Evaluation of Different Brands of Cefixime Available in Pakistan by Newly Developed Spectrophotometric Method

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Authors' contributions

This work was carried out in collaboration among all authors. Author MLM got idea, designed research methodology, authors URM, BS, data interpretation and manuscript final reading and approval. Authors GK, MQ literature review and author HML literature search, and English language setting, author SMU article drafting. Author NK literature review and critical analysis, Author DD research design and methodology and author SAL final approval of the idea and approval of the draft. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

A fast, simple and valued method is developed to observe the quality and quantity of different pharmaceutical brands of cefixime. A spectrophotometric method has been developed for analysis of cefixime (CFX) by reacting with 4-dimethylamino benzaldehyde (DAB) as derivatizing agent. The molar absorptivity of CFX-DAB, newly synthesized derivative was calculated as 3.2×10^5 L.mole^{-1}cm^{-1} and λ maximum was 393 nm. The calibration curve was developed in range of 5-25 µg.mL^{-1} as this concentration followed beers law. The derivatization reaction is stable and didn't show any difference in absorbance with radiation interaction for up to one day. The percentage recovery of CFX was checked and calculated in different pharmaceuticals was within 95 to 99.5% with RSD

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value calculated in between RSD 0.69-0.96% (n=3), respectively. This newly developed and validated procedure was proved to be accurate and precise for the analysis of CFX. This method was successfully applied to check amount of CFX from 7 different brands of pharmaceutical preparations commercially available in Pakistan.

1. INTRODUCTION

The cefixime (CFX) chemically known as (6R, 7R)-7-[2-(2-amino, 4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, 7^2-(Z)-[O-(carboxy methyl)oxime]trihydrate [1]. It was derived from fungus *cephalosporiumacremonium*. The chemical structure is given in Fig. 1. It is indicated in various infections caused by bacterial species i.e. Anaerobes, Enterobacteriaceae etc., Gram-negative species i.e. H. influenzae, B. Catarrhalis, E. coli, N. gonorrhoeae, klebsiella, Serratiamarcescens, Haemophilus, Providencia, and Meningococcus i.e. β-lactamase. It is known to be safe and efficacious drug. [2,3]. CFX is thought to be used for drug resistant bacteria [4]. Due to the frequent prescribing of CFX, it is necessitated to analyze from dosage form and from biological fluids as well. In this regard many methods for analysis i.e. (microbiological and analytical) have been developed to analyze CFX from different pharmaceutical dosage forms [5]. CFX is analyzed quantitatively from bulk materials and different dosage forms by different techniques i.e. spectrofluorimetric [6-8], spectrophotometric [9-11], colorimetric, HPLC-CE [12], voltammetry [13-15], HPLC-MS [2,16] and HPLC methods [17-19]. In this paper, an easy and fast method is reported for analysis of CFX using derivatization at spectrophotometer. 4-dimethylamino benzaldehyde (DAB) has been utilized as derivatizing agent. The method was applied successfully for analysis of CFX from dosage forms with excellent percentage recovery i.e. 95 to 99.5%. The new method was successfully applied to check amount of CFX from 7 different brands of pharmaceutical preparations commercially available in Pakistan.

2. EXPERIMENTAL

2.1 Materials and Reagents

Cefixime (CFX) was purchased from GlaxoSmithKline (GSK) Pvt. Ltd. Karachi, 4-dimethylaminobenzaldehyde (DAB) and acetic acid were purchased from E. Merck, Germany, sodium acetate from Fluka, Switzerland and ethanol from BDH, (U.K). Hydrochloric acid, ammonium chloride, ammonia solution, sodium carbonate and bicarbonate were purchased from Sigma-Aldrich Pvt. Ltd.

2.2 Instruments and Apparatus

The spectrophotometric studies were carried out with a double beam spectrophotometer UV 1601 (Shimadzu Corporation, Japan) and Perkin Elmer, Lamda 25 UV/Visible spectrometer (USA) with dual silica 1-cm cuvettes. The pH was checked using Orion model 420, a pH meter with glass electrode and combined reference electrode made by Orion Research Inc Boston USA.

