

COMMENTARIES

Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Metronidazole

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ABSTRACT: Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing metronidazole are reviewed. Metronidazole can be assigned to Biopharmaceutics Classification System Class I. Most BE studies that were identified reported the investigated formulations to be bioequivalent, indicating the risk of bioinequivalence to be low. Formulations showing differences in bioavailability showed dissimilarities in *in vitro* dissolution profiles. Furthermore, metronidazole has a wide therapeutic index. It is concluded that a biowaiver for solid IR formulations is justified, provided: (a) the test product and its comparator are both rapidly dissolving; (b) meet similarity of the dissolution profiles at pH 1.2, 4.5, and 6.8; (c) the test product contains only excipients present in IR drug products approved in International Conference on Harmonisation (ICH) or associated countries in the same dosage form; and (d) if the test product contains sorbitol, sodium laurilsulfate, or propylene glycol. The test product needs to be qualitatively and quantitatively identical to its comparator with respect to these excipients. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association *J Pharm Sci* 100:1618–1627, 2011

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INTRODUCTION

A monograph based on literature data is presented on metronidazole, with respect to its biopharmaceutical properties and the risk of waiving *in vivo* bioequivalence (BE) testing for the approval of new immediate release (IR) solid oral dosage forms containing metronidazole, including both reformulated products and new multisource products. The purpose and scope of this series of monographs were discussed

previously.¹ Briefly, the aim is to evaluate all pertinent data available from literature sources to assess the risk of a biowaiver decision and recommend whether a biowaiver is advisable or not. Risks considered are both the chance of an incorrect biowaiver decision and the assessment of its consequences on public health and individual patient risks. This systematic approach to recommend or to advise against a biowaiver decision is referred to in the recently published World Health Organization (WHO) Guideline.² These monographs do not intend to simply apply the WHO,² United States Food and Drug Administration (FDA),³ and/or European Medicines Agency (EMA) Guidance,⁴ but aim also as a critical evaluation of these and other countries' regulatory documents. Biowaiver monographs have already been published for several active pharmaceutical ingredients (APIs), also available online at www.fip.org/bcs.⁵

EXPERIMENTAL

Literature data were obtained from Web of Science, PubMed, and Micromedex databases up to December 12, 2009. The keywords used for searching were as follows: metronidazole, intestine absorption, linear absorption, absolute bioavailability, human bioavailability, bioequivalence, log *P*, solubility, permeability, and lipophilicity.

GENERAL CHARACTERISTICS

Name

INN: Metronidazole.^{6,7} Its structure is shown in Figure 1.

Therapeutic Indications and Dose

Metronidazole is classified in the WHO Essential Medicines List as antiamoebic, anti-giardiasis, and antibacterial.⁸ It is used in combination with other antibiotics and either bismuth compounds or proton pump inhibitors for treatment of peptic ulcer disease caused by *Helicobacter pylori*.⁹⁻¹² Because of its activity against anaerobic bacteria, metronidazole has also been employed in the treatment of periodontal disease.^{13,14} Approved indications include treatment of trichomoniasis, vaginitis, and urethritis caused

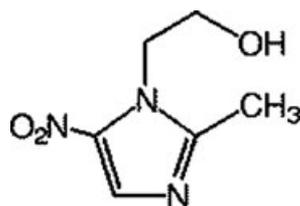


Figure 1. Structure of metronidazole; molecular weight: 171.16.

by *Gardnerella vaginalis*, giardiasis, amoebiasis, and infections caused by anaerobic bacteria,¹⁵ which comprise intraabdominal infections, skin and skin structure infections, gynecologic infections, bacterial septicemia, bone and joint infections, central nervous system infections, lower respiratory tract infections, and endocarditis.¹⁶ Depending on the indication, the dosage regimen can vary from 250 mg three times daily for 7 days to 750 mg three times daily for 10 days. Single doses of 2 g can also be used.¹⁶ Daily doses can be as high as 2.5 g.⁹

