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PhD Thesis Abstract

KINETIC AND PHARMACOKINETIC STUDIES FOR CARBAMAZEPINE

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Key words: kinetic models, pharmacokinetic models, pharmacokinetic analysis, pharmacokinetic drug-drug interaction, carbamazepine, 10,11-epoxy-carbamazepine, ivabradine, zolpidem, lansoprazol.

INTRODUCTION

The aim of this work was the study of carbamazepine pharmacokinetics after oral administration of immediate or prolonged release pharmaceutical formulations and analysis of some of its pharmacokinetic drug interactions. A special attention was paid to some pharmacokinetic aspects which are not described well enough in literature.

The pharmacokinetics of carbamazepine is relatively well studied. In literature there are bioavailability and pharmacokinetic studies of carbamazepine from pharmaceutical formulations with oral administration or studies regarding the pharmacokinetic drug interactions of carbamazepine. However, only in a few of these studies there is a kinetic, mathematic approach of carbamazepine pharmacokinetics or of its pharmacokinetic drug interactions.

One aspect insufficiently analyzed/discussed is the absorption process of carbamazepine. The carbamazepine has a low water solubility (μg/ml), whereas it is administered in oral doses of 150-600 mg. In other words, the drug dissolution in the environmental liquid at absorption place may be a limiting step in the global absorption process of carbamazepine. However, there are on the market a lot of pharmaceutical preparations with carbamazepine with immediate or prolonged release, meaning that even the pharmaceutical formulation may control the drug release and consequently, the absorption.

Carbamazepine is a potent inducer of cytochrome P450 enzymes responsible by drug metabolism (specially CYP3A4), thus it may interact/interfere with these drugs. After a drug-drug interaction, some pharmacokinetic parameters of the interfered drug may be changed.

The drug plasma levels are lowered after the pharmacokinetic interaction and the therapeutic effect may be also decreased. The pharmacokinetic study of drug interactions of carbamazepine is able to reveal the quantitative effect of drug interaction on the drug pharmacokinetic and may explain the eventual changes in treatment efficacy of other drugs co-administered with carbamazepine.

The experimental part of the thesis have four main chapters.

In the first chapter of experimental part there are presented the analytical methods used for quantification of carbamazepine and its active metabolite, 10,11-epoxy-carbamazepine, in biological samples. There are also presented the validated analytical methods used for other drugs those pharmacokinetic interaction with carbamazepine is studied.

In the second chapter of the experimental part the kinetic release of carbamazepine from pharmaceutical formulations is analyzed, using both simple and complex kinetic models.

The third chapter of experimental part presents the pharmacokinetics of carbamazepine and its active metabolite after single oral dose administration from either immediate or prolonged release pharmaceutical formulations. For this analysis there were used multiples methods of analysis (pharmacokinetic and statistical), like compartmental and non-compartmental pharmacokinetic analysis and there was assessed the bioequivalence between pharmaceutical formulations with carbamazepine.

The last chapter of the experimental part presents the pharmacokinetic interactions of carbamazepine with drugs like ivabradine, zolpidem and lansoprazol. Like previously mentioned, pharmacokinetic (non-compartmental and compartmental analysis) and statistical methods were applied.

EXPERIMENTAL PART

4 ANALYTICAL AND BIOANALYTICAL METHODS IN PHARMACOKINETIC STUDIES

The analysis of drug pharmacokinetics may be done only after knowing the drug levels in biological matrices. For an accurate kinetic analysis, one needs an analytical method with some performance parameters previously analyzed, operations described in the analytical method validation guidelines.

There were developed and validated analytical methods for determination of plasma levels of carbamazepine, 10,11-epoxy-carbamazepine, ivabradine, zolpidem and lansoprazol from human plasma. In the process of analytical method development, some parameters like the retention/analysis time, the sensitivity, selectivity and analyte recovery were optimized.

5 KINETIC ANALYSIS OF CARBAMAZEPINE FROM PROLONGED RELEASE PHARMACEUTICAL FORMULATIONS

In this chapter is analyzed the in vitro kinetic release of carbamazepine from pharmaceutical formulations from Romanian market. There were analyzed 9 formulations form 4 different companies.

On the process of kinetic analysis, there were first tested some simple models, like 1st order or zero order kinetics. Finally, more complex models were build and used for analysis, models consisted in combinations of two 1st and/or zero order kinetic processes.

All the products with carbamazepine, prolonged release formulations available on Romanian market were analyzed (Table 17).

Table 17. Pharmaceutical formulations containing carbamazepine analyzed.

No.	Formulation	Drug concentration	Producer	Lot / Expiry
1	Finlepsin 200 R	200 mg	AWD.Pharma GmbH, Germany	5H176 ; 08-2008
2	Finlepsin 400 R	400 mg	AWD.Pharma GmbH, Germany	6F302A ; 08-2009
3	Tegretol 200 CR	200 mg	Novartis, Switzerland	T5161 ; 05-2008
4	Tegretol 400 CR	400 mg	Novartis, Switzerland	T5199 ; 05-2008
5	Neurotop 300 R	300 mg	Gerot, Germany	260158 ; 11-2010
6	Neurotop 600 R	600 mg	Gerot, Germany	260242 ; 02-2011
7	Timonil 150 R	150 mg	Desitin Arzneimittel GmbH, Germany	05007665; 09-2008
8	Timonil 300 R	300 mg	Desitin Arzneimittel GmbH, Germany	06002003; 01-2011
9	Timonil 600 R	600 mg	Desitin Arzneimittel GmbH, Germany	05005477; 05-2010

The mean dissolution profiles of carbamazepine from prolonged release are presented in Fig. 51.

