

Evaluation of In Vitro Equivalence for Drugs Containing BCS Class II Compound Ketoprofen

e-mail: sovdep2007@yandex.ru

I. E. Shohin^{1,2,*}, J. I. Kulinich^{1,2}, G. V. Ramenskaya^{1,2}, and G. F. Vasilenko²¹Sechenov First Moscow State Medical University, 8-2, Trubetskaya Street

119991, Moscow, Russia

²Scientific Center for Expertise of Medical Products, Moscow, Russia

ABSTRACT

This paper describes the evaluation of the in vitro equivalence of capsules containing a BCS Class II compound, ketoprofen, marketed in Russia under biowaiver conditions and the innovator product. The in vitro equivalence test was carried out according to WHO Technical Report Series, No. 937, Annex 8. Dissolution profiles of test and reference (innovator) ketoprofen capsules are considered equivalent at pH 6.8 without statistical treatment, equivalent at pH 4.5 ($f_1 = 3$ and $f_2 = 80$), and not equivalent at pH 1.2 ($f_1 = 22$ and $f_2 = 41$). Generally, the evaluated capsules did not meet biowaiver criteria for drugs containing BCS Class II API, possibly due to the effect of surfactant (sodium lauryl sulfate) contained in the test preparation on the solubility.

INTRODUCTION

The dissolution test, at first exclusively a quality control test, is now emerging as a surrogate equivalence test for certain categories of orally administered pharmaceutical products. For some solid dosage forms containing active pharmaceutical ingredients (API) with suitable biopharmaceutical properties, in vitro dissolution profile similarity can be used to establish equivalence of the test product with the reference product. Such studies, used to approve equivalence other than through in vivo equivalence testing, are called "biowaivers" (1). A biowaiver includes dissolution kinetics studies called "in vitro equivalence tests." This term is used in the WHO Technical Report Series, No. 937, Annex 7, and means a dissolution test that includes comparison of the dissolution profile between test product and reference product in three media: pH 1.2, pH 4.5, and pH 6.8. It should be noted that quality control dissolution test conditions differ from in vitro equivalence tests and are not applicable for this purpose (2).

Ketoprofen is an ibuprofen-type, nonsteroidal anti-inflammatory drug (NSAID) regarded as a nonselective cyclooxygenase (COX) inhibitor. Ketoprofen is indicated for the treatment of mild to moderate pain related to dysmenorrhea, headache, migraine, postoperative and dental pain, and in the management of spondylitis, osteoarthritis, rheumatoid arthritis, and soft tissue disorders (3). The chemical name of ketoprofen is (RS)-2-(3-benzoylphenyl) propanoic acid. Its structure is shown in Figure 1.

Solubility

Ketoprofen is described as slightly soluble in water (4, 5). Experimental solubility at pH 2.0 is 0.205 mg/mL (6), and

the lowest solubility in aqueous solution is 0.010 mg/mL (7). Within the gastrointestinal pH range, ketoprofen is an ionized compound (weak acid). The pK_a of ketoprofen is approximately 4.45 at 25 °C (8). Thus, ketoprofen solubility increases with pH and is about 40.76 mg/mL at pH 6.8 (9). Russia has two Marketing Authorizations (MA) for ketoprofen as immediate-release capsules at a strength of 50 mg and three MAs for ketoprofen as immediate-release tablets at strengths of 25 and 100 mg (10). Thus, the dose/solubility (D/S) ratio for ketoprofen is less than 250 mL for the highest dose marketed in Russia, although it dissolves in 250 mL of buffer solution at pH 6.8. Therefore, ketoprofen is a "low solubility" drug according to WHO Guidance.

Permeability

When an API is absorbed to an extent of 85% or more, it is considered "highly permeable." The absolute bioavailability of ketoprofen is 90%; T_{max} is 1–2 h after oral administration. The permeability obtained by the method of intestinal perfusion in vivo ($P_{eff\ in\ vivo}$) is 8.7×10^{-4} cm/s (6). In a Caco-2 cell culture study, P_{app} of ketoprofen is 40.6×10^{-6} cm/s. Its intestinal perfusion in rats ($P_{eff\ in\ situ}$) is 1.9×10^{-4} cm/s (11). Therefore, ketoprofen is a compound with high permeability according WHO Guidance.

Biopharmaceutical Classification

Ketoprofen is not listed in the WHO Model list and is not classified according to BCS by WHO (12). Taking ketoprofen solubility (low) and permeability (high) into account, ketoprofen is assigned to BCS Class II according to WHO Guidances.

