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# Novel approach to bioequivalence assessment based on physiologically motivated model

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ABSTRACT

The study was conducted to exemplify an approach capable of obtaining a new insight into bioequivalence (BE) assessment, by the use of a physiologically motivated model.

Data from an oral BE study of two piroxicam (PXM) products was used as an example. The BE study was carried out with 24 healthy European subjects according to a two-sequence crossover-randomized design. The test and reference formulations were a PXM generic formulation (LaborMed Pharma, Romania) and Feldene® (Pfizer, USA), respectively. Plasma concentrations of PXM were monitored by a validated high-performance liquid chromatography over a period of 144 h after administration. After the structure of the optimal model was selected, parameters that characterized the whole-body disposition behavior of PXM in the subjects were derived. The paired Student's *t*-test and Wilkoxon's test were performed on the derived parameters.

The null hypothesis of no differences in the parameters of the whole-body disposition behavior of PXM related to the test and reference product was not rejected at 5% level of significance. This result suggested that the compared products were bioequivalent and could be used interchangeably in clinical setting. The presented approach might show a new way, worth incorporating in future BE guidelines.



## 1. Introduction

The design, performance, and evaluation of bioequivalence (BE) studies have received significant attention from academia, pharmaceutical industry, and health authorities over the past quarter century, see e.g. (Bois et al., 1994; Steinijans et al., 1995; Tozer et al., 1996; Tozer and Hauck, 1997; Rescigno and Powers, 1998; Marzo, 1999; Testa, 2000; Food and Drug Administration, 2001a,b; Chen et al., 2001; Patterson and Jones, 2002; Williams et al., 2002; Hu et al., 2004). After single-dose administration of oral products, BE estimators are primarily expressed by summary variables, such as an area under the plasma concentration–time curve (AUC), as a robust estimator of the extent of drug bioavailability, and a plasma drug maximum concentration ($C_{max}$), as an imperfect estimator of the rate of drug bioavailability (Blume et al., 2005; European Medicines Agency Evaluation of Medicines for Human Use Questions & Answers on the Bioavailability and Bioequivalence Guideline, 2006; Benet et al., 2008; Haidar et al., 2008). The given variables have become surrogate indicators of therapeutic outcome.

At present, there is a belief by many theoreticians and experimentalists in the BE area that not only modification of current techniques but also new techniques are needed (Rescigno and Powers, 1998; Marzo, 1999; Chen et al., 2001). The importance and novelty of this work is that it presents a new approach to bioequivalence assessment, based on physiologically realistic models of pharmacokinetic behavior of drugs in the body, not on summary metrics commonly used in practice (AUC, $C_{max}$). The present study tests the possibility of applying an approach based on a physiologically motivated model to BE assessment. Namely, the model that incorporates approximations of the following single processes: disintegration, dissolution, multi-fraction gastric emptying, time-delayed disposition for absorption, absorption, and entero-hepatic cycling (EHC). Influences of the specified processes on the drug whole-body disposition behavior are not taken into consideration in the conventional techniques for the BE assessment, which are centered in summary variables such as AUC and $C_{max}$. Summary variables filter out information conveyed by the influence of the specified single processes, consequently the definition of bioequivalence in terms of the summary variables exhibits problems (Tozer

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et al., 1996; Rescigno and Powers, 1998). To include influences of physiological factors to bioequivalence assessment is important mainly in the case of testing formulations of drugs with a high degree of variability of absorption from the gastrointestinal tract, where omitting the considered influences might lead to incorrect conclusions. The approach presented in this study was applied to a BE assessment of two piroxicam (PXM) formulations. PXM products were used on purpose, as the PXM whole-body disposition behavior in humans is complex, plasma PXM concentration–time profiles often exhibit two or more multiple well resolved peaks after the PXM oral administration, and PXM is known as a subject to EHC (Benveniste et al., 1990). PXM is a member of the oxicam group of non-steroidal anti-inflammatory drugs. It is recognized for its value as a potent chemo-preventative and anti-tumor agent, and moreover as a potent agent in treatment of rheumatoid arthritis, osteoarthritis and other joint diseases (Kelloff et al., 1994).

The approach presented was designed from viewpoints of the theory linear time-invariant dynamic systems (Kailath, 1980; Ďurišová and Dedík, 1997, 2005; Dedík and Ďurišová, 2004, 2008; Dedík et al., 2007; Tvrdonova et al., 2008) and physiologically motivated models (Andersen et al., 2006; Tvrdonova et al., 2008). In this study, a dynamic system is considered a means of describing how one state of a dynamic process develops into another state over the course of time.

## 2. Materials and methods

### 2.1. Dosage forms

Product 1: the reference formulation, 20 mg PXM tablets (Pfizer, Feldene®). Product 2: the test formulation, 20 mg PXM capsules (LaborMed Pharma Romania, PXM Generic formulation).

