolam with oral lorazepam. Maximum score is 15, with scores ≥ 4 indicating withdrawal.

COMFORT-B scores were available for 77/79 patients (97.5%). All the available scores are shown in Figure 9.3, with a median of three scores per patient per day. From a total of 1,122 COMFORT-B scores, 136 incidences of oversedation and 150 incidences of undersedation were determined, in combination with the NISS, during the 96-hour study period. Only 44 of the incidences of undersedation were accounted for by COMFORT-B scores of at least 23 and the other 106 by a COMFORT-B score between 11-22 and a NISS of one.

In some patients the COMFORT-B scores, in combination with the NISS, were outside the adequate sedation range and these are presented in Figure 9.4. This figure shows that the incidence of oversedation increased after substitution with lorazepam. During the two days before substitution, 13 and 16 patients, respectively, experienced oversedation compared to 39 and 30 patients in the two days after substitution. Undersedation decreased from 28 and 21 patients before lorazepam initiation to 16 and 13 patients after the start of lorazepam.
A total of 34 patients (43.0%) received one or more rescue administrations of midazolam before substitution, compared to 19 patients (24.1%) after substitution, with a 95% confidence interval (95% CI) of -0.06-0.77, p=0.096. Furthermore, 29 patients (36.7%) received rescue administrations of other sedatives before substitution compared to 21 patients (26.6%) after substitution (95% CI -0.18-0.94, p=0.178). In total, 50 patients (63.3%) received rescue administrations before substitution and 34 patients (43.0%) after substitution with a median of two administrations in both periods. During the 48-hour post substitution period, 56 patients (70.9%) continued their sedative or analgesic co-medication. Co-medication was decreased in 44 patients and increased in three patients.

Regarding the safety of the substitution, no apnoeas were reported and no flumazenil was prescribed during the 96 hours around the conversion.
Adherence to the conversion protocol was variable. The median midazolam/lorazepam dose ratio was 11.4 (range 1.31-22.6) and 62.0% of the ratios were between 10 and 14. In 45.6% of the patients, midazolam was tapered in a timeframe of 24 hours from substitution, in agreement with the protocol. In 32.9%, intravenous midazolam was discontinued before 24 hours and in 21.5%, simultaneous administration of intravenous midazolam and oral lorazepam continued for more than 24 hours.

**DISCUSSION**

Our midazolam to lorazepam switch protocol to prevent IWS appeared to be effective in the majority of patients, as no increase in the occurrence of IWS was detected. Nevertheless, at least 20% of patients still experienced withdrawal symptoms, while almost 40% showed signs of oversedation in the early stages after conversion.

Based upon the available SOS scores, the incidence of IWS was similar before and after conversion to lorazepam. A limitation is that only about half of the patients were scored for withdrawal, making the results hard to extrapolate. When we assume that the exhibition of IWS symptoms is a trigger to start collecting SOS scores, the absence of SOS scores may be seen as a sign that the patients were doing well, but this needs to be verified in a prospective setting. Furthermore, the SOS scale cannot discriminate between opioid and benzodiazepine withdrawal. This means that the reported IWS cannot unequivocally be
attributed to benzodiazepine withdrawal, especially in the eight patients where morphine was tapered simultaneously. Nevertheless, we did not observe an increase in IWS after the conversion to lorazepam.

The incidence of IWS in critically ill children has been reported to range from 13%-87% (8-19). This large variation was the result of small sample sizes, a large variety in often unvalidated assessment methods and non-standardised or absent sedation protocols and weaning regimens. Identified risk factors for IWS are cumulative doses of midazolam greater than 40 mg/kg (8, 11), infusion of opioids and benzodiazepines for more than five days (8, 11, 13), and midazolam infusion rates above 0.35-0.42 mg/kg/h (18, 19). Taking into consideration the clinical patient characteristics, such as the high cumulative doses of midazolam and long PICU stays, it becomes apparent that the patients in our cohort were at high risk for developing IWS. In our retrospective cohort, based upon the available SOS scores, IWS was diagnosed in one-fifth of the patients, both before and after substitution.

The majority of the collected COMFORT-B scores were within the target range for adequate sedation, with a tendency towards more oversedation post-substitution. This could suggest supratherapeutic dosages of sedatives, especially during the first 24 hours in which midazolam and lorazepam were simultaneously administered. To put these findings into perspective, COMFORT-B scores of nine and 10 could be the result of a comfortably asleep child with normal muscle and facial tone and is not necessarily indicative of an unsafe situation. Considering it may take a number of days to reach steady-state plasma levels of lorazepam due to its long half-life, it seems rational to start with lorazepam while phasing out midazolam to ensure adequate exposure. The absence of apnoeas and flumazenil administration during the study period provides evidence that the combined blood levels of benzodiazepines were not within the toxic range. It is notable that several patients experienced both oversedation and withdrawal after substitution, which illustrates the complexity of managing IWS. The comparison of rescue administrations of midazolam and other sedatives yielded no statistically significant results.

The lorazepam dose calculation was based on the relative half-life and potency of lorazepam versus midazolam, as determined in adult patients, and irrespective of individual patient characteristics. Lorazepam is primarily metabolised through conjugation with glucuronic acid by multiple hepatic UDP-glucuronosyltransferase enzymes, to inactive metabolites. The maturation rates of involved enzyme systems differ between the subtypes, but may well extend beyond the age of two years, based upon gene expression data and in vivo experiments (20, 21). Paediatric pharmacokinetic data after the oral administration of lorazepam are unavailable. At the moment, there are insufficient data available to establish an age-dependent conversion factor. Midazolam pharmacokinetics in paediatric patients are well studied and are highly dependent on CYP3A4 activity. High blood levels of midazolam might be caused by delayed clearance due to immature metabolism at a neonatal age (22), ongoing inflammation and critical illness (23), co-medication, accumulation of its active metabolites after prolonged use (19) or renal insufficiency (24). None of these factors are currently considered in the dose calculation.

