

COMPARATIVE ASSESSMENT OF THE *IN VITRO* PERFORMANCE FOR MODIFIED RELEASE TABLETS CONTAINING PENTOXIFYLLINE USING PADDLE METHOD AND FLOW-THROUGH CELLS

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Manuscript received: March 2015

Abstract

The paper analyzed the impact of hydrodynamic differences existing between the two types of compendial apparatuses on the *in vitro* performance of modified release oral solid dosage forms containing pentoxifylline. The experimental conditions were selected based on the provisions of dissolution test 5 of the United States Pharmacopoeia, with previous reports suggesting the feasibility of *in vitro/in vivo* correlations. Despite the distinct kinetics probably induced by swelling and erosion differences, the mean release profiles were similar for the evaluated products and compliant with the same acceptance limits. Considering the high water solubility of the active pharmaceutical ingredient, the sink conditions requirements were fulfilled for the flow-through design even when the stationary volume of the cells was considered. A new criterion for selection of calibration products for performance verification test was suggested.

Rezumat

Lucrarea a analizat impactul diferențelor hidrodinamice existente între două tipuri de aparate compendiale asupra performanței *in vitro* pentru forme farmaceutice solide orale cu cedare modificată conținând pentoxifilină. Condițiile experimentale au fost selectate pe baza recomandărilor incluse în testul de dizolvare 5 din Farmacopeea Statelor Unite ale Americii, pentru care rapoarte anterioare au sugerat fezabilitatea corelațiilor *in vitro in vivo*. În pofida cineticilor distincte induse probabil de diferențe de hidratare și eroziune, profilele medii de cedare obținute pentru produsele evaluate au fost similare și au îndeplinit aceleași criterii de acceptare. Considerând solubilitatea mare în apă a substanței active, cerințele referitoare la condițiile *sink* au fost îndeplinite pentru procedura în flux continuu, chiar și prin considerarea volumului staționar al celulelor. A fost sugerat un nou criteriu de selecție a produselor de calibrare pentru testul de verificare a performanței.

Keywords: pentoxifylline, modified release, *in-vitro* release, paddle method, flow-through cells

Introduction

Initially identified as the column type method [1], the experimental approach using the so called flow-through cells has several advantages compared to the standard paddle or basket apparatus. The most frequently cited ones are the capacity to provide theoretically unlimited volume of dissolution media, beyond the design-limited capacities of the 1000, 2000 or 4000 mL round bottom vessels described in compendia or regulatory documentation [2]. Combined with the wide range of testing times and flow rates [3], this further provides adequate sink conditions for *in vitro* release testing of all dosage

forms containing low-solubility active pharmaceutical ingredients, when used in an open loop setup. For a formulation that will evolve in a continuously changing environment such as the gastro-intestinal tract, media change can be induced without significant hydrodynamic alterations. Several types of cells have been specifically developed as adaption for the shape, composition characteristics and mechanism of release of various types of formulations, outlining the versatility of this method [4]. New applications are continuously reported, especially for novel and special dosage forms [5, 6]. Nonetheless, the immediate release

conventional solid oral dosage forms can be assessed using the flow-through cells according to the revised version of the chapter <1092> of the United States Pharmacopoeia (USP) [7]. The analysis of the outlet fractions collected immediately after the debut of the pulsating flow are able to provide essential information on the dissolution phenomena occurring after the initial contact between the tablet or capsule and the medium. The assumed biorelevance of the generated *in vitro* dissolution or release data [3] is further increased by the ability to set a flow rate similar to the one reported *in vivo* for the relevant fluids, e.g. 2-3 mL/min [8] for the intestinal regions or 35 mL/min for the large coronary vessels [9].

