ORIGINAL PAPER

NON-LINEAR AND LINEARIZED IVIV CORRELATIONS FOR TABLETS CONTAINING A LARGE MOLECULE POLAR COMPOUND

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Abstract. *The present paper verifies possible biorelevance for dissolution tests in gastric and intestinal simulated media for immediate release oral pharmaceutical formulation containing methotrexate, a large molecule polar compound, in terms of establishing in vitro–in vivo reliable correlations (IVIVC) and successful predicting the in vivo performance for tested formulations. Five dissolution media have been chosen to model composition of gastrointestinal tract contents before and after meal intake. Results indicated that pH changes during the transfer from simulated gastric to simulated intestinal fluid slightly increases methotrexate solubilisation, whereas presence of bile salts seems to have no significant effect on methotrexate release kinetics. In vivo pharmacokinetics was obtained after a single dose oral administration of 10 mg, methotrexate to 24 subjects in fasting conditions. Correlation between in vitro dissolution and in vivo absorption (Level A IVIVC) was not linear but fairly logarithmic for all dissolution experiments. A novel approach for development of IVIVC for immediate release formulations containing polar molecules correlates in vitro dissolution with in vivo elimination resulting a linear relationship. Evaluations implied that methotrexate case represents an example of possible extrapolation of in vitro in vivo correlations from extended release formulations in case of lipophilic drugs to immediate release formulations containing polar molecules..*

Keywords: *methotrexate, biorelevant dissolution media, in vitro in vivo correlations*

1. INTRODUCTION

Dissolution testing was implemented as a tool for quality testing of solid pharmaceutical forms, mainly tablets and capsules. Later the acquired data needed to be correlated to in vivo pharmacokinetic data, and the concept of "in vitro-in vivo correlation" (IVIVC) was proposed. It is to note that hypothesis concerning a linear ivivc are valid mainly for slow release lipophilic drugs.

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The attempts to establish the IVIV correlation showed that compendial dissolution media easy to prepare and costly efficient is insufficient [1, 2] and sometimes fail as predictors of in vivo dissolution and consequently pharmacokinetics of tested drugs.

After the development of biorelevant media, reproduction of in vivo performance of drug formulations led to a more reliable in vitro evaluation tool.

Development of the Biopharmaceutics Classification System (BCS) [3] clarified partially the problems revealing that only for a part of active substances correlation is possible. Biorelevant dissolution testing was proved valuable in predicting of in vivo behaviour of highly permeable drugs BCS classes II and III [4-8].

Paper cheks possible biorelevance for dissolution tests in gastric and intestinal simulated media for immediate release oral pharmaceutical formulation containing methotrexate, in terms of establishing in vitro–in vivo reliable correlations (IVIVC) and successful predicting the *in vivo* performance for tested formulations.

Methotrexate is a weak dicarboxylic acid with polar molecule (logP=-1.8) [9], acting as a folate antimetabolite in the treatment of certain neoplastic diseases, severe psoriasis and adult rheumatoid arthritis. Chemically, methotrexate is *N*-[4-[[(2,4-diamino-6-pteridinyl) methyl]methylamino]benzoyl]-L-glutamic acid. The structural formula is shown in Fig. 1.

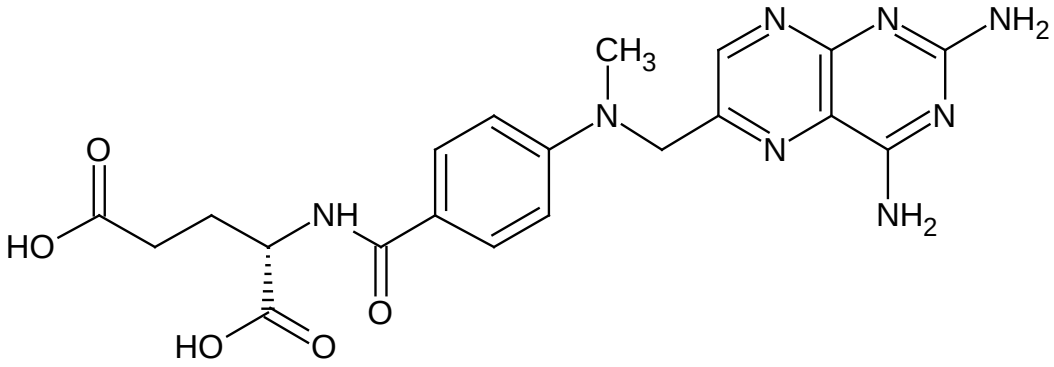

Figure 1. Methotrexate chemical structure.