This retrospective analysis of a weaning strategy reflects clinical practice in patients in a complex, intensive care setting. We acknowledge that our study had several limitations. Although COMFORT-B scores were taken regularly, we found that SOS scores were underreported. In addition, the lorazepam dose calculation in some patients was based upon the midazolam dosage rate at the moment of conversion instead of the
cumulative dose of the last 24 hours, resulting in different dosing strategies. Since 2017, a lorazepam extemporaneous oral liquid of 1 mg/ml has been available (25). As a result, oral administration of injection fluid is no longer applied and capsules are no longer used. The dose conversion is now checked by the attending pharmacist. One further limitation was that the concomitant use of other central nervous depressants was common during PICU stays in our study and this hindered the attribution of the observations to the conversion from midazolam to lorazepam.

In the past two decades, considerable progress has been made in recognising the need for weaning-off sedation strategies in PICUs. Risk factors for the development of IWS have been identified and scoring systems have been validated and implemented to monitor the patients. This study was the first to specifically address the use of oral lorazepam in the weaning-off sedation strategy in PICU patients.

CONCLUSION

The weaning protocol for sedatives using lorazepam did not increase the incidence of IWS and appeared to be safe. A better understanding of the factors that explain variations in both pharmacokinetics and pharmacodynamics may help us to further tailor weaning strategies to the individual patient.

ACKNOWLEDGEMENTS

The authors would like to thank Professor D Tibboel for his contributions to the manuscript.
REFERENCES


Summarizing discussion
Children deserve access to medicines that have been specifically developed and researched for use in young patients. The measures that were put in place with the Paediatric Regulation have ensured that paediatric medicine development became an integral part of the overall development of medicines. However, for off-patent medicines, these measures have not been sufficient.

The research combined in this thesis aims to improve paediatric pharmacotherapy by developing a standardised approach for the design and evaluation of pharmacy-compounded oral liquids of off-patent medicines. A multidisciplinary approach was sought with the intention to establish a framework for current and future paediatric formulation development, combining the expertise of pharmacists of the Laboratory of Dutch Pharmacists (LNA) and the Erasmus MC hospital pharmacy, and of clinicians of the Sophia Children's Hospital. Part 1 of this thesis consisted of a general introduction to the topic and an exploration of unmet needs and common practices relating to paediatric formulations in clinical practice. In part 2 we described the formulation development of two compounds chosen to represent both water-soluble and water-insoluble drugs, and for which an unmet need existed in paediatric practice. We presented in vitro methods to simulate in vivo performance of the developed liquids. In part 3 the results of the clinical studies were presented, in which the developed liquids were evaluated in both adult volunteers as well as the paediatric target population.

**MAIN FINDINGS**

**Part 1: A large gap still exists between paediatric needs and the availability of medicines with an age-appropriate formulation. Pharmacy-compounded, unlicensed formulations remain essential to fulfil these needs. Manipulation of oral dosage forms is common practice and there is a need for improvement of information provision regarding manipulation towards parents/caregivers.**

Part 1 of this thesis was funded by the Royal Dutch Pharmacists Association as part of the research programme of 2017. We identified the issues surrounding paediatric formulations in daily clinical practice, with the aim to guide future paediatric formulation development, and improve current information provision to parents and healthcare professionals regarding paediatric drug administration. Based on the dispensing data of the Sophia Children’s Hospital, we identified a profound gap in the availability of age-appropriate formulations, especially for neonates and infants at the intensive care, for which 42% of the dispensed products were considered unsuitable, according to the acceptability matrix from the ‘Reflection paper on formulations of choice for the paediatric population’ (1). Our data show that pharmacy compounding in the treatment of paediatric patients remains essential, as more than half of the dispensed products did not have a marketing authorisation. A survey across Dutch paediatric hospital pharmacies revealed that the use of pharmacy-compounded products was widespread, and that almost half of the most commonly used compounded products in the Netherlands were not included in the EMA inventory of paediatric needs.

As part of the suitability assessment, exposure to potential toxic excipients was calculated based upon dosage and excipients concentration and compared with EMA limits for safe exposure. We found that possible toxic exposure was not limited to only neonatal ICU patients, but was relevant in children up to the age of four years. Efforts should be made to reduce the exposure to potentially harmful excipients, by avoiding or substituting non-
essential medicines, and improving the composition of essential medicines.

In chapter 3 we identified the problems in drug administrations to children, experienced by both parents/caregivers, as well as by nurses, by determining the extent, reasons and methods used for drug manipulation. The gap in availability of age-appropriate formulations was reflected in the results from this chapter. Manipulation of oral dosage forms was common practice among both parents/caregivers as well as nurses in a paediatric hospital, with a similar prevalence of 30% in the outpatient setting versus 37% in the inpatient setting. Manipulation by parents/caregivers occurred mainly to achieve taste and dose adjustment, whilst nurses most often used manipulation for administration through a feeding tube and size reduction. This difference probably results from the more extensive formulary of the inpatient pharmacy, which allows for more precise dosing with compounded liquids and capsules of different strengths, and the higher prevalence of feeding tubes in the inpatient setting.

