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Research Paper

Comparison of WHO and US FDA biowaiver dissolution test conditions using bioequivalent doxycycline hyclate drug products

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Abstract

Objectives The dissolution characteristics of immediate-release doxycycline hyclate products with certified in-vivo bioequivalence to the innovator product were tested with a view to possible application of biowaiver-based approval.

Methods Five products were tested using US Pharmacopeia Apparatus 2: Antodox 100 mg hard gelatin capsules, Doxycyclin AL 100 T tablets, Doxycyclin-ratiopharm 100 soft gelatin capsules, Doxycyclin STADA 100 mg tablets and Doxy-Wolff 100 mg tablets. Three compendial buffers were used as dissolution media: simulated gastric fluid without pepsin, pH 1.2, acetate buffer, pH 4.5, and simulated intestinal fluid without pancreatin, pH 6.8. Results were obtained at two paddle speeds recommended for biowaiver applications: 75 rpm (World Health Organization; WHO) and 50 rpm (US Food and Drug Administration; US FDA).

Key findings The results for the tablets and hard gelatin capsules indicate that a paddle speed of 75 rpm is more representative than 50 rpm, since 75 rpm generates dissolution profiles corresponding more closely to the in-vivo profiles than those at 50 rpm. For evaluating soft gelatin capsule formulations with lipid fill, both US FDA and WHO methods were found to be over-discriminating.

Conclusions Bioequivalence of immediate-release doxycycline hyclate tablets and hard gelatin capsules, but not soft gelatin capsules, can be evaluated *in vitro* using the biowaiver dissolution test conditions specified by the WHO.

Keywords bioequivalence; Biopharmaceutics Classification System (BCS); biowaiver; doxycycline hyclate; US FDA guidance; WHO guidance

Introduction

The term 'biowaiver' is defined by the World Health Organization (WHO) as 'approval of a generic solid oral formulation of an active pharmaceutical ingredient (API) based on strictly defined dissolution criteria as a surrogate for an *in vivo* bioequivalence test'.[1] Approval of a generic product on the basis of the biowaiver procedure enables sponsors to confirm bioequivalence of the products against the comparator and to make post-approval changes without having to conduct pharmacokinetic studies.[1,2] The concept of biowaiver can be traced back to the proposal by the US Food and Drug Administration (US FDA) in 2000 to waive in-vivo bioavailability and bioequivalence studies,[2] which in turn stemmed from the Biopharmaceutics Classification System (BCS) published in 1995.[3] In order to qualify for the biowaiver procedure under the US FDA guidance, the API has to conform to BCS Class I solubility and permeability characteristics.[1,2] Recently, the WHO issued a biowaiver guidance which expanded the range of applicability.[1] The WHO guidance not only allows biowaiver of the drug products containing APIs from BCS Class I, but also opens the possibility for drug products containing APIs from BCS Class III and certain APIs from BCS Class II to qualify for biowaiver-based approval. Importantly, the dissolution test conditions stipulated in these two guidelines are not identical: a paddle speed of 75 rpm is recommended by the WHO but 50 rpm by the US FDA.

The current study compared suitability of the biowaiver dissolution test conditions from the WHO[1] and US FDA[2] guidelines. Doxycycline hyclate was chosen as a model compound because: (i) it belongs to BCS Class I;[4] (ii) it has a wide therapeutic range and thus its use is relatively safe;[5] and (iii) no cases of bioinequivalence have been reported in the literature resulting from either differences in formulations or timing of administration in

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relation to food intake.[6–8] Doxycycline therefore fits the key eligibility criteria for a biowaiver-based approval under both US FDA and WHO guidelines.

The dissolution behaviour of five doxycycline hyclate products with marketing authorisation in Germany was evaluated with the paddle method using the rotational speeds recommended by the WHO and the US FDA. Since all products had been approved on the basis of pharmacokinetically proven bioequivalence, it can be assumed that dissolution tests that indicate bioequivalence are the more appropriate test conditions for assessing biowaiver *in vitro*.

