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Spectrophotometric determination of ceftriaxone using 4-dimethylaminobenzaldehyde

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Abstract

A new spectrophotometric method has been developed for the determination of the potent antibiotic ceftriaxone (CF) by derivatization with 4-dimethylaminobenzaldehyde (DAB). The derivative indicated molar absorptivity of 5.3 x 10^3 L mol^{-1} cm^{-1} at λ_{max} 397 nm and obeyed the Beer's law within 20-100 μgmL^{-1}. The color reaction was highly stable and did not show any change in absorbance up to 24 h. The method was successfully applied for the determination of CF from various pharmaceutical preparations available in local market. The amounts of CF found in various pharmaceutical preparations were within 237.4-990 mg $ampoule^{-1}$ with standard deviation (SD) ± 0.0004-0.044 (n=3) respectively.

Keywords: *Ceftriaxone, 4-dimethylaminobenzaldehyde; spectrophotometry.*

Introduction

Ceftriaxone (CF) (6*R*,7*R*,*Z*)-7-(2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid is a third-generation cephalosporin antibiotic. Like other third-generation cephalosporins, it has broad spectrum activity against Gram negative and Gram positive bacteria [1]. CF is often used (in combination with macrolide and/or aminoglycoside antibiotics) for the treatment of community-acquired pneumonia. It is also a drug of choice for the treatment of bacterial meningitis. In pediatrics, it is commonly used in febrile infants. It has also been used in the treatment of leptospirosis [2], lyme disease and gonorrhea. It is also used as a routine prophylactic antibiotic for the patients undergoing orthopedic surgery [3]. Several analytical methods have been reported for the analysis of CF, based on spectrophotometric [4-10], derivative spectrophotometric [11], FIA [12], flourimetric [13, 14], thin layer chromatographic [15-17], ion selective electrodes [18], ion exchange chromatographic [19], high performance liquid chromatographic [20, 21], ion-pair liquid chromatographic [22] and polarographic [23, 24] techniques. For spectrometric analysis, the determination is carried out using suitable reagents such as metol-chromium(VI) reagent, mixture of Fe(III) and hexacyanoferate(III) ions, leuco crystal violet and 3-methyl-2-benzothiazoline hydrazone hydrochloride and ferric chloride [4-10, 25]. The derivatization either increases the molar absorptivity or produces bathochromic shift in the absorbance. Therefore, 4-dimethylaminobenzaldehyde, (DAB) is reported for the first time as a derivatizing reagent for spectrophotometric determination of CF in pharmaceutical preparations.

Experimental

Materials and reagents

All the chemicals and reagents used were of analytical grades. The double distilled water was used throughout the study. Pure ceftriaxone (CF), 4-dimethylaminobenzaldehyde (DAB) and acetic acid were obtained from E. Merck (Germany), sodium acetate from Fluka (Switzerland) and ethanol from BDH (U.K) were used. Buffer solutions between pH 1-10 at unit internal were prepared from hydrochloric acid (0.1M), potassium chloride (1M), acetic acid (1M), ammonium acetate (1M), sodium acetate (1M), sodium carbonate (saturated solution), sodium bicarbonate

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(1M), potassium chloride (1M), ammonium chloride (1M) and ammonia solution (1M).

The solution of DAB (2% w/v) was prepared in ethanol (100mL). The spectrophotometric studies were carried out with a double beam spectrophotometer UV-1601 (Shimadzu Corporation, Japan) with fused silica 1cm cells.

General procedure

The aqueous solution (0.2-1.0mL) containing CF (200-1000 µg) was transferred to 10mL calibrated flask and were added 3mL DAB (2% in ethanol w/v) and acetate buffer pH 5 (1mL). The contents were heated on water bath at 90 ^{0}C for 15 min. The solutions were cooled at room temperature and the volume was made up to the mark with ethanol. The absorbance was measured at 397 nm against reagent blank which was prepared in a similar way only omitting the addition of CF.

UV method (Un-derivatized)

The solution (0.2-1.0mL) containing CF (20-100µg) was transferred to 10mL calibrated flask and the volume was made up to the mark with ethanol. The absorbance was measured at 272 nm against ethanol. The molar absorptivity was calculated as $3.4x10^4$ L mol^{-1} cm^{-1}.

