International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 17, Issue 1, 2025

Original Article

QUALITY ASSESSMENT OF VARIOUS BRANDS OF CEFIXIME 400 MG CAPSULES AVAILABLE IN YEMENI MARKET

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Received: 06 Jun 2024, Revised and Accepted: 29 Nov 2024

ABSTRACT

Objective: The study aimed to assess the quality control of different brands of cefixime 400 mg capsule brands available in Yemeni drug market.

Methods: The pharmacopeial specifications of five cefixime 400 mg capsule brands available in Yemeni drug market, including two domestic brands, were assessed in this study. Assessment included assay content, capsule weight variation and disintegration. In addition, drug dissolution and antimicrobial activity test were assessed.

Results: Out of five brands, three brands of cefixime 400 mg capsules passed official specified assay tests according to United States Pharmacopeia (USP) specifications. The five brands showed a similar profile of weight variation and drug disintegration that were within the limits. However, the results of drug content for five brands showed in range 78.78-104.46 % in which three brands (B, D and E) in compliance to USP specifications (90-110%) and two brands (A and C) not compliance to USP specification. Also, the results of dissolution profile were in range 86.2-109.8% for four brands (A, B, D and E) that compliance to USP specifications, and 73.56% for brand C that was not compliance to USP specifications.

Conclusion: Based on the results obtained in this study, the drug content for three brands (B, D, E) is within the pharmacopeial limit, but the drug content of (A, C) is out of the limit, while the capsule disintegration, weight variations, and antibacterial activity in all tested brands are within the pharmacopeial limit. The dissolution profile for four brands (A, B, D, E) is within the pharmacopeial limit, but the dissolution profile for (C) is less than the allowed limit.

Keywords: Cefixime, 400-mg capsules, Quality, Specification, Yemeni market

DOI: https://dx.doi.org/10.22159/ijap.2025v17i1.51714 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Pharmacopeial evaluation of tablet or capsule is the illegal transportation of products or substances or across an international border in violation of applicable laws or other regulations. Smuggled products are usually of low price but with no doubt, have lower efficacy than those introduced legally and, hence, are risky on patients. In addition, they have a great negative impact on country economy. In several developing countries, drug quality is a source of concern. There is a general feeling and there is a high incidence of drug preparations which are not of acceptable quality. World Health Organization (WHO) reported that the quality of medicines varies greatly, particularly in low-income countries, both in manufacturing and in the distribution system. In many of these countries, 20 % to 30 % of samples collected from markets failed in their quality tests. For example, the percentage of drugs that failed quality control testing was found to be 92 % in the private sector of Chad [1].

Cefixime, an antibiotic, is a third-generation cephalosporin. Cefixime is highly stable in the presence of beta-lactamase enzymes. As a result, many organisms resistant to penicillin's and some cephalosporins due to the presence of beta-lactamases, may be susceptible to cefixime. The antibacterial effect of cefixime results from inhibition of mucopeptide synthesis in the bacterial cell wall [2, 3]. Cefixime use in the treatment of uncomplicated urinary tract infections, otitis media, pharyngitis and tonsillitis, acute bronchitis and acute exacerbations of chronic bronchitis and uncomplicated gonorrhea (cervical/urethral) [2, 3].

Biochemical and pharmacological quality control tests for different brands of pharmaceutical products that contain the same active ingredients are vital steps to confirm therapeutic equivalence for such products. Additionally, oral dosage forms depend profoundly on dissolution studies *in vitro* to predict their bioavailability *in vivo* [1, 4]. Furthermore, the official and unofficial quality control tests *in vivo-in vitro* are required to confirm the safety and efficacy of any pharmaceutical product [5].

