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In Vitro Testing of Ciprofloxacin Formulations and Preliminary Study on BCS Biowaiver

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ABSTRACT

The purpose of this study was to compare the technical quality of ciprofloxacin tablets and examine the feasibility of biopharmaceutical classification system (BCS) biowaiver. Ciprofloxacin is a synthetic quinolone derivative anti-infective agent that has been classified as a Class 3 substance according to the biopharmaceutics classification system. Due to the importance of ciprofloxacin as an antibiotic for widely resistant bacteria and the importance of price in a community basis, different ciprofloxacin products available on the market were analyzed. The possibility of extending biowaivers to ciprofloxacin was also examined. Waiver for Class 2 and Class 3 drugs is sometimes scientifically justified. Ciprofloxacin has properties that are intermediate between BCS Classes 2 and 3, as the drug is highly soluble below pH 6 and poorly soluble above this pH. Ten generic brands and the innovator brand were compared on friability, hardness, average weight, content uniformity, disintegration and dissolution. *In vitro* testing indicates significant variations among some brands in terms of hardness, disintegration and dissolution. Dissolution testing met pharmacopeial requirements for all brands. However, significant variations in dissolution profiles were observed in 0.1N HCl and in phosphate buffer (pH 6.8) with no difference detected in acetate buffer (pH 4.5). The results suggest that the formulation and/or the manufacturing process affect the dissolution and thus the bioavailability of the drug products. The significance of the observed *in vitro* differences must be confirmed by an *in vivo* bioequivalence study.

Key words: ciprofloxacin, quality control, biopharmaceutical classification system, biowaiver

INTRODUCTION

The Lebanese pharmaceutical market is flooded with generic products. The quality control of drugs, which is in an international context not uniformly regulated, and quasi-absence of quality control laboratory in Lebanon raise concerns about the quality, safety and effectiveness of generic drugs on the market. Health professionals are confronted with a wide choice of multi-source generics, imported and locally produced with unproven effectiveness, safety, quality and bioequivalence. Pharmacopeial testing confirms these properties according to fixed standards. *In vitro* dissolution testing can also be used in some cases not only to determine the quality of the pharmaceutical products but also to demonstrate bioequivalence to the brand name product.

Due to the importance of ciprofloxacin as an antibiotic for widely resistant bacteria[1] and the importance of price in a community basis, all ciprofloxacin products available in Lebanon were analyzed. Ciprofloxacin is a synthetic quinolone derivative anti-infective agent. It was first discovered in 1960 and then marketed under the brand Ciprobay. It is widely prescribed because of its safety, good tolerance and broad antibacterial spectrum with minimal resistance pattern. Generics are available on the Lebanese market at significant price differences, e.g. one generic costs 25 times less than the brand product. Although physicians may have serious concerns as to the efficacy of the different products, they sometimes prescribe cheaper products due to economical constraints. Our objective was to compare the quality of locally produced and imported products, including the innovator product, available on the Lebanese market and to examine the possibility of waiver for *in vivo* bioequivalence study.

In recent years the possibility to allow waivers of *in vivo* bioequivalence studies or "biowaivers" for individual substances has met considerable interest. A biowaiver implies that bioequivalence studies are waived by health

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authorities and hence the product is considered bioequivalent to its reference product by carrying out an *in vitro* study. Such waivers have the potential to both decrease the cost and improve the quality of medicines.

Currently, biowaiver is allowed only for immediate-release product of BCS Class 1 drug substances (highly soluble and highly permeable) that exhibit rapid *in vitro* dissolution[2]. Thus, for such products, demonstration of similar *in vitro* dissolution profiles using the recommended test methods would provide sufficient assurance of rapid *in vivo* dissolution, thereby ensuring human *in vivo* bioequivalence[3].

The possibility of extending biowaivers to other BCS Classes drugs is under scrutiny. Waiver for Class 2 and Class 3 drugs is sometimes scientifically justified[4]. *In vivo* bioequivalence data of different formulations would support such an extension. Ciprofloxacin has properties that are intermediate between BCS Classes 2 and 3[5], as the drug is highly soluble below a pH of 6 and poorly soluble above this pH[6].