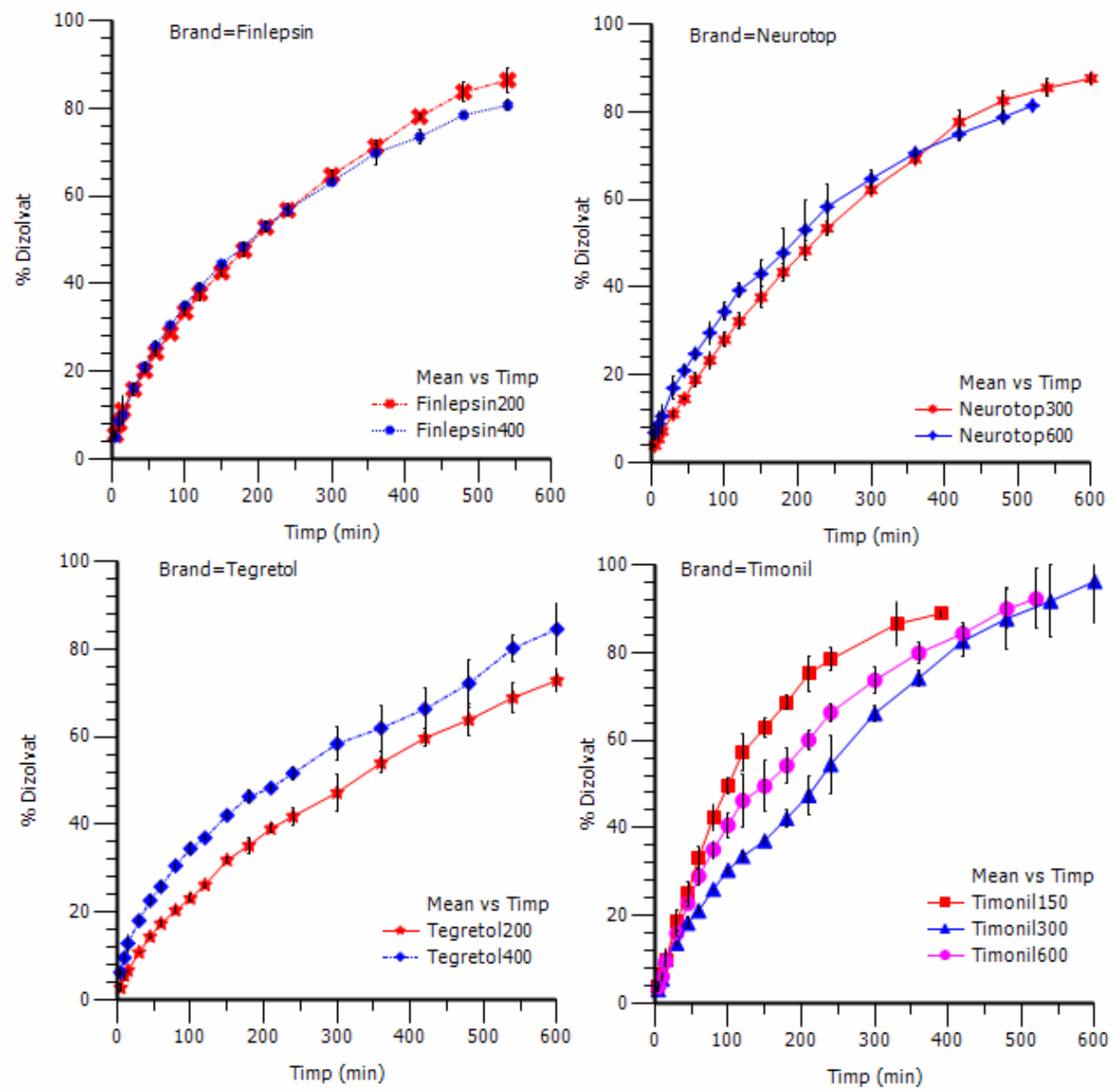


Fig. 51. Mean dissolution profiles of carbamazepine from analyzed products

In the first analysis step, 11 mechanistic models (including variants of them) were employed (Table 20).

Table 20. Mechanistic models used for analysis of dissolution profiles of carbamazepine from pharmaceutical formulations.

Model	Kinetics	Model parameters
M1	1st order	k_{ced}
M2	1sr order +lag time	k_{ced}, tlag
M3	Zero order	t_{ced} (k_{ced})
M4	Zero order + lag time	t_{ced} (k_{ced}), tlag
M5	Higuchi	k_{ced}
M6	Higuchi +lag time	k_{ced}, tlag
M7	Hopenberg; n=2	k_{ced}
M8	Hopenberg; n=3	k_{ced}
M9	Hixon Crowell	k_{ced}
M10	Peppas	k_{ced}, n
M11	Peppas +lag time	k_{ced}, n, tlag

The best model describing the kinetic release of carbamazepine from analysed products is Peppas, however, for Timonil 150 product the dissolution profile is beste described by a 1st order kinetics.

Even if the Peppas kinetic model was found appropriate to describe the kinetic release of carbamazepine from the most analyzed products, it is a semi-empirical model, indicating in fact the presence of multiple parallel/successive elementary processes. Thus, in the second part of the study, the were built and used complex kinetic models.

Eight complex kinetic models were used for kinetic analysis of carbamazepine from pharmaceutical preparations with prolonged release. Each model contains two individual kinetic processes (zero order or 1st order), which occurs either independent each other or in a successive manner (Table 22).

Table 22. Complex kinetic models used for kinetic analysis of carbamazepine release form pharmaceutical formulations.

Model	Kinetics	Model parameters
M12	1st order + 1st order , independents	k_{cedA}, k_{cedB}, f, tlagB
M13	1st order + 1st order, independents	k_{cedA}, k_{cedB}, f, tlagA, tlagB
M14	1st order + 1st order succesivess	k_{cedA}, k_{cedB}, f, tlagB
M15	1st order + 1st order, succesives	k_{cedA}, k_{cedB}, f, tlagA, tlagB
M16	Zero order + 1st order, independents	k_{ced0}, k_{ced1}, f, tlag1
M17	Zero order + 1st order, independents	k_{ced0}, k_{ced1}, f, tlag0, tlag1
M18	1st order + Zero order, independents	k_{ced1}, k_{ced0}, f, tlag0
M19	1st order + Zero order, independents	k_{ced1}, k_{ced0}, f, tlag1, tlag0

where k_{cedA}, k_{cedB} are 1st order constants, A and B are the two 1st order kinetic processes, k_{ced0} and k_{ced1} are the constants of the zero and 1st order processes, f is the dose fraction released by a specific kinetic process, tlagA, tlagB, tlag0, tlag1 – the corresponding lag-times of the kinetic processes.

For each pharmaceutical process analyzed, the optimum kinetic model was chosen, based on Akaike criteria value (minimum Akaike value for a better fit) and the corresponding kinetic parameters were calculated (Table 24).

Table 24. The best kinetic model found for each pharmaceutical formulation analyzed and its corresponding parameters

Product	Kinetic model	Model parameters
Finlepsin 200	M12	k_{cedA}= 0.103 min^{-1}; f=0.13, k_{cedB}= 0.00323 min^{-1}; tlagB = 19.5 min
Finlepsin 400	M12	k_{cedA}= 0.077 min^{-1}; f=0.16, k_{cedB}= 0.00308 min^{-1}; tlagB = 19.6 min
Neurotop 300	M15	k_{cedA}= 0.0069 min^{-1}; f=0.045, k_{cedB}= 0.00305 min^{-1}; tlagA = 0 min, tlagB = 6.75 min
Neurotop 600	M12	k_{cedA}= 0.146 min^{-1}; f=0.125, k_{cedB}= 0.00326 min^{-1}; tlagB = 15.0 min
Tegretol 200	M12	k_{cedA}= 0.031 min^{-1}; f=0.19, k_{cedB}= 0.00196 min^{-1}; tlagB = 66.2 min
Tegretol 400	M6	k_{ced}=3.39 min^{-1}; tlag= 1.46
Timonil 150	M12	k_{cedA}= 0.0527 min^{-1}; f=0.172, k_{cedB}= 0.0061 min^{-1}; tlagB = 21.1 min
Timonil 300	M12	k_{cedA}= 0.019 min^{-1}; f=0.34, k_{cedB}= 0.00427 min^{-1}; tlagB = 140.3 min
Timonil 600	M13	k_{cedA}= 0.0115 min^{-1}; f=0.59, k_{cedB}= 0.00411 min^{-1}; tlagA=0, tlagB = 167.8 min

5.7 CONCLUSIONS

There were realized the dissolution tests for 9 pharmaceutical preparations with prolonged release containing carbamazepine.

The optimum dissolution conditions were found and applied.

The mean (n=3) dissolution profiles of carbamazepine were obtained for each of the 9 pharmaceutical products tested, those being further analyzed by using 11 simple kinetic models and 8 complex kinetic models.

From the tested mechanistic models, the one best describing the release process of carbamazepine from tested products counts in two 1st order processes (one starting right after the beginning of the experiment, and the other, slower, starting later).

6 PHARMACOKINETICS OF CARBAMAZEPINE AND 10,11-EPOXY-CARBAMAZEPINE AFTER SINGLE DOSE ADMINISTRATION ON HEALTHY VOLUNTHEERS

In this chapter is studied the pharmacokinetics of carbamazepine and of its active metabolite, 10,11-epoxy-carbamazepine, administered as a single oral dose of carbamazepine in form of either immediate (IR) or prolonged/extended (ER) release pharmaceutical formulations.

6.1 PHARMACOKINETICS OF CARBAMAZEPINE AND 10,11-EPOXY-CARBAMAZEPINE AFTER ADMINISTRATION OF IMMEDIATE RELEASE PHARMACEUTICAL FORMULATIONS

6.1.1 Subjects

24 healthy subjects took part of the study

6.1.2 Study design

The clinical study was done in two periods, cross-over. In each period the 24 volunteers received, in an alternate way, a single oral dose of 400 mg carbamazepine (immediate release, 2x200 mg dose) from two commercial preparations: one generic formulation (T - Test) and the reference formulation (Tegretol 200, producer Novartis, Switzerland; R – Reference). The wash-out period between treatment was two weeks.

Blood samples (5 ml) were taken at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120 and 144 hours after dose administration.

The mean plasma levels of carbamazepine, for each pharmaceutical product administered, are shown in Fig. 54.

