

BIOAVAILABILITY OF VALPROATE SUPPOSITORIES FORMULATED FOR PAEDIATRIC USE

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Abstract

Considering that the rectal route might eliminate the time-dependent changes of valproic acid kinetics and starting from the need to develop suppositories for paediatric use, in our previous studies three types of valproate suppositories were selected for further *in vivo* investigation. In this paper, the experimental pharmacokinetic study conducted on rabbits is presented, in order to evaluate the relevant *in vivo* pharmacokinetic parameters. The parameters obtained after a single rectal dose over a period of 8 h, were analysed in each case by the non-compartmental pharmacokinetic model. The similarity of the three release profiles previously found *in vitro* has not fully confirmed the *in vivo* parameters, which shows that the *in vivo* pharmacokinetic test still remains necessary in case of studies on the development of suppositories formulation.

Rezumat

Având în vedere că administrarea rectală poate elimina modificările dependente de timp ale farmacocineticii acidului valproic și pornind de la necesitatea dezvoltării de supozitoare rectale de uz pediatric, s-au selectat în studii anterioare trei formulări de supozitoare cu săruri ale acidului valproic care au fost propuse în continuare pentru studiul *in vivo*. În această lucrare este prezentat studiul farmacocinetic pe iepuri, în scopul de a evalua parametrii farmacocinetici relevanți *in vivo*. Parametrii obținuți după o singură doză rectală pe o perioadă de 8 ore, au fost analizați în fiecare caz prin modelul farmacocinetic noncompartimental. Similaritatea celor trei profiluri de cedare determinată anterior *in vitro* nu a fost pe deplin confirmată *in vivo*, de unde reiese că testul farmacocinetic *in vivo* rămâne necesar în cazul studiilor de dezvoltare a formulării supozitoarelor.

Keywords: valproic acid, sodium valproate, paediatric suppositories, bioavailability, pharmacokinetic study

Introduction

Anticonvulsant therapy in paediatric practice provides certain challenges due to several particular aspects of the pharmacokinetics and pharmacotoxicology of antiepileptic medication: narrow therapeutic index [12] – usually requiring adjustment of the drug dose according to the pharmacokinetic criterion based on its concentration in the plasma [10]; low compliance – which imposes the need of using products tailored for children [1]; variability in pharmacological response – which requires clinical monitoring of medication [6]; risk of drug interactions [14] – taking into account the intercurrent diseases conditions

and the enzymatic inhibition or induction ability of compounds [8] (e.g. valproic acid and sodium valproate, or carbamazepine and phenobarbital) [2]. Valproic acid is still widely used, at least in Romania and the most commonly orally administered, as an antiepileptic drug and also as a mood stabilizer [3]. Rectal dosing of valproic acid is considered an effective treatment for *status epilepticus* [11], seizure maintenance therapy and neuropathic pain [5]. The use of rectal preparations is highly useful and effective especially in children that are unable to take oral medications because of repeated vomiting, gastrointestinal surgery and *status epilepticus* associated

with lack of venous access [17]. Normal circadian alterations in plasma pharmacokinetics, which occur after the oral administration of valproate in the morning or in the evening, do not occur with rectal dosing [5, 18]. Therefore, the rectal administration may have an advantage to eliminate the time-dependent changes of valproic acid kinetics [18]. All these real advantages justify the studies which aim the formulation of valproate (valproic acid, sodium valproate) in the form of suppositories. Although, such studies have been published since 1980 [7, 9, 16] and even if the results of these studies are still cited as useful alternatives for the oral route [5], there are not yet authorized industrial products on the market [13, 19]. This consequence is due, in our opinion, on one side to the technological difficulties

generated by the properties of the active substance (valproic acid – an oily liquid, sodium valproate – a hygroscopic powder) [9, 13] and on the other side to the difficulties in standardizing a pharmacokinetic model on laboratory animals [16].

In our previous studies, starting from the need to develop suppositories with valproate for paediatric use, 18 formulations have been evaluated with regard to their *in vitro* kinetic release [4]. Three of those formulations were selected to be subjected to our further investigations (Figure 1) based on the following criteria: content of active pharmaceutical ingredient (API) in different form (acid or salt) simultaneously with similar *in vitro* release profiles and fast release kinetics.