2.2.1 Analytical procedure for determining CFX

In this work simple and inexpensive laboratory chemicals have been used to form the stable derivative. The ethanol solution (500-1000 µL) containing CFX (5-25µg mL-1) was transferred to 5 ml volumetric flask followed by addition of DAB (2% in ethanol w/v) up to 500µL. In last addition of 500 µL of acetate buffer (pH 5). The contents were heated on water bath at 95 ^{0}C for at least 20 minutes. After heating, the solution was cooled at room temperature and the volumes were adjusted to mark with ethanol. The absorbance was measured at 393 nm. For checking absorption of solution against blank/solvent which was run. The reaction is given in Fig. 2. The molar absorpitivity of CFX alone was observed as 3.4 x 10^4 L mole^{-1} cm^{-1}. CFX or with 4-dimethylaminobenzaldehyde (DAB) to form an imine derivative which absorbs at 393, nm maximally with bathochromic shift having molar absorptivity of 3.2 x 10^5L.mole^{-1} cm^{-1}.

3. RESULTS AND DISCUSSION

3.1 Optimization of Parameters

3.1.1 Analytical wavelength

For the analysis of drugs, the wavelength of maximum absorbance plays very important role.

It is compulsory to ensure that derivatizing reagent should not absorb close to the region where the drug-derivative absorbs. This may cause inaccuracy in absorption of the drug the reason is that, derivatizing reagent is added in excess to complete the reaction quantitatively. That's why it is mandatory to select the wavelength where the analyte derivative shows maximum absorbance value and the derivatizing reagent indicates minimum absorbance. The absorbance value of 5 µg.mL^{-1} of CFX-DAB derivative was recorded at different wavelengths between 230-550nm after heating for 20 minutes at 95 ^{0}C in presence of buffer with pH 5. It was noted that the maximum absorbance occurs at 393 nm against reagent blank, therefore, the wavelength of 393 nm was selected as ʎ-max.

3.1.2 Effect of concentration of derivatizing agent

The effects of adding various amounts of DAB ethanolic solution i.e. 0.5-3 ml on absorbance of 5 µg.mL^{-1} CFX, was checked. The amount equal to 0.5 ml DAB (ethanolic solution) when added to 5 µg.mL^{-1} CFX have given maximum absorption at λ max 393 nm.

3.1.3 Effect of heating time on derivatization

The effect of time on absorbance of 5 µg.mL^{-1} CFX solution in presence of 2% DAB solution was checked at 393 nm from 0-30 min with an interval of 5 min. Maximum absorbance was observed after heating for 20 min. That's why 20 minutes was considered as optimal heating time of derivatization.

3.1.4 Effect of solvents for derivatization

The effect of various solvents was checked to know the best solvent for derivatization reaction such as ethanol, 1-propanol, 1-butanol, amyl alcohol, isoamyl alcohol, acetonitrile, ethyl acetate, toluene, nitrobenzene and carbon tetrachloride on the absorbance of 500 µL of CFX 5 µg.mL^{-1}. All the other reaction conditions were kept same for this study. It was observed that the best solvent for this study was ethanol. In ethanol all the drug standards were dissolved for the analysis. All the results are given in Table 1.

3.1.5 Effect of pH

The effect of adding 0.5 mL of buffers of different pH values i.e. 1-10 was checked on the absorbance of 5 µg.mL-1 CFX solution with all the conditions already set. It was observed thatthe absorbance increased gradually from pH 1 to pH 5. Addition of buffer above pH 5 observed to produce precipitation as shown in Fig. 3. Therefore, the acetate buffer of pH 5 was selected as optimum.

3.2 Interference Study

The effect of various chemicals such as mannitol, sorbitol, sucrose, lactose, glucose, glactose and fructose was investigated to check whether the method was selective or not. The amount of these chemicals were increased up to 10 times of that of CFX and it was observed that none of these substances varied the absorbance in range more than ± 0.5% as shown in Table 2. The allowable limit of interference was ± 5%. This study proved that the newly developed method was very much selective for CFX analysis. This study was checked by same method as given in Maheshwari et al., [3].