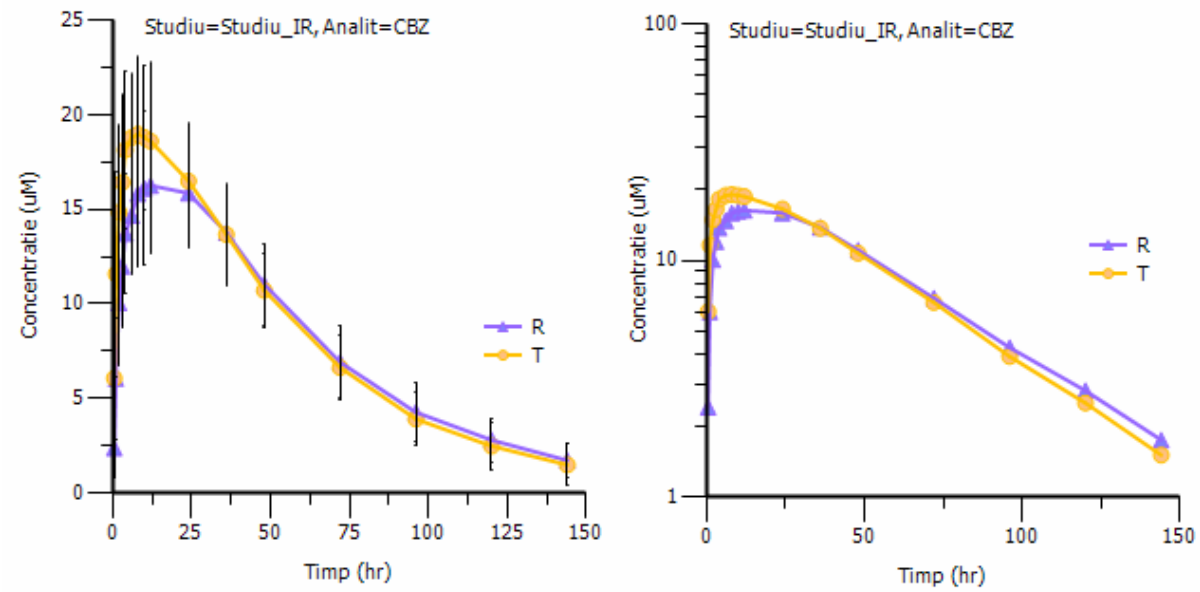


Fig. 54. Mean plasma levels of carbamazepine after a single oral dose administration of 400 mg carbamazepine in 24 healthy volunteers, as Test and Reference products, immediate release formulations

6.1.4 Non-compartmental pharmacokinetic analysis

The mean values of pharmacokinetic parameters of carbamazepine and its active metabolite are shown in Tables 35 and 36.

Table 35 Mean values of pharmacokinetic parameters of carbamazepine administered as Test and Reference products, immediate release

		Treatment					
		Reference			Test		
Parameter	Units	Mean	SD	CV%	Mean	SD	CV%
Cmax	µmol/L	17.28	3.86	22.3	20.31	4.04	19.8
Tmax	hr	13.42	6.53	48.6	7.83	5.78	73.7
ASClast	hr*µmol/L	1165.2	225.5	19.3	1197.8	216.3	18.0
ASCinf	hr*µmol/L	1267.2	265.5	20.9	1292.4	288.4	22.3

		Treatment					
		Reference			Test		
Parameter	Units	Mean	SD	CV%	Mean	SD	CV%
ASC_%Extrap	%	7.70	3.56	46.2	6.57	4.95	75.2
Lambda_z	1/hr	0.02	0.00	23.3	0.02	0.01	27.7
t1/2	hr	36.25	8.04	22.1	34.13	11.46	33.5
TMR	hr	58.59	10.68	18.2	53.95	14.19	26.3
Vz_F	L	71.32	15.49	21.7	64.72	14.69	22.7
Cl_F	L/hr	1.39	0.27	19.6	1.37	0.27	19.5

Table 36 Mean values of pharmacokinetic parameters of 10,11-epoxy-carbamazepine administered as Test and Reference products, immediate release

		Treatment					
		Reference			Test		
Parameter	Units	Mean	SD	CV%	Mean	SD	CV%
Cmax	µmol/L	1.09	0.43	40.0	1.19	0.43	36.3
Tmax	hr	36.50	6.60	18.0	31.00	8.61	27.7
ASClast	hr*µmol/L	78.1	26.5	34.0	84.9	25.7	30.3
ASCinf	hr*µmol/L	82.9	27.0	32.5	89.1	26.3	29.5
ASC_%Extrap	%	6.21	3.53	56.8	4.93	2.56	51.9
Lambda_z	1/hr	0.02	0.01	21.1	0.03	0.01	22.4
t1/2	hr	29.31	6.81	23.2	27.30	5.58	20.4
TMR	hr	62.50	9.50	15.2	56.94	7.93	13.9
Vz_F	L	972.3	429.6	44.1	826.4	327.8	39.6
Cl_F	L/hr	22.33	6.39	28.6	20.54	5.60	27.2

The ANOVA test was applied for comparison of pharmacokinetic parameters of carbamazepine and its metabolite for products Test and Reference (ANOVA 2-ways, sources of variation subject and formulation, log-transformed values, p=0.05). The results of statistical analysis are shown in Table 37 and 38.

Table 37 The results of ANOVA statistical evaluation of pharmacokinetic parameters of carbamazepine administered as Test and Reference products, immediate release.

		Way					
		Subject			Treatment		
Parameter	Units	No_DF	F_stat	P*	No_DF	F_stat	P*
Ln(Cmax)	µmol/L	23	9.03	0.000001	1	39.5	0.000002
Ln(Tmax)	hr	23	2.93	0.006325	1	28.7	0.000019
Ln(ASClast)	hr*µmol/L	23	21.8	0.000000	1	3.63	0.069375
Ln(ASCinf)	hr*µmol/L	23	19.2	0.000000	1	0.936	0.343390
Ln(Lambda_z)	1/hr	23	2.62	0.012438	1	2.11	0.160316

		Way					
		Subject			Treatment		
Parameter	Units	No_DF	F_stat	P*	No_DF	F_stat	P*
Ln(t1/2)	hr	23	2.62	0.012438	1	2.11	0.160316
Ln(TMR)	hr	23	7.08	0.000007	1	9.14	0.006044
Ln(Vz_F)	L	23	2.67	0.011174	1	3.93	0.059356
Ln(Cl_F)	L/hr	23	19.2	0.000000	1	0.936	0.343390

*significant for p<0.05

Table 38 The results of ANOVA statistical evaluation of pharmacokinetic parameters of 10,11-epoxy-carbamazepine after carbamazepine administration as Test and Reference products, immediate release.

		Way					
		Subject			Treatment		
Parameter	Units	No_DF	F_stat	P*	No_DF	F_stat	P*
Ln(Cmax)	µmol/L	23	24.0	0.000000	1	11.8	0.002272
Ln(Tmax)	hr	23	0.764	0.737660	1	5.80	0.024427
Ln(ASClast)	hr*µmol/L	23	39.1	0.000000	1	21.3	0.000121
Ln(ASCinf)	hr*µmol/L	23	35.8	0.000000	1	15.4	0.000676
Ln(Lambda_z)	1/hr	23	5.41	0.000073	1	3.84	0.062231
Ln(t1/2)	hr	23	5.41	0.000073	1	3.84	0.062231
Ln(TMR)	hr	23	9.86	0.000000	1	25.7	0.000039
Ln(Vz_F)	L	23	22.2	0.000000	1	15.8	0.000605
Ln(Cl_F)	L/hr	23	35.8	0.000000	1	15.4	0.000676

*significant for p<0.05

For carbamazepine, for all compared pharmacokinetic parameters there are significant differences between subjects (inter-individual variability). The differences related to administered pharmaceutical formulation were revealed for maximum concentration (Cmax), time of maxim (Tmax), and mean residence time (MRT). All these parameters are strongly correlated with the formulation characteristics, thus explaining the observed differences. In a similar was one can observe the differences between parameters for metabolite. However, in this case significant differences were obtained also for areas under the curve, the apparent clearance and the apparent distribution volume, the last two being related to carbamazepine bioavailability (F).

6.1.6 Pharmacokinetic modeling of carbamazepine and of its active metabolite

For pharmacokinetic analysis, the Phoenix software was employed.

6.1.6.1 Pharmacokinetic modeling of carbamazepine

In the first step of pharmacokinetic analysis, there were used only the plasma levels of carbamazepine and there were applied 8 kinetic models. The differences between these models consisted in the absorption kinetics (1st or zero order), a lag time for absorption and the distribution compartments for carbamazepine For all models, the elimination of carbamazepine was considered to be a 1st order kinetic process. The characteristics of the built models are presented in Table 40.

