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Research Article

IN VITRO CEFIXIME DISSOLUTION IN PHARMACOPEIA-RECOMMENDED MEDIUM AND SIMULATED GASTROINTESTINAL FLUIDS: A COMPARATIVE STUDY

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ABSTRACT

Objectives: The aim of this study was to compare *in vitro* dissolution of cefixime in a pharmacopeial-recommended medium and in simulated gastrointestinal fluids.

Methods: Before dissolution testing, the drug content in the tested materials was determined by ultraviolet spectrophotometer. The dissolution media used in this study were recommended by the United States Pharmacopeia (USP) as well as four different media that mimic gastric and intestinal fluids in fed and fasted states. The tested materials included the pure drug and two 0.2-g capsule brands (original and test).

Results: The pharmacopeial medium showed no difference in both extent and rate of the drug dissolution between the tested materials. In the contrary, the difference was significant when the simulated fluids were used. Moreover, it was found that the simulated intestinal fluid (SIF) of fed state showed 21–32% decrement in the drug dissolution compared to that of the corresponding fasted-state simulated fluid. Indeed, this finding agreed those of *in vivo* bioavailability studies published in literature.

Conclusion: The SIF is much more valid as a medium for *in vitro* testing of cefixime capsule than the one recommended by the USP.

Keywords: Cefixime, Simulated gastrointestinal fluids, Dissolution, Fed, Fasted, Pharmacopeia.

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INTRODUCTION

Cefixime trihydrate is the third-generation cephalosporin antibiotic. Chemically, it is a 5-Thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, 7-[(2 amin o-4thiazolyl){(carboxymethoxy) imino] acetyle} amino] 3ethynyl-8-oxo-trihydrate [1-3]. It is widely prescribed to treat otitis media, respiratory tract infections, and uncomplicated urinary tract infections [4]. The drug is sparingly soluble in water and has an acidic nature with pH of 2.6–4.1 for a solution of 0.0007 g/ml in water [1]. Cefixime is administered only orally and is available as capsule, tablet, and oral suspension [2].

Cefixime has a low bioavailability when given orally (22–54%) [5-7]. Due to its poor solubility and permeability, the drug is considered as a Class IV drug according to biopharmaceutical classification system. Therefore, problems in either drug dissolution or absorption or both contribute to its poor bioavailability [8,9]. In addition, certain studies indicated that food can decrease its bioavailability following oral administration of the capsule by approximately 15% based on area under the curve and 25% based on peak plasma concentrations [10].

In vitro dissolution test of cefixime capsules, as recommended by the United States Pharmacopeia (USP), is carried out in phosphate buffer pH 7.2 as dissolution medium [1]. Although it was reported that a dilute hydrochloric acid-based solution at pH 1–2 can simulate gastric fluid, and phosphate-buffered solution at pH 6.8 can mimic intestinal fluid, dissolution media that are more closely representing physiological conditions may provide more accurate results [11]. There are many types of simulated gastrointestinal fluids which have been reported for *in vitro* drug dissolution of many drugs [12-15].

To the best of our knowledge, all previous studies that focused on cefixime dissolution either as a pure drug or a pharmaceutical product used the pharmacopeia-recommended phosphate buffer as dissolution medium and no one has studied the dissolution behavior of the drug in simulated gastrointestinal fluids. This was the novelty of the present work to give data of cefixime dissolution behavior in simulated fluids and compare it with *in vivo* bioavailability data reported in literature. Accordingly, the present work will give results that determine whether the medium recommended by the pharmacopeia or the simulated gastrointestinal fluids would be more matching to *in vivo* results, in particular, the impact of food on the bioavailability of the drug.

METHODS

Instruments

USP dissolution apparatus II (Erweka® DT6, Germany) as well as ultraviolet (UV) spectrophotometer (Shimadzu, UV-1800, Japan) were used in this study.

Materials

The reference standard of cefixime was a gift from Global-Pharma Co., Yemen. The original as well as the test brands of 0.2-g capsule of cefixime were purchased from the local drug market in Yemen. All other materials were at least of analytical grade.

