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IN VITRO – IN VIVO CORRELATION OF PENTOXIFYLLINE: A COMPREHENSIVE KINETIC ANALYSIS

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ABSTRACT. This study presents an analysis of *In Vitro-In Vivo* Correlation (IVIVC) for pentoxifylline modified-release tablets, with a focus on deriving robust predictive models. *In vitro* dissolution tests were conducted in three pH media (1.2, 4.5, and 6.8) to simulate various gastrointestinal conditions. Data was collected under fed conditions from a bioequivalence (BE) study. The modeling and calculations were performed using Phoenix WinNonlin® version 8.4 software, enabling estimation of drug absorption kinetics. A Level A IVIVC model was employed for each *in vivo* data to establish a direct and reliable correlation between the *in vitro* dissolution profiles and the *in vivo* pharmacokinetic data. The determination coefficient (R^2) exceeded 0.97, demonstrating a high degree of predictability and robustness in the established IVIVC.

Keywords: *In Vitro – In Vivo Correlations (IVIVCs), pentoxifylline, in vitro dissolution, bioequivalence, Level A Correlation*

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INTRODUCTION

In the field of pharmaceutical sciences, establishing a robust *In Vitro-In Vivo* Correlation (IVIVC) is pivotal for the development and regulatory approval of drug formulations and also for lifecycle management [1–5]. The continuous enhancement of drug development remains a key objective, with the industry committed to pioneering innovative approaches to achieve this goal. Within the increasing pressures to expedite product development timelines, it is imperative to foster integrated collaboration among scientists from diverse disciplines, including analytical chemistry, formulation science, clinical pharmacology, and other pharmaceutical fields. Such interdisciplinary teamwork is crucial to ensure the success of pharmaceutical products at every stage of development [2,6]. A critical tool is the establishment of IVIVC, a predictive model that bridges the gap between laboratory dissolution data and *in vivo* drug performance, offering a scientific basis for understanding drug behavior within the human body [7,8]. Both academia and the pharmaceutical industry, along with regulatory bodies, have concentrated efforts on utilizing IVIVC to address a variety of objectives [7–9]. IVIVC plays an important role in the development and post approval changes of a drug product [2,4,6] but also limiting the risk of *in vivo* failure [10].

In vitro release, typically illustrated through dissolution profiles in biorelevant or bio-predictive media, contrasts with *in vivo* release, which is primarily determined by pharmacokinetic studies measuring plasma drug concentration or the amount of drug absorbed [2,4,10]. Observed *in vivo* differences in the rate and extent of drug absorption between two pharmaceutically equivalent solid oral products may be attributed to variations in their *in vivo* dissolution profiles [11].

Over the years, IVIVC has seen significant evolution, driven by advances in analytical techniques and a deeper understanding of drug dissolution and absorption dynamics [2,10]. Originally applied to immediate release formulations, the scope of IVIVC has now expanded to include modified-release products, driven by advancements in dissolution testing methodologies and computational modelling [8].

Despite these advancements, the field of IVIVC faces several challenges, including variability in dissolution testing methods, inter-individual physiological differences, and the inherent complexities of modified-release formulations [3,7,9]. Most of the published studies still focus on early-stage applications rather than regulatory purposes, reflecting a gap in the adoption of advanced technologies [1–3,8,12].

The legislative framework for *In Vitro-In Vivo* Correlation (IVIVC) across Europe [13,14], USA [15], and Canada [16] is guided by a common scientific principle that seeks to establish a predictive mathematical model linking *in vitro*

drug release to *in vivo* pharmacokinetic responses [4,9]. While specific regulatory guidelines may vary, they all emphasize the importance of IVIVC in drug development and approval processes. This includes setting dissolution specifications, supporting bioequivalence studies, and facilitating drug product modifications post-approval [4,9,17]. The harmonization efforts by international regulatory bodies aim to streamline the IVIVC application across different jurisdictions, ensuring consistency and predictability in drug development and assessment [6].