While acknowledging the major advantages of the USP apparatus 4 [10, 11], it should be pointed out that considerable work has been done for standardization and performance verification [12-14]. The calibrator prednisone and salicylic acid tablets [12, 13] or crystals of drugs [15] have been used for assessing the comparability of dissolution rates generated in usually implemented testing conditions. The obtained differences have been traced to the concentration gradients established between the solid - liquid interface of the drug particles and the bulk medium [16]. The compliance with the sink requirements for the routine quality control procedures should be analyzed based upon momentary conditions, i.e. by correlating the actual exposed surface area with the inner volume of the cells (19 and 8 mL, for 22.6 and 12 mm diameter, respectively). The aim of the current paper was to comparatively evaluate the *in vitro* performance of several modified release oral solid dosage forms containing pentoxifylline in two dose strengths, using the paddle method and flow-through cells design in simple test conditions. One of the compendial set-ups described in the current USP special monograph was used or adapted and the applicability of the acceptance specification was screened.

Materials and Methods

The *in-vitro* dissolution testing procedure was adapted from the methodology described as the test 5 in the USP. Briefly, the paddle method was used at 50 rpm, on a SR8 Plus Dissolution Station (Hanson Research Inc., US). The release media was 900 mL purified water degassed according to compendial guidelines. After introduction of the dosage forms and initiation of the stirring, samples of 5 mL were collected at 30, 60, 120, 180, 240, 300 and 360 through resident cannulas with immersed 10 µm polypropylene filters. Replacement procedure was conducted using fresh media, degassed and heated at the testing temperature of $37 \pm 0.5^\circ\text{C}$.

For the USP apparatus 4, the large tablet cells (22.6 mm inner diameter) were selected, considering the dimensions of the dosage units and the expected increase in volume, due to the swelling process of the matrix forming macromolecular agent. The cells were connected with a HKP 720 piston pump, within an Erweka DFZ720 testing system with 7 positions (Erweka GmbH, Germany). The open-loop design was adopted with a flow rate of 4 mL/min at $37 \pm 0.5^\circ\text{C}$. The inlet cone of 40° was filled with 7 g of glass beads having a mean diameter 1 mm. The film coated tablets were placed on V shape holders, preventing the contact with the glass beads. Glass fiber filters of 2.7 µm were inserted in the outlet collection head. The dissolution media, as well as the sample volume and collection schedule was the same as previously described.

The experimental protocol involving the use of the two compendial apparatus was applied to 5 modified releases solid oral dosage forms containing pentoxifylline, the available information on their qualitative composition being displayed in Table I. The products were purchased from the local pharmacies and used without any previous treatment. Each test was performed on 6 dosage units.

Table I

The identification and qualitative composition of the evaluated modified release products

Assigned code	A	B	C	D	E*
<i>Pentoxifylline (mg)</i>	400	400	400	600	400
Hydroxypropyl methylcellulose	✓	✓	✓	✓	-
Hydroxyethyl cellulose	-	-	-	-	✓
Atomised lactose	-	✓	-	-	-
Mannitol	✓	-	-	-	-
Corn starch	✓	-	-	-	-
Povidone	✓	-	✓	✓	✓
Colloidal silicone dioxide	-	✓	-	-	-
Talc	-	✓	✓	✓	✓
Magnesium stearate	✓	✓	✓	✓	✓
Coating film	✓	✓	✓	✓	✓
Batch no.	3040070481	2135743	3050113	3280912	40C341

* the reference listed drug

The analysis of the collected samples was performed spectrophotometrically, at 274 nm with no prior dilution, using an Agilent 8453 Pharmaceutical UV-visible system (Agilent Instruments, Germany) with the Advanced module UV-Visible ChemStation software (version B.04.01). For the assessment of the mean *in vitro* release profiles, correction of the dissolved amounts at each time point were performed, based on preceding sampling collections and on actual eluting volume. The labeled claimed content was used for calculations. In the case of flow-through cells, the results were expressed as cumulative and fractional release.

The analytical standard of pentoxifylline was purchased from Sigma and used as received. The purified water was generated on a SGW Ultraclear

UV Plus™ system (Germany), with a conductivity lower than 0.2 $\mu\text{S}/\text{cm}$ and a total organic compound content below 30 pbb.