The most unexpected result from the survey described in chapter 3 was the low dissemination of information regarding the correct method of manipulation from the pharmacy towards both parents/caregivers and nurses. Even though this information is available to pharmacies in the Dutch reference work Oralia VTGM, and within the hospital to the nurses through every workstation, only half of the interviewed parents/caregivers stated to have received their information from the pharmacy, and only 28% of the nurses consulted the pharmacy-provided information. Aside from this finding, many of the recommendations in the Oralia VTGM are based on practical experience, rather than research.

Part 2 The concept to develop two types of formulations, for water-soluble and water-insoluble drug compounds, appeared fertile for improving the availability of age-appropriate, paediatric formulations for off-patent drugs. Amlodipine and lorazepam can be compounded into stable oral clear solutions using simple techniques and safe excipients.

The second part of this thesis presented results from the ZonMw project that aimed to integrate pharmaceutical development of paediatric formulations and the consecutive clinical testing in the target population. Because of the need for flexible dosing and ease of administration, oral liquids were the preferred dosage form to be developed, and amlodipine and lorazepam were chosen to serve as proof of concept, and because of the unmet need in paediatric practice. As evidenced by chapter four and five of this thesis, the close cooperation with the LNA resulted in two feasible new formulations, both using safe and readily available excipients, requiring simple compounding techniques, and providing good stability when stored refrigerated.

Next to the pharmaceutical development, we explored the use of biopharmaceutical methods to predict in vivo performance of medicines in paediatric populations, facilitated by the University of Bath. With experiments designed to reflect clinical practice in the Sophia Children’s Hospital, the impact of patient related factors on drug performance was studied, using drug solubility in paediatric biorelevant media and biorelevant dissolution. Ideally, these in vitro predictive methods, combined with in silico models, will in the future replace in vivo experiments and clinical trials in paediatric patients.
Part 3 The theoretical approach from part two resulted in clinically useful formulations. The amlodipine oral solution is bioequivalent to amlodipine tablets, and both the amlodipine and lorazepam oral solutions provide high oral bioavailability.

The third part of this thesis consists largely of clinical research. As part of the ZonMw project, both formulations were further studied in the target population to assess pharmacokinetic parameters, safety issues and acceptability. For amlodipine, we chose to first compare the performance of the oral solution to originator tablets in adult volunteers in a bioequivalence study. As expected, the oral solution and tablets were bioequivalent, with only a statistically different time to maximum concentration. With the slow and passive absorption of amlodipine, this difference is expected to have no clinical relevant effect on blood pressure control. The consecutive population pharmacokinetic study in paediatric patients confirmed the oral solution to be a good treatment option for younger paediatric patients with adequate acceptability. The population pharmacokinetic study of the lorazepam oral solution in paediatric intensive care patients was the first study to evaluate oral lorazepam in paediatric patients. Using a population pharmacokinetic approach and non-linear mixed effects modelling, we demonstrated high oral bioavailability of 80% for the lorazepam oral solution.

METHODOLOGICAL CONSIDERATIONS

Strengths and limitations

The major strengths of the studies included in this thesis relate to

- the large datasets collected at the Sophia Children’s Hospital, representing the entire paediatric age range and all major and minor specialties
- the multidisciplinary approach, combining the expertise of pharmacist and paediatricians, and based on clinical practice of the largest paediatric hospital of The Netherlands
- the conformity of the results of the clinical trials with our expectations and available literature

One of the main strengths of this thesis generates from the multidisciplinary approach, which ultimately resulted in the development of two paediatric oral solutions, which are supported by clinical data from the target population, and can be considered standard of care following incorporation into the Formulary of Dutch Pharmacists (FNA). The composition, method of preparation and shelf-life make both oral solutions suitable for large-scale production as well as extemporaneous compounding. The formulation design and validation was supported by the experts of the LNA, and the Department of Pharmaceutical Technology and Biopharmacy of the University of Groningen. The collaboration with the University of Bath showed that in vitro biopharmaceutical tools can be useful for studying drug performance in children. The straightforward experimental setups make it possible to address numerous different administration scenarios, which would not be feasible or ethical in pharmacokinetic studies in children.
The clinical phase of the ZonMw project was designed to perform patient-based research in the target paediatric population, aiming to elucidate pharmacokinetic, acceptability and safety parameters of the developed oral solutions. Both paediatric trials were designed in close collaboration with the clinicians, and the lorazepam trial profited from the well-established clinical research structure of the paediatric intensive care unit. We were able to include patients in a difficult setting, and as young as 4 weeks old. Furthermore, the clinical trial results were in accordance with our expectations based on the physical-chemical characteristics of the compounds and previously reported studies in both adults and children.

The most important general limitations of the studies included in this thesis relate to

- gaps in the knowledge base regarding acceptability of medicines to paediatric populations
- a knowledge gap concerning gastro-intestinal physiology in paediatric patients, limiting the predictive value of the biopharmaceutical in vitro experiments
- due to refusal of the parents (amlodipine) and absence of an arterial line (lorazepam), inclusion rates in the paediatric trials were low

Guidance issued by the European Medicines Agency states that patient acceptability must be an integral part of paediatric formulation development and be described in the paediatric investigation plan (PIP) (2), but before this guidance came into effect in 2014, there was no requirement for medicines to be demonstrated to be acceptable to children. The evidence base concerning what is acceptable to paediatric patients is therefore limited and standard methods or criteria that define what is considered acceptable have not been determined (3). The suitability assessment in chapter 2 is based on the acceptability matrix from the ‘Reflection paper on formulations of choice for the paediatric population’ by the EMA (1), but the matrix was based on expert opinion rather than sound scientific evidence, which limits the validity of the results.