Therapeutic Index and Toxicity

Metronidazole is, in general, very well tolerated, has a wide therapeutic index,¹⁰ and its serum and tissue concentrations do not require routine determination.¹⁷ The most common adverse reactions reported occur in the gastrointestinal (GI) tract, particularly nausea, anorexia, diarrhea, epigastric distress, constipation, and abdominal cramps.^{9,18,19} Less frequent untoward effects in the digestive tract include an unpleasant metallic taste and vomiting.^{9,18,19} Occasionally, dysuria, cystitis, dry mouth, dry vulva and vagina, feeling of pelvic pressure, vaginal burning, rash, headache, and insomnia may occur.^{18,19} Incoordination, dizziness, vertigo, encephalopathy, convulsion, and ataxia are rare neurotoxic effects that warrant discontinuation of metronidazole.^{9,18,19} Temporary neutropenia may occur, and raised liver enzyme values, cholestatic hepatitis, and jaundice have occasionally been reported.⁹

CHEMICAL PROPERTIES

Salt, Esters, and Polymorphs

The British Pharmacopoeia, the European Pharmacopoeia, the International Pharmacopoeia, and the US Pharmacopoeia have monographs for metronidazole base and metronidazole benzoate²⁰⁻²³; the Brazilian Pharmacopoeia has a monograph for metronidazole base only.²⁴ Metronidazole base is used for gel, injections, tablets, and suppositories,²⁰⁻²⁴ whereas metronidazole benzoate is formulated as oral suspensions.²⁰ Metronidazole hydrochloride is used for injections.⁹ This monograph refers to metronidazole base only. Polymorphism has not been reported for the metronidazole base.

Solubility

Metronidazole's solubility in water was reported as 10 mg/mL at 20°C²⁵ and 10.5 mg/mL at 25°C.²⁶ Another source reported a solubility of 64.8 mg/mL at room temperature and pH 1.2, decreasing to around 10 mg/mL at pH values between 2.5 and 8.0.^{27,28} Lindenberg et al.²⁹ conducted solubility experiments at 37°C in buffers at pH 1.2, 4.5, and 6.8; the values

Table 1. Solubility at 37°C of Metronidazole from Literature Data³⁰ and the Corresponding Dose/Solubility (*D/S*) Ratio's for Different Definitions of *D*

pH	Medium	Solubility at 37°C (mg/mL)	D/S ^a (mL)	
			500 (mg) ^b	2000 (mg) ^c
1.0	Clark-Lubs buffer	30.6	16	65
3.0	McIlvaine buffer	14.1	35	142
5.0	McIlvaine buffer	12.8	39	156
7.0	McIlvaine buffer	11.6	43	172

^a Critical limit: 250 mL.

^b Highest strength of IR solid oral dosage forms on US market and also highest tablet strength of WHO List of Essential Medicines.

^c Highest single dose administered.

were not reported, but metronidazole was concluded to be highly soluble at a dose of 500 mg. Table 1 shows the data reported by Ogata et al.³⁰ at 37°C in the pH range 1.0–7.0, being the most relevant for Biopharmaceutics Classification System (BCS).

Partition Coefficient

At 25°C, log *P* values of 0.75²⁶ and $-0.02^{31,32}$ were reported in *n*-octanol/water and in *n*-octanol/0.1 M sodium diphosphate buffer (pH 7.4), respectively. A log *D* value of -0.27 at pH 5.0 has also been reported.³¹

pKa

Metronidazole is a basic compound with pKa value of 2.62.^{18,27}

Available Dosage Forms Strengths

The WHO Essential Medicines List includes metronidazole IR tablets with strengths ranging from 200 to 500 mg.⁸ Tablets of higher strengths are not known to be marketed (see Table 2).

PHARMACOKINETIC PROPERTIES

Absorption and Bioavailability

Recognized textbooks and reviews report metronidazole to be rapidly absorbed with a bioavailability (BA) of higher than 90% and approaching toward 100%.^{9,17,19,33} Pharmacokinetic studies reported in the literature support the presence of high BA. In a study with eight healthy male volunteers receiving metronidazole orally as a 400-mg tablet and intravenously (i.v.), the fraction absorbed was reported to be higher than 0.98.³⁴ Another study consisted of four separate experiments in which metronidazole and tinidazole were compared after i.v. and oral administration. Five volunteers took part in the study, and the BA of the 500 mg metronidazole tablets was reported to be 111%.³⁵ Metronidazole pharmacokinetics was also studied in five healthy women after single oral dose versus i.v.; the mean oral BA was reported as $100 \pm 5\%$.³⁶ In another study, the pharmacokinetics of metronidazole was measured in eight con-

senting patients. Single doses of 0.5, 1, 1.5, and 2 g were administered i.v. by a zero-order infusion pump; seven of the eight patients received a second, identical, oral dose 1 week later, and the oral BA of metronidazole was calculated to be approximate 100%.³⁷ Only one study of metronidazole tablets was identified reporting a BA below the critical limit of 85%; in that crossover study in 11 volunteers, 400 mg tablets from two manufacturers were compared against an 800 mg i.v. dose. Both unchanged metronidazole and its hydroxy metabolite were measured by high-performance liquid chromatography. The BA of the two tablet brands was reported to be 80.4% and 84.1%, respectively.³⁸