Table 40 Pharmacokinetic models for carbamazepine, 1st step of analysis

Model	**Absorption kinetics**	**Absorption lag time**	**Distribution, number of compartments**
M1	1st order	No	1
M2	1st order	Yes	1
M3	1st order	No	2
M4	1st order	Yes	2
M5	Zero order	No	1
M6	Zero order	Yes	1
M7	Zero order	No	2
M8	Zero order	Yes	2

For both Reference and Test products, from the eight tested models, the best is model 4 (1st order absorption kinetics with lag time, bicompartmental distribution and 1st order elimination kinetics).

The correlation between calculated versus model predicted concentrations of carbamazepine, corresponding to models M1 and M4 are presented in Fig. 57. One can observe a better correlation for model M4.

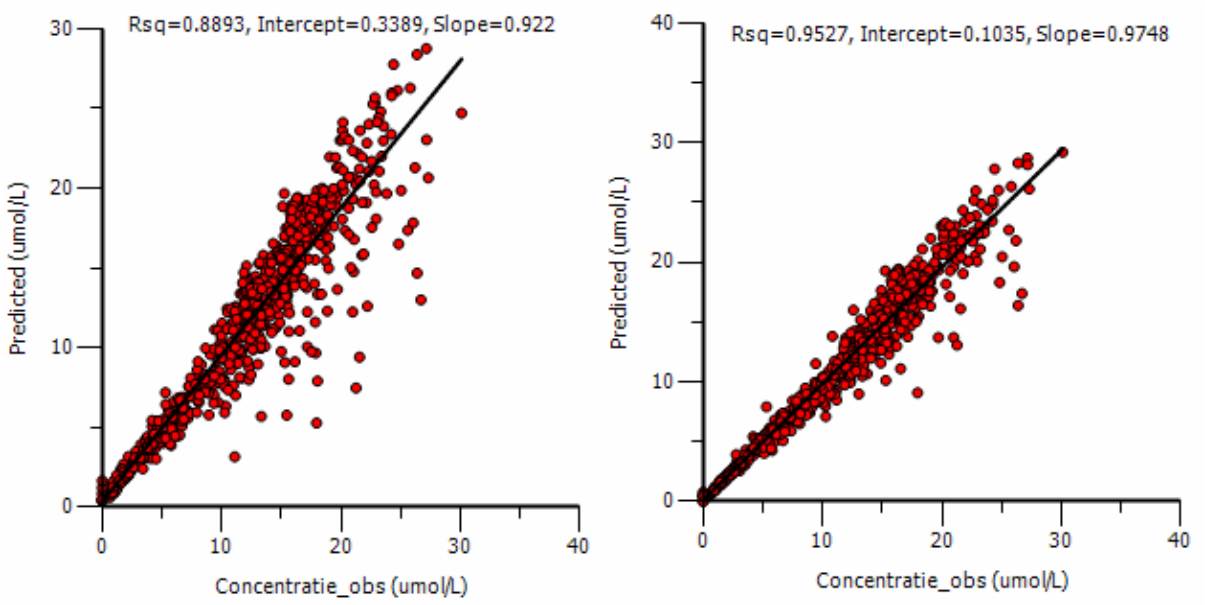


Fig. 57. The correlation between calculated versus experimental concentration of carbamazepine, obtained for models M1 and M4.

In the second step of analysis of pharmacokinetic modeling, the starting point was model M4 previously identified, which was further developed considering a complex absorption kinetics (two different processes, 1st and/or zero order), Table 41. The models derived from model M4 were noted with M41-M44.

Table 41 Pharmacokinetic models for carbamazepine, second step of the pharmacokinetic study

Model	**Absorption kinetics**	**Absorption lag time**	**Distribution, number of compartments**
M4 (previously identified)	1st order	Yes	2
M41	1st order + 1st order, independents	Yes	2
M42	1st order + 1st order, successive	Yes	2
M43	Zero order + 1st order, independents	Yes	2
M44	Zero order + 1st order, successive	Yes	2

The four models derivate from M4 were fitted to experimental data, and the Akaike index values were compared. For both analyzed products, the best fit is obtained for model M41.

However, after reconsidering the absorption kinetics of carbamazepine is important to check again if some previous selecting decisions are still valid. Thus, a third step of pharmacokinetic analysis was run, starting from model M41 and building another three kinetic models – M411, M412 and M413 (Table 42).

Table 42 Pharmacokinetic models for carbamazepine, third step of analysis

Model	**Absorption kinetics**	**Absorption lag time**	**Distribution, number of compartments**
M41(previous selected)	1st order + 1st order, independents	Yes	2
M411	1st order + 1st order, independents	No	2
M412	1st order + 1st order, independents	Yes	1
M413	1st order + 1st order, independents	No	1

The three models derived from M41 were fitted to experimental data, and the Akaike values compared. The best model (best fitting) was found to be M412, this being selected as the representative model describing the carbamazepine pharmacokinetics (Fig. 62).

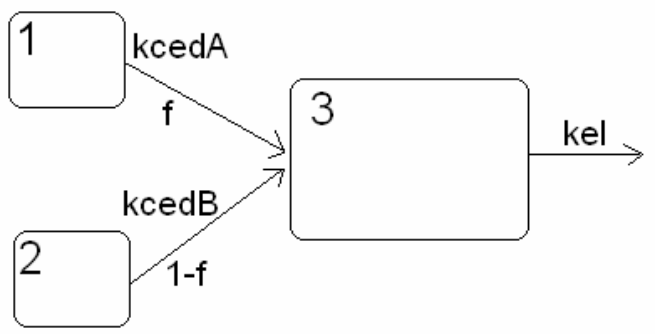


Fig. 62 Schema of the model M412, used for pharmacokinetic analysis of carbamazepine

6.1.6.2 Pharmacokinetic modeling of carbamazepine and 10,11-epoxy-carbamazepine

In the fourth step of pharmacokinetic analysis of carbamazepine and 10,11-epoxy-carbamazepine, 8 new models were created (M412-1....M412-8), containing the compartments and kinetic processes for metabolite (Table 43).

Table 43 Pharmacokinetic models for carbamazepine (CBZ) and 10,11-epoxy-carbamazepine (ECBZ), fourth step of analysis

Model	Absorption kinetics	Absorption lag-time	CBZ, no. of compartments	ECBZ, no. of compartments	Exclusive elimination of CBZ through ECBZ	Hepatic passage effect
M412-1	1st order + 1st order, independents	Yes	1	1	No	No
M412-2				1	Yes	No
M412-3				1	No	Yes
M412-4				1	Yes	Yes
M412-5				2	No	No
M412-6				2	Yes	No
M412-7				2	No	Yes
M412-8				2	Yes	Yes

The 8 models derived from model M412 were fitted to experimental data (simultaneous fitting of plasma levels of carbamazepine and 10,11-epoxy-carbamazepine), and the Akaike values were compared for identification of the best model.

For both pharmaceutical products, the model M412-3 was found to best describe the experimental data and was used as representative model for pharmacokinetics of carbamazepine and 10,11-epoxy-carbamazepine.

6.1.6.3 Utilization of graphs method for pharmacokinetic analysis of carbamazepine and 10,11-epoxy-carbamazepine

In this study we compared the results obtained for a dataset for carbamazepine and metabolite by using the same complex kinetic model, in two forms: differential equations and analytical equations obtained by graphs method.

For analysis, the mean plasma levels of carbamazepine and its metabolite were used.

A kinetic model considering a 1st order kinetic process for carbamazepine absorption, with hepatic passage effect and distribution for both analytes was used. Carbamazepine is eliminated from the body by both systemic metabolism and other ways.

The differential equations built are presented in Fig. 68 and are applicable correlated to the value of current time in comparison with lag-time:

$$Eq1\begin{cases}\text{If time} < \text{Tlag}: \\ \frac{\partial A}{\partial t} = 0 \\ \frac{\partial B}{\partial t} = 0 \\ \frac{\partial C}{\partial t} = 0 \\ \frac{\partial D}{\partial t} = 0 \\ \frac{\partial E}{\partial t} = 0 \\ C_B = 0 \\ C_D = 0 \end{cases} \qquad Eq2\begin{cases}\text{If time} \geq \text{Tlag}: \\ \frac{\partial A}{\partial t} = -k01*A - k02*A \\ \frac{\partial B}{\partial t} = k01*A + k31*C - k13*B - k10*B - k12*B \\ \frac{\partial C}{\partial t} = k13*B - k31*C \\ \frac{\partial D}{\partial t} = k02*A + k12*B + k24*E - k42*D - k20*D \\ \frac{\partial E}{\partial t} = k42*D - k24*E \\ C_B = \frac{B}{Vd} \\ C_D = \frac{D}{Vd} \end{cases}$$

Fig. 68 The differential equations of the pharmacokinetic model of carbamazepine and 10,11-epoxy-carbamazepine Equation 1 is used when time<Tlag, equation 2 is used when time ≥Tlag

All the pharmacokinetic calculations (using either differential or analytical equations) were made using WinNonlin software

In this approach a new mechanism equivalent to the model from Figure 1 is drawn considering that every substance is transforming in a final product with a constant rate, even it is zero (Fig. 69-a). The consumption flow graph is the image of the new mechanism with the γ decreased from every output transmittance. (Fig 69-b).