The solubility and dissolution experiments presented in chapter 6 explore biopharmaceutical tools that can be used to predict in vivo drug performance. Ideally, the results obtained from in vitro dissolution experiments would be integrated into more complex in silico prediction models. This physiologically based pharmacokinetic (PBPK) modelling and simulation is already commonly used in formulation development/bridging for adult medicines and provides a promising tool for paediatric in vivo drug performance prediction, provided we gain a better understanding of the developmental changes of the gastrointestinal tract in the paediatric population (4). Furthermore, validation of the biopharmaceutical methods requires rich PK data, which are often not available.

In the lorazepam trial, removal of the arterial line to prevent infection and/or discharge to the general ward often resulted in eligible patients not participating in the study. For the amlodipine trial, refusal by the parents due to the burden of study procedures was common. This led to lower than expected inclusion rates, which is commonly referred to as Lasagna’s Law, where “the incidence of patient availability sharply decreases when a clinical trial begins and returns to its original level as soon as the trial is completed” (5).
Study endpoints and feasible trial design

Initially, the amlodipine paediatric trial was designed to compare formulation performance of tablets and our oral solution. From *in vitro* studies and adult data we already knew that the oral pharmacokinetics of amlodipine are minimally influenced by the dosage form (6), which was confirmed in our bioequivalence study in healthy adults. Also, ICH E11 clearly states that relative bioavailability comparisons of paediatric formulations with the adult oral formulation should be done in adults, unless the drug is unsafe in healthy volunteers, the PK of the compound is different in patients, or the PK of the compound is different in children (7). Since amlodipine is absorbed by slow passive diffusion across the intestinal membrane, differences in intestinal drug absorption between adults and paediatric patients are unlikely. A comparison of formulation performance in paediatric patients was therefore in hindsight not indicated. With an amendment, we changed the focus of the trial to elucidation of the pharmacokinetic parameters of amlodipine in children, with secondary endpoints regarding acceptability, pharmacodynamics (blood pressure) and clinical covariates, but ultimately only acceptability was a formulation specific outcome. During the conduct of the study, it became clear that the study procedures and switching to study medication were considered a burden to many of the eligible patients and parents, and were reasons not to participate in the trial. Consequently, inclusion of study participants did not reach the goal of 20 patients.

Pharmacokinetic data of amlodipine in children under the age of six years are still warranted, but are formulation independent, which we were not aware of at the start of the project. This provides the opportunity to collect them in less invasive manner, for instance, from renal transplant patients that regularly undergo blood sampling for therapeutic drug monitoring of immunosuppressants. To collect pharmacokinetic data from the youngest patients, study procedures could be limited to collection of capillary blood samples, which is for many patients a regular procedure with a low burden.

The lorazepam trial was well designed for its purpose of determining oral bioavailability, which we accomplished with inclusion of only eight patients. Even though there were no indications that lorazepam would perform different in paediatric patients compared to adults, we have now confirmed this in a relatively non-invasive trial in the relevant population. From personal experience, inclusion rates could have been improved with a slightly different approach, which was implemented with the second study amendment. Introducing the study to the parents became easier when the lorazepam oral solution became standard of care and replaced the previously compounded 0.1 mg capsules. Initially, the study was designed to include only patients who were yet to start with lorazepam, but this was actually no requirement for the determination of oral bioavailability when using non-linear mixed effects modelling. The single administration of an intravenous dose was no objection for any of the parents. Unfortunately, the presence of an arterial line proved to be essential for the successful collection of blood samples, and was a factor we could not influence. It shows how complicated clinical research in paediatric patients can be. The acceptability of the oral solution could not be assessed in this population, as all patients received it through a feeding tube. It is expected that the formulation will incidentally be applied in the outpatients setting, where this formulation property will become more relevant.
RECOMMENDATIONS

Recommendations for practice

Even though no immediate risks were identified in the survey regarding manipulation, pharmacist should improve their efforts in proactively informing parents/caregivers about drug manipulation and administration, and this should include both verbal as well as written information. The Royal Dutch Pharmacists Association could support this effort with the development of patient-oriented, generic information leaflets regarding manipulation techniques, most importantly dose adjustment of solid dosage forms.

Recommendations for policy

As shown in the amlodipine trial, the availability a suitable formulation can greatly improve the ease of drug administration to children, and subsequently, have an influence on treatment outcome. It is essential that pharmacists keep investing in the development of suitable formulations for paediatric patients, in collaboration with paediatricians, the LNA and compounding pharmacies within The Netherlands. The special interest group ‘paediatrics’ of the Dutch Association of Hospital Pharmacists should take the lead in this.

The efforts of the European Pharmacopoeia Commission in the compilation of a pan-European Paediatric Formulary should be highly supported. Information collected in the Formulary of Dutch Pharmacists could be valuable. Further financial support from the European Union could accelerate the efforts and is necessary for standardisation, validation and filling the gaps in information, and would, in our opinion, be well-spent.

Standard methods or criteria that define what is considered acceptable to children have not been determined (3). The approach that was chosen in chapter eight to study the acceptability of the amlodipine oral liquid is generally considered suitable, but the lack of standardisation makes comparing results difficult. A lack of knowledge about what is currently considered to be acceptable to paediatric patients hinders the development of acceptable, age-appropriate medicines. Therefore, EMA guidance on how to perform and interpret acceptability studies in paediatric patients is highly warranted.

Recommendations for future research

The oral solutions presented in part 2 of this thesis were meant to serve as proof of concept, and the drug substances were chosen to represent water-soluble and water-insoluble compounds. The approach that was chosen to process the poorly water-soluble lorazepam, using a mixture of organic solvents, should be tested for other drug substances with poor aqueous solubility. The readily available and cheap excipients, and the relatively easy compounding method, could possibly provide a solution for a large range of difficult to process drug substances. Compound selection should focus on BCS class II and class IV drugs.