Biowaiver Eligibility for BCS Class II API

The BCS Guidance of the United States Department of Health and Human Services, Food and Drug

*Corresponding author.

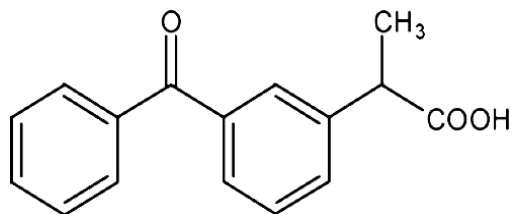


Figure 1. Structure of ketoprofen.

Administration (FDA) recommends the biowaiver only for drug products containing Class I compounds (13). Discussions at scientific workshops after the guidance became available and in subsequent publications suggested that biowaivers could be extended to drug products containing Class II and III APIs. WHO Technical Report Series, No. 937, Annex 8 states that biowaivers can apply to Class II weak acids, which are highly soluble at pH 6.8 but not at pH 1.2 or pH 4.5. Ketoprofen fulfills these criteria as described above. Therefore, ketoprofen in vitro equivalence may be evaluated under biowaiver conditions for BCS Class II.

MATERIALS AND METHODS

Chemicals

Analytical grade concentrated hydrochloric acid, glacial acetic acid, potassium dihydrogen phosphate, disodium hydrogen phosphate dodecahydrate, and potassium chloride were used.

Innovator ketoprofen immediate-release capsules, used as reference product, and a generic version (test product) in strengths of 50 mg marketed in Russia were evaluated.

Apparatus and Procedure

All dissolution studies were performed using USP Apparatus 2 (Erweka DT 600, Frankfurt, Germany) at 75 rpm. Dissolution media were USP buffer solutions pH 1.2 (hydrochloric acid solution), pH 4.5 (acetate buffer solution), and pH 6.8 (phosphate buffer solution) at 37 ± 0.5 °C. Dissolution medium volume was 500 mL. Twelve capsules of each preparation were studied to evaluate statistical significance of the results. In all experiments, 5-mL sample aliquots were withdrawn at 10, 15, 20, 30, and 45 min using micropipettes and immediately replaced with equal volumes of fresh medium at the same temperature to maintain constant total volume during the test. All samples were filtered through 0.45- μ m membrane filters. Because the absorbance values of undiluted samples were greater than 1, 4.5 mL of corresponding dissolution medium was added to 0.5 mL of each sample to obtain valid results. Diluted samples were mixed using a Vortex mixer for 15 s.

Drug release was evaluated spectrophotometrically using a UV-vis spectrophotometer (Agilent 8453, Santa Clara, CA, USA) at 260 nm using the corresponding dissolution medium as a reference. Ketoprofen CRS solution at 0.01 mg/mL in the corresponding dissolution medium was used as reference standard solution.

Table 1. Excipients Contained in (1) Reference and (2) Test Drugs

Excipient	Evaluated Drug
Gelatin	1, 2
Indigo carmine FD&C blue #2	2
Patent blue V	1
Silica colloidal	1, 2
Croscarmellose sodium	2
Lactose	1, 2
Magnesium stearate	1
Sodium lauryl sulfate	2
Povidone	2
Titanium dioxide	1, 2
Cellulose	2

Dissolution profile comparisons were made according WHO Guidances (1) calculating f_1 and f_2 factors. Statistical treatment was carried out using Microsoft Excel software.

Excipients

The excipients contained in the evaluated drug products are shown in Table 1.

RESULTS AND DISCUSSION

Biowaiver criteria for drugs containing BCS Class II APIs with weak acid properties and high solubility at pH 6.8 but not at pH 1.2 and pH 4.5 (1) are:

1. The dosage form is rapidly dissolving (85% in 30 min or less) in pH 6.8 buffer (only).
2. The test product exhibits similar dissolution profiles, as determined by the f_2 value or equivalent statistical evaluation, to those of the reference product at the three pH values (pH 1.2, 4.5, and 6.8).

Furthermore, for test products containing Class II APIs with dose/solubility ratios of 250 mL or less at pH 6.8, the excipients should be critically evaluated in terms of type and amounts (e.g., of surfactants) in the formulation.

Both evaluated drug products are “rapidly dissolving” (see Table 2) at pH 6.8 because the API release at time point 30 min is greater than 85%.