Oral doses of 250, 500, 750, and 2000 mg gave maximum plasma concentrations (C_{max}) of 6, 12, 20, and 40 $\mu\text{g/mL}$,^{9,16,39–41} with time to C_{max} (t_{max}) ranging from 0.25 to 4 h.^{16,19,33–36,39–41} Pharmacokinetics is linear up to a dose of 2000 mg.¹⁹ Administration with food may delay, but does not reduce the absorption of metronidazole.⁹ In a study in which the BE of a formulation containing metronidazole, bismuth bisacalcitrate, and tetracycline combined in one capsule was evaluated in fed and fasting conditions, and was also compared with the same drugs administered in separate capsules in fasted conditions, it was concluded that food slightly decreases the rate of absorption of metronidazole because the lower limit of the 90% confidence interval (CI) for C_{max} , was 74%. With respect to formulation, metronidazole capsule was bioequivalent to the fixed-dose combination product.⁴²

Gastric acid blockade following a 5-day administration of omeprazole did not modify the pharmacokinetics and metabolic profile of orally administered metronidazole in healthy male volunteers.⁴³ Neither C_{max} nor t_{max} of a 400-mg metronidazole tablet was affected by the increased gastric pH produced by omeprazole pretreatment,^{34,43} indicating also that this drug does not affect the movement of metronidazole through the stomach.

The influence of concomitant oral administration of adsorbing drugs on the BA of metronidazole was also evaluated.⁴⁴ Adsorbing drugs included an antidiarrheal mixture (kaolin and pectin), an antacid mixture (aluminum hydroxide and simethicone), and

Table 2. Excipients^a Present in Metronidazole IR Solid Oral Drug Products^b with a Marketing Authorization (MA) in a Number of Countries^c and the Minimal and Maximal Amount of That Excipient Present Pro Dosage Unit in Solid Oral Drug Products with an MA in the US^d

Excipient	Drug products containing that excipient with an MA granted by the named country	Range present in solid oral dosage forms with an MA in the US (mg)
Calcium carbonate	DE (1)	8.6–350
Calcium hydrogen phosphate	BR (2,3) DE (4) DK (5,6) FI(7) FR (8) IE (9,10) IL (11) IS (12,13) NO (14,15) SE (16) UK (17)	109–636
Cellulose, microcrystalline	DE (18–28) DK (29,30) FI (31–34) IS (35) NL (36–38) NO (39) NZ (40,41) SE (42) UK (43–45) US (46–52)	4.6–1385 ^e
Cellulose, powdered	DE (1,19,20,22–26)	44–170
Croscarmellose sodium	DE (1,21) DK (30) FI (31) IS (35) NL (36) NO (39) SE (42) UK (44,45)	2–180
Crospovidone	DE (19,20,22–26) US (48,50,51)	4.4–792 ^e
Gelatin	CA (53) DE (27,28) DK (29) FI (32,33) NL (38) UK (43) US (54)	1–756 ^e
Glucose	DE (1)	184–904
Hydroxypropylcellulose	UK (44) US (46,49,52)	4–132
Hypromellose	BR (2,3) DE (4,18,27,55) DK (5,6,29,30) FI (7) FR (8,56) IE (9,10) IL (57) IS (12,13,35) NL (36) NO(14,15,39) SE (16,42) UK (44,45) US (46)	0.8–537
Kaolin	DE (1)	8–30
Lactose	CA (53) DE (1,19–27,58) DK (29,30) FI (31–34) IE (59,60) IS (35) NL (36–38) NO (39,61) NZ (40,41) SE (42) UK (43–45) US (47,49,52)	23–1020 ^e
Macrogols	BR (2,3) DE (1,4,18–20,22–27,55,58) DK (5,6,30) FI (7) FR (8,56) IE (9,10) IL (57) IS (12,13,35) NL (36) NO (14,15,39) SE (16,42) UK (44,45) US (46)	0.12–961 ^e
Magnesium stearate	BR (2,3) CA (53) DE (1,4,18–28,55,58) DK (5,6,29,30) ES (62) FI (7,31–34) FR (8,56) IE (9,10,59,60) IL (11,57) IS (12,13,35) NL (36–38) NO (14,15,39,61) NZ (40,41) SE (16,42) UK (17,43–45) US (47,48,54)	0.15–401 ^e
Methylcellulose	DE (27)	14–184
Montanglycol wax	DE (1)	0.03–0.06
Polacrillin potassium	CA (53)	3–46
Povidone	BR (2,3) DE (1,4,18–26,55,58) DK (5,6,30) ES (62) FI (7,31,34) FR (8,56) IE (9,10,59,60) IL (11,57) IS (12,13,35) NL (36,37) NO (14,15,39) NZ (40,41) SE (16,42) UK (17,45) US (47)	0.17–80
Propylene glycol	DK (29) US (54)	1.5–148
Silica	CA (53) DE (1,19,20,22–26,58) FI (32) IE (59,60) IL (11) UK (44) US (48–52)	0.50–100
Simeticone	DE (27)	0.0004–5.7
Sodium laurilsulfate	CA (53)	0.65–52
Sodium starch glycolate	DE(28) FI(32) IL (11) NZ (41) US (47,49,52)	2–876 ^e
Sorbic acid	DE(27)	0.93
Sorbitol	BR (3) FI(32)	5–337
Starch	BR (2,3) DE (4,18,27,28,55,58) DK (5,6,29,30) ES (62) FI (7,31–34) FR (8,56) IE (9,10,59,60) IL (11,57) IS (12,13,35) NL(36–38) NO (14,15,39) NZ (40,41) SE (16,42) UK (17,43–45) US (54)	0.44–1135 ^e