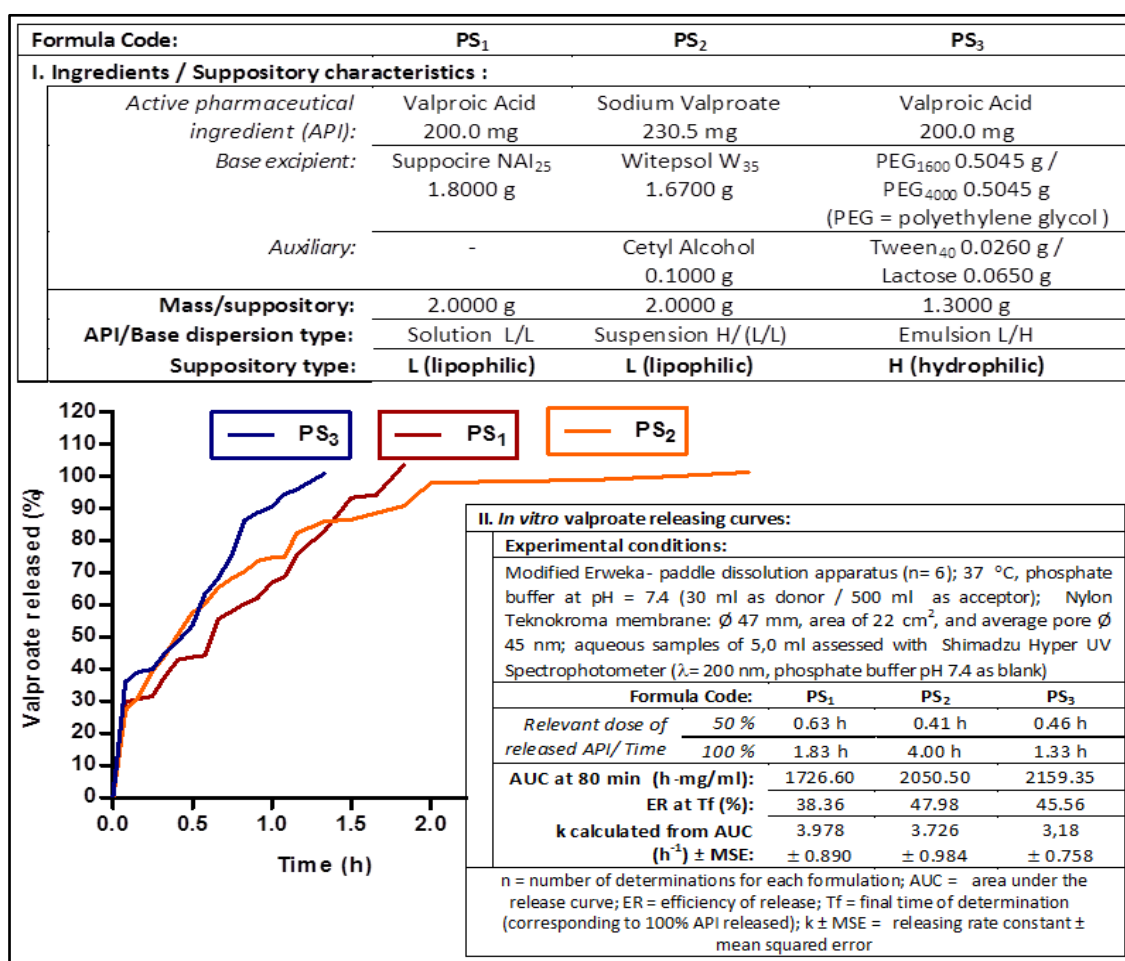


Figure 1.

Paediatric suppositories selected based on previous *in vitro* studies

Considering the practical difficulties and the possible error sources, in this study we present an experimental pharmacokinetic model with the following objectives: to evaluate the bioavailability of the previous selected suppositories formulations on the basis of their relevant *in vivo* pharmacokinetic parameters; to assess the extent to which the *in vitro*

release studies and the *in vivo* pharmacokinetic measurements converge to the same conclusions.