Standard calibration curve

A standard stock solution of 100 µg/ml of cefixime (anhydrous) solution was prepared by dissolving and dilution of an amount of cefixime trihydrate reference standard (equals 0.05 g of cefixime anhydrous) in a mixture of methanol:water (70:30). Serial dilution of that stock solution was made to produce six standard solutions with a range of concentrations 2–12 µg/ml. The UV absorbances of solutions were then measured at 254 nm [1]. The linearity and regression equation of the calibration curve were then determined.

Drug content

Three tested materials including pure cefixime, the original brand, and test brands of 0.2-g capsule of the drug were investigated. Three different theoretical concentrations (Ct) of 5, 6, and 12 µg/ml of each tested

material were prepared in a mixture of methanol:water (70:30) and investigated by UV spectrophotometry at 254 nm. The UV absorbances of those solutions were introduced into the standard calibration equation to calculate the responding practical concentrations (Cp). The content percentage of the drug in each solution was determined as follows:

$$\text{Drug content \%} = 100 * Cp/Ct.$$

The drug content in each tested material was then recorder as average±standard deviation. According to USP, the limit of cefixime anhydrous content is 90–110% [1].

In vitro dissolution tests

Using USP dissolution apparatus II, the *in vitro* dissolution tests were performed at 310±173.5°K for 45 min. The dissolution medium was 900 ml of either the USP dissolution medium of 0.05 mol/l phosphate buffer pH 7.2 [1] or one of the simulated gastrointestinal fluids as follows:

- Simulated gastric fluid (SGF) fasted state: (pH 2.1) It consisting of hydrochloric acid 0.01–0.05 mol/l+Sodium lauryl sulfate 2.5 g+Sodium chloride 2 g+Distilled water q.s. to 1000 ml [11].
- SGF fed state: It was a long-life milk (pH 5) [11].
- Simulated intestinal fluid (SIF) fasted state: (pH 6.8) It consisting of potassium dihydrogen phosphate 0.029 mol/l+Sodium hydroxide qs to pH 6.8+Sodium taurocholate (bile salt) 5×10^{-3}mol/l+Lecithin 1.5×10^{-3}mol/l+Potassium chloride 0.22×10^{-3} mol/l+Distilled water q.s. to 1000 ml [11].
- SIF fed state (pH 5): It consisting of acetic acid 0.144 mol/l+Sodium hydroxide q.s. to pH 5+Sodium taurocholate (bile salt) 15×10^{-3} mol/l+Lecithin 4×10^{-3} mol/l+Potassium chloride 0.19 mol/l+Distilled water q.s. to 1000 ml [11].

In each dissolution test, three 10 ml aliquots were withdrawn at 15, 30, and 45 min intervals and filtered. Fresh three 10 ml of dissolution medium was added to compensate for the withdrawn volumes. About 3 ml of the filtered solution was made up to 10 ml with methanol and investigated by UV spectrophotometer at 254 nm. A mixture of 3 ml of fresh dissolution medium made up to 10 ml of methanol was used as blank solution.

When longtime milk was used as dissolution medium, the milk proteins were separated as follows: To each 10 ml aliquot withdrawn, 2 ml of ethanol was added to the sample which was then subjected by freezing at 273°K for 1 h followed by centrifugation at 4000 round/min for 15 min. The supernatant was discarded and 7 ml methanol was added to the residue and again centrifuged at 4000 rpm for 15 min. To the resultant supernatant, water was added up to 10 ml; the UV absorbance at 254 nm was measured.

The Cp was calculated by introducing the sample absorbance into the standard calibration equation and cumulative percentage drug release was determined as described earlier in "drug content." According to USP limit [2], not <75% should dissolve in 45 min.

To assess, the difference in drug release rate between original (reference) and test brands in the similarity factor (f_2) was calculated as follows [16]:

$$f_2 = 50.\log\{[1+(1/n)\sum_{t=1}.(R_t-T_t)^2]^{-0.5}.100\}$$

Where, f_2=Similarity factor, n=number of time points, R_t=% drug dissolved (reference brand), T_t=% drug dissolved (test product). If f_2 >50, the profiles were considered similar.