The paediatric population remains a difficult population to study. Clinical trials are expensive, and resources should be allocated wisely. Many trials fail or are not completed, and the reasons for that are several (8). It is very likely that paediatric drug development will benefit from European collaboration, as envisioned by the Connect4Children collaborative network for European clinical trials for children, which aims to generate a
sustainable infrastructure that optimises the delivery of clinical trials in children.

In vitro biopharmaceutical techniques, combined with in silico models, have the potential to replace in vivo experiments and clinical trials, but there is still a knowledge gap concerning GI physiology in paediatric patients. Aside from the factors influencing in vivo dissolution, specific research is still required on the factors influencing permeability, mainly the ontogeny of metabolizing enzymes and drug transporters, to better predict oral drug absorption in this population. Access to existing paediatric rich pharmacokinetic data is required to validate the biopharmaceutical tools.

In this thesis we have shown that for off-patent medicines, for which there is no economics basis for licensing, pharmacy compounding may offer a highly feasible solution to provide acceptable and dose flexible pharmacotherapy for children.
REFERENCES


Samenvatting
Geneesmiddelonderzoek bij kinderen heeft lange tijd te weinig aandacht gehad. Tot ver in de 20e eeuw was men van mening dat kinderen niet zouden mogen deelnemen aan klinisch geneesmiddelonderzoek, met name vanwege ethische bezwaren. Tegenwoordig is de algemene consensus dat kinderen recht hebben op toegang tot geneesmiddelen die specifiek voor hen ontwikkeld en onderzocht zijn. Dat neemt niet weg dat er nog talloze obstakels te overbruggen zijn. Met name de heterogeniteit binnen de vaak toch al kleine studiepopulatie, maakt het opzetten van goede kindergeneesmiddelonderzoeken een uitdaging. Daarnaast maken de veelal kleine doelpopulaties het economisch onaantrekkelijk voor bedrijven om te investeren in geneesmiddelregistraties specifiek voor kinderen.

Om dit probleem aan te pakken werd, in navolging van de Verenigde Staten, in 2006 in de Europese Unie de Paediatric Regulation aangenomen, wat ertoe heeft geleid dat in de jaren 2007-2016 meer dan 260 nieuwe geneesmiddelen en indicaties voor gebruik door kinderen zijn goedgekeurd door de European Medicines Agency (EMA). Farmaceutische bedrijven beschouwen de ontwikkeling van kindergeneesmiddelen nu als integraal onderdeel van het ontwikkeltraject van een geneesmiddel. Hier tegenover staat dat de ontwikkeling van middelen die uit patent zijn, is achtergebleven.

Er zijn veel ‘oudere’ geneesmiddelen die een belangrijke plaats hebben in de behandeling van kinderen, maar vaak is hiervan geen geschikte toedieningsvorm beschikbaar. Met name de acceptatie door de patiënt (o.a. op basis van smaak) en de dosisflexibiliteit vormen vaak een probleem. Apothekers kunnen in een dergelijk geval zelf een geneesmiddel bereiden, zogenaamde magistrale bereidingen. Dit heeft vaak de voorkeur boven het manipuleren van bestaande toedieningsvormen, zoals het vermalen van tabletten, of toediening met dranken of voeding. Magistrale bereidingen worden in Nederland meestal volgens standaardvoorschriften gemaakt (Formularium der Nederlandse Apothekers (FNA)) hoewel dat niet verplicht is. FNA-voorschriften worden farmaceutisch-technisch uitgebreid onderzocht en wanneer deze onder de juiste omstandigheden worden bereid kan de kwaliteit gegarandeerd worden. Buiten het FNA worden er echter nog talloze niet-gestandaardiseerde bereidingen toegepast die qua samenstelling sterk kunnen verschillen tussen de verschillende kinderziekenhuizen. Het is de vraag of het ontwerp van deze producten optimaal is voor toepassing bij kinderen.

**Deel 1 Kinderformuleringen in de dagelijkse klinische praktijk**

In hoofdstuk 2 van dit proefschrift hebben we in kaart gebracht welke plaats de apotheekbereiding inneemt in de behandeling van klinische patiënten van het Erasmus MC Sophia Kinderziekenhuis. Met name neonaten, zowel prematuur als aterm geboren, werden vaak behandeld met eigen bereidingen, die meer dan de helft van de afgeleverde geneesmiddelen vormden. Ook werd duidelijk dat er in Nederland veel eigen bereidingen worden toegepast die niet in de EMA inventory of paediatric needs zijn opgenomen, terwijl deze middelen dus kennelijk wel nodig zijn. Naast de focus op apotheekbereidingen hebben we in dit hoofdstuk ook onderzocht hoe groot de blootstelling aan potentieel schadelijke hulpstoffen was bij klinische patiënten, met een focus op vloeibare geneesmiddelen. Hieruit bleek dat er verbeteringen te behalen vielen door middel van substitutie van bepaalde producten en het verbeteren van de samenstelling van bepaalde eigen bereidingen.

Als er geen goede toedieningsvorm beschikbaar is, wordt vaak teruggevallen op
manipulatie van de toedieningsvorm door ouders en/of zorgverleners, bijvoorbeeld door tabletten te vermalen, ze op te oplossen, te breken of ze vermengd met melk of eten toe te dienen. De consequenties van het manipuleren op de effectiviteit en veiligheid van het geneesmiddel zijn niet duidelijk of soms zelfs bewezen schadelijk. Uit het onderzoek beschreven in hoofdstuk 3 onder poliklinische patiënten in het Erasmus MC Sophia Kinderziekenhuis bleek 45% van de ondervraagde ouders orale medicatie te manipuleren voor toediening. In de praktijk zijn instructies aan ouders over manipulatie-mogelijkheden vaak beperkt, niet uniform en veelal niet goed onderbouwd. Eén van de aanbevelingen die uit dit proefschrift volgen is dan ook om deze informatievoorziening door apothekers te verbeteren, en waar nodig deze informatie ook te genereren.