Oral absorption of methotrexate appears to be dose dependent. At doses of 30 mg/m^2 or less (for patients receiving treatment for psoriasis, or rheumatoid arthritis or low dose antineoplastic therapy), methotrexate is generally well absorbed, having a mean bioavailability of approx. 60% and $t_{1/2}$ between 3 to 10 hours. The absorption of doses greater than 80 mg/m^2 is less significant, possibly as a result of a saturation effect, and $t_{1/2}$ is 8 to 15 hours. Peak serum levels are reached within one to two hours [10].

Based on the Biopharmaceutics Drug Disposition Classification System (BDDCS), methotrexate is considered a Class III drug (high solubility and poor metabolism) [11]. In Biopharmaceutics Classification System (*BCS), methotrexate* is categorized as a class III or class IV compound [3].

The permeability of methotrexate is the rate-controlling drug absorption factor. In addition, class III drugs exhibit a high variability of rate and extent of absorbed drug. Since the dissolution is immediate, the variation is due to alteration of gastrointestinal (GI) physiological properties and membrane permeation rather than dosage form factors.

2. MATERIALS AND METHODS

2.1. MATERIALS

Methotrexate reference standard was purchased from Sigma-Aldrich.

Methotrexate 2.5 mg tablets, were purchased commercially.

Physiological compounds - granular Lecithin, (Acros Organics) Pepsin (Fluka) and Sodium taurocholate 97% (Sigma) were used for the preparation of biorelevant media.

Acetic acid, sodium chloride (NaCl), sodium dihydrogen phosphate monohydrate and NaOH pellets were all of analytical grade and purchased from Merck KGaA (Darmstadt, Germany). 37% hydrochloric acid (conc. HCl) was obtained from Riedel-de Haën.

2.2. METHODS

Synthetic Dissolution Media

Synthetic dissolution media for comparative approach consisted in the followings [4].

Simulated Gastric Fluid SGF

Fasted State Simulated Intestinal Fluid blank (FaSSIF blank)

Fed State Simulated Intestinal Fluid blank (FeSSIF blank)

Biorelevant Dissolution Media

A set of two biorelevant media was used, presented in recently published articles and proved to be representative for fasting state conditions in the small intestine (FaSSIF) and simulated postprandial conditions in the small intestine (FeSSIF). Preparation of both synthethic and biorelevant dissolution media was done in accordance with the published methods [4, 12].

Dissolution Studies

Drug release experiments were performed with USP Apparatus 2 (Paddle), DT 800H, Erweka, Germany. Each vessel was filled with 500 mL/1000 mL of media, and an agitation speed of 50 rpm was used for all dissolution studies. Experiments were run in triplicate. Samples (5 mL) were removed after 5, 10, 15, 20, 30, 45, 60, and 90 min using a glass syringe, then filtered through a 0.45-μm Teflon® filter and immediately diluted with methanol. Quantification of Methotrexate was achieved by using a validated HPLC method.

Clinical Study

In vivo data were obtained in a bioequivalence study comparing Methotrexate (Lederle) with a generic formulation. A single dose of 10 mg methotrexate (4*2.5 mg tablets) was administered to 24 subjects in a cross-over two-period, two sequences bioequivalence study in fasting conditions, with a washout period of 6 days between Phase I and Phase II. Plasma levels were measured at 0 and 0.25, 0.5, 0.75, 1, 1.5,2, 2.5, 3, 4, 6, 8, 10, 12, 24 and 48 h. The study was approved by National Ethics Committee and National Medicines Agency. Plasma levels of active substance methotrexate were determined using a validated Liquid Chromatographic method.

Computerized methods for estimation of parameters

Estimation of pharmacokinetic parameters was performed using subroutines of the software KINETICA 4.2 and TOPFIT 2.0. Data were fitted with a monocompartmental oral absorption – elimination model.