### 2.2. Study design

A standard, randomized, single-dose, fasting-state, two-period, crossover BE study (Food and Drug Administration, 2001b; Patterson and Jones, 2002; Williams et al., 2002; Hu et al., 2004; Blume et al., 2005; European Medicines Agency Evaluation of Medicines for Human Use Questions & Answers on the Bioavailability and Bioequivalence Guideline, 2006; Haidar et al., 2008; Benet et al., 2008) was conducted in 24 healthy female and male Romanian volunteers between the age of 18 and 45 years. The volunteers gave written informed consent to participation in the study, after they were informed of the nature and implications of the study. The BE study followed the tenets of the Declarations of Helsinki promulgated in 1964 and was approved by the Institutional Ethic Committee and Regulatory authorities of Biopharmacy and Pharmacology Research, S.A., Bucharest, Romania. A complete medical history and physical examination were given to each volunteer prior to the BE study. The volunteers reported to the clinical laboratory in the morning after an overnight fast. They were randomly assigned to treatment sequences to receive the reference formulation followed by the test formulation with a two-week washout period between doses. In each sequence, blood samples were withdrawn from the forearm vein and were collected by indwelling catheter into heparinized evacuated tubes. Plasma was removed by centrifugation at 4 °C and the plasma was stored in glass vials at −70 °C until analysis. Pre-dose blood samples were taken. Thereafter the volunteers received 20 mg of PXM either in Product 1 or Product 2 with 180 ml of room-temperature water. Blood samples for determination of plasma PXM concentrations were taken at predose (−1 h) and then 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 24, 72, 144 h post-dose. Subjects arrived in the evening (8 p.m.) at the clinical site. After the overnight fast they received the drug, at 8 a.m. in the morning. They remained in the clinic 24 h after drug administration. They received the standard meal on the first day at 12 a.m. and 5 p.m. Water intake was non-restricted. Other liquids were not allowed. Second day after blood sampling at 8 a.m., they left the clinical site and came back every day at 8 a.m. for blood sampling. Avoidance of alcohol consumption was recommended for the outside clinic period.

### 2.3. Analysis of PXM in plasma

The plasma samples were assayed by a validated high-pressure liquid chromatographic (HPLC) method. The HPLC system consisted of a Waters model 712 injector (Waters Assoc., USA), a model 600 solvent delivery system, and a model 486 UV detector. Kromasil® 100-5C18 reversed-phase column (15 cm × 0.46 cm) kept in a thermostat at 45 °C was used. The mobile phase consisted of 60% trifluoroacetic acid 0.1% and 40% acetonitrile:methanol (4:1, v/v) mixture.

The flow rate was 1.0 ml/min. The detection of PXM was performed at the wavelength $\lambda = 330$ nm. The lower limit of quantification was 0.1 μg/ml for PXM. The intra- and inter-assay relative standard deviations ranged from 0.86% to 5.33% and 3.55% to 4.88%, respectively.

### 2.4. Model construction and validation

The following subsections contain theoretical foundations for the approach exemplified in this study and address readers interested in mathematical considerations. The foundations presented are important because they legitimize the approach used here; however the understanding of the given details is not essential to appreciate the results obtained in this study. Therefore, readers who wish to skip the subsections may do so with little loss in understanding the approach and results presented.

To select the optimal model of the PXM whole-body disposition behavior in the subjects enrolled, the following means were used: (1) tools of mathematical modeling and analysis based on the LDS theory, implemented in the software CTDB (Clinical Trials DataBase) (Dedík and Ďurišová, 2004); (2) the measured plasma PXM concentration–time profiles of the subjects enrolled. Starting from a nominal model structure that was *a priori* designed to make sense anatomically and physiologically, nested rival model structures were proposed. Performance of the nested model structures was evaluated by extensive simulations and goodness-of-fit tests over all subjects enrolled, until no improvement of a model performance could be achieved, and until the best compromise between goodness-of-fit and parsimony was reached. The verification of nested models consisted of the following steps: (i) the goodness-of-fit was tested numerically, using the minimum of the square criterion $\varepsilon$,

$$\varepsilon = \sum_{j=1}^{m} (C(t_j) - C_M(t_j))^2, \quad (1)$$

where $m$ is the number of sampling points, $C_M(t)$ is a model-based prediction of a plasma PXM concentration–time profile, and $C(t)$ is a measured plasma PXM concentration–time profile; (ii) goodness-of-fit was assessed visually by plotting the profile $C_M(t)$ versus the profile $C(t)$, and by inspecting these graphs; (iii) nested rival models were compared, using Akaike's information criterion (AIC). The parsimonious model was the one with the smallest AIC score (Hosmer and Lemeshow, 2000).