In the United States, both innovator and generic drug companies employ IVIVC models in their regulatory submissions, particularly for oral extended-release (ER) drug products [7].

An IVIVC model should be applied strictly for interpolation within the data range used during its development, ensuring adherence to validated parameters and avoiding extrapolation beyond these bounds [13]. International regulatory guidelines provide comprehensive protocols for both internal and external validation of IVIVC models [18].

According to the United States Pharmacopeia (USP) and Food and Drug Administration (FDA) guidelines, IVIVC models are classified into three levels: A, B, and C, with Level A representing the highest standard of correlation. Level A IVIVC models establish a direct and point-to-point relationship between the *in vitro* dissolution and the *in vivo* input rates, providing the most comprehensive and predictive correlation. This high degree of correlation underscores the reliability and applicability of the IVIVC model in predicting *in vivo* drug behavior based on *in vitro* dissolution data. This level of correlation is particularly beneficial for obtaining biowaivers, which can expedite the drug approval process by reducing the need for extensive clinical trials. Biowaivers are often not granted for Level B, C, and multiple C correlations [3–7,15].

Regulatory authorities recommend IVIVC-based biowaivers for modified-release dosage forms, particularly those with extended release characteristics [2,15]. The Biopharmaceutics Classification System (BCS) is a scientific framework that classifies drug substances into four categories based on their aqueous solubility and intestinal permeability. BCS class I drugs exhibit high solubility and high permeability, while class II drugs have low solubility but high permeability. Class III drugs are highly soluble but poorly permeable, and class IV drugs are characterized by both low solubility and low permeability. This classification helps predict the rate-limiting step for drug absorption, either solubility/dissolution or permeability, and is instrumental in guiding regulatory decisions for *in vitro-in vivo* correlation (IVIVC) studies. For oral dosage forms, as emphasized by the FDA, it is feasible to correlate *in vitro* and *in vivo* data when the absorption of the drug substance is constrained by the dissolution

rate [15]. Consequently, establishing IVIVC is more straightforward for BCS class II compounds and, in certain cases, BCS class III compounds, as well as extended-release (ER) formulations [3].

Pentoxifylline, chemically known as 3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1H-purine-2,6-dione or 1-(5-oxohexyl)-3,7-dimethylxanthin, is a methylxanthine derivative with significant therapeutic potential (Figure 1) [19]. This compound is characterized by its molecular formula $C_{13}H_{18}N_4O_3$ and a molecular weight of 278.3 g/mol [20,21].

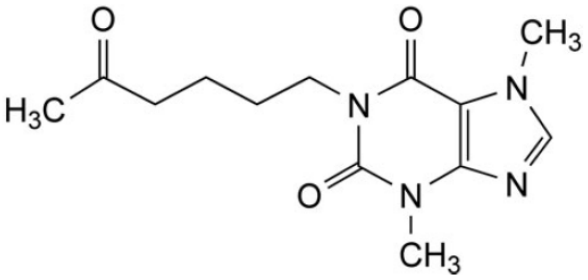

Figure 1. Chemical structure of pentoxifylline
(IUPAC name 3,7-Dimethyl-1-(5-oxohexyl) purine-2,6-dione)

Pentoxifylline, an anti-haemorrhagic medication utilized in the treatment of intermittent claudication but with much broader potential for protecting vascular health and optimising tissue perfusion [21], undergoes significant hepatic metabolism, resulting in a rapid decrease in therapeutic levels [18,22].

Following oral administration, pentoxifylline is well-absorbed, reaching peak plasma concentrations within an hour, although its bioavailability is subject to a non-linear, dose-dependent first-pass effect. Metabolized in the liver and erythrocytes, pentoxifylline and its active metabolites are primarily excreted *via* the urine, with a minor proportion eliminated through feces. These pharmacokinetic attributes underscore pentoxifylline's therapeutic potential and the necessity for precise formulation to optimize its clinical efficacy.