In Yemen as well as other poor countries, the price of drugs is the main factor in determining patient's access to health care; where many people put off the use of needed medicines due to the high cost of branded products. Moreover, few studies have been conducted so far to evaluate drugs' quality control in Yemen [5-9]. Therefore, further studies should be conducted in this field to evaluate the quality control tests for locally and internationally manufactured drugs, to ensure the quality and efficacy of the pharmaceutical products, and to offer suitable substitutions to patients. Even though there are many different brands of cefixime available in the Yemeni market and the clinical use of cefixime is highly increased among the public, there is no quality control study has been conducted on this field in Yemen. The findings of this study can be used as a source of information to drug regulatory authorities and drug manufacturers in Yemen. There are about 17 brands of this antibiotic, most of which are in form of 400-mg tablets and 5 brands in form of 400-mg capsules, including 2 domestic brands available in Yemeni drug market. There is no published study regarding the assessment of post-marketing quality tests of these products conducted in Yemen. Accordingly, this study aimed to evaluate the quality control of different brands of cefixime 400 mg capsules available in the Yemeni market [10].

MATERIALS AND METHODS

Material

Cefixime reference standard was a gift from Modern-Pharma Co, Yemen. Brands: Five brands of cefixime that were two national, one original, and two Arabian brands were purchased from the drug markets. Other materials used were: Methanol (Comp Hi media Laboratories, country of India). Distilled water: (comp. Hi media laboratories).

Instrumentations

HPLC (High-Performance Liquid Chromatography; Waters, model: Pump, 1525, Detector, 2998, Germany) and an Inertsil® ODS-3V C_{18} column (250 mm×4.6 mm, 5 µm), Japan was used. Electric balance (Mettler Toledo, USA), Mixture (JJ-1mixer, China). Water bath (Pharma test, Germany), Centrifuge (China), pH meter (Metrohm, Germany), Disintegration tester (Pharma test. Germany), Dissolution tester (DT 600, Erweka, Germany) and Filter paper (AU 480, Beckman Coulter, American).

Methods

The quality control tests (Official), including assay content, weight variation, disintegration time, dissolution profile and antimicrobial activity, were studied *in vitro* for comparison between five commercial brands of cefixime 400 mg capsules that available in Yemeni markets.

Sample (brands) collection

Five brands of cefixime 400 mg capsule were obtained from different selling pharmacies in Sana´a City, Yemen. The study samples were collected from October 8th to October 28th, 2022. All of the selected brands of cefixime were capsules. The five brands were checked for their strength, batch number, manufacturing and expiry dates, as well as they, were coded from A to E (table 1). The standard procedures were used during all analytical processes. The study samples were stored at 25 °C according to the manufacturers' instructions before evaluation. All chemicals used were of analytical grade.

Table 1: List of the tested commercial cefixime 400 mg capsule in yemen

Brands name	Country	Manufacturing company	Code	Strength	Batch. no	Mf. date	Ex. date
Suprax	Jordan	(Hikma)	A	400 mg	220042	01/2022	01/2025
Goxime.	Yemen	(Global Pharma.)	B	400 mg	21685	11/2021	11/2024
Rofix	Yemen	(RFA)	C	400 mg	22B009	08/2022	08/2025
Magnacef	Jordan	(Pan. Pharm.)	D	400 mg	W376	03/2022	03/2024
Fixoral	Syria	(Alpha)	E	400 mg	21427 A	04/2022	011/2024

Physicochemical tests measurement

Weight variation

Twenty hard gelatin capsules were usually weighed individually, and the contents were removed. The emptied shells were individually weighed, and the net weight of the contents was calculated by subtracting the weight of the shell from the respective gross weight. The content of active ingredient in each capsule may be determined by calculation based on the per cent drug content in the formulation. The average of weight variations for all brands was calculated.

The variation of weight for all brands capsules should not deviate from±5 % according to USP [11].

According to USP [11], the capsules complied the specification if no more than 2 capsules deviate by more than twice the limits which are±10% (if average capsule powder weight is<130 mg), ±7.5% (If average capsule powder weight is 130–324 mg), ±5% (if average capsule powder weight is>324 mg).