Fig. 1 depicts the computational scheme of the structure of the physiologically motivated model of the PXM whole-body disposition behavior, selected as optimal in the subjects enrolled. The model structure was described in detail in study (Tvrdonova et al.,

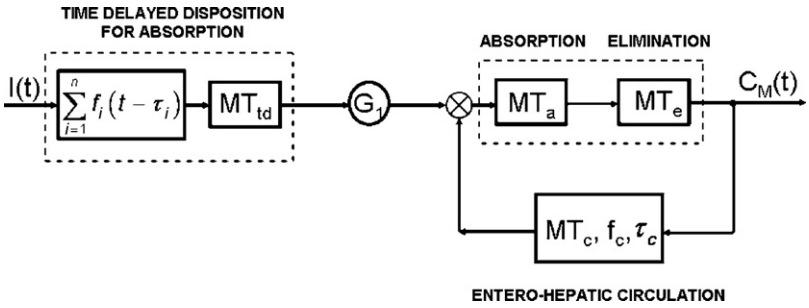


**Fig. 1.** Physiologically motivated model of whole-body disposition behavior of a drug. $I(t)$ is the drug input into the body in a single oral dose; $\tau_i$ for $i = 1, \ldots, n$ are time-delay parameters; $f_i$, $i = 1, \ldots, n$ are fractions of a drug dose; $n$ is the number of fractions of a drug dose; $MT_{TD}$ and $G_1$, respectively are the mean-time parameter and gain of the subsystem TD that formalizes disintegration, dissolution, and gastric emptying processes; the subsystem AE formalizes absorption and elimination; $MT_a$ and $MT_e$, respectively are the mean-time parameters of the absorption and elimination process; $MT_c$, $f_c$, and $\tau_c$, respectively are the mean-time parameter of entero-hepatic circulation, the fraction of a drug dose undergoing entero-hepatic circulation, and time-delay parameter of entero-hepatic circulation; $C_M(t)$ is the model-based prediction of plasma concentration–time profile of a drug.

2008), therefore only a brief model outline is given here. The crucial assumption behind the model is that the processes dominantly involved in the PXM whole-body disposition behavior (absorption, distribution, elimination) can be adequately formalized as a linear dynamic system H with time-invariant parameters. The scheme of the system H shown in Fig. 1 consists of two parts. The first part contains the subsystem TD that formalizes the time-delayed disposition for absorption, namely disintegration, dissolution, and multi-fraction gastric emptying. The subsystem TD consists of $n$, $i = 1, \ldots, n$, branches, coming from stomach to an absorption site. The branch $i$ is characterized by the time-delay parameter $\tau_i$ and the fraction $f_i$ of the PXM dose. $\tau_i$ is the finite time taken for the fraction $f_i$ to transit from stomach to a site of absorption. The subsystem TD is characterized by the mean-time parameter $MT_{TD}$ and mean residence time $MRT_{TD}$. The second part of the scheme in Fig. 1 consists of one forward branch and one backward branch, containing the subsystems AE and EHC, respectively. The subsystem AE accounts for absorption and elimination. The mean-time parameters $MT_a$ and $MT_e$, respectively are characteristics of the absorption and elimination processes. The subsystem *EHC* accounts for EHC. It encompasses: (1) the block with the mean-time parameter $MT_c$, i.e. the mean-time parameter of the EHC process, (2) the quantity $f_c$, $f_c < 1$, i.e. the fraction of the PXM dose undergoing EHC, and (3) the time delay $\tau_c$ of the EHC process.