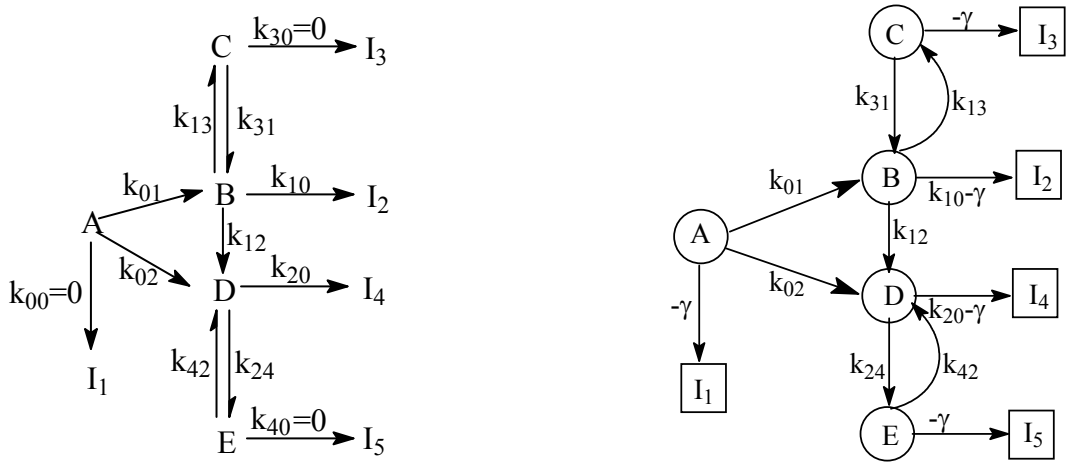


Fig 69. The equivalent mechanism (a) and consumption flow graph (b)

One has identified all forward paths (FW) and by adding its gains (FWG, for example $FWGl = k_{01}k_{12}(k_{20}-\gamma)k_{31}k_{42}$), one obtains the gain of the consumption flow graph (GC), which, according to the definition, is equal with the value of the secular determinant:

$$\Delta = CG = \sum FWGi = (k_{01}+k_{02}-\gamma)\cdot[(-\gamma)(k_{12}+k_{10}-\gamma)+k_{13}(-\gamma)+k_{31}(k_{12}+k_{10}-\gamma)]\cdot$$
$$\cdot[(-\gamma)(k_{20}-\gamma)+k_{42}(k_{20}-\gamma)+k_{24}(-\gamma)]=0$$
$$\Delta = (k_{01}+k_{02}-\gamma)[\gamma^2-\gamma(k_{12}+k_{13}+k_{10}+k_{31})+k_{31}(k_{12}+k_{10})][\gamma^2-\gamma(k_{20}+k_{24}+k_{42})+k_{20}k_{42}]=0$$

From the above equation it can be found the exponential factors and the expressions of consumption determinants (Δc):

$\gamma_1 = k_{01}+k_{02;}$ γ_2, γ_3 (the solutions of the first square equation from the last product of Δ), and γ_4, γ_5 (the solutions of the second square equation from the last product of Δ).

The formation flow graph for B (Fig. 70) is depicted from the consumption flow graph, considering the species of interest being a target one (a final product); the output edges of B species are rejected and a new input node is added (source S which represents the initial conditions). From Fig. 69-b it is clear that no any connections emerge from the node D to the node B, thus the D species will not appear in the formation flow graph of B species.

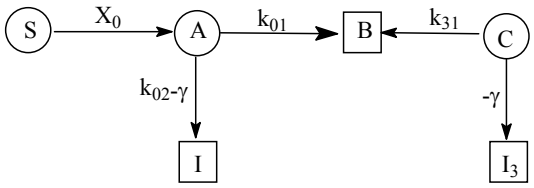


Fig 70. The formation flow graph for B species

In Fig. 70, I denotes the output node of A species whose transmittance is the sum of all its outgoing edge transmittances when the node D is missing. The analytical solution for B species is:

$$C_B = \frac{X_0 k_{01}(k_{31}-\gamma_1)e^{-\gamma_1 t}}{(\gamma_2-\gamma_1)(\gamma_3-\gamma_1)} + \frac{X_0 k_{01}(k_{31}-\gamma_2)e^{-\gamma_2 t}}{(\gamma_1-\gamma_2)(\gamma_3-\gamma_2)} + \frac{X_0 k_{01}(k_{31}-\gamma_3)e^{-\gamma_4 t}}{(\gamma_1-\gamma_3)(\gamma_2-\gamma_3)}$$

The formation flow graph for D is (Fig. 71):

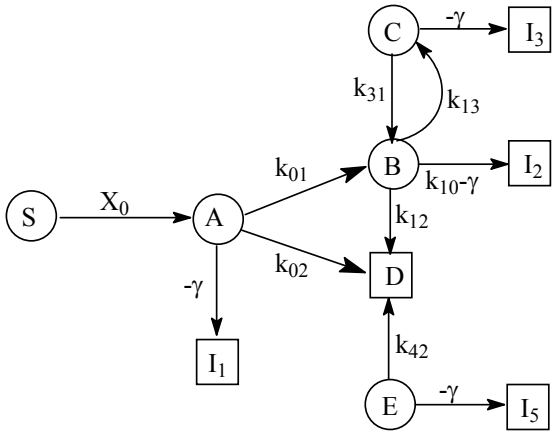


Fig. 71. The formation flow graph for D species (10,11-epoxy-carbamazepine)

The analytical solution for D specie is:

$$C_D = \frac{X_0 \cdot (k_{01} \cdot k_{12} \cdot (k_{31}-\gamma_1) \cdot (k_{42}-\gamma_1) + k_{02} \cdot (k_{42}-\gamma_1) \cdot [\gamma_1^2 - \gamma_1(k_{10}+k_{12}+k_{13}+k_{31}) + k_{31} \cdot (k_{10}+k_{12})]) \cdot e^{-\gamma_1 t}}{(\gamma_2-\gamma_1)(\gamma_3-\gamma_1)(\gamma_4-\gamma_1)(\gamma_5-\gamma_1)} +$$
$$\frac{X_0 \cdot k_{01} \cdot k_{12} \cdot (k_{31}-\gamma_2) \cdot (k_{42}-\gamma_2) \cdot e^{-\gamma_2 t}}{(\gamma_1-\gamma_2)(\gamma_3-\gamma_2)(\gamma_4-\gamma_2)(\gamma_5-\gamma_2)} + \frac{X_0 \cdot k_{01} \cdot k_{12} \cdot (k_{31}-\gamma_3) \cdot (k_{42}-\gamma_3) \cdot e^{-\gamma_3 t}}{(\gamma_1-\gamma_3)(\gamma_2-\gamma_3)(\gamma_4-\gamma_3)(\gamma_5-\gamma_3)} +$$
$$\frac{X_0 \cdot (k_{01} \cdot k_{12} \cdot (k_{31}-\gamma_4) \cdot (k_{42}-\gamma_4) + k_{02} \cdot (k_{42}-\gamma_4) \cdot [\gamma_4^2 - \gamma_4(k_{10}+k_{12}+k_{13}+k_{31}) + k_{31} \cdot (k_{10}+k_{12})]) \cdot e^{-\gamma_4 t}}{(\gamma_1-\gamma_4)(\gamma_2-\gamma_4)(\gamma_3-\gamma_4)(\gamma_5-\gamma_4)} +$$
$$\frac{X_0 \cdot (k_{01} \cdot k_{12} \cdot (k_{31}-\gamma_4) \cdot (k_{42}-\gamma_5) + k_{02} \cdot (k_{42}-\gamma_5) \cdot [\gamma_4^2 - \gamma_4(k_{10}+k_{12}+k_{13}+k_{31}) + k_{31} \cdot (k_{10}+k_{12})]) \cdot e^{-\gamma_5 t}}{(\gamma_1-\gamma_5)(\gamma_2-\gamma_5)(\gamma_3-\gamma_5)(\gamma_4-\gamma_5)}$$

The analytical equations obtained for C and D species were implemented in a Java software, and the corresponding pharmacokinetic parameters were obtained after fitting.