Content uniformity assay

The contents uniformity test of the capsules was carried out using HPLC. 25 ml of 0.4 M tetrabutylammonium hydroxide solution diluted with water to 1000 ml, and adjusted with 1.5 M phosphoric acid to a pH of 6.5 as buffer and acetonitrile in ratio (75:25) was used as mobile phase. The flow rate of the mobile phase was adjusted to that the retention time of cefixime is 10 min. and the injection volume of the sample was 10 µl. Cefixime detection wavelength was set at 254 nm. The acceptable limit for drug content, chemical compliance and content of active ingredients uniformity tests were carried out in accordance with the standard method specified in USP [11].

Standard calibration curve

A stock standard solution of 0.1 mg/ml was prepared by dissolving 100 mg of cefixime reference standard in 50 ml of methanol and complete the volume to 100 ml with 0.1 N HCl to prepare standard stock solution of concentration (1 mg/m). Transfer 5 ml of stock solution to another 50 ml volumetric flask and complete the volume to 5 ml with mobile phase (25 ml of 0.4 M tetrabutylammonium hydroxide solution diluted with water to 1000 ml, and adjusted with 1.5 M phosphoric acid to a pH of 6.5 as buffer and acetonitrile in ratio (75:25)) to prepare solution of concentration (100 µg/ml). Serial dilutions were employed to produce 6 standard solutions with concentrations 5-30 µg/ml. Chromatographic process was performed under the stated chromatographic conditions. Calibration curves related to the obtained peak areas of the drug to the corresponding concentrations were made, and the regression equations were calculated. Measure the (High-Performance Liquid Chromatography) HPLC chromatogram at 245 nm for six concentrations and repeat each one three times and write the required chromatographic data.

Test solution

Ten capsules content of each brand were weighed and then an amount of the powder theoretically equivalent to 100 mg of cefixime was dissolved in 50 ml of the methanol and complete the volume to 100 ml with 0.1 N HCl and filter,10 ml of previous solution was taken into 100 ml volumetric flask and the volume was completed with mobile phase solution (buffer and acetonitrile in ratio (75:25)) to provide a solution with a theoretical concentration (Ct) of (7.5, 17.5, 27.5 µg/ml). The HPLC chromatogram of the resultant solution was measured at 254 nm. The test was performed in triplicate and the average peak area was then introduced in the regression equation to calculate the practical concentration (Cp). Drug content was eventually calculated from the following equation

$$\text{Drug content } \% = \frac{\text{Cp}}{\text{Ct}} \text{X100}$$

Where Cp will be the practical concentration and Ct were the theoretical concentration (7.5, 17.5, 27.5 µg/ml).

The drug content of any brand which was within the limit of (90-110%) will be considered complied to USP specifications [11].

Disintegration

Six capsules of each brand were investigated using a disintegration apparatus. The disintegration medium used was 900 ml 0.1 N HCl at 37±1 °C. The time taken to break all capsules with complete disappearance of capsule from the mesh was determined. According to USP, capsule brand complies specifications if all six capsules disintegrate within no more than 30 min [10, 11].

Dissolution

Dissolution test (*in vitro* drug release) was used as a part of the *in vitro-in vivo* correlation. The dissolution test parameters should be set to be identical to the human body's conditions [12]. The dissolution study was conducted using the USP dissolution apparatus II (paddle apparatus), (Ewerka, Type DT6, Germany). 900 ml of 0.1M HCL was used as the dissolution medium for the first hour followed by phosphate buffer pH 6.8 maintained at 37 °C±2 °C with a rotation speed of 75 rpm. At intervals of 30 min, 10 ml samples were withdrawn using a pipette and replaced with equal volumes of fresh solution. The withdrawn samples were filtered with Whatman filter paper (No. 1) and suitably diluted. The diluted

filtrates were analysed on a HPLC ((HPLC; Waters, model: Pump, 1525, Detector, 2998, Germany)) at a wavelength of 254 nm. For calculation of cefixime sample concentration, the peak area of the sample was introduced into the regression equation of calibration curve and the percentage of drug release was computed [13].