Table 2. Dissolution Amount for Evaluated Drugs

Medium	Test Product		Reference Product	
	% dissolved (X) 15 min	% dissolved (X) 30 min	% dissolved (X) 15 min	% dissolved (X) 30 min
pH 6.8	98.0	99.4	95.0	99.5

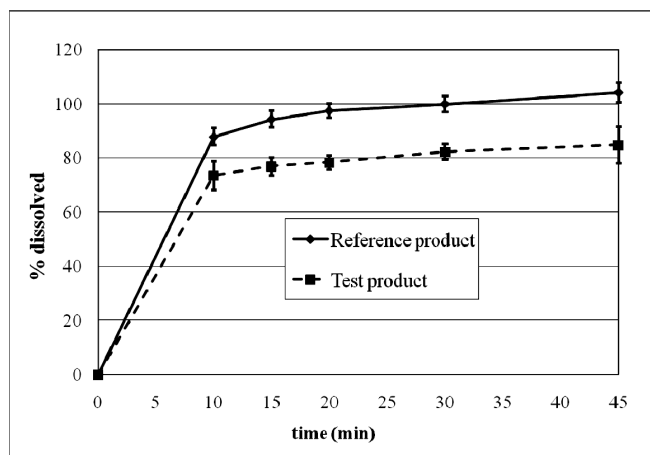


Figure 2. Dissolution profiles of test and reference drugs at pH 1.2.

Dissolution profiles and corresponding data are shown in Figures 2–4 and Table 3. Dissolution profiles in pH 6.8 buffer are similar without statistical treatment (greater than 85% released after 15 min). For pH 4.5, the similarity factor f_2 value is 80 and factor f_1 is 3; therefore, the dissolution profile of the test product is similar to that of the reference product at this pH. Dissolution profiles in pH 1.2 buffer are considered not similar because the calculated factors ($f_2 = 41$ and $f_1 = 22$) do not meet the acceptance criteria ($50 \leq f_2 \leq 100$; $0 \leq f_1 \leq 15$). The surfactant (sodium lauryl sulfate) contained in the test preparation possibly increased the ketoprofen solubility, which caused the dissolution profile dissimilarity. The addition of sodium lauryl sulfate in concentrations of 0.5–2% significantly increases the solubility of ketoprofen, especially at low pH, from about 5 to about 30 times that at pH 4.6, respectively (9). Therefore, the presence of surfactants increases the release rate of ketoprofen and improves its dissolution. However, such differences in test and reference drug products do not seem to be critical, because improved dissolution in acidic medium with sodium lauryl sulfate

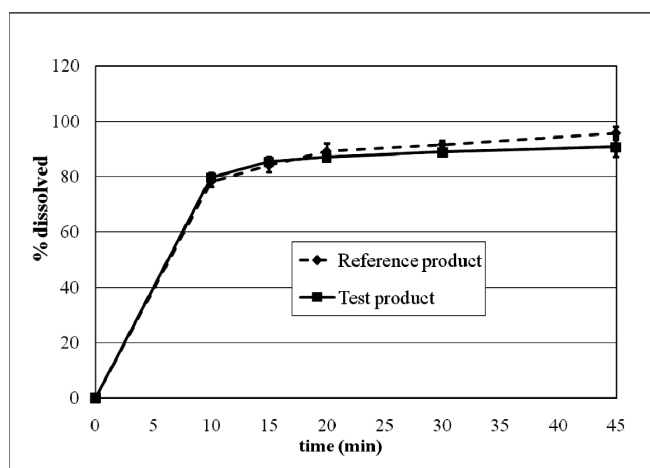


Figure 3. Dissolution profiles of test and reference drugs at pH 4.5.

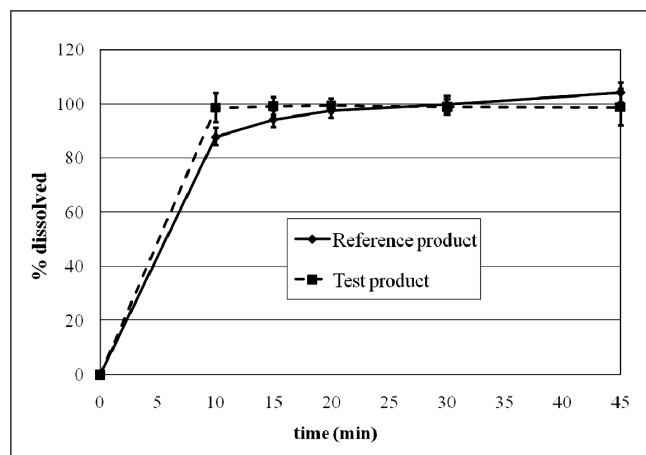


Figure 4. Dissolution profiles of test and reference drugs at pH 6.8.

would not cause bioequivalence of evaluated products, only in vitro inequivalence. In addition, because of the high permeability of ketoprofen, its low solubility at pH 1.2 does not pose a serious risk for bioequivalence. This acidic medium may be “over discriminatory” for in vitro equivalence evaluation of ketoprofen drug products.