Materials and Methods

Preparation of the suppositories. In order to enable the experimental pharmacokinetic study, it was necessary to adapt the shape and size of previous

selected paediatric formulations to the rabbit rectal administration. For this reason, each suppository formulation was prepared by fusion moulding

method so that it contained 20 mg of valproic acid or the equivalent of sodium valproate.

Table IComposition of suppositories proposed for the *in vivo* study

Formula:		FS ₁	FS ₂	FS ₃
Ingredients/suppository	API:	20.00 mg Valproic Acid (Oakaville, Canada)	23.05 mg Sodium Valproate (Sigma-Aldorich, India)	20.00 mg Valproic Acid (Oakaville, Canada)
	Base excipient:	Suppocire [®] NAI ₂₅ (Gattefossé, France)	Witepsol [®] W ₃₅ (Huls AG Trisdorf, Germany)	Polyethylene glycols - 1:1 PEG ₁₆₀₀ /PEG ₄₀₀₀ (Loba Chemie Wien Fischamend, Austria; Scharlau Chemie, Germany)
	Auxiliary:	-	5% Cetylic Alcohol (BASF Ludwigshafen, Germany)	2% Tween ₄₀ 5% Lactose (BASF Ludwigshafen, Germany)
Mass/suppository:		1.0000 g	1.0000 g	1.0000 g
Suppository type:		Lipophilic	Lipophilic	Hydrophilic

In vivo pharmacokinetic experimental method (in rabbits):

The study was conducted under the Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes [20] and it was approved by the Research Ethics Committee of the University of Medicine and Pharmacy of Tîrgu Mureş, Romania.

Experimental animals. A homogeneous group of 18 female rabbits (each weighing 2.0 ± 0.05 kg) were used and kept according to EU guidelines: in special cages, in a 12 h cycle, appropriate microclimate, having free access to water (*ad libitum*). The rabbits were randomised in 3 groups of 6 rabbits each and they were kept unfed for 12 hours (the night before the start of the experiment), but having free access to water (*ad libitum*).

Experimental protocol. Drug administration was carried out rectally, for each rabbit, in a single dose of 20 mg (10 mg/kg b.w.) equivalent of valproic acid dose. Immediately after administration, the anus of

each animal was blocked using an adhesive patch for 1 hour in order to avoid leakage of the melted suppository base containing the active substance following the start of the defecation reflex as a result of rectal irritation and to prevent the oral absorption of suppositories, taking into account the fact that the rabbits are coprophagic animals. Blood samples were taken from the marginal vein of the ear, 1 mL of blood per sampling time as follows: 0 (blank); 0.5; 1; 1.5; 2; 4; 8 hours.

Preparation of plasma samples. After the harvesting in polypropylene tubes pre-treated with K₃EDTA, the blood was immediately centrifuged in a refrigerated centrifuge (at 4°C), at 3500 rpm for 10 minutes. The resulting plasma was transferred to polypropylene tubes and stored at -20°C until analysis.

Analysis of plasma samples was performed by a HPLC (LC/MS) method [15], according to the experimental conditions which are mentioned in Table II.

Table II

Experimental conditions of HPLC valproate determination

Extraction procedure:	
0.2 mL of plasma sample were transferred into polypropylene tube; 0.6 mL of methanol were added and the sample was mixed for 10 seconds using vortex mixer; after 10 minutes, it was centrifuged for 10 minutes at 10,000 rpm and 200 µL of supernatant were transferred into the chromatographic vials; 2 µL of supernatant were injected into the liquid chromatography system; quantification was carried out by the peak area method.	
Optimized HPLC-MS system parameters [15]:	
LC separation:	MS detection:
- instrument: Agilent 1100 Series HPLC - column: Zorbax SB-C18 (100 x 3 mm, 3.5 µm) - mobile phase: A: Acetic Acid 0.1% B: Acetonitrile A:B = 60:40 - analysis conditions: 4.0 min (time); 1.0 mL/min (flow); 45°C (temp); 2 µL (injected volume).	- instrument: Agilent 6410 Series Triple Quad LC/MS - ionization: ESI, negative - detected ions: mode MS2 SIM (143.1 m/z derived from 144.2 m/z valproic acid); unit resolution; fragmentation 100; Dwell 200; - source parameters: 50 psi (nebulizer pressure); 10 L/min (gas flow); 350°C (drying temperature); 4000 V (voltage).