Results and Discussion

The mean *in vitro* dissolution profiles indicated similar release rates between the flow through design and to the standard paddle method (Figure 1). Except for product B, the fractions of pentoxifylline dissolved in the aqueous media were higher for the USP apparatus 4, attaining a maximum difference of 5% at 240 min. This may be explained by the lack of soluble polyol filler, which is able to create aqueous pores and increase the swelling of the matrix forming macromolecular agent.

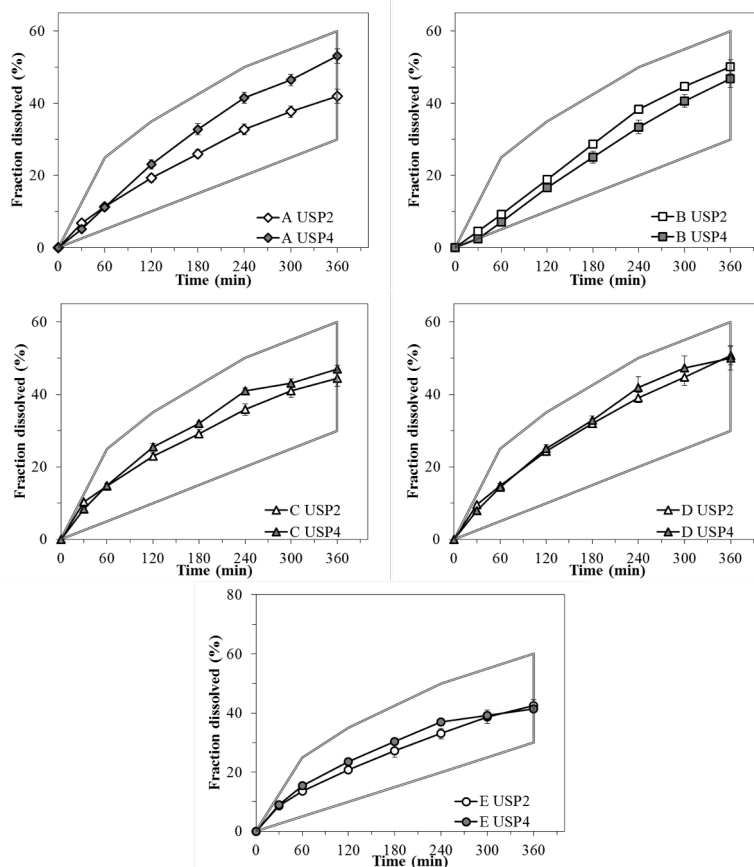


Figure 1.

The mean *in vitro* dissolution profiles of pentoxifylline from modified release film coated tablets ($n = 6$, mean $\pm$ standard deviation; double lines represent the lower and upper acceptance limits, according to the USP specific monograph; USP2 - paddle method, USP4 - flow-through cells)

On the other hand, the association of povidone and mannitol for product A seemed to accelerate the swelling for the paddle method, with released fractions gradually increasing up to 11% at the end of the testing period (Figure 2). The erosion phenomenon was considerable, leading to a rapid exposure of the inner layers of the modified release

formulations. It must be pointed out that preliminary assessment of the testing conditions confirmed that the release is influenced to a considerable extent by the position of the matrix tablets. Positioning of the dosage unit on top of the glass beads didn't alter significantly the diffusion across the forming gel barrier, despite the

supplementary mechanical obstruction of the contact with the media. Nevertheless, when the reference listed drug was clamped within the tablet holder and not placed perpendicular on top of it, a

burst in the release rate was observed. The increased erosion of the gel by the wired holder generated almost complete dissolution in less than 180 min.

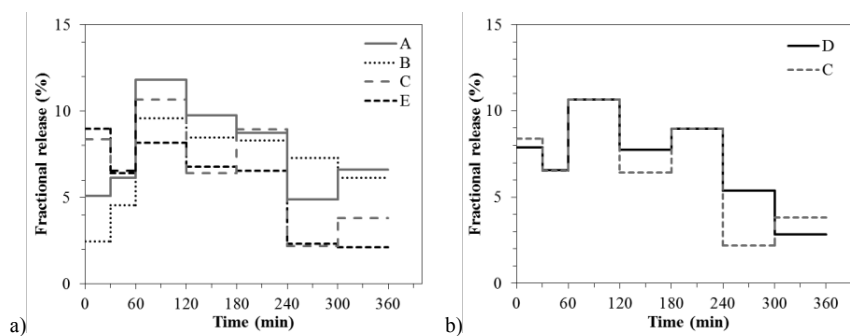


Figure 2.