MATERIALS AND METHODS

Ciprofloxacin formulations were purchased from randomly selected pharmacies in Lebanon. Information on the country of manufacturing is summarized in Table 1.

Acetonitrile (HPLC grade), triethylamine, phosphoric acid (analytical grade) were purchased from Sigma, (St Louis, MO). United State Pharmacopeia (USP) standards Ciprofloxacin Hydrochloride, and its three degradation products-ethylenediamine, desfluorociprofloxacin and fluoroquinolonic acid-were used to prepare 200 µg/mL stock solutions in their respective mobile phases. These stock solutions were used to build the calibration curves. Triplicates were assayed for every sample.

Table 1. Ciprofloxacin tablets available on the Lebanese Market

Brand	Strength (mg)	Country	Price (LP)	Price (US $)
Ciprobay	500	Germany	48114	32.08
CE	500	Egypt	16000	10.67
CG	500	Greece	19880	13.25
CJ1	500	Jordan	28735	19.16
CJ2	500	Jordan	21350	14.23
CJ3	500	Jordan	21051	14.03
CK	500	KSA	26400	17.60
CL	500	Lebanon	13650	9.10
CS1	500	Spain	30000	20.00
CS2	500	Spain	31200	20.80
CSY	500	Syria	1900	1.27

I. *Content Uniformity Testing and Impurities Content*

Ciprofloxacin tablets were analyzed for their drug and impurities content as described in USP 30[7]. Ten tablets of each of the tested brands were individually triturated to fine particles using a mortar and pestle. The obtained powder was then transferred to a 100 mL volumetric flask and diluted to volume with water. An aliquot of 10 mL was filtered through a 0.45 µm syringe filter and diluted with mobile phase to obtain an equivalent solution of about 200 µg of ciprofloxacin per mL. A 20 µL sample was injected into the HPLC.

All formulations were analyzed in triplicate using a sensitive and reproducible HPLC method modified in our laboratory[7]. The HPLC system consisted of LC-10 pump (Shimadzu, Kototo, Japan), a variable ultraviolet detector monitor (Shimadzu, SPD-10) and a Chromatopac Shimadzu (C-R8A) integrator. Separation was done using a pre-packed stainless-steel column (150 mm × 4.6 mm i.d.) filled with uBondapack C18 10 µm Silica (Waters, Milford, MA) and the flow rate was 1.5 mL/min. The precision of the assay method was determined by calculating the relative standard deviation (inter- and intra-days) of the peak areas obtained after repeated injections (n = 3) of standard solutions.

For the separation of ciprofloxacin and the degradation products, ethylenediamine and desfluorociprofloxacin, a mobile phase consisting of water: acetonitrile: triethylamine (83:16:1) adjusted to a pH of 3.4 with concentrated phosphoric acid was used. The UV detector was set to 278 nm.

For the detection of the degradation product fluoroquinolonic acid, a mobile phase consisting of water: acetonitrile (40:60) and 0.1% phosphoric acid was used. The UV detector was set to 330 nm.

The percentage of each impurity peak in the chromatogram obtained from the assay preparation was calculated using the formula: $100r_i / r_t$ in which r_i is the response of each impurity peak; and r_t is the sum of the responses of all the peaks.

II. *Tablet Weighing*

Twenty tablets of each brand were used to study weight variation using digital balance (Precisa 125A, Dietikon, Sweitzerland).

III. *Friability*

A sample of ten tablets was placed into a friabilator (Pharma Test PTFE, Hainburg, Germany). All loose dust was then removed from the tablets before weighing. A maximum mean weight loss of not more than 1.0% is considered acceptable.

IV. *Hardness*

The hardness of the tablets was measured six times using a hardness tester (Pharma Test PTR, Hainburg, Germany).

V. *Disintegration Testing*

The disintegration time of the tablets was investigated using a disintegration apparatus (Pharma Test PTZ, Hainburg, Germany) with 900 mL of distilled water as immersion fluid at a temperature of 37°C ± 0.5°C.