Table 2 continued on next page.

cholestyramine. The antidiarrheal mixture was found to affect the BA of metronidazole insignificantly; however, its BA was significantly reduced with the antacid mixture and with the anion exchange resin cholestyramine.

Permeability

According to Simms-Cendan,¹⁰ metronidazole is passively transported through mammalian cells. No studies about metronidazole permeability in Caco-2 cells were identified.

A mucosal permeability study of several drugs, including metronidazole, was performed in the equine jejunum³² and metronidazole exhibited unusual high permeability when compared with the other drugs

(cephalexin, marbofloxacin, and fluconazole), with effective permeability (P_{eff}) around 9×10^{-5} cm/s. According to the authors, the high permeability of metronidazole may be due to a direct cytotoxic effect on the mucosal epithelial cells, and also to absorption by paracellular transport, in addition to transcellular transport.

Distribution, Metabolism, and Elimination

Metronidazole is widely distributed and appears in most body tissues and fluids.^{9,16,33} Less than 20% of the circulating metronidazole is bound to plasma proteins.^{9,16,19,33,39} The distribution volume ranges from 0.51 to 1.1 L/kg.^{19,33,39,45} Metronidazole is metabolized in the liver by side-chain

Table 2. Continued

Excipient	Drug products containing that excipient with an MA granted by the named country	Range present in solid oral dosage forms with an MA in the US (mg)
Starch, pregelatinized	DE (19,20,22–26,58) IE (59,60)	6.6–600
Starch, modified	DE (1)	23–50
Stearic acid	DE (58) US (46–49,52)	0.9–72 ^e
Sucrose	DE (1,21)	12–900
Talc	DE (1,4,27,58) DK (6,29) FI (7) IE (9) IL (11) IS (13) NO (15)	0.25–220 ^e
Vegetable oil, hydrogenated	US (50)	2–261