Materials and Methods

Materials

Doxycycline hyclate products tested in this study were all obtained from Phoenix Pharmahandel Aktiengesellschaft & Co KG (Hanau, Germany). All drug products had successfully passed an in-vivo bioequivalence study to obtain a marketing authorisation. The compositions of the formulations are shown in Table 1. The pharmacokinetic profiles are shown in Figure 1.[9–12] The chemicals used in this study were all of analytical grade. Doxycycline hyclate reference substance (99% pure) and maleic acid were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Sodium taurocholate was from Prodotti Chimici e Alimentari SpA (Basaluzzo, Italy). Egg phosphatidylcholine (Lipoid E PC) was a gift from Lipoid GmbH (Ludwigshafen, Germany). Sodium chloride, sodium acetate trihydrate, sodium dihydrogen phosphate monohydrate and sodium hydroxide pellets were purchased from Merck KGaA (Darmstadt, Germany). Hydrochloric acid (37%) was from Hedinger GmbH & Co. KG (Stuttgart, Germany) and glacial acetic acid was purchased from Caesar & Loretz GmbH (Hilden, Germany).

Quantitative analysis of doxycycline hyclate

Quantitative analysis of doxycycline hyclate was performed using UV spectrophotometry.[13] The method was slightly modified from the pharmacopoeia by adjusting the wavelength to 268 nm (the maximum absorbance in all three buffers used in the dissolution tests).

Media preparation

The detailed compositions of simulated gastric fluid without pepsin (SGF_{sp}), acetate buffer and simulated intestinal fluid without pancreatin (SIF_{sp}) have been described previously.[14] They were slightly modified by replacing potassium dihydrogen phosphate with sodium dihydrogen phosphate at an equimolar concentration. The composition of the biorelevant medium – fasted state simulated intestinal fluid – an updated version (FaSSIF-V2) – has been described by Jantratid *et al*.[15]

Solubility study

Since the literature[8] and preliminary results indicated that doxycycline hyclate is highly soluble in aqueous media, it was only necessary to confirm that the dose to solubility (D : S) ratio was 250 ml or less (one glass of water). The shake-flask method was used to determine the solubility of doxycycline hyclate. Approximately 40 mg doxycycline hyclate reference substance was weighed and placed into a 20 ml scintillation vial and 20 ml test medium (SGF_{sp} adjusted to pH 1.0, SGF_{sp}, pH 1.2, acetate buffer, pH 4.5, SIF_{sp}, pH 6.8, SIF_{sp} adjusted to pH 7.5) added. The ratio of drug substance to buffer used for these determinations corresponded to ingesting 461.6 mg doxycycline hyclate, double the highest dose strength of doxycycline hyclate products (230.8 mg),[8] with just under 250 ml fluid. The samples were shaken at 37°C on an orbital shaker (Heidolph Polymax 1040, Schwabach, Germany). Samples were taken at 12 and 24 h, filtered through 0.45 μm PTFE filters and analysed by UV spectrophotometry. All solubility experiments were performed in triplicate.

In-vitro dissolution study

In line with WHO and US FDA guidelines, dissolution tests were all performed using the USP Apparatus 2 (paddle assembly) and three standard dissolution media (SGF_{sp}, acetate buffer, pH 4.5 and SIF_{sp}). The medium volume was 500 ml per vessel, and the temperature was 37 ± 0.5°C. The essential difference between WHO and US FDA methods is the paddle speed: 75 rpm (WHO)[1] vs 50 rpm (US FDA).[2] Samples were taken after 5, 10, 15, 20, 30 and 45 min. Sampling was performed manually using a 5 ml glass syringe connected to a stainless steel sampling device, with a cylindrical polyethylene

Table 1 Composition of doxycycline hyclate products with marketing authorisation in Germany. The dose of doxycycline hyclate in each case was 115.4 mg