Pharmacokinetics

The primary bioavailability parameters (peak plasma concentration of valproic acid – C_{max} , peak concentration time – T_{max} and plasma concentration – time curve) were obtained from the plasma concentration of valproic acid measured after administration. The area under the plasma concentration – time curve (AUC_{tot}) was calculated according to the trapezoidal rule for the observed blood levels from 0 to 8 h after administration. To obtain the auxiliary parameters ($T_{1/2}$ – the half-life; MRT – the mean residence time – the average time the molecule remains in the body; $AUC_{0 \rightarrow \infty}$ – an extrapolated value which according to the rules for bioanalytical assays, may not exceed 20% in order to be accepted [21]), plasma level data were analysed by the non-compartmental model, using Kinetica (Thermo Electron Corporation) as specific pharmacokinetic software.

Statistical analysis. The descriptive statistics analysis were performed by the ANOVA and Turkey-Kramer *post hoc* test, and the differences were assumed to be significant when $p < 0.05$.

Results and Discussion

Development of the pharmaceutical formulations.

Three formulations of suppositories (coded of “F series”) containing 20 mg of valproic acid or the equivalent of sodium valproate/suppository (Table I) in different suppository bases were prepared so as to be possible to administer rectally the dose of 10 mg/kg to rabbits. Their composition is similar to the suppositories for children (coded of “P series”)

selected previously based on their *in vitro* release characteristics (Figure 1). Therefore, the suppositories FS₁ (similar to PS₁) contain valproic acid dissolved in Suppocire NAI 25 as lipophilic excipient (a semi-synthetic glyceride base comprising saturated C12-C18 triglyceride fatty acids with melting range of 33°C to 35°C and a hydroxyl index of 20); the suppositories FS₂ (similar to PS₂) contain sodium valproate dispersed in Witepsol W₃₅, also a lipophilic excipient (fatty acids with hydroxyl values of 40 - 50 and melting point in range of 33.5 - 35.5°C, less sensitive to shock cooling) with 5% cetyl alcohol as lipophilic emulsifier agent (hydroxyl values 228 - 233 and melting point of 47 - 50°C, HLB1-2); the suppositories FS₃ (similar to PS₃) contain valproic acid dispersed in polyethylene glycol – 1:1 PEG₁₆₀₀ (hydroxyl values of 68 - 82)/ PEG₄₀₀₀ (hydroxyl values of 30 - 36) as hydrophilic excipient, with 2% Tween 40 as hydrophilic emulsifier agent (hydroxyl values of 85 - 100, HLB = 15.6) and 5% lactose monohydrate (as absorbent powder).

Besides their role in the technological development of suppositories, both the hydroxyl groups of the excipients and especially the emulsifiers could also act as promoters of the biological absorption [7].

The experimental results of the in vivo pharmacokinetic study. The primary data of the pharmacokinetic study obtained for each rabbit (Figures 2-4) were determined as follows: area under the curve vs. time after administration of a single suppository, maximum plasma concentration (peak) of valproic acid (C_{max}) and the peak concentration time (T_{max}).