The values of kinetic parameters of CBZ and ECBZ, their estimation precision and model diagnostics data, obtained using numerical integration method and analytical equations are presented in Table 47.

Table 47 Comparison between kinetic parameters of CBZ and ECBZ obtained by using numerical integration method and analytical solutions

		Numerical solutions			Analytical solutions			
Parameter	Units	Value	Standard error	CV%	Value	Standard error	CV%	Difference (%)
k_{10}	hr^{-1}	0.195368	0.152337	78.79	0.195	0.152131	78.02	-0.16
k_{20}	hr^{-1}	0.240881	0.332125	132.77	0.245881	0.314893	128.07	2.1
k_{12}	hr^{-1}	0.013134	0.014161	101.43	0.013134	0.013915	105.95	0
k_{13}	hr^{-1}	0.422876	0.121555	28.91	0.423	0.111382	26.33	0.3
k_{31}	hr^{-1}	0.107113	0.021891	20.49	0.107	0.020134	18.82	-0.1
k_{01}	hr^{-1}	0.025387	0.017670	69.35	0.025387	0.016654	65.6	0
k_{02}	hr^{-1}	$1.43\ 10^{-4}$	0.000494	474.81	$1.78\ 10^{-4}$	0.00035	196.63	24.5
k_{24}	hr^{-1}	0.788298	0.960750	123.44	0.907298	0.952438	104.98	15
k_{42}	hr^{-1}	0.349274	1.326444	368.28	0.375274	1.314581	350.3	7.4
V	L	4218.811	1240.08	29.19	4218.811	1220.32	28.93	0
Tlag	hr	0.559831	0.117656	21.09	0.56043	0.108542	19.38	0.11
Model diagnostics								
SSR*		1.4123			1.121			
Corr**		0.9911			0.9923			
AIC***		32.357			32.352			

* sum of squares of residuals, **correlation observed-predicted data, ***Akaike information criteria

7 PHARMACOKINETIC DRUG INTERACTIONS OF CARBAMAZEPINE

Three pharmacokinetic drug-drug interactions were run, between carbamazepine with ivabradine, zolpidem and lansoprazol. All these drugs are substrates of CYP3A4 enzyme, which is induced by carbamazepine. Due to induction of drug metabolism, the carbamazepine may affect the plasma levels and pharmacokinetics of ivabradine, zolpidem and lansoprazol.

The aim of the study was to analyze these three interactions regarding their magnitude and the possible clinical significance.

7.1 THE STUDY OF PHARMACOKINETIC INTERACTION BETWEEN IVABRADINE AND CARBAMAZEPINE

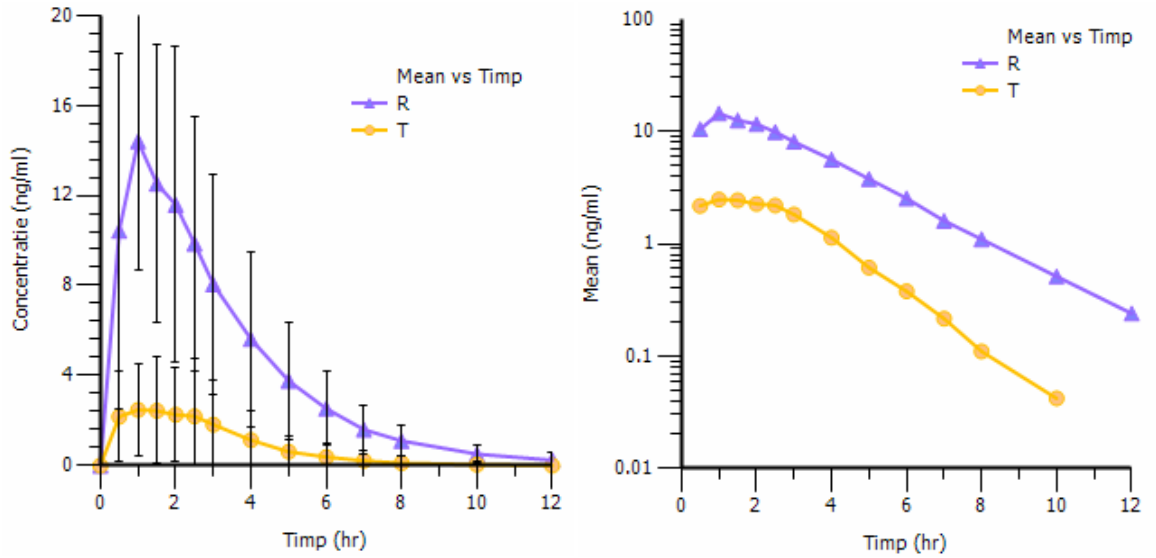
7.1.3 Study design

The study was run in two periods. In the first period (Reference, R), each volunteer received a single oral dose of 10 mg ivabradine (immediate release tablets, Corlentor product, Les laboratories Servier, France). Blood samples (5 ml) were drawn at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10 and 12 hours after drug administration. After blood sampling, each volunteer received for 9 days, a single daily dose of 400 mg carbamazepine (controlled release tablets, product Tegretol 400 CR, producer Novartis Pharma, Germany).

After the treatment with carbamazepine, in the second study period, a dose of 10 mg ivabradine was administered, along with 400 mg carbamazepine. A new series of blood samples were drawn. The second period of the study, when a pharmacokinetic interaction between ivabradine and carbamazepine may occur, will be further noted with Test (T).

The mean concentrations of ivabradine for each treatment period are presented in Fig. 84. One can observe a significant decrease of plasma levels of ivabradine in Test period, when it

is administered with carbamazepine. In log graph one can also observe the slightly change in terminal slope, this being a marker of changes of systemic clearance of the drug.



a b

Fig. 84 Mean plasma levels of ivabradine administered alone of in combination with carbamazepine, Cartesian graph (a) and log graph (b) (R = ivabradine alone, T=ivabradine+carbamazepine)

7.1.5 Pharmacokinetic drug interaction analysis by non-compartmental analysis

The mean values of pharmacokinetic parameter of ivabradine administered alone of with carbamazepine are presented in Table 66.

Table 66 Mean values of pharmacokinetic parameters of ivabradine administered alone (Reference, R) or with carbamazepine (Test, T).

		Treatment					
		Reference			Test		
Parameter	**Units**	**Mean**	**SD**	**CV%**	**Mean**	**SD**	**CV%**
Cmax	ng/ml	16.25	7.30	44.94	3.66	2.76	75.45
Tmax	hr	0.97	0.47	48.22	1.19	0.89	74.80
ASClast	hr*ng/ml	51.62	27.21	52.70	9.51	8.74	91.94
ASCinf	hr*ng/ml	52.81	27.46	52.00	10.33	8.79	85.12
ASC_%Extrap	%	2.63	1.51	57.22	11.90	7.64	64.20
Lambda_z	1/hr	0.39	0.07	18.10	0.45	0.13	27.82
t1/2	hr	1.85	0.35	19.01	1.69	0.68	40.34
TMR	hr	3.12	0.52	16.64	3.03	1.01	33.25
Vz_F	L	623.04	291.40	46.77	3678.90	2610.12	70.95
Cl_F	L/hr	238.70	114.86	48.12	1492.49	793.58	53.17

One can observe different values for the most of the pharmacokinetic parameters of ivabradine administered in the two periods of treatment, alone or in combination with carbamazepine.

A statistical ANOVA test was run to detect any significant differences between the pharmacokinetic parameters of ivabradine (2-ways ANOVA, with subject and treatment as sources of variation, p=0.05). The results of statistical test are presented in Table 67.