Statistical study

The Statistical Package for Social Sciences (IBM SPSS) version 30.0 was used to perform the statistical analysis. Single-way analysis of variance (ANOVA) was used for comparing the quality assessment tests results of the five brands of cefixime 400 mg capsule that indicated a significant difference in all the tests results when the *p*-values less than 0.05.

RESULTS

In the present study, the quality of five brands of cefixime 400 mg capsules was assessed. The capsule brands investigated were five officially registered brands, including two local and three international brands, which are available in Yemeni drug market.

Identification tests revealed that all brands contained "cefixime" compared to reference standard of the drug. The stated drug may contain in those brands.

Regarding drug content in the tested brands investigated by HPLC, it was found that the standard calibration curve (fig. 1) for that test was with R^2 = 0.9998, which confirmed the validity of the test and correlation of the response of the instrument to small changes in drug concentration.

Fig. 2 below represents that the correlation coefficient (R^2) = 0.9998) was not more than 01 and not less than 0.9, but R^2 between linearity limit (0.9 – 01). This linearity indicates that the HPLC system used for measuring different concentrations of cefixime is calibrated and appraises.

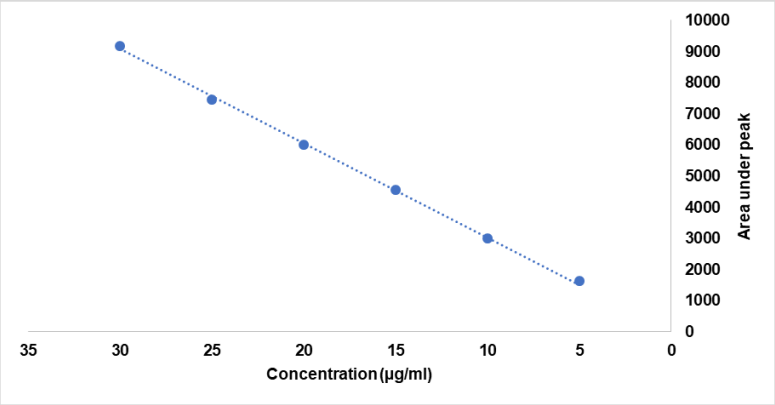

Fig. 1: Calibration curves for the HPLC determination of cefixime (5-30 µg/ml) using buffer and acetonitrile (95:5), adjusted with phosphoric acid to a pH of 3.8 as the mobile phase at adjusted flow rate to produce Rt 10 min. and UV detection at 245 nm

The five tested brands showed that the drug contents were ranged from 78.78-104.46 %, which were not compliance to USP specification (90-110%) for two brands (A and C) with 89.73% and 78.78%, respectively, and in compliance to USP specification (90-110%) for three brands (B, D and E) with 99.05%, and 103.88% and 104.46%, respectively

Similarly, the results of capsule disintegration and weight variation of all the five brands were within allowed limits (tables 2, fig. 2). However, the results of capsule dissolution of four brands (A, B, D and E) were within the allowed limit more than 80% of concentration after 30 min with 108.14%, 87.13%, 109.82% and 86.20%, respectively, and for one brand (C) were out of the limit that less than 80% of the concentration after 30 min with 73.56% (table 2, fig. 2).

In addition, the results of antibacterial activity test for the five brands of cefixime were acceptable according to the USP limits in which the zone inhibition must be more than 12 mm. In comparison with five brands, the C brand had more antibacterial activity than the four other brands (table 3, fig. 2). These results indicate that brand C had a good antibacterial effect; although it had bad quality control results specially in assay content and dissolution profile. This means that the bad results may be due to a manufacturing defect or due to bad storage or bad transportation.