The mean values of the fractional release for the modified release film-coated tablets containing pentoxifylline ($n = 6$), in the flow-through design: a) 400 mg multisource products; b) 400 and 600 mg strengths of the same generic product

The mechanism of release was dependent upon the nature of excipients, varying between a typical Fickian profile and combined diffusion and erosion processes [17]. For the product available in two different strengths, the similarity of the qualitative composition and probably the proportionality of their quantity generated the same kinetic model, but distinct between the two experimental setups. The mean profiles induced by the paddle method were adequately fitted by the Korsmeyer-Peppas model (non-Fickian transport), whereas the data from the

flow-through design were described by a Weibull function (data not shown).

The compendial pair-wise model-independent procedure was applied for assessing the differences in release [18, 19, 23]. Considering the value of the f_2 metric (Table II), the *in-vitro* similarity was concluded between the reference listed drug and the generic products. Moreover, the two different strengths of the same product with consistent kinetic model showed no significant difference, making the proportionality waiver approaches feasible.

Table II

The results of pair-wise comparison procedures applied to the mean dissolution profiles (f_1 , difference factor; f_2 , similarity factor)

Compared products	Reference	E				D
Method	Test	A	B	C	D	C
Paddle method (900 mL, 50 rpm)	f_1	4.65	16.8*	7.6	16.66*	8.51
	f_2	89.21	66.55	83.18	65.81	74.25
Flow-through cells (1440 mL, 4 mL/min)	f_1	17.7*	19.14*	9.34	14.25	4.78
	f_2	62.28	63.08	75.22	65.92	83.32

* values outside the acceptance interval for *in vitro* similarity, 0 to 15

Although the use of water as a medium is not generally recommended, due to the lack of biorelevance, variable quality between laboratories and absence of buffer capacity, the high solubility of the active pharmaceutical ingredient provided full compliance with the sink requirements, throughout the test. For the flow-through cells, this conclusion is maintained even if the momentary volume of the inner compartment is considered (19 mL). A previous report [20] indicated that *in vitro* - *in vivo* correlations can be achieved even when these simple conditions are adopted.

All mean *in vitro* release met the requirements with regard to the acceptance limits of the fraction

dissolved at 60, 120, 240 and 360 min. This conclusion was valid independent on the experimental design and consistent with the small difference induced by the assumed hydrodynamic difference between the two compendial apparatuses. It may be assumed that the rate limiting step of the global process is not significantly altered. Notably, the upper and lower acceptance limits described two distinct dissolution models, i.e. Fickian release and zero order kinetics (Figure 3). Most probably, the intermediate compliance region can accommodate composition variables generating combined mechanisms, as confirmed by the current experimental data.

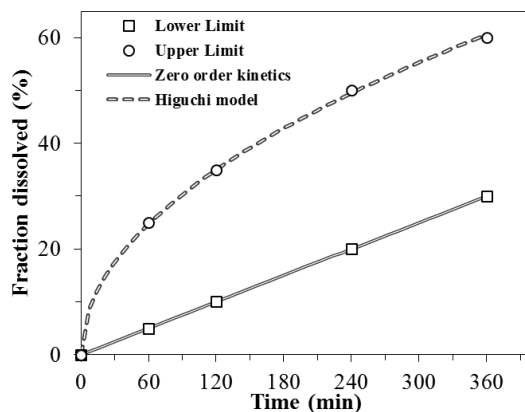


Figure 3.