Analysis of CF from pharmaceutical preparations

Thirty samples of different pharmaceutical companies were collected and analyzed for the contents of CF. The sample (0.1g) from each of the CF preparations, Acmex (Acme Laboratories (PVT) Ltd. Lahore, Pakistan), Aczon (Global pharmaceuticals Islamabad, Pakistan), Bestrix (Asian Pharmaceuticals Karachi, Pakistan), Broadced (Kalbe Pharma International (PVT) Ltd. Lahore, Pakistan), Cef-3 (Shazal'z Pharmaceuticals Rawalpindi, Pakistan), Cefcin (Cirin Pharmaceuticals (PVT) Ltd. Rawalpindi, Pakistan), Cefin (Macter International (PVT) Ltd. Karachi, Pakistan), Cefotrim (Pharmedic Laboratories (PVT) Ltd. Lahore, Pakistan), Ceftison (Qureshi Pharma (PVT) Ltd. Karachi, Pakistan), Ceftrex (Polyfine Chempharma (PVT) Ltd. Peshawar, Pakistan), Ceftridex (Rex Pharmaceuticals Karachi, Pakistan), Cefxone (Bosch Pharmaceuticals (PVT) Ltd. Karachi, Pakistan), Cepox (MBL Pharma Karachi, Pakistan), Cerixon (Genesis Pharmaceutical (PVT) Ltd. Lahore, Pakistan), Chef (Al-Habib Pharmaceuticals / Al-Habib Corporation Karachi, Pakistan), Chroncef (English Pharmaceuticals Industries Lahore, Pakistan), C-trox (Mediceena Pharma (PVT) Ltd. Lahore, Pakistan), Cyforon (Ali Gohar Pharmaceuticals (PVT) Ltd. Karachi, Pakistan), Dayzone (High-Q International Karachi, Pakistan), Eftriax (Pharmatec (PVT) Ltd. Karchi, Pakistan), Efxone (Candid Pharmaceuticals Lahore, Pakistan), Elxone (Ethel Pharma International Karachi, Pakistan), Farcef (Pulse Pharmaceuticals Lahore, Pakistan), Fotamin (Vision Pharmaceuticals Islamabad, Pakistan), Inocef (Barrett Hodgson (PVT) Ltd. Karachi, Pakistan), Lozon (Zesion Pharmaceuticals (PVT) Ltd. Islamabad, Pakistan), Macxone (Macquins International Karachi, Pakistan), Maxcef (Indus Pharma (PVT) Ltd. Karachi, Pakistan), Oxidil (Sami Pharmaceuticals (PVT) Ltd. Karachi, Pakistan) and Rociphin (Roche (PVT) Ltd. Karachi, Pakistan) was dissolved in 100mL of distilled water. The solution (0.6mL) was transferred to 10mL calibrated flask and the content of CF was determined following the general procedure as described in section B.

% Recovery of CF from pharmaceutical samples by standard addition technique

CF powder (0.1g) was dissolved in 100mL water. Two portions, each consisting 0.6mL were taken in two different 10mL calibrated flask. One was added with 0.2mL containing 200µg CF solution and the derivatization procedure was followed for both solutions as described in B.

The % recoveries were calculated from the increase in the absorbance with added standard.