Influence of food on dissolution

The influence (F_i) of food on cefixime dissolution was calculated as accuracy as follows:

$$F = 100*(\sum \text{Release}_{fed} - \sum\text{Release}_{fasted})/\sum\text{Release}_{fed}$$

Where, $\sum$ Release_{fed} was the cumulative drug release (0–45 min) in the fed-state medium; $\sum\text{Release}_{fasted}$ was the cumulative drug release (0–45 min) in the fasted-state medium.

RESULTS

Standard calibration curve

The standard calibration curve obtained by UV analysis at 254 nm of cefixime in methanol:water (70:30), as shown in Fig. 1, was linear with linearity coefficient of 0.9994. This finding indicated the optimum reproducibility of the UV spectrophotometer technique to be used to quantify the drug. The regression equation of the curve (y=0.0531×−0.0024) was used to determine the content of the drug in the tested materials as well as in the samples withdrawn from the *in vitro* dissolution tests.

Drug content

As shown in Table 1, pure drug as well as the original and test brands showed drug content ranged from 99.8 to 103% which complied with the USP limit. This finding indicated that the drug content in all three tested materials did not have an impact on differences in amount of drug released during the *in vitro* dissolution tests of the pure drug or the two tested brands.

In vitro dissolution

As demonstrated in Table 2, the pure drug as well as the original and test brands showed cumulative drug release (0–45 min) in the USP recommended medium in the range (81.1–94.8%) which were within the USP limit of ≥75%. Furthermore, the similarity factor in rate of drug release between original and test brands was >50 which indicated proper similarity between the two brands. Based on these findings, a primary conclusion might arise that there were no significant differences in the dissolution extent or behavior between the original and test brands. However, with the using of simulated gastrointestinal fluids as dissolution medium, the differences were indeed obvious. In SGFs (fasted or fed states), the cumulative drug release percentage of the pure drugs and the tested brands decreased to the range (40.5–47.6%) in fasted state and (41.8–65.1%) in fed state which were both obviously lower than that obtained in the USP dissolution medium. Such decrement may be attributed to the acidic nature of the drug [1]

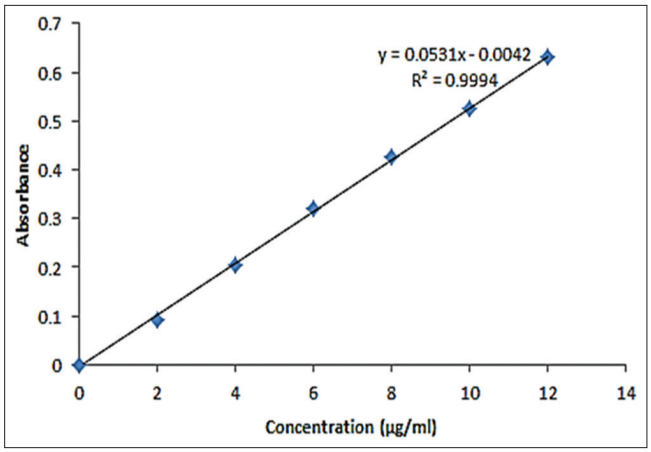

Fig. 1: Standard calibration curve of cefixime in methanol:water (70:30) at 254 nm; sample size=6 concentrations (at triplicate runs)

Table 1: Drug content of cefixime in capsule brands

Test	Drug content % (average±standard deviation)
Pure drug	103^{Δ}±0.023
Original brand	101^{Δ}±0.145
Test brand	99.8^{Δ}±1.067

Sample size for each test=3 (at triplicate runs), for each test, $^{\Delta}$: Complied to USP drug content limit (90–110%)

Table 2: Cumulative drug release % (0–45 min) of cefixime in different dissolution media