1, Vagimid[®]-Dragees überzogene Tabletten (Mono); 2, FLAGYL[®] Comprimidol 250 mg; 3, FLAGYL[®] Comprimidol 400 mg; 4, Flagyl[®] 400 mg Filmtabletten (Mono); 5, Flagyl, filmovertrukne tabletter (200 mg); 6, Flagyl, filmovertrukne tabletter (400 mg); 7, FLAGYL 400 mg tabletti, kalvopäällysteinen; 8, FLAGYL 250 mg cp pellic; 9, Flagyl 400 mg tablets; 10, Flagyl 200 mg tablets; 11, Metrogyl[®] tablets; 12, Flagyl 200 mg töflur; 13, Flagyl 400 mg töflur; 14, Flagyl 200 mg tablett, filmdrasjert; 15, Flagyl 400 mg tablett, filmdrasjert; 16, Flagyl 200/400 mg tablett; 17, Flagyl 200/400 mg tablets; 18, Clont[®] 400 mg Filmtabletten (Mono); 19, Metronidazol 400 mg Drossapharm tabletten (Mono); 20, Metronidazol AL 400 mg tabletten (Mono); 21, Metronidazol Artesan–Drossapharm tabletten (Mono); 22, Metronidazol-CT 400 mg tabletten (Mono); 23, Metronidazol HEXAL 400 mg tabletten (Mono); 24, Metronidazol-ratiopharm[®] 400 mg tabletten (Mono); 25, Metronidazol Sandoz[®] 400 mg tabletten (Mono); 26, Metronidazol STADA[®] 400 mg tabletten (Mono); 27, Vagimid[®] 500 Filmtabletten (Mono); 28, Vagimid[®] tabletten (Mono); 29, Metronidazol “DAK,” filmovertrukne tabletter (250/500 mg); 30, Metronidazol “Actavis,” filmovertrukne tabletter (250/500 mg); 31, Metronidazol Actavis 500 mg tabletti; 32, Trikozol 200/400 mg tabletti; 33, Metronidazol Epipharm 200 mg tabletti; 34, Metronidazol Epipharm 400 mg tabletti; 35, Metronidazol Actavis 250/500 mg filmuhúðaðar töflur; 36, Metronidazol Actavis 250/500 mg, omhulde tabletten; 37, Metronidazol Lagap, tabletten 500 mg; 38, Metronidazol Lagap, tabletten 250 mg; 39, Metronidazol Actavis 500 mg tablett; 40, TRICHOZOLE 200 mg tablets; 41, TRICHOZOLE 400 mg tablets; 42, Metronidazol Actavis 250/500 mg filmdragerade tabletter; 43, Metrolyl* (Metronidazole) tablets BP 200 mg; 44, Metronidazole Tablets 200/400 mg (Actavis UK Ltd); 45, Metronidazole tablets 500 mg (Actavis UK Ltd); 46, Flagyl (Metronidazole) tablet, film coated (250/500 mg) [G.D. Searle LLC]; 47, Metronidazole (Metronidazole) tablet (250/500 mg) [IVAX Pharmaceuticals, Inc.]; 48, Metronidazole (Metronidazole) tablet (250/500 mg) [Mutual Pharmaceutical Company, Inc.]; 49, Metronidazole (Metronidazole) tablet (250/500 mg) [TEVA PHARMACEUTICALS USA]; 50, Metronidazole (Metronidazole) tablet (250/500 mg) [Watson Labs]; 51, METRONIDAZOLE tablet (500 mg) [Apac Packaging]; 52, METRONIDAZOLE tablet (250/500 mg) [UDL Laboratories, Inc.]; 53, Pr-FLAGYL[®] (Metronidazole)(500 mg capsule); 54, METRONIDAZOLE capsule (375 mg) [Pliva Inc.]; 55, Clont[®] 250 mg Filmtabletten (Mono); 56, FLAGYL 500 mg cp pellic; 57, FLAGYL 250 mg tablets; 58, Arilin[®] 250/500 mg Filmtabletten (Mono); 59, Metronidazole tablets BP 200 mg; 60, Metronide 200 mg tablets; 61, PRECOSA, 250 mg kapsel, hard; 62, Flagyl 250 comprimidol.

^a Colorants and ingredients present in the printing ink are not included. Coating substances are excluded if in the SPC they are stated separately from the other inactive ingredients.

^b Excluded are: oral suspensions.

^c Abbreviations of countries, see text.

^d Source of data: FDA's Inactive Ingredient Database, <http://www.fda.gov/Drugs/InformationOnDrugs/ucm113978.htm> (version date 06-01-2010).