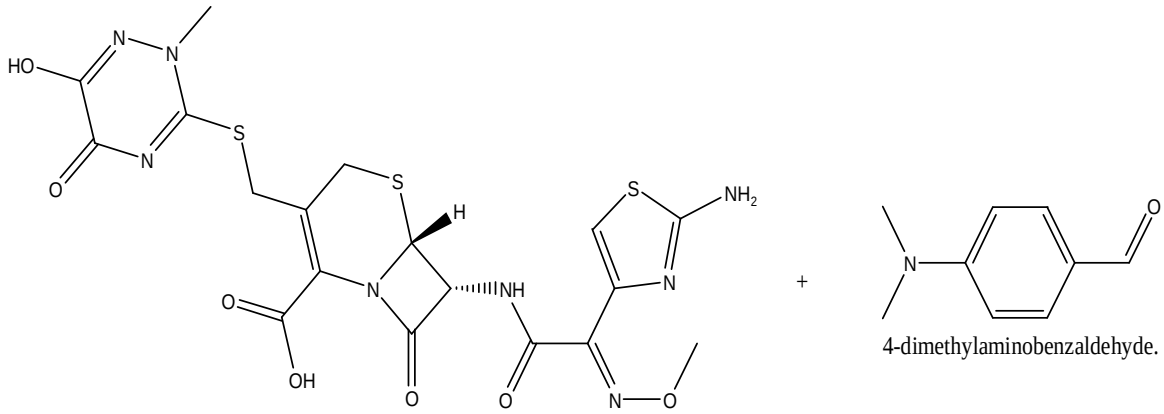
Results and Discussion

Ceftriaxone (CF) reacts with 4-dimethylaminobenzaldehyde (DAB) to form an azomethine derivative 7 - (2 - { 2 -[(4-Dimethylamino-benzylidene) –amino]–thiazol–4–yl } – 2 -methoxyiminocetylamino)-3-(6-hydroxy – 2 – methyl l - 5-oxo-2,5-dihydro -[1,2,4] triazin -3-ylsulfanylmethyl)-8-oxo-5-thia-1-aza-bicyclo [4.2.0]oct-2-ene-2-carboxylic acid (CF-DAB) (Fig. 1) which has maximum absorbance (λ_{max}) at 397 nm with molar absorptivity of $5.3x10^3$ L mol $^{-1}$ cm^{-1}. DAB was then tested as a derivatizing reagent for the spectrophotometric determination of CF. The effects of pH, effect of reagent (DAB), heating time and temperature on the formation of (CF-DAB) derivative were studied.

Optimization of analytical parameters

Absorption spectra for wavelength selection

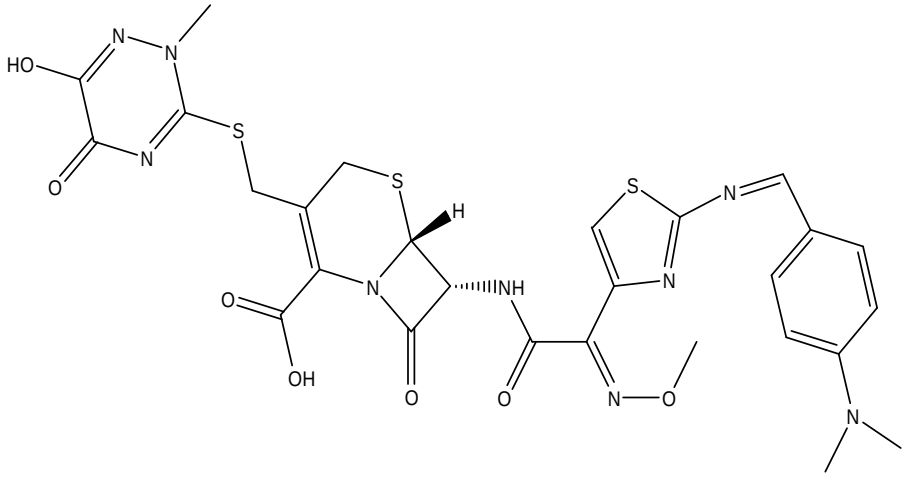
For the quantitative analysis, the wavelength of maximum absorbance plays an important role. It is necessary to select the wavelength where the derivatizing reagent indicates minimum absorbance and the analyte derivative shows maximum absorbance value.



(6R,7R,Z)-7-(2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid

+

4-dimethylaminobenzaldehyde.



7-(2-{2-[(4-Dimethylamino-benzylidene)-amino]-thiazol-4-yl}-2-methoxyimino-acetylamino)-3-(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-[1,2,4]triazin-3-ylsulfanylmethyl)-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Figure 1. **Formation of CF derivative using 4-dimethyl aminobenzaldehyde as derivatizing reagent**

The absorbance value of 20 µg mL^{-1} of CF as DAB derivative was recorded at different wavelengths between 250-500 nm after heating for 15 min at 90^0C using buffer pH 5. It is evident that the maximum absorbance occurred in visible region at 397 nm against reagent blank and was selected as optimum.

Effect of reagent concentration

The effects of adding various amounts of DAB solution on absorbance of 20µg mL^{-1} CF was examined (Fig. 2). The concentration of reagent DAB was varied between 1-6mL of 2% in ethanol with an interval of 1mL. A similar absorbance was observed with addition of 2 and 3mL and the addition of 3mL (2% w/v) DAB solution was selected.