Table 67 Results of ANOVA statistical test for comparison of pharmacokinetic parameters of ivabradine administered alone or in combination with carbamazepine

		Hypothesis					
		Subject			Treatment		
Parameter	**Units**	**No_DF**	**F_stat**	**P***	**No_DF**	**F_stat**	**P***
Ln(Cmax)	ng/ml	17	3.30	0.009155	1	134	0.000000
Ln(Tmax)	hr	17	1.04	0.468179	1	0.0905	0.767184
Ln(ASClast)	hr*ng/ml	17	3.56	0.006230	1	175	0.000000
Ln(ASCinf)	hr*ng/ml	17	3.61	0.005728	1	181	0.000000
Ln(Lambda_z)	1/hr	17	1.78	0.123064	1	3.07	0.097543
Ln(t1/2)	hr	17	1.78	0.123064	1	3.07	0.097543
Ln(TMR)	hr	17	1.63	0.160802	1	0.766	0.393574
Ln(Vz_F)	L	17	3.28	0.009389	1	126	0.000000
Ln(Cl_F)	L/hr	17	3.61	0.005728	1	181	0.000000

*significant for $p<0.05$

Excepting Lambda_z, $t_{1/2}$, Tmax and TMR, one can observe significant differences between all the pharmacokinetic parameters of ivabradine analyzed, proving the intense pharmacokinetic interaction between ivabradine and carbamazepine.

7.1.7 Pharmacokinetic drug interaction analysis by compartmental analysis

The principle of analysis is that some pharmacokinetic parameters of ivabradine (clearance CL and the relative bioavailability F) may be changed due to drug interaction. A schematic presentation of the kinetic models is shown in Table 69.

Table 69 Kinetic models built for analysis of the interaction mechanism between ivabradine and carbamazepine

Model no.	**tlag**	**k_{01}**	**Cl_R**	**Cl_T**	**Frel**
M1	**X**	**X**	**FIX**		**FIX**
M2	**X**	**X**	**X**	**X**	**FIX**
M3	**X**	**X**	**FIX**		**X**
M4	**X**	**X**	**X**	**X**	**X**

In the previous table, tlag means the lag time until absorption starts, k_{01} is the absorption process rate constant (1^{st} order kinetics), Cl is the clearance, Vd is the drug distribution volume and Frel is the relative bioavailability of ivabradine administered with carbamazepine in comparison with ivabradine administered alone. The R and T are correlated with the study period (R=Reference - ivabradine administered alone, T=Test – ivabradine administered with carbamazepine). The "FIX" notation means that a certain parameter did not change due to drug interaction, having the same value in the two study periods, and "X" notations meant that a certain parameter may have different values between treatments. For example, Model 2 consider that the observed pharmacokinetic interaction appears due to clearance changing between periods, but the Model 4 considers that two parameters are changed due to interaction: both clearance and the relative bioavailability.

7.1.7.3 Kinetic modeling results

The smallest value for Akaike index (best fit, best model) was obtained for model M4, considering that the observed pharmacokinetic interaction appears due to changes both of clearance (systemic metabolism) and relative bioavailability (pre-systemic metabolism).

For example, some typical fittings of models 1-4 to a subject data (Subject 5) are presented in Fig. 86.

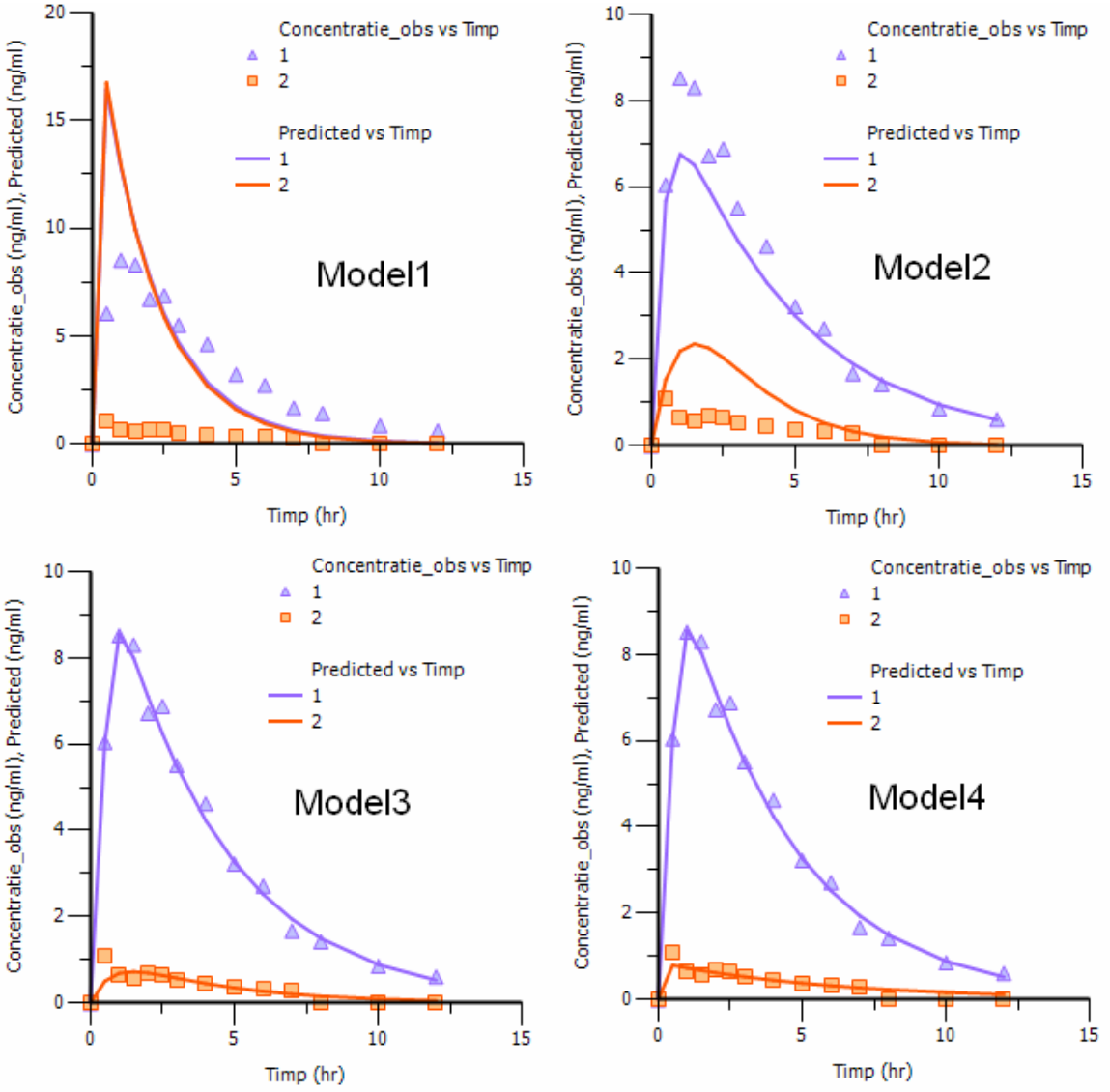


Fig. 86. Fittings of the models 1-4 to Subject 5 experimental data (1=Reference, 2=Test)

7.1.8 CONCLUSIONS

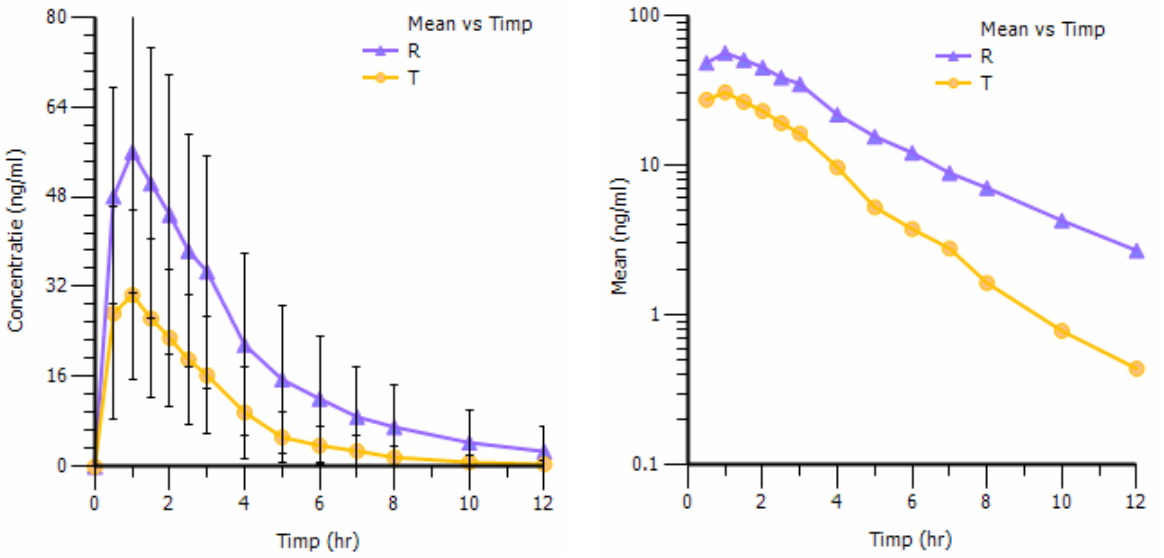
The pharmacokinetic interaction between ivabradine and carbamazepine was studied.