Product	Lot (expiry date)	Excipients
Antodox 100 mg hard gelatin capsules (Juta Pharma, Flensburg, Germany)	R0600220 (12/08)	Sucrose, maize starch, crospovidone, Eudragit E 100, talc, gelatin
Doxycyclin AL 100 T tablets (Aliud Pharma GmbH & Co. KG, Laichingen, Germany)	64123B (08/09)	Microcrystalline cellulose, lactose monohydrous, magnesium stearate, maize starch, croscarmellose sodium, ricinus oil, silica
Doxycyclin-ratiopharm 100 soft gelatin capsules (Ratiopharm GmbH, Ulm, Germany)	H10134 (02/10)	Yellow beeswax, hydrogenated soybean oil, partially hydrolysed soybean oil, triglyceride, soy lecithin, ethyl vanillin, acetanisole, sorbitol, mannitol, hydrolysed starch, glycerol, gelatin
Doxycyclin STADA 100 mg tablets (STADApharm GmbH, Bad Vilbel, Germany)	6172 (03/10)	Microcrystalline cellulose, croscarmellose sodium, gelatin, monohydrous lactose, macrogol 6000, magnesium stearate, maize starch, Eudragit E 100, silica, talc
Doxy-Wolff 100 mg tablets (Dr. August Wolff GmbH & Co. KG, Arzneimittel, Bielefeld, Germany)	704501 (04/12)	Aluminum hydroxide, Eudragit E, gelatin, monohydrous lactose, macrogol 6000, magnesium stearate, maize starch, silica, talc