Deel 2 Farmaceutische ontwikkeling en in vitro evaluatie

Er is een grote behoefte aan goed onderzochte, kindvriendelijke, orale geneesmiddelen, die bij voorkeur een grote dosisflexibiliteit hebben. In het kader van het programma Priority Medicines voor Kinderen heeft ZonMW hiervoor een subsidie verstrekt, waarmee de ontwikkeling van twee dranken is bekostigd. Uitgangspunt was hierbij dat de formuleringen toepasbaar zouden zijn voor meerdere geneesmiddelen. Amlodipine en lorazepam zijn vervolgens gekozen als modelstoffen voor water-oplosbare en niet water-oplosbare geneesmiddelen.

In samenwerking met het Laboratorium der Nederlandse Apothekers (LNA) werd gestart met de farmaceutische ontwikkeling van twee dranken, rekening houdend met de beperkte hoeveelheid hulpstoffen die veilig gebruikt kunnen worden en specifieke aspecten zoals smaak (acceptatie). Om doseerfouten van potente middelen te voorkomen gaat de voorkeur uit naar een heldere drank boven een suspensie, omdat bij een suspensie omschudden nodig is voor dosis homogeniteit. In de praktijk zijn ernstige fouten voorgekomen bij toepassing van inhomogene suspensies. Op basis van de fysisch-chemische eigenschappen (oplosbaarheid, pKa) van het geneesmiddel is gekeken welke oplosvloeistoffen mogelijk waren, welke pH nagestreefd moest worden en welke hulpstoffen daarbij noodzakelijk waren. Vervolgens is houdbaarheidsonderzoek uitgevoerd met gevalideerde analysemethodes. Dit heeft uiteindelijk geresulteerd in de ontwikkeling van een amlodipinedrank van 0,5 mg/ml (hoofdstuk 4) en een lorazepamdrank van 1 mg/ml (hoofdstuk 5).

Naast de ontwikkeling van de twee dranken is in samenwerking met de Universiteit van Bath onderzoek gedaan naar in vitro modellen die de blootstelling aan orale geneesmiddelen bij kinderen kunnen voorspellen. Hierbij is de vrijgifte van twee geneesmiddelen onderzocht in nagebootste vloeistoffen uit het maagdarmkanaal. Hiermee kan een voorspelling worden gedaan over de uiteindelijke blootstelling bij toediening aan patiënten. Het is de bedoeling dat, in de toekomst, deze modellen het in vivo onderzoek bij kinderen grotendeels overbodig maken.

Deel 3 Klinische toepassing van de formuleringen

Om de blootstelling aan twee verschillende varianten van hetzelfde geneesmiddel te vergelijken wordt bio-equivalentieonderzoek bij volwassen vrijwilligers uitgevoerd. De farmacokinetische parameters area under the curve en de maximale plasmaconcentratie na een eenmalige dosis van het onderzoeksmedicijn en een referentiemiddel worden vergeleken, als het verschil binnen bepaalde grenzen valt worden de middelen
beschouwd als bio-equivalent. Uit de bio-equivalentiestudie met amlodipine beschreven in hoofdstuk 7 bleek dat de drank en tabletten gelijkwaardig waren. Voor lorazepam is geen bio-equivalentieonderzoek uitgevoerd, omdat er geen relevant, bij kinderen toegepast product was om mee te vergelijken. Gezien de fysisch-chemische eigenschappen van lorazepam is er ook geen groot verschil te verwachten tussen verschillende producten.

De volgende fase was de toepassing van de dranken bij kinderen, waarbij farmacokinetiek (PK), farmacodynamiek (PD), bijwerkingen en de acceptatie in kaart gebracht werden. Voor amlodipine werd onderzoek uitgevoerd bij patiënten (6 maanden-11 jaar) met hypertensie, voor lorazepam bij kinder-IC-patiënten (0-11 jaar). Alle geïncludeerde patiënten gebruikten het geneesmiddel om klinische redenen.

Met software om patiëntendata te modelleren (Non-lineair Mixed Effects Modeling, NONMEM®) was het mogelijk om ook met beperkte datasets en wisselende bloedafnametijdstippen resultaten te genereren. Deze lieten zien dat beide dranken voorzagen in adequate bloedspiegels, er werden geen ernstige bijwerkingen waargenomen gerelateerd aan de dranken en ze werden goed geaccepteerd door de doelgroep. Bij de lorazepamstudie was de doelgroep een kwetsbare, instabiele groep op de IC met veel co-morbiditeit en co-medicatie, maar ouders bleken toch open te staan voor deelname van hun kind aan onderzoek.