3. RESULTS AND DISCUSSION

3.1. IN VITRO DISSOLUTION

Effect of dissolution medium composition on rate and extent of release

Dissolution in simulated gastric fluid (pH 1.2) was rapid with a linear time course in first 30 minutes and complete after 45 minutes. Apparently, methotrexate solubilisation is slightly increased by pH changes during the transfer from simulated gastric to simulated intestinal fluid, complete dissolution being achieved in 30 minutes at pH 5.0 and 6.5. Presence of bile salts did not influence the solubility of methotrexate, its inclusion in micelles did not determined a more rapid dissolution. At intestinal level, although food is slightly lowering the pH and substantially increasing bile salts concentration, methotrexate release remained similar to preprandial conditions (complete dissolution in 30 minutes).

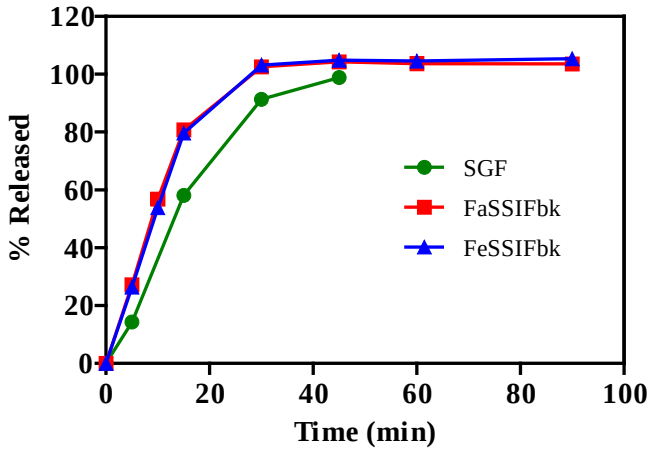

Figure 2. Comparative release of methotrexate in synthetic dissolution media.

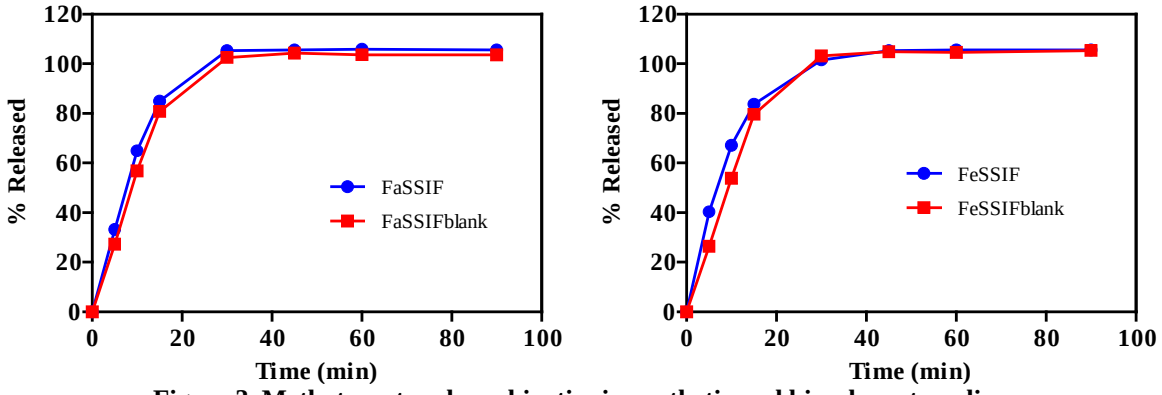

Figure 3. Methotrexate release kinetics in synthetic and biorelevant media.

3.2. IN VIVO PHARMACOKINETICS

Examination of the individual plasma levels curves (Fig. 4) reveals a variability concerning c_{max}, t_{max}, $t_{1/2}$ area under curves. Since in vitro dissolution is highly reproducible, the in vivo variability has to be connected mainly with in vivo release and absorption. Due to the high variability of individual pharmacokinetic profiles a reliable approach for correlation of in vitro release to in vivo data is the use of in vivo mean curve.