Classified under the BCS as a Class II compound, pentoxifylline is characterized by low solubility but high permeability, indicating that its absorption is predominantly solubility-limited [19].

IVIVC plays a crucial role in the application of biowaivers, thereby expediting the drug approval process and reducing the necessity for human bioequivalence studies [11].

The primary objective of constructing an IVIVC is to validate that the *in vitro* dissolution characteristics are predictive of the *in vivo* pharmacokinetic performance of the drug across various release rates [5]. This correlation is

crucial for ensuring that the predictive model remains reliable and robust, enabling the facilitation of manufacturing modifications without compromising the drug's therapeutic efficacy and safety [6,23]. The successful establishment of a Level A IVIVC thus provides a powerful tool for both regulatory assessment and the optimization of drug formulation development processes [6,10].

The development of Level A IVIVC typically involves multiple drug formulations exhibiting a range of dissolution rates, including slow, medium, and fast release profiles. These formulations undergo comprehensive *in vitro* dissolution testing to generate dissolution profiles, followed by *in vivo* pharmacokinetic studies to obtain plasma concentration profiles. Advanced deconvolution techniques are subsequently employed to accurately determine the *in vivo* absorption profiles from the plasma concentration data [4–6,15,23,24].

In this study, the focus is on the development of IVIVC model for Pentoxifylline modified-release tablets, using such a Level A IVIVC, by evaluating various dissolution media and considering physiological variables.

This research serves as an initial exploratory study, aiming not at the development of an industrial-scale IVIVC, but to serve as a support for subsequent, more comprehensive studies in this area. Accordingly, the *in vitro* experiments were conducted on two modified-release formulations of pentoxifylline, namely, the reference product Trental 400 mg modified-release tablets (R), Sanofi Aventis, France, and the prototype generic formulation Pentoxifylline 400 mg modified-release tablets (T) developed by Antibiotice SA, Iaşi, Romania.

RESULTS AND DISCUSSION

In vitro dissolution data analysis

The *in vitro* dissolution characteristics of two modified-release formulations of pentoxifylline were assessed through *in vitro* dissolution tests, in three distinct pH media: 1.2, 4.5, and 6.8. These pH conditions were carefully chosen to simulate the physiological conditions of the gastrointestinal (GI) tract: pH 1.2 represents the acidic gastric environment, pH 4.5 simulates the early intestinal transit, and pH 6.8 corresponds to the later stages of intestinal digestion [25].

The detailed methodology and sample preparation protocols are outlined in the experimental section. The percentage release of pentoxifylline was quantified, with results presented in Figure 2 as mean values ± standard deviation (SD) of six replicates.

The results presented in Figure 2 demonstrate that the *in vitro* release of pentoxifylline from the generic modified-release product developed by Antibiotice SA and the reference product are independent of pH, as evidenced

by the almost superposable dissolution profiles across all three media. This pH independence represents a significant advantage, as the chosen dissolution media simulate a range of physiological and nutritional conditions encountered in the gastrointestinal (GI) tract. Specifically, pH 1.2 mimics the fasting state in the stomach (pH 1–3), while pH 4.5 simulates the fed state in the stomach (pH 4–6, depending on food composition). Lastly, pH 6.8 represents the pH of the small intestine, consisting in the duodenum (pH 5–6.5), jejunum (pH 6–7), and ileum (pH 7–8), as well as portions of the large intestine such as the cecum (pH 5.5–7) and colon (pH 6–7.5) [25]. This consistency in drug release across varying pH conditions ensures reliable drug performance regardless of the fed or fasting state, reflecting a stable release mechanism suitable for the diverse physiological environments of the GI tract. Such properties enhance the predictability of the modified-release formulation and align well with the criteria for robust IVIVC models.