**Table 1**
Reference formulation of piroxicam (Feldene® 20 mg).

| Subject | $MT_{TD}$ (h) | $n$ | $MT_c$ (h) | $\tau_c$ (h) | $f_c$ | $MT_{ae}$ (h) | $MRT_{TD}$ (h) | MRT (h) | $\underline{Q}_{cl}$ (ml/h) | $Q_{cl}$ (ml/h) |
|---|---|---|---|---|---|---|---|---|---|---|
| I | 0.261 | 6 | 1.338 | 11.118 | 0.800 | 2.470 | 5.357 | 48.315 | 1174.20 | 262.055 |
| II | 0.345 | 6 | 2.076 | 6.823 | 0.867 | 2.399 | 4.281 | 51.904 | 1375.00 | 220.167 |
| III | 0.476 | 5 | 1.698 | 8.281 | 0.881 | 2.300 | 4.257 | 54.989 | 1472.80 | 227.324 |
| IV | 0.251 | 5 | 3.716 | 10.017 | 0.819 | 3.152 | 4.307 | 51.598 | 1175.90 | 253.743 |
| V | 0.494 | 5 | 1.625 | 6.892 | 0.907 | 1.473 | 4.486 | 56.290 | 1786.50 | 219.974 |
| VI | 0.570 | 6 | 3.934 | 7.234 | 0.878 | 2.164 | 5.043 | 55.788 | 2353.60 | 376.364 |
| VII | 0.228 | 5 | 2.277 | 10.303 | 0.848 | 2.214 | 4.750 | 53.176 | 959.92 | 178.763 |
| VIII | 0.248 | 5 | 1.500 | 6.694 | 0.818 | 2.442 | 4.143 | 43.840 | 1613.20 | 325.521 |
| IX | 0.348 | 6 | 2.249 | 7.559 | 0.835 | 1.679 | 4.574 | 47.379 | 1740.60 | 312.402 |
| X | 0.542 | 6 | 1.578 | 7.797 | 0.841 | 1.632 | 4.620 | 47.476 | 2202.50 | 387.747 |
| XI | 0.261 | 5 | 1.284 | 9.962 | 0.788 | 3.039 | 5.116 | 46.592 | 1852.00 | 432.339 |
| XII | 0.501 | 6 | 4.516 | 5.633 | 0.882 | 1.493 | 5.352 | 54.369 | 2133.30 | 314.564 |
| XIII | 0.284 | 6 | 3.771 | 8.295 | 0.811 | 2.082 | 6.142 | 48.953 | 1047.50 | 226.809 |
| XIV | 0.274 | 6 | 3.867 | 6.257 | 0.855 | 2.347 | 4.784 | 51.757 | 1211.80 | 209.688 |
| XV | 0.351 | 5 | 3.009 | 7.727 | 0.740 | 3.232 | 4.193 | 40.130 | 1456.70 | 393.082 |
| XVI | 0.115 | 5 | 3.545 | 9.020 | 0.796 | 2.624 | 4.272 | 47.271 | 1132.20 | 260.078 |
| XVII | 0.229 | 6 | 3.840 | 5.855 | 0.791 | 2.798 | 4.531 | 43.696 | 1104.60 | 250.941 |
| XVIII | 0.212 | 6 | 5.201 | 8.017 | 0.858 | 2.469 | 5.039 | 55.022 | 1048.10 | 195.542 |
| XIX | 0.224 | 6 | 3.074 | 6.085 | 0.833 | 2.716 | 4.497 | 48.157 | 1324.80 | 248.139 |
| XX | 0.224 | 5 | 3.073 | 8.005 | 0.845 | 3.210 | 3.805 | 52.282 | 1292.80 | 242.307 |
| XXI | 0.150 | 6 | 2.877 | 10.705 | 0.866 | 2.821 | 4.832 | 56.748 | 1067.20 | 198.491 |
| XXII | 0.414 | 5 | 3.661 | 8.075 | 0.873 | 2.367 | 4.632 | 55.607 | 1328.10 | 219.443 |
| XXIII | 0.279 | 5 | 3.738 | 8.901 | 0.859 | 3.478 | 3.679 | 55.255 | 936.84 | 178.412 |
| XXIV | 0.223 | 6 | 1.262 | 7.875 | 0.824 | 1.950 | 5.403 | 46.100 | 1572.90 | 298.418 |
| Mean[a] | 0.313 | 6[c] | 2.863 | 8.047 | 0.838 | 2.440 | 4.671 | 50.528 | 1431.80 | 268.013 |
| SD[b] | 0.102 | | 0.884 | 1.209 | 0.027 | 0.428 | 0.429 | 2.892 | 353.46 | 70.276 |

$MT_{TD}$—mean time of the subsystem that formalizes time-delayed disposition for absorption; $n$—number of the fraction of piroxicam dose disposable for absorption; $MT_c$—mean time of the subsystem that formalizes entero-hepatic circulation; $\tau_c$—time delay of the subsystem that formalizes entero-hepatic circulation; $f_c$—the fraction of piroxicam dose undergoing entero-hepatic circulation; $MT_{ae}$—mean time of the subsystems that formalizes absorption and elimination; $MRT_{TD}$—mean residence time of the subsystem that formalizes time-delayed disposition for absorption; MRT—mean residence time of the entire system that formalizes the whole-body disposition behavior of piroxicam; $\underline{Q}_{cl}$—appearent oral clearance estimated using a model that did not incorporate entero-hepatic circulation; $Q_{cl}$—apparent oral clearance estimated using a model that incorporated entero-hepatic circulation.

[a] Arithmetic mean.

[b] Standard deviation.

[c] Median of the number of fractions of piroxicam dose.