VI. *Dissolution Testing*

The dissolution profiles of the tablets were investigated using USP dissolution Apparatus 2 (Pharma Test PTWS, Hainburg, Germany) at 50 rpm for 30 min. One tablet was placed in each of the six vessels filled with 900 mL of 0.01N hydrochloric acid at 37 ± 0.5°C. UV absorbance was measured every 5 minutes using a spectrophotometer (Jenway 6405UV/Vis, Essex, England) set at 276 nm and previously calibrated. Absorbance values collected after diluting the samples were converted to percentages of tablet dissolved by dividing by the standard UV absorbance.

Dissolution profiles were also investigated in three solutions: 0.1 N HCl, acetate buffer (pH = 4.5), phosphate buffer (pH = 6.8). According to current US FDA criteria, a *rapidly dissolving* drug products is one for which no less than 85% of labeled amount dissolves within 30 minutes and a *very rapidly dissolving* product is one for which no less than 85% of labeled amount dissolves within 15 minutes[2].

RESULTS

The drug content of all tablets was between 93.47 and 99.61%, (Table 2). All preparations were within pharmacopeial limits[6], i.e. ciprofloxacin tablets contain ciprofloxacin hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ciprofloxacin.

Not more than 0.2% of ciprofloxacin ethylenediamine analog or of any other individual impurity peak was found. The sum of all the impurity peaks was not more than 0.5% of the total peaks (Table 2).

I. *Physical Parameters*

Table 3 summarizes the physical properties of the tablets. Average weights range from 0.7317 to 0.9363. The relative standard deviations for tablet weight variation are between 0.0409 and 4.977. All tablets succeeded friability test as the percentage weight loss after the test was below 1%.

Hardness significantly differs from one brand to another, ranging from 94 to 283 N as shown on Figure 1.

Disintegration testing reveals significant differences among the brands, with Ciprobay exhibiting the most rapid disintegration and CSY and CS1 the highest distintegration times (Figure 2).

II. *Dissolution Testing in 0.01N HCl*

Figure 3 shows the dissolution profiles of tablets in 0.01N HCl. In all cases, the amount of ciprofloxacin released in 30 minutes was not less than 80% of the labeled amount. Most products may be considered as *very rapidly*

Table 2. Uniformity of dosage units and percentage of impurities, EDA: ethylenediamine, DFC: desfluorociprofloxacin, FQA: fluoroquinolonic acid

	% of the labeled amount	RSD	Percentage of impurities			
			EDA	DFC	FQA	TOTAL
Ciprobay	99.61	3.38	0.06684	0.00864	0.00252	0.078
CE	95.27	3.93	0.08002	0	0.02241	0.10243
CG	98.69	5.63	0.07012	0	0.00323	0.07335
CJ1	95.45	5.01	0.06533	0.00778	0.02304	0.09615
CJ2	93.89	2.44	0.08978	0	0.05352	0.1433
CJ3	93.87	1.66	0.06612	0	0.03557	0.10169
CK	97.55	2.11	0.14421	0	0.01756	0.16177
CL	94.22	2.80	0.05649	0.00738	0.05487	0.11874
CS1	94.22	3.67	0.09719	0	0.04054	0.13773
CS2	98.83	2.07	0.12355	0	0.03221	0.15577
CSY	93.47	3.99	0.13274	0	0.04911	0.18185

Table 3. Physical characteristics of tablets

	Average weight (g)	SD	RSD (%)	weight loss (%)	Average diameter (cm)	SD	Disintegration time (min)	Hardness (N)	SD
Ciprobay	0.7738	0.0004	0.0455	0	3.78	0.0052	1.75	179.2	6.02
CE	0.9363	0.0002	0.0226	0.009	4.82	0.0052	12.33	173.6	7.43
CG	0.7668	0.0003	0.0425	0.022	3.99	0.0041	1.58	206.8	12.6
CJ1	0.7705	0.0363	4.9779	0.057	3.64	0.0041	2.50	182	14.8
CJ2	0.7784	0.0003	0.0409	0.005	4.19	0.0052	2.28	202.7	23.9
CJ3	0.7642	0.0082	1.0702	0	3.8	0.0052	3.67	282.7	21.33
CK	0.8232	0.0012	0.1431	0.017	3.76	0.0084	6.00	174.25	14.8
CL	0.7272	0.0026	0.3596	0.006	3.56	0.0063	3.00	93.75	13.6
CS1	0.8282	0.001	0.1225	0.083	3.98	0	14.10	150.7	7.08
CS2	0.7667	0.0048	0.6246	0.012	3.81	0.0041	8.00	195.5	30
CSY	0.7317	0.0062	0.8568	0.098	3.35	0.0105	14.30	271	18.47

dissolving as more than 85% of the labeled amounts of the drug substance dissolves within 15 minutes. The only exceptions were CE, CL, CS1 and CSY.