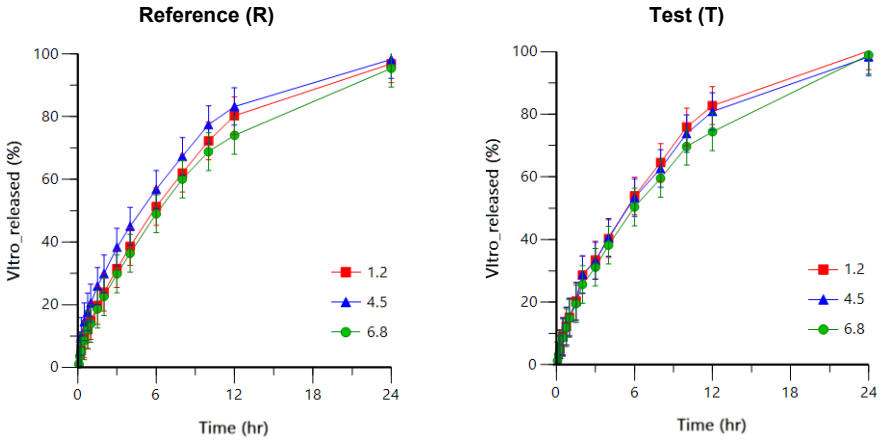

Figure 2. Dissolution profiles of pentoxifylline 400 mg modified-release tablets (Reference and Test) in three pH conditions (1.2, 4.5, and 6.8)

In vivo data

The *in vivo* data used to establish the IVIVC for pentoxifylline were derived using mathematical deconvolution techniques. This method takes the known output function, represented by the mean plasma concentrations, to back-calculate the input function, which reflects the absorption kinetics of pentoxifylline [24]. As a result, the time-dependent relative fraction of pentoxifylline absorbed from the administration site was determined and is illustrated in Figure 3. The corresponding bioequivalence study was carried out at the Center for Drug Evaluation, Antibiotice SA, Iași, Romania.

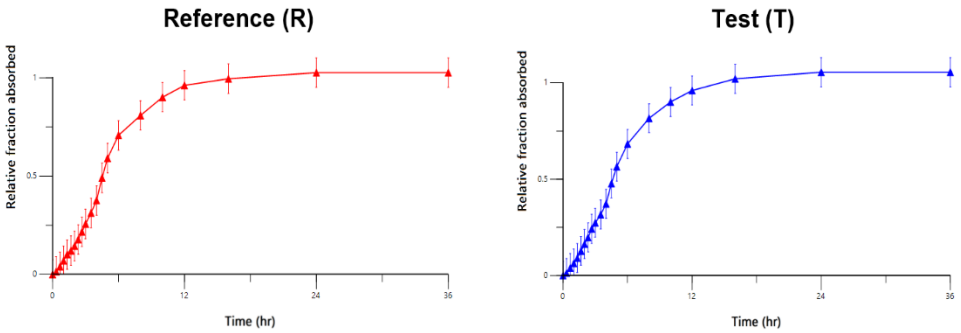

Figure 3. The relative fraction of pentoxifylline absorbed following the oral administration of a single 400 mg dose of the Reference and Test formulations under fed conditions of the *in vivo* clinical trial

In vitro – in vivo correlation

The *in vitro–in vivo* correlations (IVIVCs) were evaluated to establish a Level A correlation. Absorption profiles of Pentoxifylline 400 mg modified-release tablets were derived from individual plasma drug concentration *versus* time profiles obtained during bioequivalence study. These absorption profiles were assessed under fed conditions.

By correlating the percentage of *in vivo* absorbed pentoxifylline with the *in vitro* dissolution percentages, an IVIVC was established. The regression analysis, including slopes, intercepts, and determination coefficients for the IVIVCs, is detailed in Table 1.

The IVIVC plots from Figure 4 illustrate the relationship between *in vitro* dissolution and *in vivo* absorption of pentoxifylline 400 mg modified-release tablets under fed conditions of subjects enrolled in the clinical trial, evaluated using the deconvolution approach across three pH values for the dissolution media: 1.2, 4.5, and 6.8.