^e The upper range value reported is unusually high for solid oral dosage forms and the authors doubt its correctness.

oxidation, producing 1-(β -hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (about 30%–65% of the activity of metronidazole) and 2-methyl-5-nitroimidazole-1-yl-acetic acid (not active), and by glucuronide conjugation.^{9,16} The major route of elimination of metronidazole and its metabolites is via urine, in which 60%–80% of the dose is excreted (6%–18% as unchanged drug). The fecal excretion accounts for only 6%–15% of the dose. The elimination half-life of the parent compound ranges from 6 to 14 h, with an average value of 8.5 h, and it is about 9.7 h for the hydroxy metabolite.^{9,10,19,33,39} An evaluation of gender differences in the disposition of metronidazole carried out during a BE study suggests that metronidazole clearance in women is about 12% higher than in men, although these differences are probably of no clinical relevance.⁴⁶

DOSAGE FORM PERFORMANCE

Excipients

Excipients present in IR metronidazole tablets with a Marketing Authorization (MA) in Brazil (BR),¹⁵ Canada (CA),⁴⁷ Germany (DE),⁴⁸ Denmark (DK),⁴⁹ Spain (ES),⁵⁰ Finland (FI),⁵¹ France (FR),⁵² Ireland (IE),⁵³ Iceland (IS),⁵⁴ Israel (IL),⁵⁵ the Netherlands (NL),⁵⁶ New Zealand (NZ),⁵⁷ Norway (NO),⁵⁸

Sweden (SE),⁵⁹ the United Kingdom (UK),⁶⁰ and the USA (US)⁶¹ are summarized in Table 2.

Excipients and Manufacturing Effects on BA

Six studies were identified, but the older studies were conducted at a time when BE was considered to have been demonstrated if no significant differences in pharmacokinetic parameters were observed. Only the recent studies applied the current BE criteria in which two products are declared bioequivalent if, with high probability, the hypothesis that their formulations are bioinequivalent can be rejected.^{2–4}

In a study published in 1978, eight commercial tablet formulations and a solution of metronidazole were administered to 10 patients as single oral 250 mg doses.⁶² The composition of the formulations was not reported. Differences ($\alpha = 0.05$) between test and reference formulations were evaluated by analysis of variance on the logarithmic transformation of the raw data for area under the plasma concentration–time curve (AUC) and C_{\max} . With respect to AUC, the commercial tablets and the reference did not show a statistical significant difference. With respect to C_{\max} , one tablet seemed not to have met the current BE criteria. The slow rate of absorption of that tablet formulation was reported to be associated with a slower dissolution in the USP rotating basket at 100 rpm using 0.1 N HCl as medium. The solution

resulted in a significantly lower AUC compared with the tablets. The authors hypothesized that the lower AUC after administration of the solution was due to the rapid GI transit of this formulation. Excipients such as sorbitol are frequently used in oral solutions and, when present in high quantities, accelerate the GI transit.⁶³ However, the hypothesis that lower AUC was excipient related can neither be rejected nor be confirmed because the composition of the product was not disclosed.

In a study reported in 1985, five commercial sugar-coated tablets (formulation not revealed) were evaluated, including the correlation between *in vitro* and *in vivo* results. Also, the effect of gastric acidity on the BA was studied.³⁰ Ten healthy male volunteers received a 250 mg metronidazole tablet orally. C_{\max} and AUC were compared, and significant differences were observed in C_{\max} as well as in AUC. The tablets were also submitted to disintegration testing in buffer pH 1.2, pH 5.0, and pH 7.2 and to several dissolution tests. Three tablets showed very slow disintegration and very slow dissolution at pH 5.0; the same tablets also showed lower C_{\max} and AUC values than the tablets that disintegrated fast or relatively fast at pH 5.0. The authors postulated that those differences could have been caused by the coatings of those tablets; with the suspicion that Eudragit E or shellac might have been present, both of which would be expected to retard the release at pH 5. A good correlation was found between the results of the dissolution test in the paddle apparatus at pH 5.0 and the AUC in the patients with high gastric acidity; other correlations were poor.

A study published in 1991 compared the BA of an unmarketed test tablet versus the reference Flagyl[®] (Rhodia, BR), both containing 400 mg of the drug. Dosage strengths of 200 and 500 mg for the test tablet were additionally included in the study.⁴¹ The composition of the tablets was not revealed. Fourteen male volunteers received the formulations according to an open randomized four-period crossover design with a 7-day washout period. The authors concluded the test product to be bioequivalent to the reference with respect to AUC and C_{\max} , although 90% of CI interval for C_{\max} did not meet current BE criteria. This may, however, have been caused by insufficient statistical power of the study. Good correlations were obtained between C_{\max} , AUC, and amount of metronidazole ingested, indicating linear pharmacokinetics.