III. *Dissolution Testing in Buffer Solutions*

Significant variations in the *in vitro* dissolution profiles were observed when the dissolution media were changed. Figures 4 to 6 show the dissolution profile of a generic (CS1) and the innovator product in 0.1N HCl (Figure 4), in acetate buffer (pH=4.5) (Figure 5) and in phosphate buffer (pH=6.8) (Figure 6). In 0.1N HCl, the brand product complies with current US FDA criteria for *very rapidly dissolving* drug products (no less than 85% dissolved in 15 minutes) whereas less than 60% of the generic product dissolved in 30 minutes. No difference was detected in acetate buffer (pH=4.5) as the two products dissolved within 15 minutes. In phosphate buffer, less than 35% of the brand product was dissolved in 30 minutes compared to about 2% for the generic one.

Dissolution profiles for the brand name product were comparable in all medium except in phosphate buffer (Figure 7). In 0.01N HCl, 0.1N HCl and acetate buffer, the brand product met the criteria for *very rapidly dissolving* product.

The generic product (CS1) exhibited a slower rate of dissolution in 0.1N HCl whereas in phosphate buffer the dissolution of the drug was almost nil (Figure 8). Only in 0.01N HCl and in acetate buffer did the generic product met the criteria for *rapidly dissolving* product (more than 85% dissolved in 30 minutes).

Table 4 shows the variations in dissolution times (T50%, T80%, T85%) between Ciprobay and CS1 in the different media. Note that the values for Ciprobay varied within a limited range irrespective of the dissolution media (except phosphate buffer) whereas wider variations were observed for CS1.

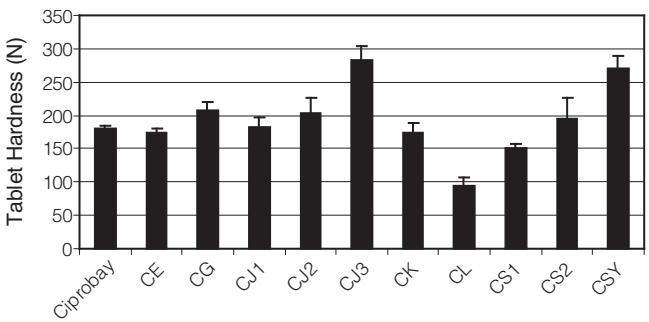

Figure 1. Tablet hardness (± SD)

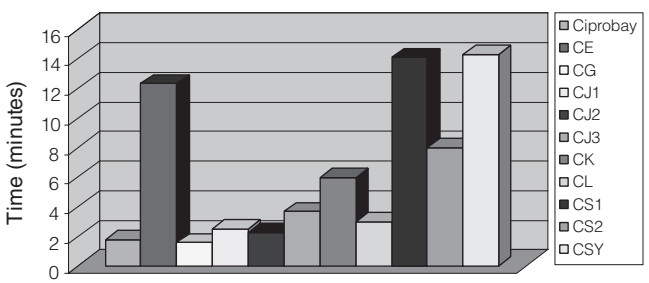

Figure 2. Disintegration times (minutes)

