Original Article

A green analytical method for the simultaneous analysis of cefixime trihydrate and ambroxol HCl based on ultraviolet derivative spectroscopy

Abstract

Context: Until date, there is no reported derivative spectrophotometric method for the combination of cefixime trihydrate (CEF) and ambroxol HCl (ABH). So an urgent need was felt to develop an ultraviolet (UV) derivative spectroscopic method, which reduces the cost of analysis on comparing with high-performance liquid chromatography or high-performance thin layer chromatography method. **Aims:** To develop and validate an economical and ecofriendly derivative spectroscopic method that avoids the use of organic solvents for simultaneous quantification of both the drugs. **Materials and Methods:** A simple method based on the derivative spectrophotometric method at zero crossing wavelengths has been developed for the simultaneous quantification of CEF and ABH. As the method depends on hydrotropic dissolution, 0.1N urea is used as the solvent, and it yields an economical and ecofriendly method. Two wavelengths 253 nm (zero crossing point (ZCP) for CEF) and 306 nm (ZCP for ABH) were selected for the quantification of ABH and CEF respectively. **Results:** The first derivative amplitude-concentration plots were linear over the range of 5–35 μg/ml and 3–10.5 μg/ml with detection limits of 0.187 and 0.0937 μg/ml and quantification limits of 0.625 and 0.312 μg/ml for CEF and ABH respectively. The percentage recovery was within the range between 99.05% and 102%. The % relative standard deviation for precision and accuracy of the method was found to be <2%. **Conclusion:** The proposed method was found to be simple, accurate and precise and can be successfully applied to the routine quality control analysis of studied drugs in their tablet formulations.

Key words:
Ambroxol HCl, cefixime trihydrate, derivative spectrophotometry, hydrotropy

Introduction

Cefixime trihydrate (CEF) ([6R,7R]-7-(2-[2-Amino-4-thiazolyl]glyoxylamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo(4.2.0)oct-2-ene-2-carboxylicacid,72-(Z)-[O-(carboxymethyl)oxime] is an orally absorbed third generation cephalosporin antibiotic that was approved by the U.S. Food and Drug Administration in 1997 for the treatment of mild to moderate bacterial infections. It has a broad antibacterial spectrum against various Gram-positive and Gram-negative bacteria, including *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Escherichia coli* and *Klebsiella pneumoniae* resistant to ampicillin, cephalexin, cefaclor and trimethoprim-sulfamethoxazole. It is used for the treatment of susceptible infections, including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis and urinary-tract infections.[1-3] A detailed literature survey has shown that cefixime has been studied either alone or in combination with other drugs by various analytical methods such as spectrophotometric,[4-8] fluorimetric,[9] voltammetric,[10,11] high performance liquid chromatography

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(HPLC),[3,12-17] high performance thin liquid chromatography (HPTLC)[18-21] and layer chromatograph-mass spectrometry (LC-MS).[22] Ambroxol hydrochloride (ABH) chemically, 4-([2-amino-3,5-dibromophenyl]-methyl)-amino] cyclohexanol hydrochloride is a mucolytic expectorant and used to reduce the viscosity of mucous secretions[23] (Budavari 1996). Methods available for the determination of ABHX alone or in combination with other drugs include ultraviolet (UV) spectroscopy,[24,25] capillary electrophoresis,[26] HPLC,[27-32] gas chromatography,[33,34] and LC-MS.[35] There are very less analytical methods reported so far for this combination, and they are based on HPLC.[21,36] To the best of our knowledge, there is no reported method available for the analysis of this combination by derivative spectroscopy. So an urgent need was felt to develop a UV derivative spectroscopic method, which reduces the cost of analysis on comparing with HPLC or HPTLC method.

Materials and Methods

Instruments and chemicals

A double beam UV-visible spectrophotometer (Shimadzu, 1800) having UV-probe software was used for the analysis. Matched and calibrated quarts cuvettes were used as sample cell. The samples of CEF and ambroxol HCl (ABH) were procured from Dr. Reddy's laboratory, India. The tablets were purchased from local market.

Analytical method development and validation

Different solvents such as methanol, water, phosphate buffer, acetate buffer etc., were used as solvents and either zero crossing points (ZCPs) were not available for either drug or for both drugs. When the solvent was shifted to 0.1N urea, ZCPs were available for both the drugs and hence selected as the solvent. Moreover, it is more preferable as it yields an economical and ecofriendly method by avoiding the use of any organic solvents at any stage in the analysis. Standard solutions of CEF (10 μg/ml) and ABH (10 μg/ml) prepared in 0.1N urea were scanned in the spectrum mode between 200 and 400 nm so as to obtain the respective zero-order spectra. It was converted into first derivative spectra selecting delta λ =4 nm and scaling factor = 1. The overlapped spectra of CEF and ABH showed the presence of the ZCP for both the drugs. That is, ZCP of ABH at which CEF showed the derivative absorbance and vice versa for CEF were noted.