Table 2: Results of weight variation, drug content, disintegration time and dissolution profile for five brands of cefixime 400 mg capsules

Brands codes	Wt. variation test (gm) (n=20)		Drug content % (n=5)	Disintegration time (min) (n=6)	Dissolution profile (%) (n=6)
	Mean±SD	The limit (5%)			
A	0.498±0.006	0.025	89.73±3.85	15:05±1:48	100.14±2.55
B	0.51± 0.009	0.026	99.05±1.51	10:56±0:53	87.13±0.37
C	0.578± 0.013	0.029	78.78±4.18	11:20±1:04	73.56±0.16
D	0.509± 0.0097	0.026	103.88±0.68	12:20±0:47	109.82±0.40
E	0.509± 0.0095	0.026	104.46±4.58	12:07±1:33	86.2±1.01

SD; standard deviation, min; minute, n; number of tested samples

Table 3: Results of antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* for five brands of cefixime 400 mg capsules

Brands code	*Staphylococcus aureus*		*Escherichia coli*	
	mean of inhibition zone (n=5)	SD	mean of inhibition zone (n=5)	SD
A	22.33	0.577	34.67	1.155
B	22.00	0.000	34.67	0.577
C	25.67	0.577	35.33	1.155
D	22.00	1.155	34.33	0.577
E	22.33	0.577	32.67	1.155

SD; standard deviation, n; number of tested samples

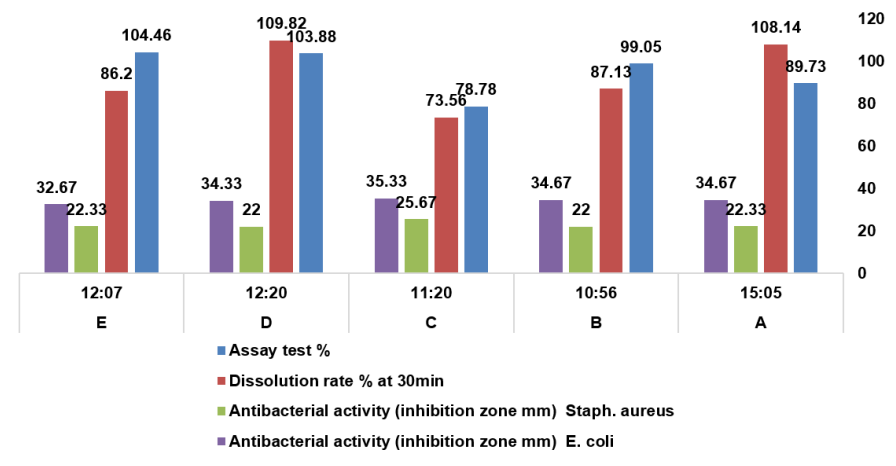

Fig. 2: Results of drug content (n=5), disintegration time (n=6), dissolution profile (n=6) and antibacterial activity against *Staphylococcus aureus* (n=5) and *Escherichia coli* (n=5) for five brands of cefixime 400 mg capsules, the results of the statistical study indicate a significant difference in all the tests results, where the *p*-values was less than 0.05, as showed in table 4

Table 4: Statistical comparison of results of quality control tests for different brands of cefixime 400 mg

Brands code	Weight variation (gm) (n=20)		Disintegration time (min) (n=6)	Assay content test % (n=10)	Dissolution rate % at 30 min (n=6)	Antibacterial activity (inhibition zone mm) (n=5)	
	Mean	SD				*Staph. aureus*	*E. coli*
A	0.498	0.006	15:05	89.73	108.14	22.33	34.67
B	0.510	0.009	10:56	99.05	87.13	22.00	34.67
C	0.578	0.013	11:20	78.78	73.56	25.67	35.33
D	0.509	0.0097	12:20	103.88	109.82	22.00	34.33
E	0.509	0.0095	12:07	104.46	86.20	22.33	32.67
(*p*-value)	3.6E-12*					7.57E-07#	

SD: Standard Deviation, n; number of tested samples, *Staph. Aureus; Staphylococcus Aureus*, *E. coli; Escherichia coli*. *ANOVA-single way-test between the results (means and SD) of Quality assessment tests (Wt. variation, drug content, disintegration time, and dissolution profile, *respectively,)* for five brands of cefixime 400 mg capsules indicated sig. variation ($p<0.05$). #ANOVA-single way-test between the results (means and SD) of Quality assessment tests antibacterial activity against *Staphylococcus aureus* and *Escherichia coli, respectively, for five* brands of cefixime 400 mg capsules indicated sig. variation.