DISCUSSION

All tested products had ciprofloxacin content within

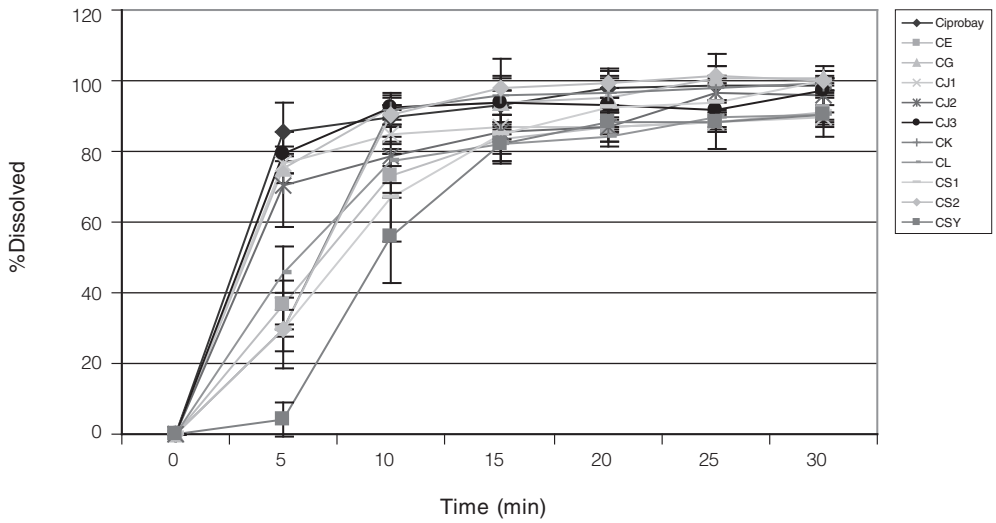

Figure 3. Dissolution profile (±SD) of ciprofloxacin tablets in 0.01N HCl

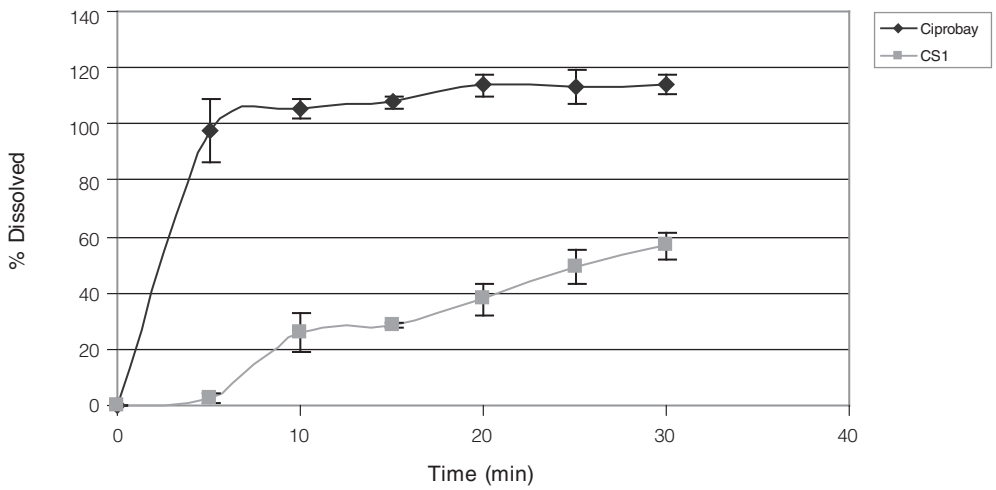

Figure 4. Dissolution profiles (±SD) of ciprofloxacin tablets in 0.1 N HCl

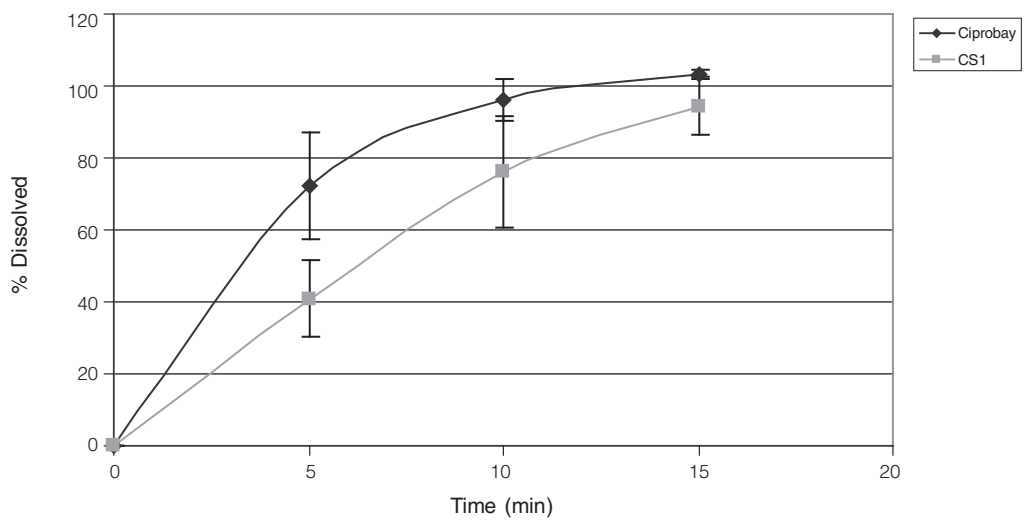