3.3 Stability of the Derivative

The stability of derivative was examined in terms of absorbance at the concentration of 5 µg.mL^{-1} CFX for about a period of 48 hours. It was observed that no change in absorbance of more than 4 % was seen up to this period that's why the new method was stable.
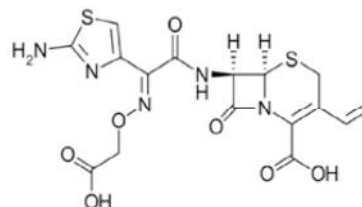

Fig.1. Chemical structure of cefixime

3.4 Calibration Graph

A linear calibration curve was obtained which obeyed the Beer's law within the concentration range 5-25 µg.mL^{-1} of CFX with R^{2} value of 0.999 as shown in Fig. 4. The sandells sensitivity (0.004) was observed at 4 µg.mL^{-1} CFX-DAB. The validity of the calibration curve was obtained by the analysis of test solution of CFX and the percent relative error was found ±1-3%.

3.5 Applicability of Method

To check the applicability of newly developed method, seven samples containing CFX were

purchased from local market and were analyzed to determine the quantity of CFX (Table 03). The mean values (g), percentage recovery and relative standard deviations are given for all the samples. Seven samples of different pharmaceutical companies which were analyzed by this method were cebosch (Bosch Pharmaceuticals Pvt. Ltd.), Maxpan (Indus
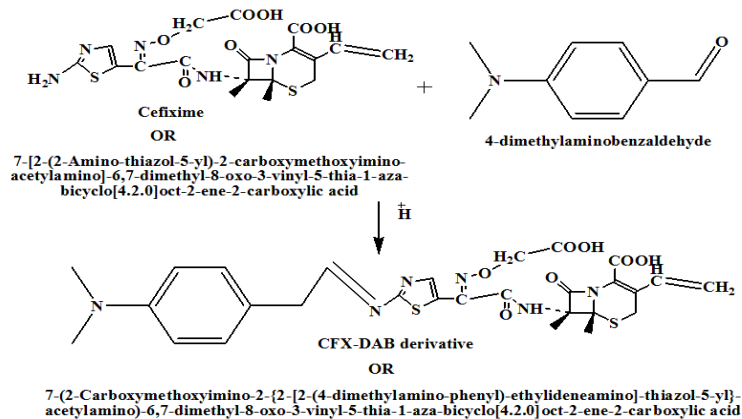

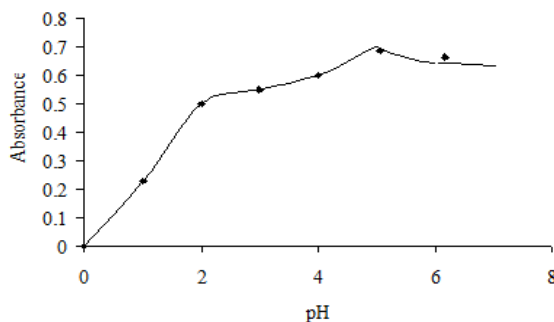**Fig. 2. Diagramtic reaction for synthesis of CFX-DAB derivative**

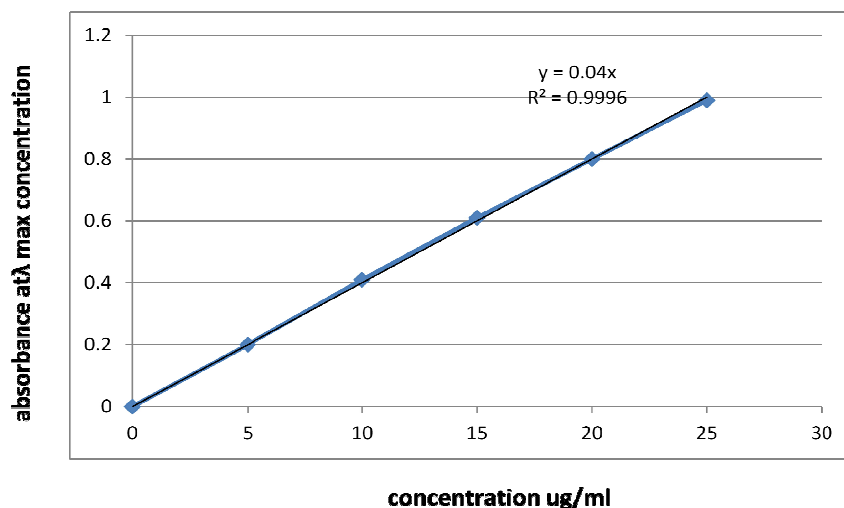**Fig. 3. Effect of Buffer pH on derivatization reaction**