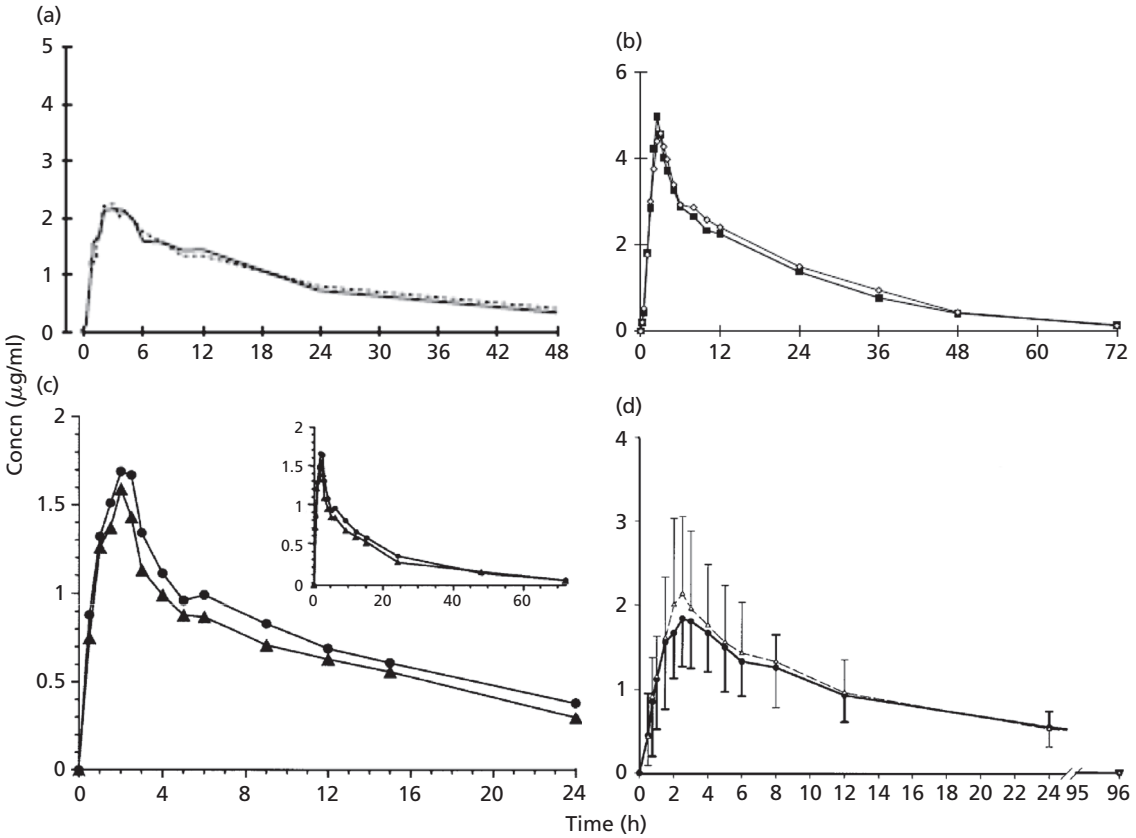

Figure 1 Mean plasma doxycycline concentration–time profiles following a single oral dose of (a) Antodox 100 mg hard gelatin capsules, where (– – –) Antodox 100 and (—) reference product;[9] (b) Doxycycline AL 100 T tablets where (■) Doxycyclin and –○– reference product;[10] (c) Doxy-Wolff 100 mg tablets, where (■) Doxy-Woltt 100 and (▲) reference product,[11] and (d) Doxycyclin-ratiopharm 100 soft gelatin capsules[12] compared with reference products, where (–○–) Doxycyclin-ratiopharm 100 and (△) reference product. (The plasma concentration–time profile of Doxycyclin STADA 100 mg tablets was not available).

filter stick at the end of each sampling device. The volume withdrawn from the vessels at each sampling time point was approximately 5 ml, which was replaced with prewarmed medium. After withdrawal, the samples were filtered through a 0.45 μm PTFE filter, suitably diluted and then analysed by UV spectrophotometry. Six replicates of each experiment were performed.

In addition, the USP Apparatus 3 (Bio-Dis, reciprocating cylinder) and a biorelevant medium, FaSSIF-V2,[15] were used for further dissolution testing of Doxycyclin-ratiopharm 100 soft gelatin capsules (SGC). Because of the poor dissolution behaviour of these capsules using the paddle method, the Bio-Dis method was used to evaluate the effect of hydrodynamics on dissolution of this product. The dissolution conditions consisted of 220 ml dissolution medium per vessel with a dip rate of 15 dips per min, at 37 ± 0.5°C. The top and bottom mesh sizes were 840 μm. Sampling and analysis were conducted as described above for the experiments with Apparatus 2.

Evaluation of the dissolution profiles

The dissolution profiles of the doxycycline hyclate products were constructed from the average (from six vessels) of the percentage of cumulative drug dissolved at each sampling time point (this diverged from the n = 12 requirements of biowaiver testing).[2] A model-independent approach including the difference (f_1) and similarity factors (f_2) was used for the dissolution profile comparison using the following equations:[1,2]

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} \times 100 \quad (1)$$

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (2)$$

where n is the number of time points, R is the dissolution value of the reference batch at time t, and T is the dissolution value of the test batch at time t.

Results

Solubility of doxycycline hyclate

To confirm the high solubility of doxycycline hyclate according to both US FDA and WHO criteria, this parameter was evaluated in five compendial media covering the pH range 1.0–7.5. In all cases, the criterion of a D : S ratio of

250 ml or less was met, based on the highest dose strength of 230.8 mg (data not shown).

Dissolution of doxycycline hyclate from tablets and hard gelatine capsules

Three doxycycline hyclate tablet products and one hard gelatin capsule (HGC) product available on the German market were dissolved in the three standard buffers using the WHO (75 rpm) and US FDA (50 rpm) paddle methods. The results are shown in Figures 2 and 3.

Doxycyclin AL 100 T (tablets) was the only doxycycline hyclate product tested that released more than 85% of the API within 15 min at both paddle speeds (75 and 50 rpm), thus qualifying as 'very rapidly dissolving'.[1]

Antodox 100 mg (HGC) and Doxy-Wolff 100 mg (tablets) released 85% or more doxycycline hyclate within 30 min at a paddle speed of 75 rpm, thus fulfilling the WHO criteria for 'rapidly dissolving'.[1] Neither product fulfilled the criteria for 'rapidly dissolving' under the US FDA conditions (paddle speed 50 rpm). For both products, 'coning' occurred at a paddle speed of 50 rpm.