In a BE study published in 2006, the plasma pharmacokinetics of metronidazole and its active hydroxy metabolite were investigated. The test formulation was Vagimid[®] Dragees (Apogepha Arzneimittel GmbH, DE).⁶⁴ The trial was conducted according to a randomized, controlled, open, within-patient crossover design with two periods and a 1-week washout in 16 healthy volunteers after the

administration of single oral doses of 500 mg. The test formulation was bioequivalent with respect to metronidazole (90% CI for C_{\max} and AUC were 1.02–1.15 and 1.02–1.12, respectively) and its metabolite (90% CI for C_{\max} and AUC were 0.92–1.05 and 0.98–1.06, respectively).

Another BE study, also published in 2006, was carried out in 12 healthy volunteers according to a two-sequence, crossover randomized design.⁶⁵ Volunteers were given a single dose of two 250 mg tablets of either the metronidazole test formulation (Amin, Iran) or the reference formulation Flagyl[®] (Pharmacia, Italy). The composition of the formulations was not reported. The 90% CI for the ratios of the logarithmically transformed AUC (0.99–1.01) and C_{\max} (0.94–1.01) values of the generic product over those of Flagyl[®] were within the limits of 0.80–1.25. It was concluded that the test product was bioequivalent to the reference. Both formulations were also submitted to the dissolution test using the rotating basket method at 100 rpm with 900 mL of 0.1 N HCl as dissolution medium and met USP 25 specifications of not less than 85% of drug content released in 60 min. The test product dissolved very rapidly ($\geq 85\%$ in 15 min) and the reference dissolve rapidly ($\geq 85\%$ in 30 min), but this difference in dissolution rate was not associated with a lack of BE.

Another study, published in 2007, compared a test formulation (EMS-Sigma Pharma, BR) with its reference Flagyl[®] (Rhodia), each containing 400 mg metronidazole.⁶⁶ The composition of the formulations was not reported. Single oral doses of each preparation were administered to 23 healthy male volunteers in a two-sequence crossover randomized design with a 7-day interval. The 90% CI were 0.85–0.97 and 0.90–1.07 for AUC and C_{\max} , respectively; hence test and reference products were bioequivalent with regard to both rate and extent of drug absorption despite the small but statistically significant difference in AUC between test and reference. No dissolution testing was performed.

Dissolution

The USP 32 specification for metronidazole tablets is not less than 85% of the labeled amount dissolved in 60 min in 900 mL 0.1 N HCl using the apparatus 1 (basket) operated at 100 rpm.²³

Five metronidazole tablets, available in BR, including the innovator and one generic product, were submitted to *in vitro* dissolution using the basket at 100 rpm in 900 mL 0.1 N HCl according to USP 23.⁶⁷ Only the innovator showed very rapid dissolution, that is, more than 85% in 15 min. The generic product and one brand product showed rapid dissolution, that is, 85% dissolved in less than 30 min. Two products showed slower dissolution rate and released less than 85% in 30 min. Because in BR, all generic products are

submitted to *in vivo* BE testing, these products must have passed, hence these differences in dissolution rate do not translate into a lack of BE.

DISCUSSION

Solubility

According to the current regulatory guidances, an API is highly soluble if its *D/S* is 250 mL or less at the pH range of 1.0–6.8^{2,4} or 1.0–7.5³ at 37°C. However, the regulatory guidances differ in their definition of *D*. The FDA regulation defines *D* as the highest dose strength,³ whereas the WHO² and the EMA⁴ regulation define *D* as the highest dose recommended by WHO and the highest single dose administered, respectively.

Table 1 shows that for all definitions of *D*, the quotient *D/S* is below its critical limit of 250 mL, hence metronidazole is highly soluble.

Permeability

Metronidazole complies with the WHO² and EMEA⁴ definition of highly permeable: not less than 85% of dose fraction absorbed. It also complies with the FDA³ definition of not less than 90% of dose fraction absorbed because most references describe its oral BA as approaching 100%. The only metronidazole intestinal permeability study found, performed in equine jejunum, showed the drug to be highly permeable.³² No permeability studies of metronidazole in Caco-2 cells were identified, but the demonstration of complete absorption in humans is preferred for BCS-based biowaiver applications.⁴ In addition, metronidazole undergoes extensive hepatic metabolism,^{68–70} indicating also high GI permeability.^{69,70} The partition coefficient of metronidazole is below the partition coefficient of metoprolol, sometimes taken as the reference compound for highly permeable.⁷¹ This suggests that the partition coefficient is not a reliable predictor for permeability.