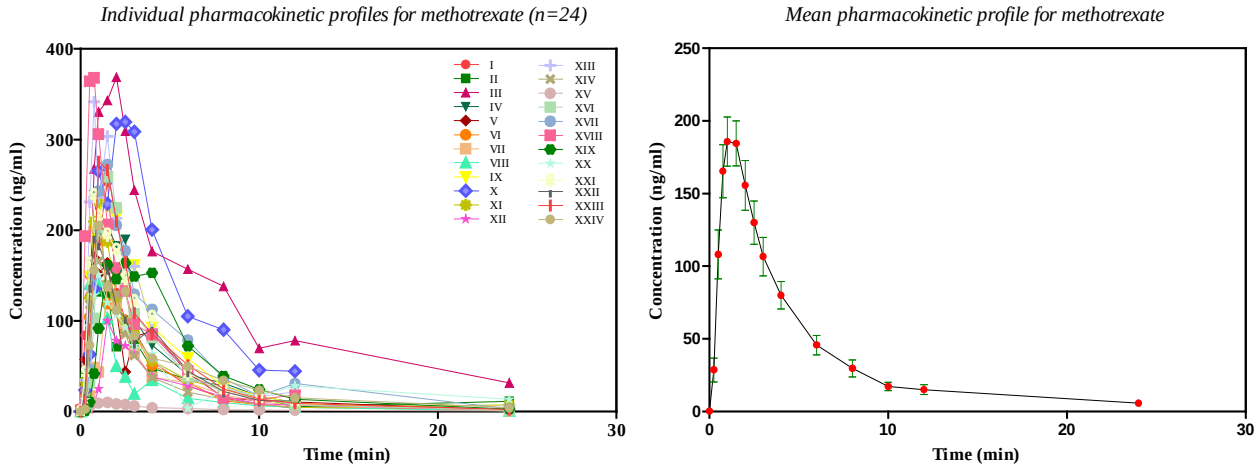

Figure 4. Methotrexate pharmacokinetics.

The mean pharmacokinetic profile (Fig. 4) is smooth and looks essentially like an absorption-distribution pharmacokinetic curve. Fitting data with solutions of compartmental models lead to good approximations of theoretical curves. Since fitting improvement by bicompartmental modeling was not significant (as proved by Akaike index and comparison of residual sum of squares by Fisher test) monocompartmental model was preferred (Fig. 5) [13, 14]. Other argument for monocompartmental model arises from the fact that metothrexate is a weak acid and consequently has a poor solubility in lipids and remains mainly in the central compartment.

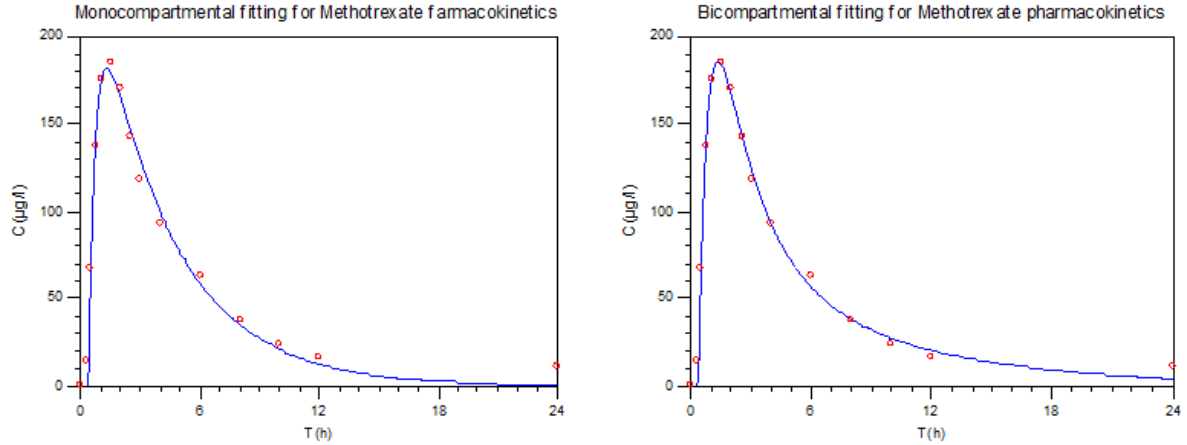

Figure 5. Modelling of methotrexate pharmacokinetics.

Time course of absorption

Following monocompartmental behaviour applying Wagner-Nelson formula [15] for calculation of fraction of methotrexate absorbed as function of time, was considered justified.

Model considers that the amount of drug in living body at a moment t is the product of plasma concentration and volume of the central compartment. The absorbed amount is the sum of amount in body at the time t and the eliminated amount in the interval (0,t). In the hypothesis of monocompartmental model, the eliminated amount is proportional with plasma concentration. Consequently the absorbed fraction of drug (FRA) at the moment would be given by formulas

$$FRA(t) = \frac{V_d c(t) + \int_0^t k_e V_d c(t)\,dt}{\int_0^\infty k_e V_d c(t)\,dt} = \frac{c(t) + \int_0^t k_e c(t)\,dt}{\int_0^\infty k_e c(t)\,dt}$$

where c is the plasma concentration, V_d volume of central compartment, k_e elimination constant.