The dissolution behaviour of Doxycyclin STADA 100 mg tablets was neither 'very rapidly dissolving' nor 'rapidly dissolving' (i.e. less than 85% of the labelled amount of the API was released within 30 min in SIF_{sp}, irrespective of the stirring rate used). 'Coning' occurred at a paddle speed of 50 rpm but was reduced at 75 rpm.

Tables 2 and 3 show the f_1 and f_2 values for the comparison of the two paddle speeds and the three dissolution media, respectively. Although a paddle speed of 50 rpm generally resulted in a slower dissolution rate than 75 rpm, the difference was only significant for Doxy-Wolff 100 mg tablets. This product showed the most pronounced coning at 50 rpm. With respect to the dissolution media, the products generally dissolved quickly in SGF_{sp}, with slower dissolution in acetate buffer and SIF_{sp}. The effect was especially notable for Antodox 100 mg HGC and Doxycyclin STADA 100 mg tablets, reaching statistical significance in both of these cases.

Dissolution testing of soft gelatin capsules

Doxycyclin-ratiopharm 100 is the only doxycycline hyclate product on the German market formulated as SGCs. As illustrated in Figure 4, even when quite elaborate methods were used (biorelevant media, Bio-Dis apparatus), release was very modest (less than 20% within 45 min).

Discussion

Doxycycline hyclate products

Doxycycline hyclate products were chosen for this study because they are eligible for biowaiver-based approval according to both WHO and US FDA criteria. Furthermore,