Preparation of stock solution and standard solution

Ten mg of each standard drug were weighed and dissolved separately in 0.1N urea to obtain stock solution (1000 μg/ml) of each drug. These solutions were diluted suitably with 0.1N urea to obtain the standard solutions of CEF and ABH.

Method validation

The method was validated for accuracy, precision, linearity, LOD, and LOQ by the following procedures**.**

Linearity

The stock solution of CEF (50 μg/ml) and ABH (50 μg/ml) were prepared in 0.1N urea. CEF solutions of various concentrations (5–35 μg/ml) were prepared by diluting appropriate volumes with 0.1N urea. In a similar manner, stock solution of ABH also diluted to prepare various concentrations (3–10.5 μg/ml).The first derivative spectra were recorded using the prepared solutions against 0.1N urea as blank.

Accuracy

The accuracy of the method was determined by calculating recoveries of CEF and ABH by standard addition method. Tablet powder equivalent to 10 mg of CEF was taken in three different volumetric flask and 80%, 100% and 120% of pure cefixime bulk drug was added respectively and diluted with 0.1N urea. Similarly, tablet powder equivalent to 3 mg of ABH was transferred into another three different 10 ml volumetric flasks and to it 80%, 100% and 120% of pure ABH bulk drug was added respectively and diluted with 0.1N urea. The amounts of CEF and ABH were estimated by measuring derivative response at the selected wavelength (306 nm for cefixime and 253 nm for ABH) and the concentrations were calculated from the computed regression equation resulting from the linearity studies. The recovery was performed in triplicate at each specified concentration level.

Precision

The intra-day precision of the proposed first derivative spectrophotometric method was determined by estimating the corresponding response 3 times on the same day for three different concentrations of CEF (10, 20, 30 μg/ml) and ABH (3, 6, 9 μg/ml). The inter-day precision was determined by estimating the corresponding response 3 times on 3 different days for the same concentrations of CEF and ABH.

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined on samples containing very low concentrations of the analyte. LOD and LOQ were estimated at a signal to noise ratio of 3:1 and 10:1 respectively by analyzing a series of dilute solutions of known concentration. The values can be calculated by equation 1 and 2, respectively.

$$LOD = 3.3\ \sigma/S \qquad \text{equation 1}$$

$$LOQ = 10\ \sigma/S \qquad \text{equation 2}$$

σ = standard deviation of the response and S = slope of calibration curve.

Analysis of the dosage form (assay)

Twenty tablets of marketed formulation, each containing 300 mg of CEF and 100 mg of ABH were used. Solution was

prepared from tablet powder so as to get a final solution containing 30 µg/ml CEF and 10 µg/ml ABH. The amount of CEF and ABH was determined by substituting derivative responses into the equation of the straight line representing the calibration curves for CEF and ABH.

Results and Discussion

Selection of zero crossing point

The first derivative spectrum of CEF has zero absorbance at 253 nm, where ABH gives the significant derivative response, while the first derivative spectrum of ABH has zero absorbance at 306 nm, where cefixime gives the significant derivative response. Therefore, 253 nm and 306 nm were selected for estimation of ABH and CEF, respectively as shown in Figure 1.

Calibration plot for cefixime trihydrate and ambroxol HCl

Cefixime trihydrate and ABH were showing a linear relationship between concentration (µg/ml) and derivative absorbance. CEF and ABH were linear in the range of 5–35 µg/ml and 3–10.5 µg/ml respectively. From the linear regression analysis, correlation coefficient value (r^2) for CEF and ABH was 0.9999 and 0.9999 respectively, which indicated the linearity of the method. From Figure 2, it was observed that with the increase in CEF concentration, the derivative response at 306 nm was increased. Similarly, the derivative response for ABH at 253 nm was increased with the increase in its concentration. The regression equation for CEF and ABH is shown in the linearity data given in Table 1.

The accuracy was determined by standard addition method. Three different levels (80%, 100% and 120%) of standards were spiked to commercial tablets in triplicate. The mean of percentage recoveries and % relative standard deviation (RSD) values were calculated and reported in Table 2. The % recoveries of CEF and ABH were found to be in the range of 99.05–101 and 99.6–100.63, respectively which are satisfactory.

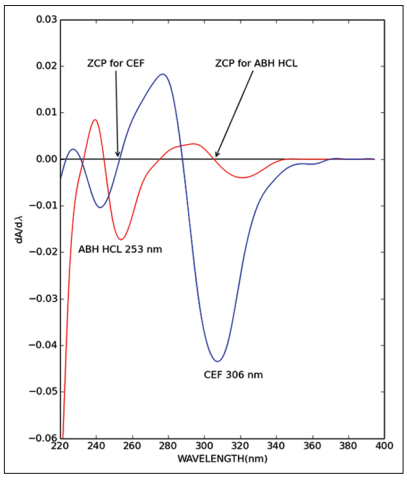

Figure 1: First order ultraviolet overlaid spectrum of cefixime trihydrate and ABH showing the zero crossing points of each drugs