De laatste jaren wordt het belang van goede toedieningsvormen van geneesmiddelen voor kinderen steeds meer erkend. Apotheekbereidingen spelen hierbij een belangrijke rol vanwege het ontbreken van handelsproducten. Optimalisatie en standaardisatie van deze bereidingen is noodzakelijk uit oogpunt van kwaliteit. Bij de ontwikkeling moet aandacht zijn voor dosisflexibiliteit en de geschiktheid voor neonaten en jonge kinderen, met name ten aanzien van hulpstoffen. Een samenwerking tussen kinderartsen en apothekers is hierbij belangrijk om de behoefte in de klinische praktijk adequaat te kunnen invullen. In dit onderzoek heeft dat geleid tot de succesvolle ontwikkeling en toepassing van amlodipine- en lorazepamdrank bij kinderen.
Summary
Drug development for children has long been a neglected area compared to adult drug development. Until late into the 20th century, the general view was that children should not participate in clinical trials, particularly because of ethical concerns. Today, the general consensus is that children are entitled to medicines that have been specifically developed and researched for them. Nevertheless, many barriers still remain. In particular, the heterogeneity within the already very small study population makes setting up good paediatric drug researches a challenge. In addition, the mostly small target populations make it economically unattractive for companies to invest in drug registrations specifically for children. To address these issues, the Paediatric Regulation was adopted in the European Union in 2006, leading to more than 260 new medicines and indications for use by children approved by the European Medicines Agency (EMA) in 2007-2016. Pharmaceutical companies now consider the development of paediatric medicines as an integral part of the development process of a medicine. On the other hand, the development of off-patent medicines lags behind.

There are many ‘older’ medicines that have an important place in the treatment of children, but often no suitable dosage form is available. In particular, acceptance by the patient (e.g. based on taste) and dose flexibility are a problem. Pharmacists can in such a case compound a medicine, so-called magistral preparations. This is often preferred over manipulating existing dosage forms, such as grinding of tablets, or administration with drinks or food. In the Netherlands, magistral preparations are usually made according to standard instructions (Formulary of Dutch Pharmacists (FNA)), although this is not mandatory. FNA products are extensively studied regarding pharmaceutical quality, and when they are prepared under the right conditions the quality can be assured. However, numerous non-standard preparations are still being used outside the FNA, which differ greatly in composition between the different children’s hospitals. The question is whether the design of these products is optimal for application in children.

Part 1 Paediatric formulations in daily clinical practice

In chapter 2 of this thesis we demonstrated the importance of pharmacy preparation in the treatment of clinical patients at the Erasmus MC Sophia Children’s Hospital. In particular, neonates, born prematurely and term, were often treated with pharmacy preparations, which accounted for more than half of the medicines dispensed. It also became clear that many pharmacy preparations used in the Netherlands are not included in the EMA inventory or paediatric needs, while these medicines are obviously needed. In addition to the focus on pharmacy preparations, in this chapter we also investigated the extent of exposure to potentially harmful excipients in clinical patients, with a focus on liquid medicines. This showed that improvements could be achieved by substituting certain products and improving the composition of certain pharmacy preparations.

If a suitable dosage form is not available, parents and/or caregivers often rely on manipulation of the dosage form, for example by grinding tablets, dissolving them, breaking them or mixing them with milk or food. The consequences of manipulating on the effectiveness and safety of the drug are not clear, or even proven to be harmful. From the research described in chapter 3 among outpatients at the Erasmus MC Sophia Children’s Hospital, 45% of the parents participating in the questionnaire indicated to manipulate oral medication for administration. In practice, instructions to parents about manipulation options are often limited, not uniform and often not well substantiated. One of the recommendations that follows from this thesis is therefore to improve this
information provision by pharmacists and, where necessary, to generate this information.

Part 2 Pharmaceutical development and in vitro evaluation

There is a great need for well-studied, child-friendly, oral drugs, which preferably have a large dose flexibility. Under the ZonMW Priority Medicines program for children, ZonMW has provided a subsidy, which has funded the development of two liquid formulations. The starting point was that the composition of the formulations would be suitable for several drugs. Amlodipine and lorazepam were then chosen as model compounds for water-soluble and non-water-soluble drugs.

In collaboration with the Laboratory of Dutch Pharmacists (LNA), the pharmaceutical development of two liquid formulations was started, taking into account the limited amount of excipients that can be safely used, and specific aspects such as taste (acceptance). In order to prevent dosing errors of potent agents, preference is given to a clear liquid over a suspension, because in a suspension shaking is necessary for dose homogeneity. In practice, serious errors have occurred with the use of inhomogeneous suspensions. On the basis of the physicochemical properties (solubility, pKa) of the compounds, we examined which solvents were possible, which pH had to be sought and which excipients were necessary. Subsequently, stability testing was performed using validated analysis methods. This ultimately resulted in the development of an amlodipine oral solution of 0.5 mg/ml (chapter 4) and a lorazepam oral solutions of 1 mg/ml (chapter 5).

In addition to the development of the two liquids, in collaboration with the University of Bath research was done into in vitro models that can predict the exposure to oral medicines in children. Here, the release of two drugs was investigated in simulated fluids from the gastrointestinal tract. This allows a prediction to be made about the drug exposure when administered to patients. The intention is that, in the future, these models will largely replace in vivo research in children.

Part 3 Clinical application of the formulations

To compare the exposure to two different variants of the same drug, bioequivalence testing is performed in adult volunteers. The pharmacokinetic parameters area under the curve and the maximum plasma concentration after a single dose of the study drug and a reference product are compared, and if the difference falls within certain limits, the products are considered bioequivalent. The bioequivalence study with amlodipine described in chapter 7 showed that the oral solution and tablets were equivalent. For lorazepam, no bioequivalence study was performed, because there was no relevant product used in children to compare with. Given the physical-chemical properties of lorazepam, no major difference can be expected between different products.

The next phase was studying the formulations in paediatric patients, in which pharmacokinetics (PK), pharmacodynamics (PD), side effects and acceptance were investigated. For amlodipine, a study was conducted in patients (6 months -11 years) with hypertension, for lorazepam in paediatric intensive care patients (0-11 years). All included patients used the drug for clinical reasons.