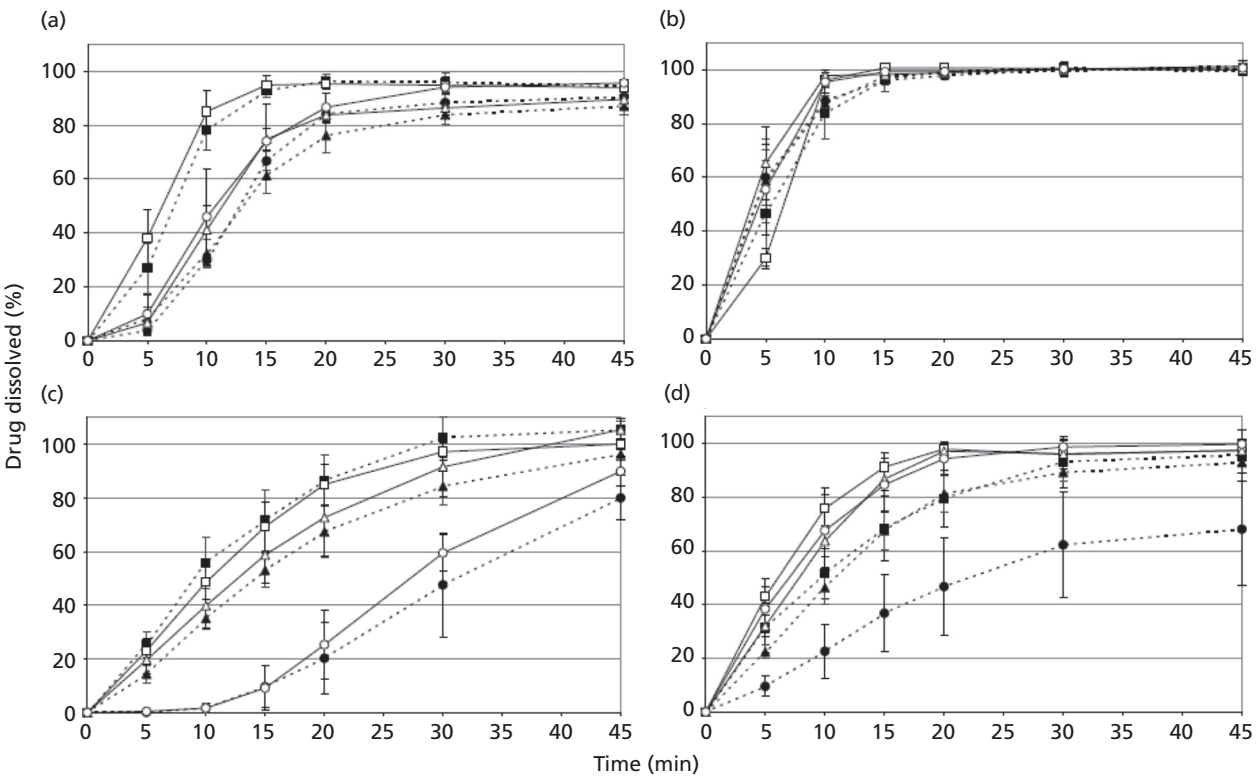

Figure 2 Dissolution profiles of immediate-release doxycycline hyclate products with a marketing authorisation in Germany: (a) Antodox 100 mg hard gelatin capsules; (b) Doxycycline AL 100 T tablets; (c) Doxycyclin STADA 100 mg tablets; (d) Doxy-Wolff 100 mg tablets. Values represent mean ± SD of the percentage of doxycycline hyclate dissolved at each sampling time point. ■, □ simulated gastric fluid without pepsin; ▲, Δ, acetate buffer; ●, ○ simulated intestinal fluid within pancreatin. Dotted lines/filled symbols indicate profiles generated by the US FDA method; continuous lines/open symbols indicate profiles generated by the WHO method.

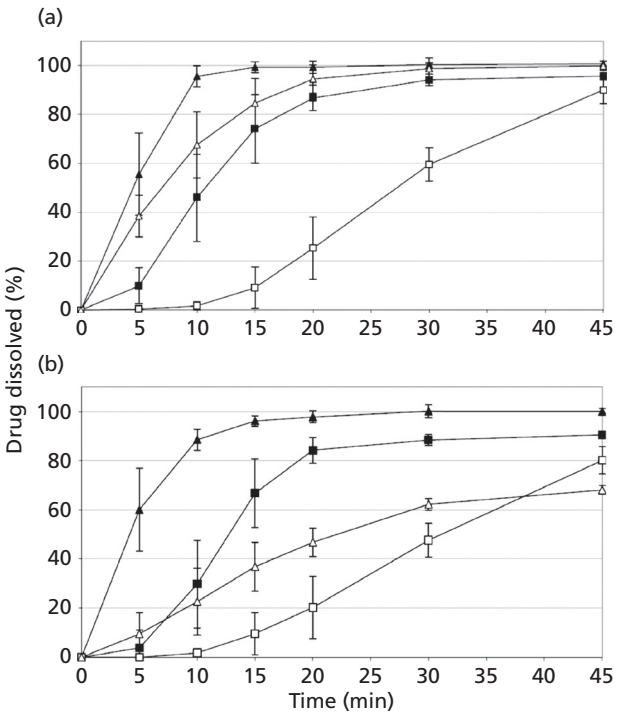

Figure 3 Dissolution profiles of immediate-release doxycycline hyclate products with a marketing authorisation in Germany in simulated intestinal fluid without pancreatin (SIF_{sp}) at (a) 75 rpm (WHO method) and (b) 50 rpm (US FDA method). Data are mean ± SD of the percentage of doxycycline hyclate dissolved at each sampling time point. ■, Antodox 100 mg hard gelatin capsules; ▲, Doxycyclin AL 100 T tablets; □, Doxycyclin STADA 100 mg tablets; Δ, Doxy-Wolff 100 mg tablets.

several multi-source products are available on the German market, enabling a more comprehensive comparison.

Dissolution test conditions

Table 4 compares the dissolution test conditions and specifications by the Japanese, US and European Pharmacopoeias [13,16,17] for doxycycline hyclate products for quality control purposes and for biowaiver purposes, indicating that quality control tests are not suitable for determining bioequivalence. In this study, biowaiver test conditions were applied. For the dissolution testing of Doxycyclin-ratiopharm 100 SGC, the Bio-Dis method with FaSSIF-V2 as the medium was also used, because preliminary results showed that this lipid-based formulation did not release satisfactorily using the paddle method and it was hypothesised that hydrodynamics might play a role in facilitating the release of the API.