The analysis results show that there is an intense pharmacokinetic interaction between ivabradine and carbamazepine, expressed by a significant reduction of plasma levels of ivabradine, thus the reduction of drug exposure (by 83%) and the reduction of drug elimination half-life.

7.2 THE STUDY OF PHARMACOKINETIC INTERACTION BETWEEN ZOLPIDEM AND CARBAMAZEPINE

The study was run in a similar way as previously described for pharmacokinetic interaction between ivabradine and carbamazepine.

The mean plasma levels of zolpidem for each treatment period (zolpidem alone or zolpidem + carbamazepine) are presented in Fig. 91.



a b

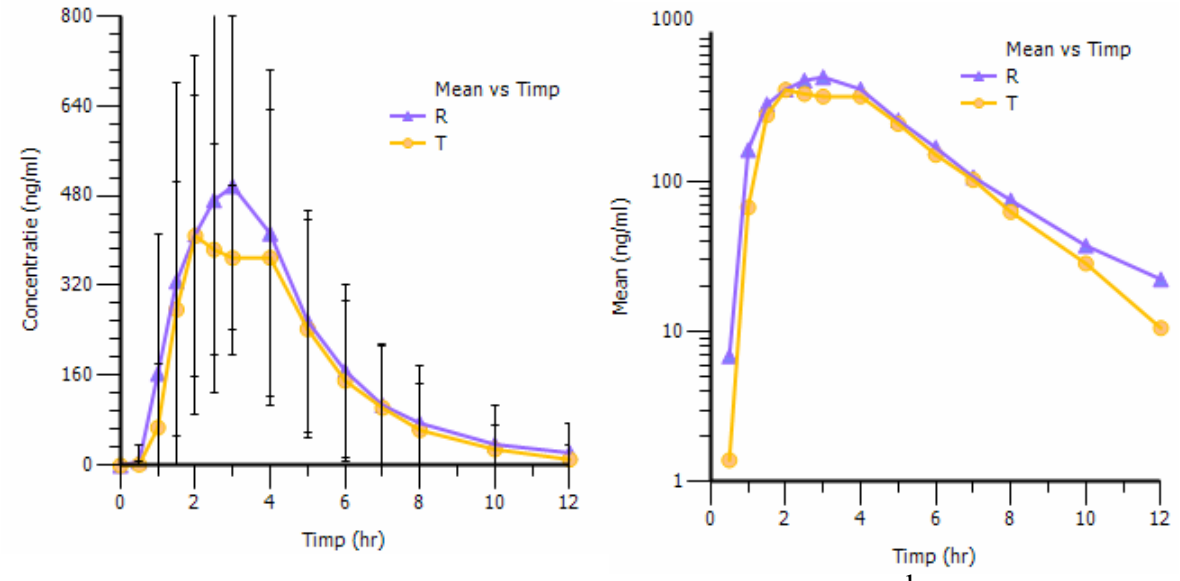
Fig. 91 Mean plasma levels of zolpidem administered alone or in combination with carbamazepine after pre-treatment with carbamazepine; Cartesian graph (a) and log graph (b) (R = zolpidem alone, T=zolpidem+carbamazepine)

The pharmacokinetic analysis demonstrated the intense drug-drug interaction between zolpidem and carbamazepine, having the same mechanism as shown in case of ivabradine: changes in both systemic and pre-systemic metabolism of zolpidem.

7.3 THE STUDY OF PHARMACOKINETIC INTERACTION BETWEEN LANSOPRAZOL AND CARBAMAZEPINE

The study was run in a similar way as previously described for pharmacokinetic interaction between ivabradine or zolpidem with carbamazepine.

The mean plasma levels of lansoprazol for each treatment period (lansoprazol alone or lansoprazol + carbamazepine) are presented in Fig. 98.



a b

Fig. 98 Mean plasma levels of lansoprazol administered alone or in combination with carbamazepine after pre-treatment with carbamazepine; Cartesian graph (a) and log graph (b) (R = lansoprazol alone, T=lansoprazol+carbamazepine)

The pharmacokinetic analysis did not reveal a significant drug-drug interaction between lansoprazol and carbamazepine, probably due to existence of alternative elimination paths for lansoprazol from the body.

8 GENERAL CONCLUSIONS

The aim of this PhD thesis was the pharmacokinetic study of carbamazepine administered as single dose in human subjects as immediate or prolonged release tablets, its release kinetics in vitro and the evaluation of some pharmacokinetic drug-drug interactions of carbamazepine.

The first chapter of experimental part describes the (bio)analytical methods used for determination of carbamazepine from pharmaceutical formulations, as well for determination of carbamazepine, 10,11-epoxy-carbamazepine, ivabradine, zolpidem and lansoprazol from human plasma. All this developed and elaborated methods have superior performances in comparison with already published papers in literature (simple sample preparation method, better sensitivity, short analysis time).

There were run in vitro dissolution tests for analysis of 9 pharmaceutical preparations with carbamazepine, prolonged release tablets. In the first step, the optimum dissolution conditions were established. By using kinetic modeling, one can obtain information about the release mechanism of drug from pharmaceutical formulations tested, that being an useful tool in the process of pharmaceutical form development. The dissolution profiles for the 9 pharmaceutical products were obtained and further analyzed using 11 simple kinetic models and 8 complex models. From the involved models, the best kinetic model describing the carbamazepine release considers the existence of two 1^{st} order kinetic processes (one starting immediately after the beginning of experiment, and the other starting later).

The pharmacokinetic of carbamazepine and 10,11-epoxy-carbamazepine (its active metabolite) was studied, from both immediate and prolonged release pharmaceutical formulations. The pharmacokinetic experiments were based on a single oral administration of pharmaceutical formulations containing carbamazepine, either as Reference or generic formulations. In the first step of analysis, the non-compartmental analysis was used, obtaining the corresponding parameters describing in a global manner the pharmacokinetics of carbamazepine and its metabolite. The calculated pharmacokinetic parameters were compared by using statistical analysis and then a bioequivalence test was run, showing that the analyzed preparations are bioequivalent and thus, interchangeable. By using kinetic modeling it was identified the best mathematical model describing the processes of

carbamazepine absorption, distribution, metabolism and elimination. The kinetic analysis was done in a few steps, starting with simple models, only for carbamazepine, and finishing with complex models allowing simultaneously fitting of plasma levels of both carbamazepine and 10,11-epoxy-carbamazepine. The best kinetic model suggests a biphasic absorption of carbamazepine (two 1st order kinetic processes), with relatively low effect of pre-systemic metabolism, elimination of carbamazepine by either metabolism to 10,11-epoxy-carbamazepine or by alternative ways. The same kinetic model was found for both immediate and prolonged release pharmaceutical formulations.

There were studied the pharmacokinetic drug-drug interactions between carbamazepine with ivabradine, zolpidem and lansoprazol, all of them substrates of cytochrome P450 CYP3A4 enzyme. Carbamazepine is an inducer of CYP3A4, so pharmacokinetic drug-drug interactions may occur, affecting the efficacy of the treatment. The pharmacokinetic experiments show that there are important drug-drug interactions between carbamazepine with ivabradine and zolpidem, the interaction mechanism being an increase of both pre-systemic and systemic metabolism of the interfered drug (as demonstrated by kinetic modeling). No significant interaction between carbamazepine and lansoprazol was detected. When co-administration of carbamazepine with either ivabradine or zolpidem occurs, the therapeutic levels of those drugs will not be reached, thus affecting the treatment efficacy.

The results of research add some new aspects regarding the pharmacokinetics of carbamazepine and its pharmacokinetic drug-drug interactions.

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