Fig. 4. Calibration curve for analysis of CFX

Table 1. Effect of addition of solvent for derivatization reaction

Solvents	Volume (mL)added	Average effect (%) CF
Ethanol	0.5	00
	1	0.25
1-Propanol	0.5	1.3
	1	1.1
2-Propanol	0.5	2.0
	1	1.1
1-Butanol	0.5	9.8
	1	6.0
Amyl Alcohol	0.5	9.8
	1	3.7
1,2-propandiol	0.5	3.1
	1	3.3
Acetonitrile	0.5	4.2
	1	4.9
Ethyl Acetate	0.5	11.1
	1	1.7
Tetra hydrofuran	0.5	71.2
	1	61.4
Water	0.5	0.1
	1	0.2

Table 2. Effect of addition of chemicals on absorbance of CFX-DAB

S.#	Chemical added	Absorbance (nm) and relative errors = (%)
1	Mannitol	0.31(0)
2	Sorbitol	.29(6.5)
3	Glucose	0.30(3.2)
4	Galactose	0.31(0)
5	Fructose	0.32(3.1)
6	Sucrose	0.30(3.2)

Table 3. Applicability of method for analysis of various pharmaceutical formulations

S.#	Drug.	Amount labeled(g) sample	Amount found (g) sample	Relative Standard deviation %	% Recovery
1.	Cefexol	0.4	0.398	(0.19)	99.0
2.	Carisef	0.4	0.398	(0.08)	99.3
3.	Cebosh	0.2	0.197	(0.17)	96.0
4.	Cefim	0.2	0.197	(0.13)	100
5.	Cefspan	0.2	0.189	(0.13)	99.3
6.	Maxpan	0.2	0.199	(0.12)	99.9
7.	Evofix	0.1	0.097	(0.15)	98.9

Pharma Pvt. Ltd.), Cefspan (Barrett Hodgson Pvt. Ltd.), Carisef (Sami Pharmaceuticals Pvt. Ltd.), Cefxol (Nabiqasim Pvt. Ltd.), Cefim (Hilton Pharmaceuticals Pvt. Ltd.) and Evofix (Pharm Evo Pvt.Ltd.). All the samples were dissolved in 100 mL of ethanol in distilled water (50% v/v). The solution (0.2 mL) was transferred to 05 mL calibrated flask and the content of CFX was determined following the general procedure as described in analytical procedure for derivatizing.

The amount of CFX from each of the sample was calculated using the external calibration curve.

4. CONCLUSION

Evaluation of drugs is necessity of time for checking quality and quantity. In this regards we present a simple and fast method to check the quality and quantity of different pharmaceutical brands of cefixime available in market of

Pakistan. A spectrophotometric method was developed for analysis of cefixime (CFX) by derivatization with 4-dimethylamino benzaldehyde (DAB). The maximum absorption was observed at λ max 393 nm. Different parameters were optimized for this method, i.e. wavelength, time of reaction, amount of solvents etc. and best one were selected from each. In last interference studies were checked with respect to common chemicals and the method was found selective among all. The method is easy, economical and valid for routine analysis and can be applied by pharmaceutical industries and hospitals for routine quality analysis. The calibration curve was found linear over a range of 5-25 $\mu g.mL^{-1}$. The percentage recovery of CFX was checked and calculated in different pharmaceuticals was within 96 to 100% with RSD value calculated in between RSD 0.08-0.19% (n=3), respectively. The method developed by this way was successfully applied to check amount of CFX from 7 different brands of pharmaceutical preparations commercially available in Pakistan. Among all the checked brands Cefim was having 100% claimed amount of Cefixim, followed by Maxpan having 99.9%. All the other products were having the Cefixim in allowable limit.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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