Table 1. *In vitro – in vivo* correlation results for pentoxifylline 400 mg modified-release tablets and statistical analysis of the correlations obtained for the clinical study under fed conditions

Dissolution media pH	IVIVC corelations					
	Reference (R)			Test (T)		
	Slope[1]	Intercept[2]	Determination Coefficient (R^2)	Slope	Intercept	Determination Coefficient (R^2)
1.2	0.6491	12.52	0.9724	0.6703	13.83	0.9752
4.5	0.6260	19.24	0.9737	0.6442	14.47	0.9730
6.8	0.6126	11.98	0.9796	0.6046	13.47	0.9818

[1] Slope – expressed in Δ% vitro released / Δ% fraction absorbed; [2] Intercept – expressed in % vitro released.

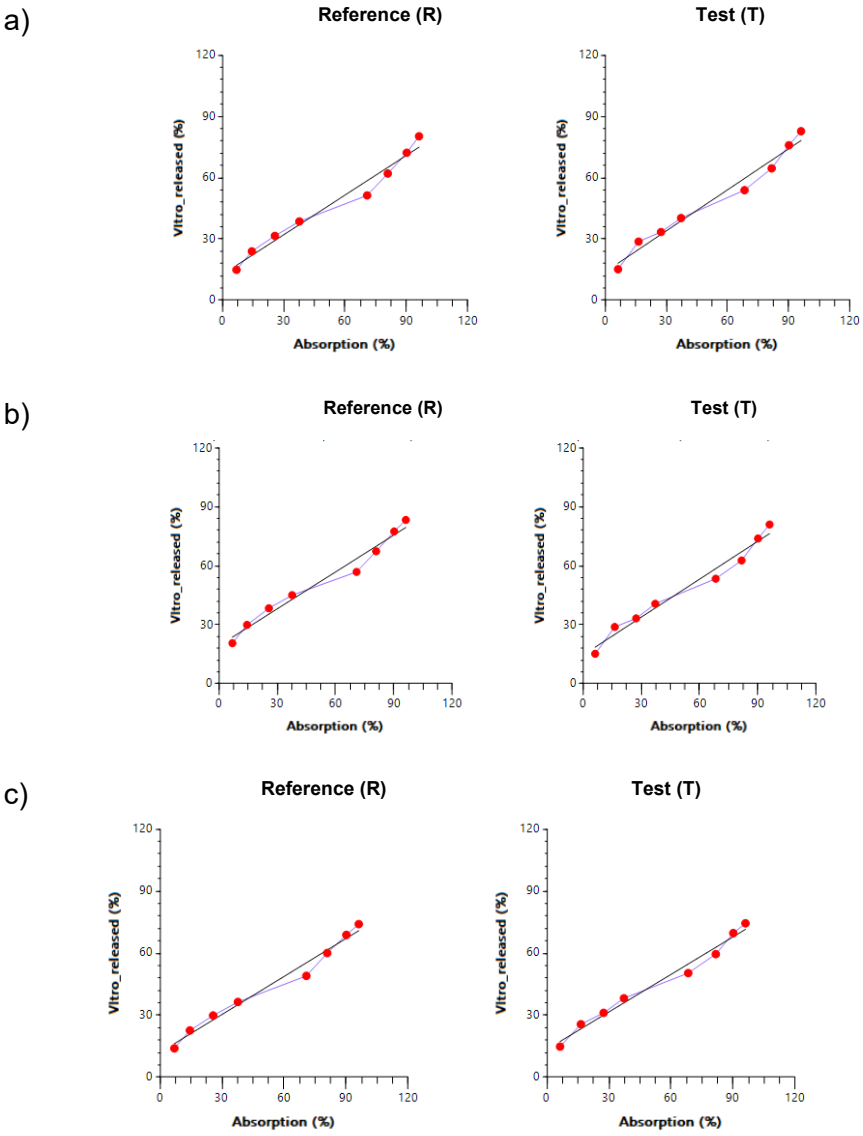

Figure 4. *In vitro – in vivo* correlation plots for pentoxifylline 400 mg modified-release tablets, generated using the deconvolution approach, at different pH levels of the dissolution media (1.2 [a], 4.5 [b], and 6.8 [c]) and under fed conditions of subjects in the clinical *in vivo* trial