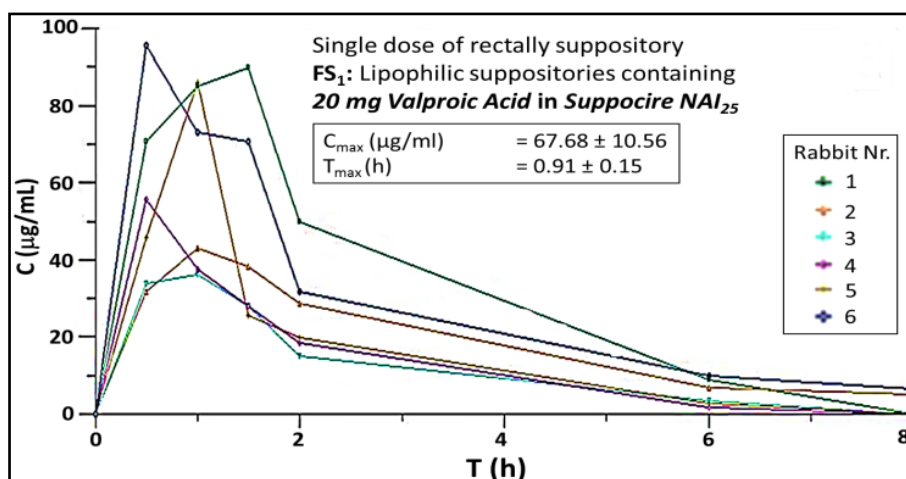


Figure 2.

Valproic acid concentration determined in rabbits after rectal administration of FS₁ in a dose of 10 mg/kg

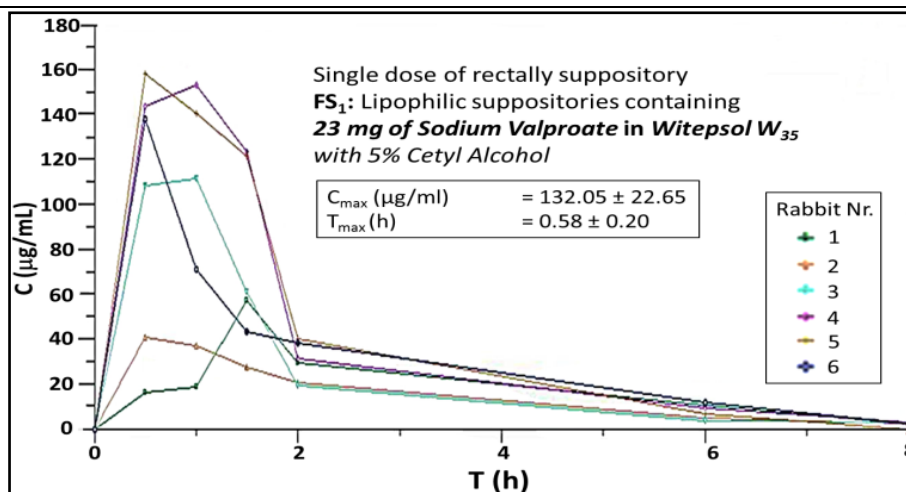


Figure 3.

Valproic acid concentration determined in rabbits after rectal administration of FS₂ in a dose of 10 mg/kg

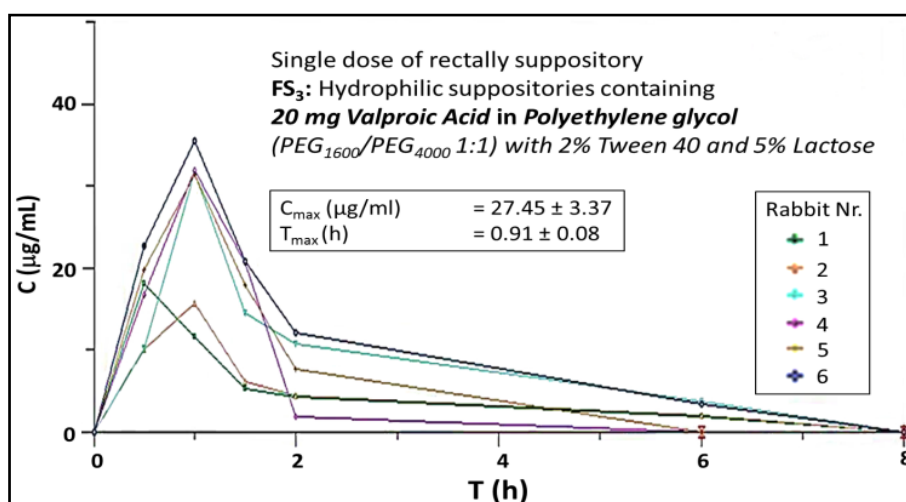


Figure 4.