Precision

The repeatability (intra-day precision) of the method was determined by intra-day (n = 3) analysis of three standard solutions of CEF and ABH at the concentration of 10, 20 and 30 µg/ml and 3, 6 and 9 µg/ml respectively. The % RSD of repeatability was <2.0 for both the drugs. Intermediate precision was determined by the inter-day (n = 3) analysis of three standard solutions of CEF and ABH at the

Table 1: Linearity data

Parameter	CEF	ABH
Zero crossing wavelength (nm)	306 nm	253 nm
Linearity range (µg/mL)	5–35	3–10.5
Slope	−0.0016	−0.0016
Intercept	−0.0003	0.0000
Correlation coefficient	0.9999	0.9999
Regression equation	y = −0.0016 x − 0.0003	y = −0.0016 x + 0.0000

CEF – Cefixime trihydrate; ABH – Ambroxol HCl

Table 2: Accuracy of the method (recovery studies)

Brand	Spiking level (%)	Drug	Theoretical content (mg)	Amount found (mg) (n=3)	Recovery (%)	% RSD
Ceftas-AL	80	CEF	18	18.35±0.130	101	0.711
		ABH	5.4	5.437±0.130	100.63	0.707
	100	CEF	20	19.83±0.037	99.16	0.189
		ABH	6.0	5.98±0.164	99.64	0.820
	120	CEF	22	21.793±0.188	99.05	0.866
		ABH	6.6	6.585±0.164	99.75	0.749

Acceptance criteria – % RSD should not be more than 2. RSD – Relative standard deviation; CEF – Cefixime trihydrate; ABH – Ambroxol HCl

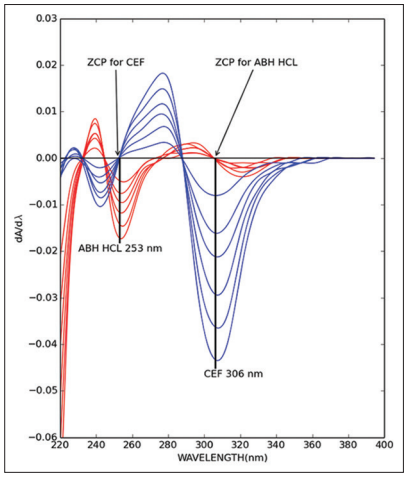

Figure 2: First order ultraviolet overlaid spectra in the linearity range of cefixime trihydrate (5–35 µg/ml) and ABH (3–10.5 µg/ml)

concentration of 10, 20 and 30 μg/ml and 3, 6 and 9 μg/ml respectively and reported in Table 3. The % RSD for inter-day analysis was <2.0 for both the drugs. These statistical data were indicative of good precision.

Limit of detection and limit of quantitation

Limit of detection and LOQ of CEF was found to be 0.187 μg/ml and 0.625 μg/ml respectively. For ABH, LOD was found to be 0.0937 μg/ml and LOQ was found to be 0.312 μg/ml.

Analysis of commercial tablets (assay)

The accuracy of proposed method was evaluated by the assay of commercially available tablets (Ceftas-AL) containing CEF (100 mg) and ABH (30 mg). The results obtained for CEF and ABH were compared with the corresponding labeled amounts and reported in Table 4. The amount of CEF and ABH found in formulation-I (Ceftas-AL) was 100.373 mg and 30.89 mg. The % RSD for assay results of the formulation (Ceftas-AL) was <2, which indicated the accuracy of the proposed method.

Conclusion

The main objective of the present work was to develop an economical and ecofriendly analytical method for the simultaneous analysis of CEF and ABH in the tablet dosage form, IPQC samples or dissolution samples. The validation study results indicated that the presence of excipients did not interfere with the analysis and hence the method can be employed for bulk drugs as well as formulations. The proposed method has some advantages as neither it requires any sophisticated instruments like HPLC or HPTLC nor costly reagents or solvents. Moreover, the solvent selected for the entire steps was urea which is a nature friendly chemical.

Table 3: Precision of the method

Drug	Theoretical amount μg/mL	Amount found±SD[a], % RSD[b] intra-day, inter-day	
CEF	10	10.25±0.173, 1.688	10.30±0.133, 1.293
	20	20.22±0.250, 1.238	20.17±0.228, 1.132
	30	29.92±0.371, 1.242	29.86±0.282, 0.946
ABH	3	3.29±0.06, 1.823	3.30±0.063, 1.914
	6	5.838±0.105, 1.798	5.84±0.108, 1.864
	9	8.701±0.100, 1.159	8.73±0.091, 1.084

Acceptance criteria – % RSD should not be more than 2. CEF – Cefixime trihydrate; ABH – Ambroxol HCl; SD – Standard deviation; RSD – Relative SD

Table 4: Assay results of commercial tablets

Formulation with label claim	Amount found in mg (AM)±SD, % RSD (*n*=3)	
Ceftas-AL	CEF	ABH
CEF=100 mg, ABH=30 mg	100.37±0.21, 1.07	30.89±0.07, 1.63

CEF – Cefixime trihydrate; ABH – Ambroxol HCl; SD – Standard deviation; RSD – Relative SD

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