The establishment of a robust IVIVC necessitates linking the pharmacokinetic profile of a drug substance with the formulation characteristics, ensuring that physiological factors do not limit drug absorption. According to the IVIVC guidelines, such correlations are particularly feasible for drugs classified under Biopharmaceutics Classification System (BCS) class II. Modified-release (MR) formulations are well-suited for IVIVC studies, as they can be designed to control drug release over an extended period, making pentoxifylline a reliable candidate for IVIVC development.

In this study, we evaluated the IVIVC of two formulations of pentoxifylline: the reference product Trental and the generic prototype of pentoxifylline 400 mg MR tablets. Data from a bioequivalence study conducted under fed conditions, alongside *in vitro* results in multiple dissolution media (at pH 1.2, 4.5, and 6.8), provides a comprehensive evaluation.

The tablet's design allows for the formation of a gel layer upon hydration, facilitating the slow release of pentoxifylline. Pentoxifylline MR tablets have demonstrated predictable and reproducible Active Pharmaceutical Ingredient (API) release, maintaining effective plasma concentrations over 24 hours with a single daily dose (400 mg), which supports a prolonged therapeutic effect.

The IVIVC was established by selecting eight common time points for both *in vivo* and *in vitro* studies (specifically 1, 2, 3, 4, 6, 8, 10 and 12 hours). The correlation, derived from data at selected time points, resulted in the establishment of six IVIVCs under fed conditions.

For an accurate IVIVC, the *in vivo* and *in vitro* profiles must exhibit similar shapes. The statistical evaluation of the IVIVC for Pentoxifylline 400 mg modified-release tablets revealed a high determination coefficient, consistently exceeding 0.97. This indicates a strong linear relationship between the *in vitro* dissolution profiles and the *in vivo* absorption data. The slope of the correlation was determined to be approximately 0.6, with intercept values ranging between 12 - 19.

The high determination coefficient, along with supportive slope and intercept values, validates the IVIVC model developed in this study. These results underscore the feasibility of using IVIVC as a reliable predictive tool for drug development and regulatory submissions, particularly for BCS class II compounds.

The findings align with current IVIVC guidelines, demonstrating that a Level A IVIVC is feasible for BCS class II drugs, characterized by low solubility and high permeability. According to these guidelines, a high determination coefficient (generally above 0.9) is essential to demonstrate the predictive power of the IVIVC. The slope and intercept values further support the robustness of the correlation, indicating that the dissolution characteristics of the modified-release formulation are effectively translated into *in vivo* absorption profiles [15].

A recent study by Taha el al (2024) was the only one identified in the scientific literature to investigate the IVIVC of pentoxifylline sustained-release (SR) formulations [18]. This study explored the role of different Flow-Through-Cell (FTC, USP IV) designs in predicting *in vivo* performance under fed and fasting conditions, proposing a closed-loop FTC system with a turbulent-flow pattern and gradient-buffer medium as the optimal setup for achieving a predictive IVIVC, with acceptable prediction error (PE%) values for pharmacokinetic parameters such as maximum plasma concentration (C_{max}) and area under the plasma concentration *versus* time curve (AUC) [18]. In contrast, our study focused on a more classical and regulatory-aligned approach to IVIVC development, using traditional dissolution testing in three pH media (1.2, 4.5, and 6.8) to simulate gastrointestinal conditions, alongside bioequivalence data obtained under fed conditions from an *in vivo* study conducted on healthy volunteers. This methodology enabled the successful establishment of six robust Level A IVIVC models with high determination coefficients ($R^2 > 0.97$), adhering to the standard requirements for biowaiver applications. In contrast to the aforementioned study, which used *in vivo* data sourced from the scientific literature, our study integrates *in vivo* data obtained in a clinical trial conducted in the same company that produced the generic formulation to be tested, ensuring better alignment with regulatory expectations.