A rapid absorption of the first half of the total available amount occurred the first hour [6] (Fig. 6). After one hour the process become slower, the second half of available methotrexate being absorbed practically in one day. The time courses of the evolutions in each phase are linear (Fig. 6).

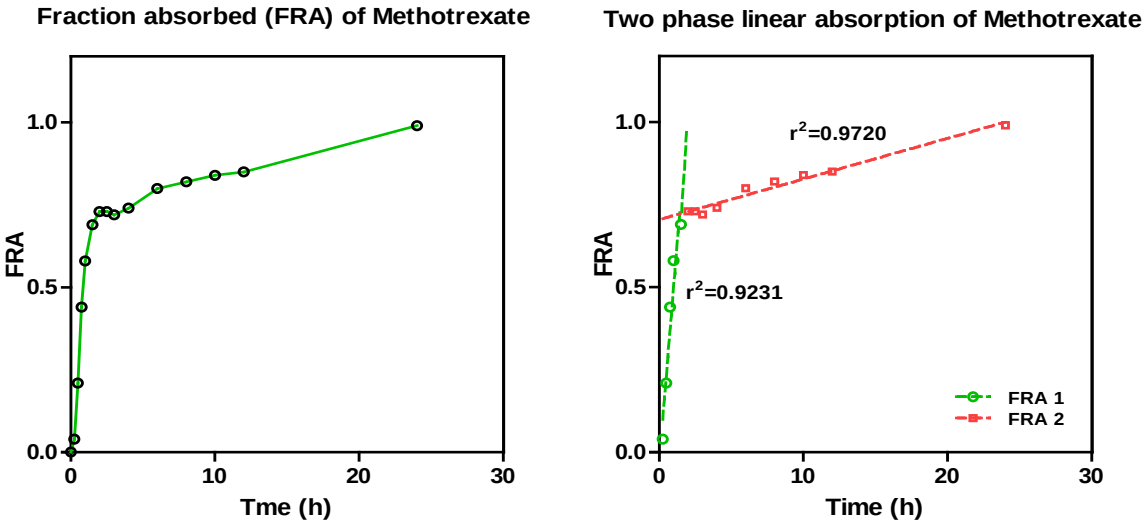

Figure 6. Evaluation of in vivo dissolution kinetics for Methotrexate by means of Wagner-Nelson method.

3.3. IN VITRO-IN VIVO CORRELATION (IVIVC)

Non-linear correlations

A Level A [16] point-to-point relationship between fraction of drug dissolved (FRD) and fraction absorbed (FRA) revealed a non linear correlation. On a logarithmic scale FRA as function of FRD gives an excellent linear correlation. The results were similar whatever the dissolution medium, suggesting that the critical parameters determining the pharmacokinetics after oral administration are connected with absorption.