Valproic acid concentration determined in rabbits after rectal administration of FS₃ in a dose of 10 mg/kg

The results of plasma level data analysed using a non-compartmental pharmacokinetic model are shown in Figures 5-7. The bioavailability parameters of

valproic acid or sodium valproate after rectal administration of suppositories are depicted in Table III:

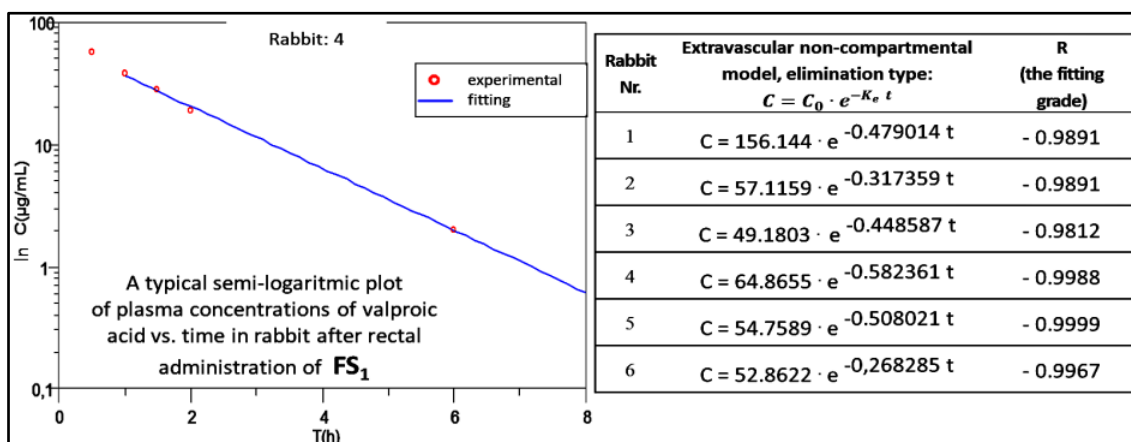


Figure 5.

Analysis of FS₁ – plasma curves by the non-compartmental model

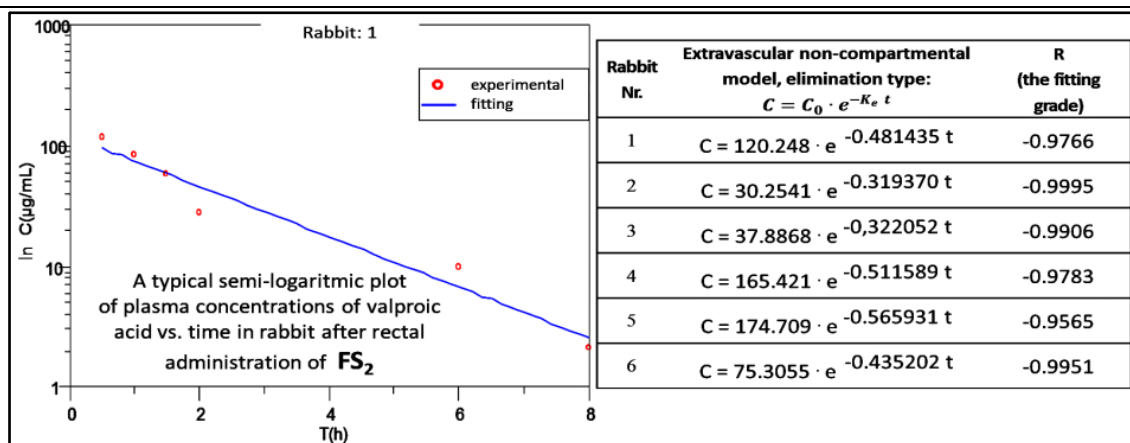


Figure 6.