Test	United States Pharmacopeia dissolution medium	Simulated gastric fluid			Simulated intestinal fluid		
		Fasted	Fed	F_i%	Fasted	Fed	Fi%
Pure drug	88.1$^{\Delta}$±1.352	45.4$^{\clubsuit}$±0.675	65.1$^{\clubsuit}$±1.004	(+) 43.4$^{\#}$±0.675	94.67$^{\Delta}$±3.002	75.1$^{\Delta}$±2.116	(−) 20.7$^{\square}$±1.145
Original brand	94.8$^{\Delta}$±6.109	47.6$^{\clubsuit}$±2.554	52.3$^{\clubsuit}$±2.312	(+) 9.9$^{\#}$±2.001	79.11$^{\Delta}$±5.101	63.2$^{\clubsuit}$±4.166	(−) 20.1$^{\square}$±3.017
Test brand	81.8$^{\Delta}$±7.003	40.5$^{\clubsuit}$±2.076	41.8$^{\clubsuit}$±3.011	(+) 3.2$^{\#}$±1.052	63.2$^{\clubsuit}$±2.124	43.1$^{\clubsuit}$±3.077	(−)31.8$^{\square}$±0.945
f2 test (original to test brand)	56.2$^{\sun}$	32.9$^{\blacksquare}$	41.4$^{\blacksquare}$	-	27.2$^{\blacksquare}$	34.8$^{\blacksquare}$	-

Sample size for each test=6 (triplicate runs); $^{\Delta}$: Complied to USP dissolution limit (≥75%); $^{\clubsuit}$: Out of USP dissolution limit; $^{\sun}$: Test brand is similar to original brand in drug release rate (f_2 <50); $^{\blacksquare}$: Test brand is not similar to original brand in drug release rate; $^{\#}$: % of food increases drug release; $^{\square}$: % Food reduces drug release

that makes most of the drug to be unionized in the acidic medium of SGF according to Henderson–Hasselbalch equation [11]. The higher dissolution extents of the drug all tested materials in the SGF fed-state medium than that in fed-state medium could be justified to the larger pH of the fed-state medium pH 5 compared to pH 2.1 according to the previously mentioned equation. This result was indeed in contrast to *in vivo* bioavailability results in literature which reported that food can decrease the drug bioavailability [8]. As a result, it could be concluded that SGFs are not the mediums that mimic the *in vivo* dissolution of the drug. In SIFs, as shown in the same table, the results of drug dissolution were different from those obtained in both USP dissolution medium and SGFs. In SIF fasted-state medium (pH 6.8), the pure drug and the original brand showed dissolution extents of 94.7% and 79.1%, respectively, which were both above the lower limit of USP dissolution of the drug (75%) while the test brand was out of that limit with a dissolution extent of 63.2%. In addition, the dissolution rate behavior was not similar between the two brands with f2 <50. In SIF fed-state medium (pH 5), the dissolution extents of all tested materials were lower than those observed in the fasted-state medium with dissolution of 75.1%, 63.2%, and 43.1%, for the pure drug, original brand, and test brand, respectively.

Similar to differences in the drug dissolution between SGF fasted- and fed-state media, the reasons of such decrement in dissolution of the drug observed with fed state compared to that of fasted-state medium could be also attributed to the lower pH of the fed-state dissolution medium.

These findings observed with SIFs with higher dissolution in fasted-state medium and lower with fed-state medium were more simulated to *in vivo* results in literature where same findings have been reported. However, to assess the similarity between in *in vitro* results of the drug dissolution using SIF fasted medium for dissolution testing observed in this study to those reported in *in vivo* studies, food influence on the drug dissolution was determined and then compared to *in vivo* studies reported in literature. With respect to SGFs, as discussed earlier, food caused increment of drug dissolution to 43.4%, 9.9%, and 3.2% for pure drug, original, and test brands, respectively. In the contrary, food decreased the drug dissolution to 20.7%, 20.1%, and 31.8% in the relevant tested materials, respectively. This finding is much more alike than reported in literature on *in vivo* bioavailability studies of the drug which stated that food decreases the drug bioavailability to 15% based on area under the curve and 25% based on peak plasma concentrations [8].

CONCLUSION

The present study concluded that using SIF fasted state as an *in vitro* dissolution medium for testing of cefixime capsules does mimic the *in vivo* conditions more than using the USP medium.

AUTHORS' CONTRIBUTIONS

The correspondent author conceived the idea and developed the theory and performed the calculations of the presented work. All authors participated in conducting experiments, discussing the results, and contributing to the last manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest of publishing this article.

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