Figure 5. Dissolution profiles (±SD) of ciprofloxacin tablets in acetate buffer pH = 4.5

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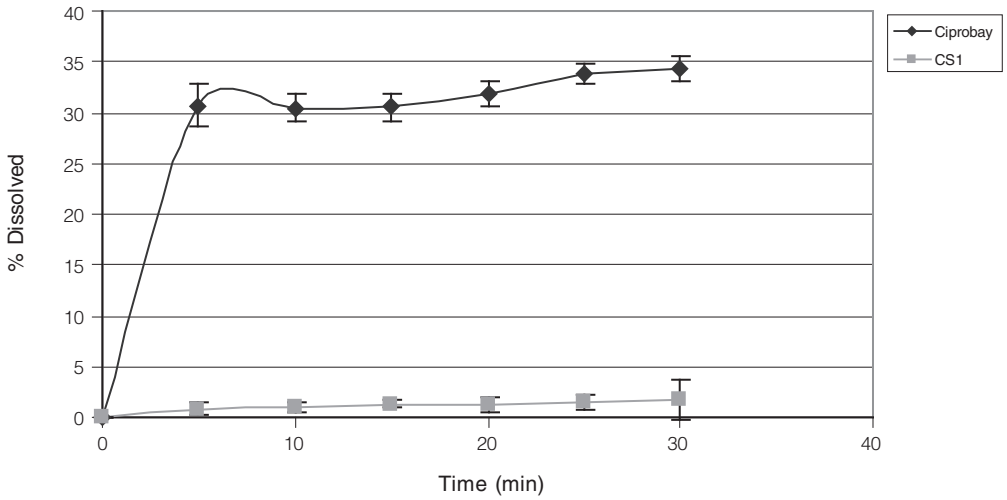

Figure 6. Dissolution profiles (±SD) of ciprofloxacin tablets in phosphate buffer pH= 6.8

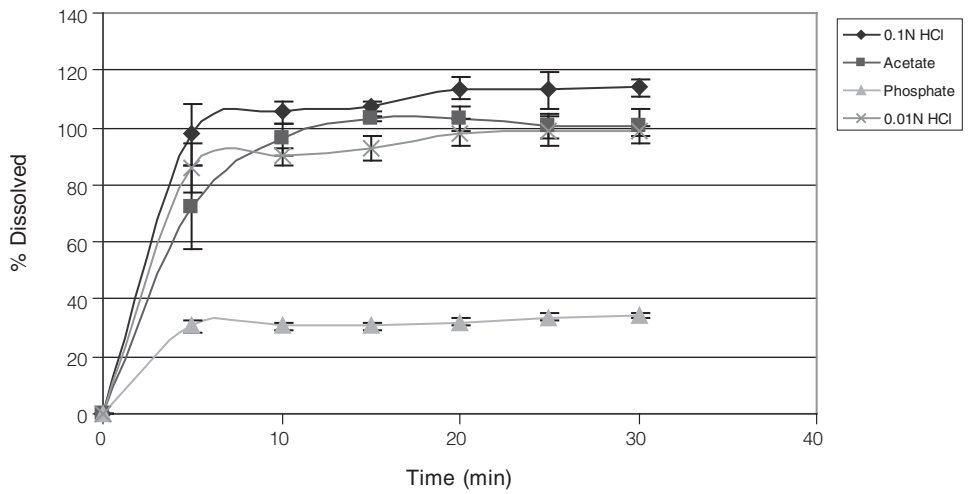

Figure 7. Dissolution profiles (±SD) of ciprofloxacin tablets (Ciprobay) in different medium.