**Table 2**
Test formulation (generic formulation of piroxicam LaborMed Pharma 20 mg).

| Subject | $MT_{TD}$ (h) | $n$ | $MT_c$ (h) | $f_c$ | $MT_{ae}$ (h) | $MRT_{TD}$ (h) | MRT (h) | $\underline{Q}_{cl}$ (ml/h) | $Q_{cl}$ (ml/h) |
|---|---|---|---|---|---|---|---|---|---|
| I | 0.626 | 5 | 1.761 | 0.849 | 1.775 | 5.304 | 52.548 | 1419.90 | 260.620 |
| II | 0.307 | 5 | 3.080 | 0.861 | 2.076 | 5.324 | 55.233 | 1327.10 | 235.405 |
| III | 0.247 | 6 | 1.975 | 0.856 | 3.814 | 5.818 | 53.264 | 1118.10 | 192.308 |
| IV | 0.316 | 5 | 1.739 | 0.782 | 3.724 | 3.513 | 42.059 | 1178.80 | 276.702 |
| V | 0.256 | 5 | 2.949 | 0.869 | 2.546 | 4.681 | 56.592 | 1145.20 | 206.526 |
| VI | 0.416 | 5 | 5.223 | 0.810 | 2.730 | 5.316 | 49.984 | 1727.70 | 393.082 |
| VII | 0.589 | 5 | 3.670 | 0.771 | 3.774 | 5.163 | 48.367 | 870.75 | 226.603 |
| VIII | 0.391 | 5 | 2.761 | 0.832 | 2.337 | 4.070 | 49.697 | 1520.30 | 298.151 |
| IX | 0.459 | 5 | 4.548 | 0.776 | 1.804 | 5.217 | 47.575 | 1322.40 | 335.233 |
| X | 0.222 | 5 | 2.278 | 0.858 | 2.417 | 3.676 | 50.875 | 1813.20 | 303.951 |
| XI | 0.264 | 5 | 7.213 | 0.796 | 2.416 | 4.613 | 49.265 | 1839.30 | 445.434 |
| XII | 0.340 | 5 | 2.939 | 0.907 | 2.395 | 5.165 | 55.189 | 2166.40 | 265.252 |
| XIII | 0.414 | 5 | 5.065 | 0.842 | 1.372 | 4.154 | 48.743 | 1480.30 | 282.646 |
| XIV | 0.570 | 5 | 1.510 | 0.847 | 1.631 | 5.526 | 52.111 | 1016.50 | 190.767 |
| XV | 0.443 | 5 | 2.588 | 0.867 | 2.453 | 4.625 | 52.641 | 1783.60 | 285.307 |
| XVI | 0.360 | 5 | 1.969 | 0.858 | 1.698 | 4.588 | 48.214 | 1450.50 | 235.960 |
| XVII | 0.381 | 5 | 1.804 | 0.840 | 4.152 | 4.041 | 52.434 | 887.70 | 174.611 |
| XVIII | 0.521 | 5 | 2.406 | 0.848 | 2.481 | 5.479 | 54.171 | 1120.10 | 214.592 |
| XIX | 0.686 | 5 | 7.446 | 0.854 | 2.517 | 4.663 | 54.094 | 1325.70 | 253.357 |
| XX | 0.265 | 5 | 2.630 | 0.894 | 1.746 | 6.229 | 59.615 | 1175.10 | 151.837 |
| XXI | 0.318 | 5 | 2.367 | 0.897 | 2.663 | 3.708 | 55.960 | 1308.00 | 180.408 |
| XXII | 0.415 | 6 | 3.880 | 0.831 | 2.588 | 4.396 | 49.683 | 1499.30 | 292.654 |
| XXIII | 0.720 | 4 | 2.102 | 0.886 | 2.774 | 5.830 | 57.931 | 989.48 | 155.666 |
| XXIV | 0.267 | 5 | 2.013 | 0.856 | 1.697 | 3.460 | 49.174 | 1896.70 | 312.891 |
| Mean[a] | 0.408 | 5[c] | 3.163 | 0.845 | 2.482 | 4.773 | 51.892 | 1390.90 | 257.082 |
| SD[b] | 0.050 | | 0.293 | 0.003 | 0.170 | 0.159 | 0.710 | 14.62 | 70.449 |

$MT_{TD}$—mean time of the subsystem that formalizes time-delayed disposition for absorption; $n$—number of the fraction of piroxicam dose disposable for absorption; $MT_c$—mean time of the subsystem that formalizes entero-hepatic circulation; $\tau_c$—time delay of the subsystem that formalizes entero-hepatic circulation; $f_c$—the fraction of piroxicam dose undergoing entero-hepatic circulation; $MT_{ae}$—mean time of the subsystems that formalizes absorption and elimination; $MRT_{TD}$—mean residence time of the subsystem that formalizes time-delayed disposition for absorption; MRT—mean residence time of the entire system that formalizes the whole-body disposition behavior of piroxicam; $\underline{Q}_{cl}$—appearent oral clearance estimated using a model that did not incorporate entero-hepatic circulation; $Q_{cl}$—apparent oral clearance estimated using a model that incorporated entero-hepatic circulation.

[a] Arithmetic mean.

[b] Standard deviation.

[c] Median of the number of fractions of piroxicam dose.