DISCUSSION

To the better of our knowledge, this study was conducted as the first one to evaluate official quality control parameters of different brands of cefixime 400 mg capsules that were available on the Yemeni market.

Weight variation: This test is one of the tests which are performed to ensure constant dosing among capsules to lessen the commonness of overdosing or underdosing. There appears to be a direct correlation between the variation in the weight of individual capsules and the corresponding variation in the drug content [13]. The amount of granules placed in the body of a capsule will ultimately determine the weight of the encapsulated products [14]. Sampled brands of cefixime capsules had weights of greater than 300 mg. Therefore, for a batch of such capsules to pass the weight variation test, not more than two of the individual weight of the capsules should deviate from the average weight by more than the percentage deviation of ±5% as recommended by USP [11]. The finding of the current study showed that all five brands (A, B, C, D and E) were passed the USP specifications. A comparable study conducted in Karachi (Pakistan) showed remarkable variations in the weight, length and diameter of cefixime capsules 400 mg [15].

Drug content: The findings show that three brands of cefixime capsules 400 mg were conformed to the specifications as stated in the USP, while two brands of cefixime were not complied to USP specifications (table 2). A comparable study conducted in Karachi (Pakistan) and Nigerian market showed no variation in drug contents of cefixime capsules 400 mg [15, 16].

Disentegration: The disintegration time is an important component in the release of the pharmaceutical active ingredient (cefixime) from the capsule. The ability of any capsule to pass the disintegration test chiefly depends on the nature and integrity of the polymer used in designing the coating for the capsule pellets. Other factors, such as the particle size of pellets and the temperature of the fluid in the disintegration apparatus, can contribute significantly to the capsules passing or failing the test [13]. In the current study, all the tested brands passed the disintegration test (table 2), indicating that the method and the polymer used in manufacturing the capsules were good and will release their contents within the stated time.

Dissolution: the dissolution rate is required as an essential criterion for drug bioavailability to confirm the drug release pattern of the dosage form[10]. Four brands in the current study released their active contents in a range of 86.20% to 109.82% within the allowed time as specified by USP, while one brand released their active contents in a percentage 73.56% not within the allowed time specified by USP. Compared to the general findings of this study, previous studies performed on cefixime 400 mg in Pakistan confirmed that all the brands of cefixime 400 mg showed satisfactory results regarding the physicochemical parameters tested as the USP specifications [15].

CONCLUSION

Based on the results obtained in this study, the drug content for three brands (B, D, E) is within the pharmacopeial limit 90-110 %, but the drug content of (A, C) is out of the limit (90-110 %); while the capsule disintegration, weight variations, and antibacterial activity in all tested brands are within pharmacopeial limit. The dissolution profile for four brands (A, B, D, E) is within the pharmacopeial limit more than 80% after 30 min, but the dissolution profile for (C) is less than the allowed limit 80 % after 30

min. The result of antibacterial for brand (C) is the best in comparing the other brands.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Dr. Ahmed Al-Ghani (Corresponding author): concept and design of the manuscript and data collection and interpretation. Dr. Mohammed Alkhawlani (2nd author): Statistical analysis. Dr. Amin Alwosabi and Dr. Abdullah Albegali (3rd and 4thauthors): experiments. Dr. Anes Thabet (5th author): Review of literature.

CONFLICTS OF INTERESTS

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

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