Modelling of *in vitro* dissolution profiles described by the lower (squares) and upper (circles) compendial acceptance limits

The comparison on *in vitro* release from various types of dosage forms using the official apparatus has been previously suggested, confirming either the feasibility for routine quality control procedures or as performance test [21]. For modified release monolithic tablets, one key factor to consider is the swelling degree, beside the contact surface area between the drug and the medium. Large size, non-disintegrable tablets may obstruct the lumen of the cell during the test, with increased shearing forces on the lateral segments. Nevertheless, the same flow rates applied to cells with 22.6 and 12 mm inner diameter leads to distinct velocities, due to different cross-sectional areas [22]. The flow-through setup is more frequently applied during the research and development phase, but its utility is continuously expanding. The Rufinamide tablets monograph with a dissolution test based on apparatus 4 was officially adopted by the USP 36 (2013). A specific product for performance verification test (PVT) is not available yet, although promising reports are available on the use of salicylic acid tablets [24]. The concluded *in vitro* similarity for apparatus 2 and 4, with feasible adoption of the same acceptance limits, is mainly driven by the high water solubility of pentoxifylline (191 mg/mL, at 37°C, [25]). This warrants a dissolution process independent on the renewal rate of medium. It is our suggestion that calibration products for PVT should be selected based upon the ratio of dose to stationary volume of the cell, e.g. complete dissolution of the labeled amount of drug can be achieved in 19 mL of media.

Conclusions

A comparative study on the *in vitro* performance of five modified release solid dosage forms containing pentoxifylline was performed using the standard paddle method and an adapted test using the flow-through cell. The mean release profiles were similar

for the generics and reference listed drug, independently on the hydrodynamic conditions of the two experimental designs. The same compendial acceptance limits can be applied for both methodological approaches. The apparent distinct kinetics were probably induced by swelling and erosion differences, traceable to the composition variables.

Acknowledgements

This work was supported by the Sectorial Operational Programme Human Resources 2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/137390 and by PN-II-PT-PCCA-2013-4-1903. The results have been partially presented at the National Congress of Pharmacy from Romania 15th edition, 10th Symposium of Biopharmacy and Pharmacokinetics, Iași, Romania, September 24-27, 2014.

References

1. Langenbucher F., Rettig H., Dissolution rate testing with the column method: Methodology and results. *Drug Develop. Ind. Pharm.*, 1977; 3(3): 241-263.
2. United States Pharmacopeial Convention Inc., Chapter <711>: Dissolution.
3. Fotaki N., Flow-through cell apparatus (USP apparatus 4): Operation and features. *Diss. Tech.*, 2011; 18(4): 46-49.
4. Brown W., Apparatus 4 flow through cell: Some thoughts on operational characteristics. *Diss. Tech.*, 2005; 12(2): 28-30.
5. Rawat A., Stippler E., Shah V.P., Burgess D.J., Validation of USP apparatus 4 method for microsphere *in vitro* release testing using Risperdal Consta. *Int. J. Pharm.*, 2011; 420(2): 198-205.
6. Brown C.K., Friedel H.D., Barker A.R., Buhse L.F., Keitel S., Cecil T.L., Kraemer J., Morris J.M., Reppas C., Stickelmeyer M.P., Yomota C., Shah V.P., FIP/AAPS joint workshop report: dissolution/*in*