Analysis of FS₂ – plasma curves by the non-compartmental model

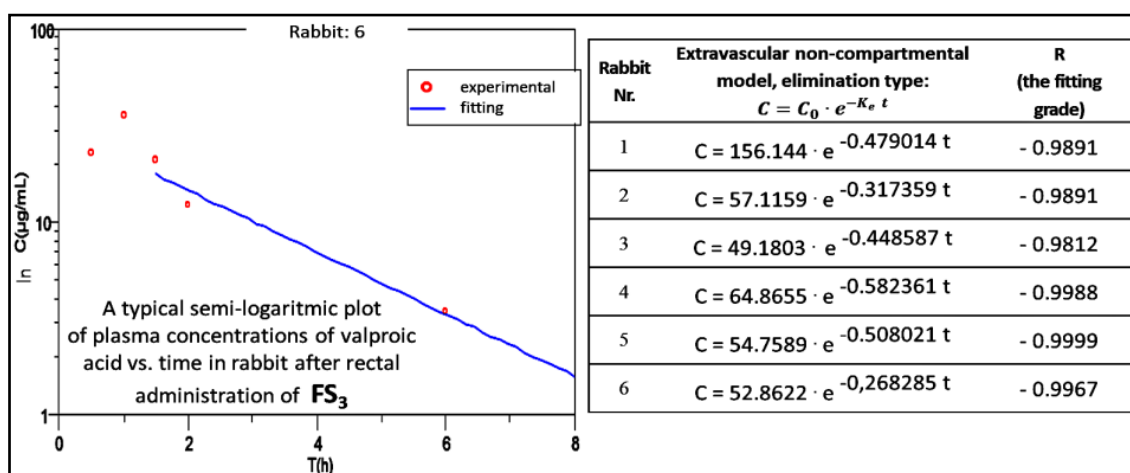


Figure 7.

Analysis of FS₃ – plasma curves by the non-compartmental model

Table III

Bioavailability of studied suppositories determined in rabbits

In vivo parameter (± MSE)	FS ₁	FS ₂	FS ₃
C_{max}^* (µg/mL)	67.68 ± 10.56	132.05 ± 22.65	27.45 ± 3.37
T_{max} (h)	0.91 ± 0.15	0.58 ± 0.20	0.91 ± 0.08
AUC_{tot}^* (h·µg/mL)	158.55 ± 28.61	215.15 ± 21.05	50.42 ± 7.65
$T_{1/2}$ (h)	1.71 ± 0.22	1.65 ± 0.16	1.90 ± 0.53
MRT (h)	2.65 ± 0.28	2.16 ± 0.08	2.86 ± 0.57

MSE = mean squared error; *p < 0.001 (FS₂ vs. FS₃, FS₁ vs. FS₃); ANOVA one-way, Turkey-Kramer *post hoc* test for multiple comparison

In vitro experimental studies were performed to assess the pharmaceutical availability of three types of valproate suppositories formulated for anti-convulsant paediatric therapy (Figure 1: P₁ - P₃), that previously were selected to be tested through an experimental pharmacokinetic study conducted on rabbits. Although the *in vitro* release test showed a faster release of valproic acid from hydrophilic excipients (Figure 1: PS₃), this behaviour was not found among the *in vivo* study results (Table III: FS₃). This fact can be explained by the already known osmotic effect of the polyethylene glycols on the rectal mucosa which delays and reduces the absorption determining in the same time a lower

local tolerance. In case of lipophilic bases (Table III: FS₁ vs. FS₂), the *in vivo* differences demonstrate that the sodium valproate, a salt with increased solubility in water, achieves in plasma higher concentrations than those of valproic acid. However, the bioavailability differences of these two lipophilic formulations are in fact not significant when they are compared with the hydrophilic formulation. Even if its dissolution process is lower, the valproic acid is extensively unionized in rectal pH (slightly acidic to neutral), a phenomenon which seems to favour its transmucosal absorption.

Conclusions

Suppositories containing lipophilic bases (valproic acid in Suppocire NAI 25 and sodium valproate in Witepsol W35 with 5% cetyl alcohol) are most suitable for rectal administration (higher plasma concentrations, higher bioavailability) than the hydrophilic formulation (based on polyethylene glycols), fact confirmed by the *in vivo* pharmacokinetic study in rabbits.

The similarity of the *in vitro* release profiles has not been fully confirmed *in vivo*, which shows that in the case of the suppositories, the *in vivo* pharmacokinetic test still remains necessary to discriminate between different formulations.

Acknowledgement

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