Comparison of paddle speed in terms of pharmacokinetic data

All the doxycycline hyclate products tested in this study have marketing authorisation in Germany, indicating that pharmacokinetic studies had demonstrated bioequivalence with a comparator product (Figure 1).[9–12] This behaviour should be appropriately reflected in the biowaiver dissolution test results. Note that SGCs are a special case in this study and are discussed separately below.

Both the WHO and US FDA procedures tend to over-discriminate among doxycycline hyclate tablets and HGC products (Figure 3). The WHO test indicated that three of the four products would be bioequivalent, whereas the FDA test indicated that only two of the four products would be bioequivalent. The f_1 and f_2 statistical evaluation Tables 2 and 3, which confirmed lack of similarity of the profiles generated by the two methods, supports the conclusion that the WHO and US FDA methods lead to different results. Since results generated with the WHO method are more consistent with the pharmacokinetic data, it seems reasonable to adopt the WHO methodology. Essentially this would mean increasing the paddle speed to 75 rpm in the US FDA dissolution procedure for the biowaiver.

Dissolution testing of soft gelatin capsules

Doxycyclin-ratiopharm 100 SGC consists mainly of lipid excipients (i.e. beeswax and soybean oil; Table 1) and was therefore viewed as a special case in this study. According to the European Pharmacopoeia, there is currently no requirement for dissolution testing of SGC for quality control purposes, and only the disintegration test is described.[17] However, as the product is intended for immediate-release, and to evaluate formulation influences on release behaviour of

Table 2 Difference factors (f_1) and similarity factors (f_2) of the dissolution data of four doxycycline hyclate products for comparison of the paddle speed (75 rpm *vs* 50 rpm)

	Antodox 100 mg HGC		Doxycyclin AL 100 T tablets		Doxycyclin STADA 100 mg tablets		Doxy-Wolff 100 mg tablets	
	f_1	f_2	f_1	f_2	f_1	f_2	f_1	f_2
SGF_{sp}, pH 1.2	NR	NR	NR	NR	5.91	68.75	19.75	37.41
	NR	NR	NR	NR	Same	Same	Different	Different
Acetate buffer, pH 4.5	9.59	56.13	NR	NR	9.63	59.37	18.34	41.79
	Same	Same	NR	NR	Same	Same	Different	Different
SIF_{sp}, pH 6.8	12.18	52.49	NR	NR	14.90	58.60	49.13	19.71
	Same	Same	NR	NR	Same	Same	Different	Different

HGC, hard gelatin capsules; NR, not required (comparison of the dissolution profiles is not required because more than 85% of the active pharmaceutical ingredient was released within 15 min); SGF_{sp}, simulated gastric fluid without pepsin; SIF_{sp}, simulated intestinal fluid without pancreatin.

Table 3 Difference factors (f_1) and similarity factors (f_2) of the dissolution data of four doxycycline hyclate products for comparison of dissolution in different media

		Antodox 100 mg HGC		Doxycyclin AL 100 T tablets		Doxycyclin STADA 100 mg tablets		Doxy-Wolff 100 mg tablets	
		f_1	f_2	f_1	f_2	f_1	f_2	f_1	f_2
75 rpm	SGF_{sp}/acetate buffer	28.28	28.76	NR	NR	12.64	52.67	NR	NR
		Different	Different	NR	NR	Same	Same	NR	NR
	Acetate buffer/SIF_{sp}	6.63	66.69	NR	NR	52.06	22.12	5.42	68.64
		Same	Same	NR	NR	Different	Different	Same	Same
	SGF_{sp}/SIF_{sp}	30.89	28.71	NR	NR	56.08	17.97	7.45	60.70
		Different	Different	NR	NR	Different	Different	Same	Same
50 rpm	SGF_{sp}/acetate buffer	27.98	29.23	NR	NR	21.63	38.78	6.39	64.50
		Different	Different	NR	NR	Different	Different	Same	Same
	Acetate buffer/SIF_{sp}	8.17	64.38	NR	NR	54.57	22.23	38.48	28.79
		Same	Same	NR	NR	Different	Different	Different	Different
	SGF_{sp}/SIF_{sp}	30.06	28.06	NR	NR	64.40	14.69	41.43	26.69
		Different	Different	NR	NR	Different	Different	Different	Different

HGC, hard gelatin capsules; NR, not required (comparison of the dissolution profiles is not required because more than 85% of the active pharmaceutical ingredient was released within 15 min); SGF_{sp}, simulated gastric fluid without pepsin; SIF_{sp}, simulated intestinal fluid without pancreatin.