With software to model patient data (Nonlinear Mixed Effects Modeling, NONMEM®), it was possible to generate results with limited data sets and changing blood sampling
times. These showed that both liquids provided adequate blood levels, no serious side effects were observed related to the study drug and the liquids were well accepted by the target group. In the lorazepam study, our patients were vulnerable, sometimes unstable ICU patients with a lot of co-morbidity and co-medication, but parents turned out to be open to participation of their child in research.

In recent years, the importance of suitable dosage forms for children has been increasingly recognized. Pharmacy preparations play an important role here because of the lack of commercial products. Optimization and standardization of these preparations is necessary to guarantee good quality. Dose flexibility and the suitability for neonates and young children, particularly with regard to excipients, should be considered in the development of formulations for children. A collaboration between paediatricians and pharmacists is important in order to adequately fill the need in clinical practice. This research has led to the successful development and application of amlodipine and lorazepam liquid formulations in children.
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Merel van Nuland  Department of Pharmacy, Erasmus MC, University Medical Center Rotterdam, The Netherlands

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Brenda C.M. de Winter  
Department of Pharmacy, Erasmus MC, University Medical Center Rotterdam, The Netherlands
About the author
LIST OF PUBLICATIONS


5. Van der Vossen AC. Marketing Authorisations under Exceptional Circumstances for Oncology Drugs. Ludwig Boltzmann Gesellschaft GmbH. 2013 Feb http://eprints.hta.lbg.ac.at/992/#
## PHD PORTFOLIO

### Courses

<table>
<thead>
<tr>
<th>Course</th>
<th>Month/Year</th>
<th>Workload</th>
<th>ECTS</th>
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<tbody>
<tr>
<td>(UMCU) Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers</td>
<td>Oct 2014</td>
<td>32 hrs</td>
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<tr>
<td>(NIHES) Biostatistics for Clinicians (EWP22)</td>
<td>Feb 2015</td>
<td>25 hrs</td>
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<td>(EUR) Biomedical English Writing and Communication</td>
<td>Mar-May 2015</td>
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<td>(EUR) Research Integrity</td>
<td>Nov 2015</td>
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<tr>
<td>(EUR) Basisdidactiek voor docenten (TtT I)</td>
<td>Jan 2016</td>
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### Seminars and workshops

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<tr>
<td>(Medical Library) Endnote</td>
<td>Aug 2014</td>
<td>4 hrs</td>
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<tr>
<td>(EUR) Open Clinica Training</td>
<td>Sept 2014</td>
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<tr>
<td>(Medical Library) Systematic Literature Retrieval (Pubmed)</td>
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<td>(Medical Library) Systematic Literature Retrieval (Other databases)</td>
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<td>Care for Pharmacy</td>
<td>Mar 2015</td>
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<tr>
<td>(LUMC) Interpreteren van PK/PD studies</td>
<td>Apr 2015</td>
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<td>TULIPS Young Researchers Day</td>
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<tr>
<td>NVT Spring Symposium ‘Pediatric drug development: a field in maturation’</td>
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<td>(EUR) Omgaan met groepen</td>
<td>Apr 2016</td>
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<tr>
<td>(EUR) Basistraining Limesurvey</td>
<td>Jun 2016</td>
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### Teaching

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<tr>
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<th>Duration</th>
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<tr>
<td>(EUR Medicine) Interacties, VO receptschrijven, Antibiotica-profylaxe en Antistolling</td>
<td>2015-present</td>
<td>24 hrs/year</td>
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<tr>
<td>(UU Pharmacy) Introduction to clinical research in children (2/year)</td>
<td>2016/2017</td>
<td>4 hrs/year</td>
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<td>Supervision Master Thesis</td>
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<tr>
<td>Kadir Akçay</td>
<td>Aug 2016 - Feb 2017</td>
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<tr>
<td>Sid Makhan</td>
<td>Feb 2017 – Jul 2017</td>
<td>2</td>
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<tr>
<td>Sandra Buljaç</td>
<td>Sept 2017 - Mar 2018</td>
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### Conferences

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<th>Date</th>
<th>Workload</th>
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<tr>
<td>7th EuPFI Conference Antwerp (Poster)</td>
<td>Sept 2015</td>
<td>32 hrs</td>
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<tr>
<td>14th International Congress of TDM and Clinical Toxicology Rotterdam</td>
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<td>8th EuPFI Conference Lisbon (Poster)</td>
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<td>FIGON/DMD (Poster)</td>
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<td>9th EuPFI Conference Warschau (Poster)</td>
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<tr>
<td>Nederlandse Ziekenhuisfarmacie dagen (Oral)</td>
<td>Nov 2017</td>
<td>24 hrs</td>
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</table>
CURRICULUM VITAE

Annette van der Vossen was born on the 11th of May 1988 in Leidschendam, and grew up in Voorburg, The Netherlands. After graduating from secondary school at Huygens Lyceum in Voorburg in 2006, she started her Pharmacy studies at Utrecht University. She obtained her bachelor’s degree in 2011 and her master’s degree in 2014. During her master’s studies, she spent six months in Vienna, Austria, to perform a research project at the Ludwig Boltzmann Institut für Health Technology Assessment.

Annette started her professional career at the Department of Pharmacy of the Erasmus MC, University Medical Center Rotterdam, were she performed the research presented in this thesis. She was supervised by Promotor prof. dr. A.G. Vulto, and co-promotor dr. L.M. Hanff. During the spring of 2017, she spent three months at the University of Bath, Department of Pharmacy and Pharmacology, for a research visit under supervision of dr. N. Fotaki.

As of July 2018 she is working a the hospital pharmacy of the Maasstadziekenhuis in Rotterdam.