- vitro* release testing of novel/special dosage forms. *AAPS PharmSciTech.*, 2011; 12(2): 782-794.
7. United States Pharmacopeial Convention Inc., In Process Revision: Chapter <1092> The dissolution procedure: Development and validation (USP38/NF33). *Pharmacop. Forum*, 2015; 40(1): 1-30.
 8. Lennernäs H., Intestinal permeability and its relevance for absorption and elimination. *Xenobiotica*, 2007; 37(10-11): 1015-1051.
 9. Seidlitz A., Nagel S., Semmling B., Grabow N., Sternberg K., Weitschies W., Biorelevant dissolution testing of drug-eluting stents: Experiences with a modified flow-through cell setup. *Diss. Tech.*, 2011; 18(4): 26-34.
 10. Gao Z., *In vitro* dissolution testing with flow-through method: a technical note. *AAPS PharmSciTech.*, 2009; 10(4): 1401-1405.
 11. Möller H., Wirbitzki E., Regulatory aspects of modified release dosage forms: special cases of dissolution testing using the flow-through system. *Boll. Chim. Farm.*, 1993; 132(4): 105-115.
 12. Nicklasson M., Wennergren B., Lindberg J., Persson C., Ahlgren R., Palm B., A collaborative *in vitro* dissolution study using the flow-through method. *Int. J. Pharm.*, 1987; 37: 195-202.
 13. Wennergren L., Lindberg L., Nicklasson M., Nilsson G., Nyberg G., Ahlgren R., A collaborative *in vitro* dissolution study: comparing the flow-through method with the USP paddle method using USP prednisone calibrator tablets. *Int. J. Pharm.*, 1989; 53: 35-41.
 14. Gjellan K., Magnusson A., Ahlgren R., Callmer K., Christensen D.F., Espmarker U., Jacobsen L., Jarring K., Lundin G., Nilsson G., Waltersson J., A collaborative study of the *in vitro* dissolution of acetylsalicylic acid gastro-resistant capsules comparing the flow-through cell method with the USP paddle method. *Int. J. Pharm.*, 1997; 151(1): 81-90.
 15. Nicklasson M., Orbe A., Lindberg J., Borgå B., Magnusson A.B., Nilsson G., Ahlgren R., Jacobsen L., A collaborative study of the *in vitro* dissolution of phenacetin crystals comparing the flow through method with the USP Paddle method. *Int. J. Pharm.*, 1991; 69(3): 255-264.
 16. Posti J., Speiser P.P., Sink conditions in the flow-through cell during dissolution. *Int. J. Pharm.*, 1980; 5(2): 101-107.
 17. Matharu A.S., Motto M.G., Patel M.R., Simonelli A.P., Dave R.H., Evaluation of hydroxypropyl methylcellulose matrix systems as swellable gastro-retentive drug delivery systems (GRDDS). *J. Pharm. Sci.*, 2011; 100(1): 150-163.
 18. Şaramet G., Rădulescu F.Ş., Bărbuceanu S.F., Miron D.S., Fiţa C.A., Mitu M.A., Stănescu A.A., Lupuliasa D., Influence of some formulation factors on the release of phenytoin sodium from hydrophilic matrix tablets. *Farmacia*, 2014; 62(6): 1230-1238.
 19. Mourad A., Miron D.S., Lupuleasa D., Karampelas O., Rădulescu F.Ş., Drăgănescu D., Roşca A.C., The impact of dissolution media on the similarity of release profiles of trimetazidine dihydrochloride from immediate release tablets. *Farmacia*, 2013; 61(3): 558-565.
 20. Ionică G., Rădulescu F., Miron D., Anuţă V., Mircioiu C., Belu I., Dissolution of pentoxifylline from extended release formulations. Researches concerning development of a biorelevant test. *Current Health Sci. J.*, 2009; 35(4): 286-289.
 21. Souliman S., Beyssac E., Cardot J.M., Denis S., Alric M., Investigation of the biopharmaceutical behavior of theophylline hydrophilic matrix tablets using USP methods and an artificial digestive system. *Drug Dev. Ind. Pharm.*, 2007; 33(4): 475-483.
 22. D'Arcy D.M., Liu B., Persoons T., Corrigan O.I., Hydrodynamic complexity induced by pulsating flow field in USP dissolution apparatus 4. *Diss. Tech.*, 2011; 18(4): 6-13.
 23. Burcea Dragomiroiu G.T.A., Miron D.S., Baloescu C., Bărcă M., Mitu M.A., Popa D.E., Rădulescu F.Ş., Comparative *in-vitro* study of immediate and modified released oral dosage forms of cefaclor. *Farmacia*, 2012; 60(3): 334-341.
 24. Eaton J.W., Tran D., Hauck W.W., Stippler E.S., Development of a performance verification test for USP apparatus 4. *Pharm. Res.*, 2012; 29(2): 345-351.
 25. Indrayanto G., Syahrani A., Soeharyono M., Lianawati T., Whyuningsih I., Aditama L., Brittain H.G., Pentoxifylline. *Analytical Profiles Drug Subst.*, 1998; 25: 295-340.