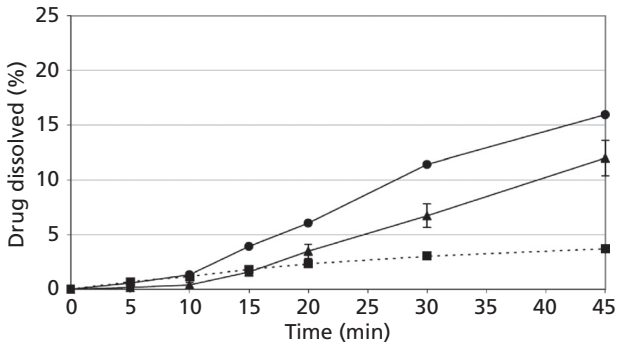

Figure 4 Dissolution profiles of Doxycyclin-ratiopharm 100 soft gelatin capsules using the US Pharmacopeia (USP) Apparatus 2 (paddle method) and USP Apparatus 3 (Bio-Dis method). Data represent mean ± SD of the percentage of doxycycline hyclate dissolved at each sampling time point. ■, USP Apparatus 2; ▲, USP Apparatus 3/blank fasted state simulated intestinal fluid – updated version (FaSSIF-V2); ●, USP Apparatus 3/ FaSSIF-V2.

BCS Class I drugs, the dissolution characteristics of Doxycyclin-ratiopharm 100 (SGC) were also evaluated in this study. On the basis of the modest release of less than 20% within 45 min (Figure 4), it appears that SGC with lipid fills are not suitable for biowaiving, at least with the current tests.

Conclusions

The dissolution test conditions proposed by the WHO (paddle speed 75 rpm)[1] appear to be more appropriate than those proposed by the US FDA (paddle speed of 50 rpm)[2] for biowaiver-based approval of HGC and tablet doxycycline hyclate products. The biowaiver dissolution test conditions are unsuitable for application to Doxycyclin-ratiopharm 100 SGC.

Acknowledgements

The authors wish to thank Juta Pharma GmbH and Aliud Pharma GmbH & Co. KG for providing plasma profiles

Table 4 Quality control and biowaiver dissolution test conditions and specifications (note that the European and Japanese Pharmacopoeias do not list specific dissolution conditions or specifications for doxycycline products)

	US Pharmacopeia 29[13]	International Pharmacopoeia 4th edn[14]	US FDA Biowaiver[2]	WHO Biowaiver[1]
Apparatus	Basket	Paddle	Paddle/basket	Paddle/ basket
Dissolution media	Water	Dissolution buffer, pH 6.8	Standard buffer, pH 1.2 or SGF_{sp}, pH 1.2 Acetate buffer, pH 4.5 Standard buffer, pH 6.8 or SIF_{sp}, pH 6.8	Standard dissolution buffer pH 1.2 Standard dissolution buffer pH 4.5 Standard dissolution buffer pH 6.8
Volume (ml)	900	500	900 ml or less	900 ml or less
Agitation (rpm)	75	75	50 (paddle) 100 (basket)	75 (paddle) 100 (basket)
Time (min)	90	30	30	30
Release (%)	> 85	≥ 80	≥ 85	≥ 85

All tests were conducted at 37 ± 0.5°C. US FDA, US Food and Drug Administration; SGF_{sp}, simulated gastric fluid without pepsin; SIF_{sp}, simulated intestinal fluid without pancreatin; WHO, World Health Organization.

(Figure 1). We also thank R. Abdel-Kader, C. Depalle, M. Hentschel, R. Heymach, E. Kastreva, T. Ranzinger and A. Voskamp for their assistance in the laboratory.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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