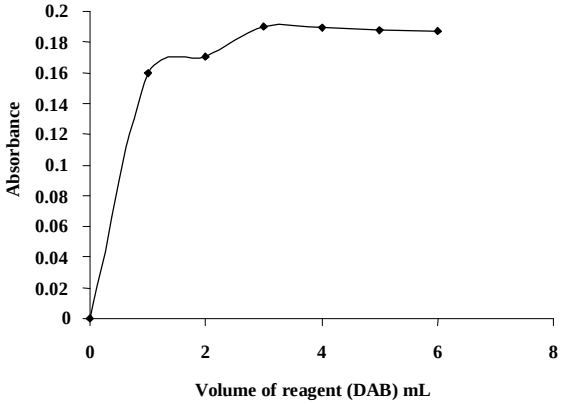


Figure 2. **Effect of volume of reagent on absorbance of CF derivative**

Effect of order of mixing the reagents

The order of adding reagents during derivatization process has important role in accuracy of results and enhancement of absorbance. In the present study, it was observed that the addition of buffer pH 5 (1mL) to 200 µg CF solution (0.2mL) followed by 3mL DAB reagent (2% w/v) resulted in a decrease in absorbance value. Taking the reagent first and then adding the buffer, followed by CF solution also had lower absorbance value. The maximum absorbance value was observed when 3mL of reagent DAB was added to the standard solution of CF followed by buffer (1mL) pH 5. The contents were then heated on water bath and the volume was adjusted to 10mL with ethanol.

Optimization of heating time and temperature for the formation of derivative

To achieve the maximum absorbance value for an analyte by the formation of stable derivative, the selection of optimum time and temperature is essential. The effect of time on the formation of derivative was checked at 397 nm from 0-30min with an interval of 5 min. A similar absorbance was observed after heating for 15 min at 90^0C and was considered as optimal.

Effect of solvents

The effect of various solvents such as methanol, 1-propanol, 1-butanol, amyl alcohol, isoamyl alcohol, acetonitrile, ethyl acetate, toluene, nitrobenzene and carbon tetrachloride on the absorbance was examined. From each of the solvents 1and 2 mL was added after the addition of 2% ethanolic solution of DAB, and 1 mL acetate buffer pH 5 followed by heating for 15 min at 90°C. The ethanol proved to be the best choice.

Effect of pH

The effect of adding 1mL of 1M buffers of pH range 1-10 on the absorbance at optimized conditions was studied.

It is evident from Fig. 3 that the absorbance increased gradually from buffer pH 1 and reaches to maximum value at pH 5. Addition of buffer of pH 8 and above produced precipitation. Therefore, the acetate buffer of pH 5 was selected as optimal.

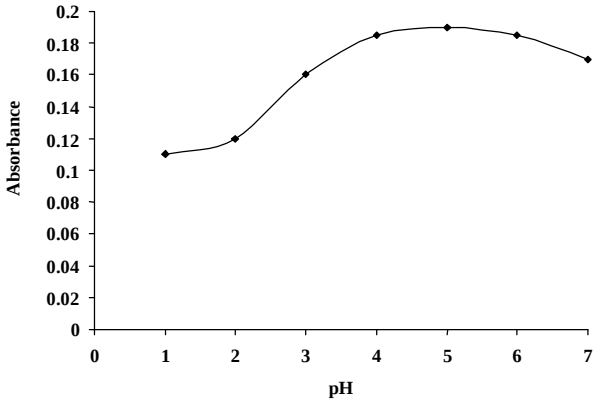


Figure 3. **Effect of pH on derivatization of CF**

Interference study

The effect of possible presence of associated materials such as mannitol, sorbitol, sucrose, glucose, galactose and fructose was investigated at 10 times the concentration of CF and it was observed that none of these substances interfered with no change in absorbance of more than ±5% (Table. 1).

Table 1. **Effects of different possible additives on the absorbance of 20 μg mL^{-1} CF derivative.**

S. No.	Chemical added	Absorbance	Relative error (%)
1.	----------	0.190	00
2.	Mannitol.	0.191	0.5
3.	Sorbitol.	0.188	-1.0
4.	Glucose	0.190	00
5.	Galactose.	0.192	1.0
6.	Fructose.	0.186	-2.1
7.	Sucrose.	0.180	-5.0

Stability of the derivative

The stability of CF-DAB derivative was examined in terms of absorbance at the concentration of 20 μg mL^{-1} CF, but no change in absorbance of more than 5% was observed within 48 h.

Calibration graph (Beer's Law)

The effect of variation in the concentration of CF on its absorbance was studied. A linear calibration curve was obtained which obeyed the Beer's law within the concentration range 20-100μg mL^{-1} of CF with coefficient of determination r^2 0.9996 (Fig. 4).