### 2.5. Calculation of characteristics of PXM whole-body disposition behavior

Subject's characteristic vectors λ (Eq. (2)), were determined employing parameters of subject's model of the PXM whole-body disposition behavior and the modules of the CTDB software for the Monte Carlo (Manno, 1999) and the Gauss–Newton (Heath, 2002) method (see details in Tvrdonova et al., 2008),

$$\lambda = (G_1, \{\tau_i, f_i\}_{i=1\ldots n}, MT_{td}, MT_a, MT_e, MT_c, f_c, \tau_c) \text{ for } t > \tau_c \quad (2)$$

under the condition given by Eq. (3).

$$\sum_{i=1}^{n} f_i = 1. \quad (3)$$

Employing these vectors, the following characteristics of the PXM whole-body disposition behavior were determined:

The hypothetical apparent clearance $\underline{Q}_{cl}$ of PXM, was determined according to Eq. (4)

$$\underline{Q}_{cl} = \frac{1}{G_1}. \quad (4)$$

$\underline{Q}_{cl}$ applies to the model that does not contain an approximation of the EHC process.

The real apparent clearance $Q_{cl}$ of PXM was calculated according to Eq. (5)

$$Q_{cl} = \frac{D}{\int_0^{t_m} C_M(t)dt}, \quad (5)$$

where $Q_{cl}$ applies to the model that contains an approximation of the EHC process.

The mean residence time $MRT_{TD}$ was calculated using Eq. (6), under the condition given by Eq. (3)

$$MRT_{TD} = \sum_{i=1}^{n} (\tau_i + MT_{TD}) f_i. \quad (6)$$

The mean time of the subsystem AE was calculated according to Eq. (7)

$$MT_{ae} = MT_a + MT_e. \quad (7)$$

The mean residence time MRT, of whole system H was calculated according to Eq. (8)

$$MRT \doteq \frac{\int_0^{t_m} t.C_M(t)dt}{\int_0^{t_m} C_M(t)dt}, \quad (8)$$

where $t_m$ is the last sampling point.

### 2.6. Statistical analysis

The paired Student's $t$-test and Wilkoxon's test were performed on the following parameters: the mean-time $MT_{TD}$, the mean-time $MT_{ae}$, the mean residence time $MRT_{TD}$, the mean time $MT_c$, the fraction $f_c$, the time delay $\tau_c$, the mean residence time MRT, the hypothetical apparent clearance $\underline{Q}_{cl}$ and the real apparent clearance $Q_{cl}$ A $p$ value of less than 0.05 was considered to indicate statistical significance (Hauck et al., 1997). The results obtained in this study were compared with those obtained for assessment quantities $AUC_0^\infty$, $AUC_0^t$, $C_{max}$, $T_{max}$, $K_{el}$, $T_{1/2}$, using the tests conventionally utilized in the BE area (Food and Drug Administration, 2001b; Chen et al., 2001).