Given that no previous studies have comprehensively evaluated IVIVCs for this drug molecule using classical approaches, our findings hold a very high degree of novelty and underscore the importance of this work in supporting the development of modified-release formulations for pentoxifylline.

Although these correlations were not intended for industrial scale-up, the promising preliminary results provide a strong foundation for further IVIVC development, potentially leading to a biowaiver application. This would reduce costs associated with additional studies required for post-approval changes, as the drug product could benefit from a biowaiver based on a Level A IVIVC, in accordance with regulatory guidelines.

CONCLUSIONS

The successful establishment of six Level A *In Vitro-In Vivo* Correlations (IVIVCs) for Pentoxifylline 400 mg modified-release tablets, with high determination coefficients ($R^2 > 0.97$), obtained from the point-to-point IVIVCs, signify a strong relationship between the *in vitro* dissolution data obtained in three pH media and *in vivo* absorption profiles. The robust IVIVC models presented here for both Reference product Trental 400 mg modified-release

tablets and for the prototype generic developed Pentoxifylline 400 mg modified-release tablets highlight the importance of controlled release formulations in achieving consistent therapeutic outcomes and underscores the utility of IVIVCs in regulatory submissions. Overall, this work contributes to the growing body of evidence supporting the use of IVIVC in drug development, particularly for modified-release formulations. It highlights the potential for IVIVCs to improve the efficiency and cost-effectiveness of bringing new pharmaceutical products to market, ultimately benefiting both industry and patients. This approach allows for the improvement of methodologies and accumulation of data critical for future IVIVC development efforts.

EXPERIMENTAL SECTION

In vitro studies

Dissolution studies were performed on 400 mg Reference and Test pentoxyfilline modified-release tablets (EvoluPharm, Auneuil, France). The dissolution tests were carried out in a rotating paddle apparatus (USP Apparatus II) from Pharma Test (type PTWS 120s, Hainburg, Germany) at 37±0.5°c and rotational speed of 50 rpm, using 900 mL of various dissolution media (media pH 1.2, 4.5 and 6.8).

A high-performance liquid chromatography (HPLC) method with UV detection was employed to identify and quantify pentoxifylline in samples obtained during the dissolution test. The analysis was performed using a Zorbax C18 column (100 mm x 3.0 mm i.d., 3.5 µm particle size) under the following conditions: the mobile phase consisted of 25% acetonitrile (ACN) and 75% 0.1% phosphoric acid (H_3PO_4); the column temperature was maintained at 25°C; the flow rate was set at 1 mL/min; the injection volume was 1 µL; detection was carried out at a wavelength of 274 nm. Under these conditions, pentoxifylline had a retention time of 1.1 minutes, with the total analysis completed within 2 minutes per sample.

The dissolution media for the *in vitro* studies were prepared using the following protocols:

- pH 1.2 Hydrochloric Acid Solution: A 0.1 N hydrochloric acid solution was prepared by dissolving 9.9 g of 37% HCl in distilled water. The solution volume was adjusted to 1 L, achieving a pH of 1.2.
- pH 4.5 Acetate Buffer Solution: To prepare the buffer, 3 g of sodium acetate trihydrate was dissolved in distilled water, followed by the addition of 14 mL of 2N acetic acid. The final volume was adjusted to 1 L with distilled water, resulting in a buffer with a pH of 4.5.

- pH 6.8 Phosphate Buffer Solution: A phosphate buffer was prepared by dissolving 6.81 g of potassium dihydrogen phosphate and 0.9 g of sodium hydroxide in distilled water. The solution volume was adjusted to 1 L to achieve a pH of 6.8.