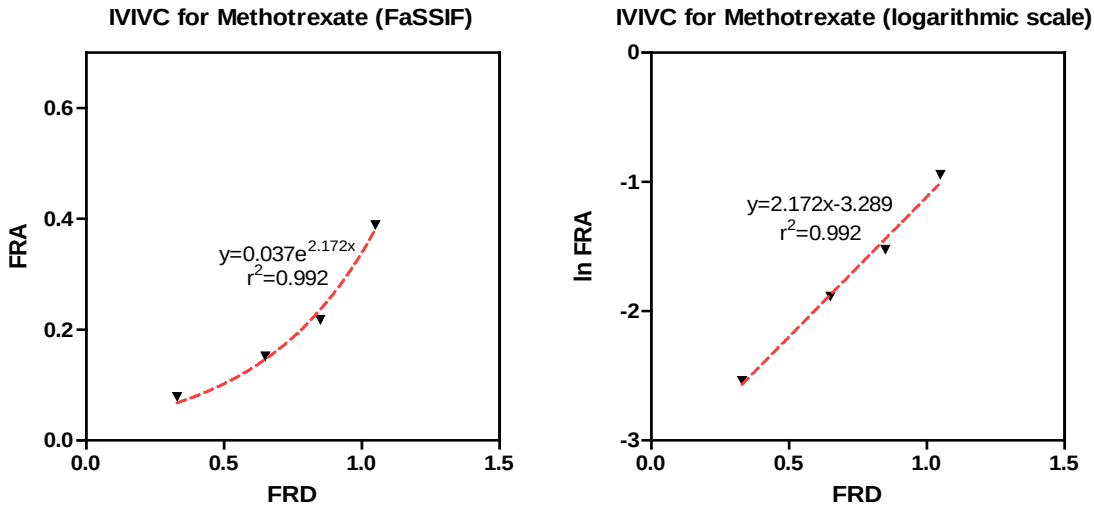

Figure 7. Level A In Vitro-In Vivo Correlation for Methotrexate.

Linear correlations following a simplified Wagner – Nelson model.

In fact, a linear correlation between FRA and FRD can be obtained in case of lipophilic drugs, when release is slow and absorption is rapid. Kinetics of the entire process is dissolution rate limited, consequently a linear relationship is to be expected.

In case of polar molecules, release is rapid and absorption is slow and the in vivo performance of the dosage form is controlled by the intrinsic biopharmaceutical properties of the drug. If supplementary we take into consideration that elimination of polar molecules is essentially renal and is rapid and $V_d c(t) \approx \int_0^t k_e V_d c(t)\,dt$, Wagner Nelson formula can be simplified to:

$$FRA(t) = \frac{V_d c(t) + \int_0^t k_e V_d c(t)\,dt}{\int_0^\infty k_e V_d c(t)\,dt} \cong \frac{\int_0^t k_e V_d c(t)\,dt}{\int_0^\infty k_e V_d c(t)\,dt} = \frac{\int_0^t k_e c(t)\,dt}{\int_0^\infty k_e c(t)\,dt} = FRE(t)$$

The IVIVC reduces therefore to a correlation between fraction of drug dissolved (FRD) and fraction of eliminated in vivo (FRE). A linear dependence between FRD and FRE was obtained (Fig. 8).

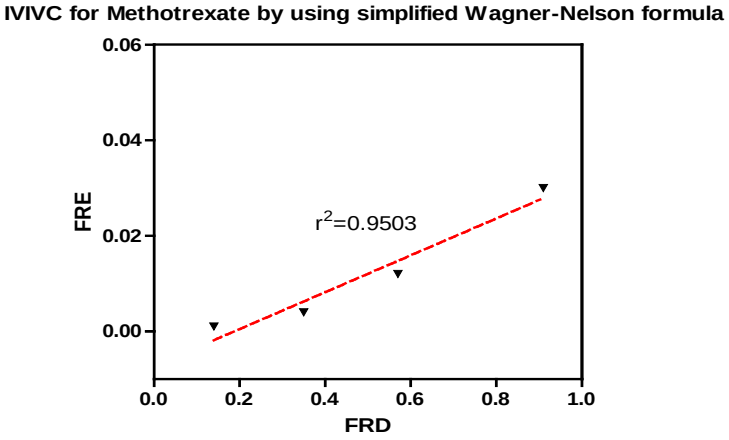

Figure 8. In Vitro-In Vivo Correlation for Methotrexate by using simplified Wagner-Nelson formula.

4. CONCLUSIONS

In vitro dissolution of methotrexate was rapid and complete, all active substance being released and solubilised within 45 minutes in all the dissolution media tested. A somewhat prolonged dissolution appeared in the case of release in SGF due to reduction of dissociation of carboxylic groups of methotrexate in acidic medium.

Calculated time course of absorption fraction revealed a two phase linear evolution of the process, one associated with dissolution process in the first hour and another connected with a pure absorption in the next one day interval.

Correlation between in vitro dissolution and in vivo absorption was not linear but fairly logarithmic.

A modification of the Wagner-Nelson formula in case of polar drugs was made by considering that after a sufficient time is possible to neglect the total amount of drug in the body at a certain time t, towards total eliminated amount in the [0,t] interval. The new model represents in fact a correlation between dissolution and elimination fractions.

The considered model for polar drug molecules proved to be fairly well applied in case of methotrexate.

Methotrexate case represents an example of possible extrapolation of in vitro in vivo correlations from extended release formulations in case of lipophilic drugs to immediate release formulations containing polar molecules.

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