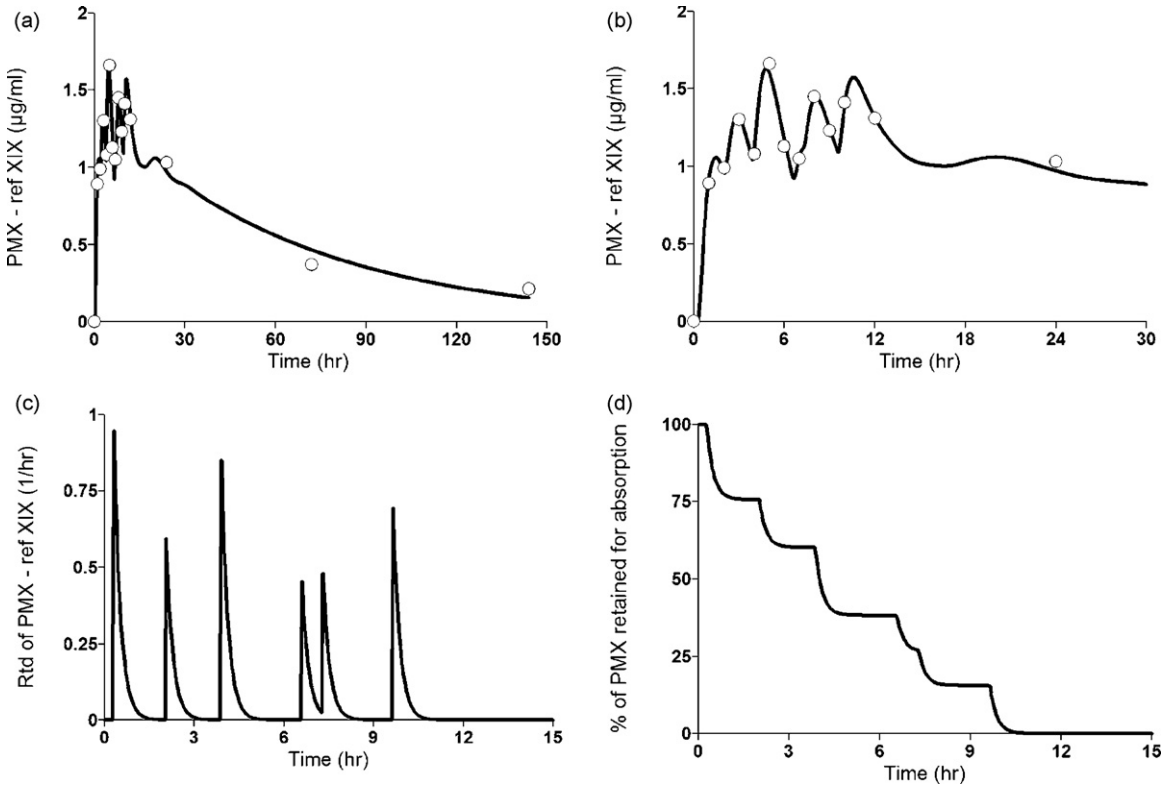


**Fig. 2.** Example of modeling results, subject XIX. Measured plasma concentration–time profile of piroxicam, administered in the single dose of 20 mg in capsules Feldene® Pfizer (circles). Model-based prediction of the measured profile (line). (a) Presentation over the whole time interval of the study; (b) presentation over the first time interval after piroxicam administration; (c) rate of time-delayed disposition of piroxicam for absorption; (d) fraction of piroxicam dose actually present in stomach and disposable for time-delayed absorption.

## 3. Results

The scheme of the physiologically motivated model selected as optimal, by physiological and statistical arguments, for all subjects enrolled and for both PXM products is shown in Fig. 1. The final estimates for the characteristics of the PXM whole-body disposition behavior are listed in Table 1 (Pfizer, Feldene®) and in Table 2 (LaborMed Pharma Romania, PXM, a generic formulation). The sensitivity and precision of the analytical methods used were sufficient for determination of plasma concentrations of PXM (Benveniste et al., 1990).

For illustrative presentations of modeling outcomes, results obtained for subject XIX were arbitrarily selected as representative. Fig. 2a depicts the plasma concentration–time profile of PXM in the specified subject after oral administration of 20 mg of PXM in the reference product (Pfizer, Feldene®) over the whole time period of the BE study. Fig. 2b shows the same profile, however over the time period of 30 h after the product administration. It follows from later figures, that outcomes of the developed model accurately described the measured plasma PXM concentration–time profile, despite the fact that the given profile was unusual and exhibited several apparent peaks. The function that approximates the rate of time-delayed disposition of PXM for absorption in subject XIX is illustrated in Fig. 2c. Six apparent peaks seen in the measured profile refer to six-fractions of the PXM dose that underwent delayed gastric emptying. The time-delayed disposition for absorption is characterized by the delayed passage of PXM from stomach to an absorption site, in the absence of mechanical obstructions. Fig. 2d shows fractions of the PXM dose (given in the reference formulation) actually present in stomach and disposable for time-delayed absorption. Analogous results obtained for subject XIX after administration of the test formulation are given in Fig. 3a–d. The results in the later figures indicate an apparent difference in the gastric emptying process after administration of the reference and test formulation, i.e. apparent differences in the number of the fractions of the PXM dose disposable for absorption (Figs. 2c and 3c), and apparent differences in percentages of the PXM dose actually present in stomach and disposable for absorption (Figs. 2d and 3d). The results analogous to those in Figs. 2a–d and 3a–d were obtained for all subjects and both products compared.

## 4. Discussion

Except for the mean-time parameter $MT_{TD}$ of the subsystem TD, no significant differences were observed between the parameters of the reference and generic PXM formulations. This finding is confirmed by slight differences observed in rates of gastric emptying processes and extents of fractions of the PXM dose disposable for absorption after administration of the reference and generic PXM formulation see Figs. 2a and b and 3a and b. It follows from Tables 1 and 2 that the fraction of the PXM dose undergoing EHC was rather high, ranging from 74% to 91% of the PXM dose. This result might account for the variability of PXM absorption (Perini et al., 2005).

Both formulations of PXM were well tolerated. Unexpected incidents did not occur. All subjects continued to the end of the BE study. No volunteer was withdrawn and no serious adverse event was found during the BE study.

The crucial assumption of linearity of the PXM whole-body disposition behavior in human body is in agreement with the fact that most drugs show linear disposition behavior at conventional therapeutic drug concentrations (Veng-Pedersen et al., 1997).