BCS Classification

Metronidazole is BCS Class I, being highly soluble and highly permeable. Kasim et al.⁷¹ defined metronidazole as BCS Class III, but they used partition coefficient data to predict the permeability, which is not a reliable predictor, nor recognized by any regulatory authority for permeability classification.

Risks for Bioequivalence Caused by Excipient and/or Manufacturing

Surprisingly for an API of BCS Class I, two reports of formulations failing to meet BE criteria were identified. But in one study,⁶² the product not meeting the BE criteria was a solution, a dosage form not covered by this monograph; in the second study,³⁰ the test

products were most probably not strictly speaking IR formulations.

Five other studies reported the investigated formulations to be bioequivalent,^{41,42,64–66} although in one study, a small but statistical significant difference in AUC between test and reference was observed.⁶⁶ These findings provide an indication that the risk of bioequivalence caused by an excipient and/or manufacturing variable(s) for metronidazole IR solid oral dosage forms is low. In addition, a survey of several countries revealed a total 62 drug products having an MA. Because one formulation may be registered as a product in several different countries, these 62 drug products do not necessarily correspond to 62 different formulations. Also, it cannot be taken for granted that every registered drug product has successfully met the present *in vivo* BE criteria against the innovator. Nevertheless, because there are so many products and because these products contain a wide range of excipients, it seems safe to conclude that the risk of bioequivalence caused by excipient(s) and/or manufacturing is low. So, generally, it can be concluded that the excipients shown in Table 2, in particular when present in a large number of products, and also present in amounts not exceeding its normal use in IR tablets, are unlikely to alter the fraction of metronidazole absorbed to the extent that the outcome of an *in vivo* BE study is changed. However, some excipients, such as sorbitol, sodium laurilsulfate, and propylene glycol, included in Table 2 are known to have a potential effect on the BA; when any of these excipients is present in the test product, the risk for bioequivalence is higher. Only when the test product with respect to these excipients is qualitatively and quantitatively identical to its reference product,⁴ the risk for bioequivalence is low.

Risk of Not Detecting Bioequivalence by *In Vitro* Testing

Few *in vivo* BE studies that are reported demonstrated bioequivalence for metronidazole tablets formulations; hence, little data are available to assess the risk of not detecting bioequivalence by *in vitro* testing. Although the study of Ogata et al.³⁰ involved probably formulations that were not strictly speaking IR, *in vitro* dissolution at pH 5.0 was able to predict bioequivalence. Furthermore, McGilveray et al.⁶² observed an association of slower rate of absorption with longer *in vitro* dissolution time in the basket at 100 rpm using 0.1 N HCl. This suggests that by comparative dissolution testing in buffers pH 1.2, pH 4.5, and pH 6.8, applying dissolution profile similarity testing on the results, any bioequivalence caused by differences in *in vivo* disintegration and/or *in vivo* dissolution could be detected. A caveat is that to date there are no regulatory accepted surrogate techniques

available that can detect bioinequivalence due to differences in GI permeability or GI motility.

Patient's Risks Associated with Bioinequivalence

According to the summary of product characteristics of Flagyl,¹⁶ single oral doses of metronidazole, up to 15 g, have been reported in suicide attempts and accidental overdoses, and the symptoms reported were limited to nausea, vomiting, and ataxia; hence, serious adverse events as consequence of higher AUC and/or C_{max} of a bioinequivalent drug product are not expected to occur.

In cases, wherein test formulations exhibit lower rate and extent of absorption as compared with the reference product, one could expect a treatment failure and/or development of microorganism resistance. However, the risk that a Class I API, formulated in an IR dosage form, meets the comparative *in vitro* dissolution criteria and yet is bioinequivalent, is minimal.

CONCLUSION

A biowaiver for solid IR formulations is justified provided: (a) the test product and its comparator are both rapidly dissolving; (b) meet similarity of the dissolution profiles at pH 1.2, 4.5, and 6.8^{2,4}; (c) the test product contains only the excipients shown in Table 2, in amounts not exceeding its normal use in IR tablets; and (d) if the test product contains sorbitol, sodium laurilsulfate, or propylene glycol, the test product needs to be qualitatively and quantitatively identical to its comparator with respect to these excipients.

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