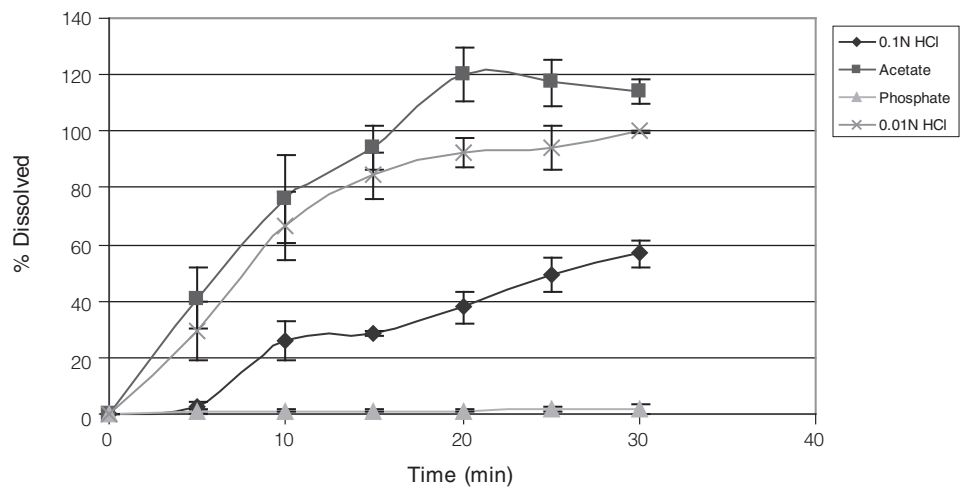

Figure 8. Dissolution profiles (±SD) of ciprofloxacin tablets (CS1) in different medium.

Table 4. Comparison of the dissolution times (T50%, T80%, T85%) in different media

Media		T50%	T80%	T85%
0.01N HCl	Ciprobay	2.82	4.31	4.85
	CS1	7.60	13.22	15.42
0.1N HCl	Ciprobay	2.10	3.80	4.11
	CS1	25.40	--	--
Acetate Buffer	Ciprobay	3.16	6.35	6.80
	CS1	6.20	10.85	12.27
Phosphate Buffer	Ciprobay	--	--	--
	CS1	--	--	--

the range allowed by the USP, which is between 90% and 110% of the labeled amount. The highest amount of ciprofloxacin was found in Ciprobay which includes 99.61% of the labeled amount, whereas the lowest amount (93.86% of the labeled amount) was found in CSY. This corresponds respectively 498.07 and 467.36 mg of active ingredient. The RSDs were less than 6% in all cases. The amount of impurities in all brands was within the allowed limits, which is less than 0.5%. Overall, the sum of all impurities was the highest in CSY, reaching 0.181% and the lowest in CG, reaching 0.073349%.

There was a 25% variation in the average weight despite similar tablet shapes. The RSD of the tablet weights was less than 5%. Weight variation after friability testing was negligible or even unchanged. The tablet hardness results suggest great variability among the brands. Since tablets are film coated to resist humidity, variation in mechanical strength may be due to the film coating. Disintegrating times show significant variation among the different brands, with CS1 and CSY having the slowest disintegration.

For all products when tested in 0.01N HCl, dissolution testing met pharmacopeial requirements, i.e. at least 80% of the drug dissolved in 30 minutes. However, significant variations were observed when the dissolution media was changed to more biorelevant ones. Dissolution profiles in 0.1N HCl and in acetate buffer were comparable for the innovator product whereas only acetate buffer was comparable to 0.01N HCl for the generic product. Moreover, testing in biorelevant media was discriminatory as it revealed dissimilarities in dissolution profiles between innovator and generic products.

These results suggest that the formulation and/or the manufacturing process affect the dissolution and thus the bioavailability of the drug product so that when properly formulated, ciprofloxacin reaches its site of absorption in a solution form. Dissolution at low pH is important as the upper GI tract (duodenum/jejunum) is the main site of absorption. The bioavailability will then be determined by its *in vivo* permeability pattern.

An *in vivo* bioequivalence study will establish whether the observed differences in the *in vitro* dissolution profile are significant *in vivo*. The *in vivo* vs. *in vitro* correlation will then justify a BCS biowaiver.

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