Terms uncommon in the pharmacokinetic literature are used in this study. In order to increase paper readability, brief expla-

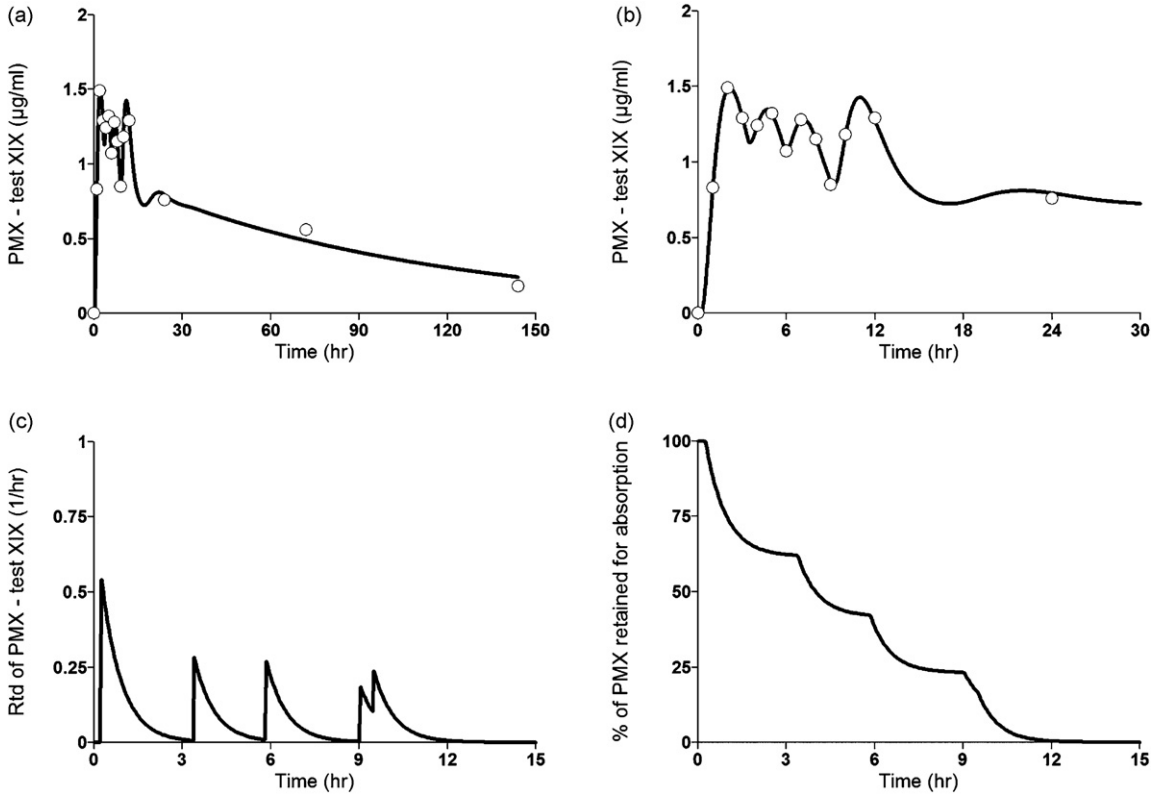


**Fig. 3.** Example of modeling results, subject XIX. Measured plasma concentration–time profile of piroxicam administered in the single dose of 20 mg in capsules LaborMed Pharma (circles). Model-based prediction of the measured profile (line). (a) Presentation over the whole time interval of the study; (b) presentation over the first time interval after piroxicam administration; (c) rate of time-delayed disposition of piroxicam for absorption; (d) fraction of piroxicam dose actually present in stomach and disposable for time-delayed absorption.

nations are given here: (1) "the system gain". The system gain is the ratio of the output and input of the system when the system approaches steady-state (Kailath, 1980; Ďurišová and Dedík, 1997, 2005; Dedík and Ďurišová, 2004, 2008; Dedík et al., 2007; Tvrdonova et al., 2008); *2)* "the time-delay parameter". Influences of time-delay parameters are known as time-lags in the pharmacokinetic literature (Nerella et al., 1993); (3)"physiologically motivated". The later term is used on purpose, with the aim to indicate that the presented model is not a physiologically based model, conventionally used in pharmacokinetics.

The obtained modeling results show that the proposed model outperforms the retrieval performance in comparison with the performance of models conventionally used in pharmacokinetics. The presented model and approach exhibit the following advantages when compared to the conventional models and approaches in the BE area: (1) the only assumption needed for the validity of the model and approach is linearity; (2) the data is analyzed on the original scale not in the log scale; (3) the efficiency of BE assessment is significantly improved by analysis of gastric emptying process. Delayed gastric emptying might be associated with slow drug absorption, a long time to reach peak concentrations, and low maximum concentrations. These effects should be taken into account in BE assessment as they can play significant role in patients with gastric emptying disorders (Nerella et al., 1993; Perini et al., 2005).

In conclusion, the most important feature of the presented model is that, based on an anatomical–physiological approach capable of considering the body as a sum of interacting parts connected anatomically by blood flow carrying the drug of interest. Mathematical modeling and simulation are challenging in the BE area, as they provide drug development professionals with powerful approaches to understand whole-body disposition behavior of a compound, and to evaluate expected performance of a compound, relative to compound competitors. The approach introduced in this study might serve as an adjunct to the traditional approaches to bioequivalence determination.

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The authors declare that there is no duality of interest associated with this manuscript.

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