The percent relative standard deviation (% RSD) for all time points fulfills all requirements ($\leq 20\%$ for first time point, $\leq 10\%$ for other time points), so obtained results are valid (see Table 2).

Table 3. Dissolution Test Results

Medium	Time (min)	Test Product		Reference Product	
		% dissolved (\bar{X})	RSD (%)	% dissolved (\bar{X})	RSD (%)
pH 1.2	10	70.8	4.0	47.2	1.7
	15	74.3	4.2	56.4	2.9
	20	76.2	5.7	63.8	3.0
	30	79.7	3.0	69.1	2.6
	45	81.8	2.1	76.4	1.1
pH 4.5	10	79.8	1.6	77.5	1.9
	15	85.0	1.7	83.7	2.5
	20	87.2	0.8	89.5	2.8
	30	88.9	0.7	91.5	1.4
	45	92.2	3.5	95.3	2.4
pH 6.8	10	96.7	3.2	87.9	5.3
	15	98.0	3.1	95.0	3.3
	20	98.9	2.6	97.3	2.6
	30	99.4	2.9	99.5	2.8
	45	99.9	3.6	104.2	6.7

CONCLUSION

The evaluated ketoprofen drug product does not fulfill biowaiver criteria for drug products containing BCS Class II APIs. Both drug products are “rapidly dissolving,” but they do not meet the criteria for dissolution profile similarity, f_1 and f_2 . The dissolution profile of the test product is similar to that of the reference product in pH 4.5 and 6.8 buffers but not similar in pH 1.2 buffer using the paddle method at 75 rpm. Thus, these products are considered in vitro inequivalent. However, other immediate-release dosage forms of ketoprofen may be evaluated using in vitro equivalence testing.

REFERENCES

1. WHO Expert Committee on Specifications for Pharmaceutical Preparations. *WHO Technical Report Series, No. 937, Annex 8. Proposal to waive in vivo bioequivalence requirements for WHO Model List of Essential Medicines immediate-release, solid oral dosage forms*; World Health Organization: Geneva, Switzerland, 2006.
2. WHO Expert Committee on Specifications for Pharmaceutical Preparations. *WHO Technical Report Series, No. 937, Annex 7. Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability*; World Health Organization: Geneva, Switzerland, 2006.
3. Ketoprofen prescribing information, Lek Pharmaceuticals d. d., Slovenia, 2007.
4. *United States Pharmacopeia and National Formulary USP 29–NF 24*; The United States Pharmacopeial Convention, Inc.: Rockville, MD, 2006.
5. *European Pharmacopoeia*, 6th ed.; European Directorate for the Quality of Medicines, Council of Europe: Strasbourg, France, 2007.
6. Tubic-Grozdanis, M.; Bolger, M.; Languith, P. Application of Gastrointestinal Simulation for Extensions for Biowaivers of Highly Permeable Compounds. *AAPS J.* **2008**, *10* (1), 213–226.
7. Therapeutic Systems Research Laboratories; Biopharmaceutics Classification System (BCS) search engine. <http://69.20.123.154/services/bcs/search.cfm> (accessed Jan 19, 2011).
8. Drug Bank database. Drug card for Ketoprofen. <http://www.drugbank.ca/drugs/DB01009> (accessed Jan 19, 2011).
9. Sheng, J.; Kasim, N. A.; Chandrasekharan, R.; Amidon, G. L. Solubilization and dissolution of insoluble weak acid, ketoprofen: Effects of pH combined with surfactant. *Eur. J. Pharm. Sci.* **2006**, *29*, 306–314.
10. FGU NCESMP Roszdravnadzor database. <http://www.regmed.ru> (accessed Jan 13, 2011).
11. Hilfinger, J. Permeability Classification... In vitro and in situ methodology. Presented at the AAPS Workshop on BE, BCS and Beyond, Bethesda, MD, May 21, 2007. <http://www.aapspharmaceutica.com/meetings/files/90/13Hilfinger.pdf> (accessed Jan 18, 2011).
12. WHO Expert Committee on Specifications for Pharmaceutical Preparations. *WHO Model List of Essential Medicines*, 15th list; World Health Organization: Geneva, Switzerland, 2007.
13. *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System; Guidance for Industry*; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). U.S. Government Printing Office: Washington, DC, August 2000.