Table 2. **Analysis of CF from pharmaceutical preparations**

S. No.	Name of drug	Amount labeled per ampoule (mg)	Amount found (mg) per ampoule (standard deviations)	±(%) Relative deviations from labeled values	Recovery (%)
01.	Acmex.	250	238.7 (0.021)	4.5	95.0
02.	Aczon.	250	247.5 (0.002)	1.0	97.8
03.	Bestrix.	250	242.5 (0.022)	3.0	100.02
04.	Broadced.	1000	990.0 (0.002)	1.0	99.3
05.	Cef-3.	250	247.5 (0.031)	1.0	98.3
06.	Cefcin.	250	247.6 (0.002)	1.0	100.2
07.	Cefin.	500	454.0 (0.040)	9.2	95.0
08.	Cefotrim.	250	237.4 (0.030)	5.0	96.0
09.	Ceftison.	250	247.5 (0.028)	1.0	95.0
10.	Ceftrex.	250	243.5 (0.015)	2.6	99.9
11.	Ceftridex.	500	485.6 (0.020)	2.8	95.3
12.	Cefxone.	250	237.8 (0.004)	4.8	98.6
13.	Cepox.	500	475.7 (0.043)	4.8	99.8
14.	Cerixon.	500	479.3 (0.044)	4.1	99.4
15.	Chef.	500	486.4 (0.022)	2.7	99.5
16.	Chroncef.	250	245.2 (0.003)	1.9	98.2
17.	C-trox.	500	490.0 (0.002)	2.0	98.0
18.	Cyforon.	500	494.1 (0.003)	1.1	97.0
19.	Dayzone.	250	247.5 (0.001)	1.0	95.8
20.	Eftriax.	500	479.1 (0.001)	4.1	99.0
21.	Efxone.	250	247.5 (0.003)	1.0	98.2
22.	Elxone.	500	482.3 (0.002)	3.5	99.0
23.	Farcef.	1000	974.0 (0.003)	2.6	96.0
24.	Fotamin.	500	493.2 (0.001)	1.3	98.0
25.	Inocef.	250	245.7 (0.005)	1.7	97.0
26.	Lozon.	500	487.5 (0.001)	2.5	99.9
27.	Macxone.	250	246.6 (0.002)	1.3	98.6
28.	Maxcef.	1000	990.0 (0.002)	1.1	100.02
29.	Oxidil.	250	245.2 (0.005)	1.9	100
30.	Rociphin.	250	247.0 (0.001)	1.2	99.7

The Sandells sensitivity (0.004) was observed at 5μg cm^{-2} CF. The validity of the calibration curve was obtained by the analysis of test solution of CF and the percent relative error was found ±1-2%.

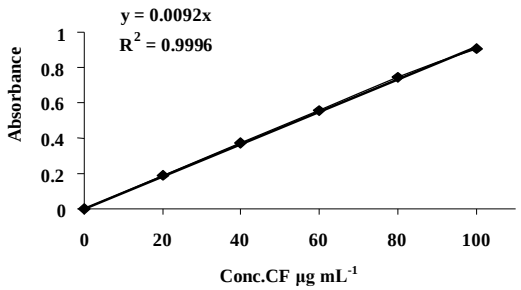


Figure 4. **Calbration curve of Ceftriaxone 0.1% using 4-dimethyl aminobenzaldehyde as derivatizing reagent**

The pharmaceutical preparations containing CF available in the local market were analyzed to determine the amount of CF quantitatively (Table. 2).

The mean observed values were within 238.7-990 mg $ampoule^{-1}$ with standard deviation (SD) within 0.0004-0.044 of thirty pharmaceutical brands (Table. 3).

Table 3. **Results of optimization, precision and accuracy**

S. No.	Parameter (s)	Selected Values
01.	Wave length λ_{max} (nm)	397
02.	Beer's law limits (μg mL^{-1})	20-100
03.	Molar absorptivity (L mol^{-1} cm^{-1})	$5.3x10^3$
04.	Sandells sensitivity (conc. at 0.004 absorbance unit) (μg cm^{-2})	5
05.	Regression equation (y)[a]. Slope (b). Intercept (a).	0.9189 0
06.	Coefficient of determination (r^2)	0.9996
07.	Standard deviation.	± 0.0004-0.044

Day to day reproducibility / repeatability

For the determination of intra and interday reproducibility of the method, the aqueous standard solution 200μg CF was taken in three different calibrated flasks (10mL) and the procedure was followed as described in section B. The above procedure was repeated for three days (n=3). The mean absorbances of intraday and interday reproducibilities were observed as 0.191 and 0.19 with (RSD) values 0.0036% and 0.095%, respectively.

Conclusions

The developed method is simple, accurate, precise, inexpensive and less time consuming in visible region after derivatization with DAB. The developed method may avoid the interferences from associated materials which may absorb in the UV region. The method was applied for the determination of CF contents from pharmaceutical preparations

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