The dissolution media were prepared by thoroughly mixing the reagents until fully dissolved, and the final pH was confirmed using a calibrated pH meter to ensure consistency and accuracy during the dissolution tests. All reagents used in the preparation of the dissolution buffer were of United States Pharmacopoeia (USP) grade.

Samples (100 µL each) were collected at specific intervals: before tablet release into the dissolution media (time 0), and subsequently at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours. The collected samples were filtered and analyzed for pentoxifylline content using an HPLC-UV method, as previously described.

In vivo data

The clinical study data, detailing plasma concentration versus time profiles, were obtained from a pilot bioequivalence study in which the test formulation was pentoxifylline 400 mg modified released tablets (developed by Antibiotice SA, Iaşi, Romania) and the reference drug was Trental® 400 mg modified release tablets (Sanofi-Aventis, Paris, France). The study was performed under fed conditions at the Centre for Drug Evaluation, Antibiotice SA, Iași, Romania. The study strictly adhered to all applicable European Union (EU) and International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) requirements and Ethical standards. The study protocol was approved by the National Ethics Committee, Romania, and by the National Agency for Medicines and Medical Devices, Romania.

Study subjects: Twenty-nine healthy male and female Caucasian subjects were screened and included in the study. All relevant study aspects were discussed with each volunteer, and written informed consent was provided by each participant prior to the commencement of the study. The study protocol clearly defined the inclusion and exclusion criteria. Only healthy volunteers who met the established criteria were selected by the investigator based on their medical history, physical examination, and clinical laboratory tests. These tests included haematology, clinical chemistry, urine analysis, urine drug screening, and pregnancy tests for female subjects. All participants were Caucasian adults aged 18 to 50 years, non-smokers or light smokers, with a body mass index (BMI) between 18.5 and 30 kg/m^2.

Drug products: All subjects received a single oral dose of 400 mg pentoxifylline, either test or reference drug, in accordance with the randomization scheme.

Study design: The study was designed as a pilot, single-center, open-label, analytically blind, randomized, single-dose, crossover study, with a wash-out period of 6 days between treatments.

At each treatment period, subjects were confined from at least 10 hours before dosing until after 24.0 hours post – dose. Follow-up ambulatory visits were scheduled for 36 hours post-dose.

The drug administration followed a standard high – fat high – calorie breakfast 30 minutes before drug administration and standardized meals at 4.0, 9.0, and 13.0 hours after drug administration. Liquid consumption was restricted from 1 hour before until 1 hour after drug administration, except 200 mL non – sparkling water for drug administration.

Blood plasma samples collection: Venous blood samples (6 mL each) were collected prior to drug administration (at 0 hours) and at predetermined time points post-dose, including 0.33, 0.67, 1.0, 1.33, 1.67, 2.0, 2.33, 2.67, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 24.0 and 36.0 hours. The collected samples were rapidly frozen until analysis at a temperature of (-25°C) ± 5°C.

Bioanalytical methodology: Quantification of the drug substance was performed using a high-throughput liquid chromatography-tandem mass spectrometry (LC/MS/MS) method, with pentoxifylline d5 serving as the internal standard. Calibration curves demonstrated linearity within the concentration range of 5–500 ng/mL.

Statistical analysis: Data analysis was conducted using Phoenix WinNonlin® software, version 8.4 (Certara, PA, USA). The results obtained for the Reference and Test products were utilized to develop the IVIVC models.

In vitro – in vivo correlations (IVIVCs)

The numerical deconvolution method was employed to evaluate the level of correlation. This approach involved deriving the absorption profile from *in vivo* data collected during clinical study. Subsequently, the percentage of the drug absorbed *in vivo* was plotted against the percentage dissolved in *in vitro* tests. The analysis and calculations were conducted using Phoenix WinNonlin® version 8.4 software (Certara, PA, USA), facilitating the estimation of drug absorption kinetics.

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