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Spectrophotometric simultaneous determination of cefixime trihydrate and ambroxol hydrochloride in tablet dosage form

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ABSTRACT

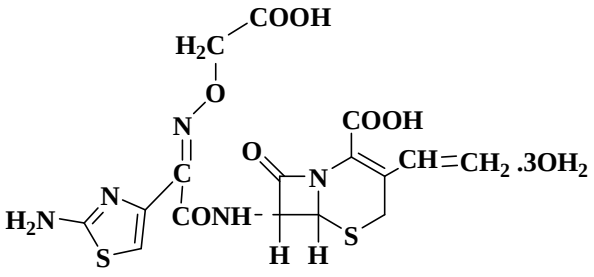
Two simple, economical, rapid, precise and accurate methods have been developed for simultaneous determination of cefixime trihydrate and ambroxol hydrochloride in bulk and in their pharmaceutical formulations. The first method based on first derivative spectrophotometry (Method A) and second method based on Vierordt's method (Method B). The amplitude in first order derivative method is zero-crossing at 238.0 nm and 275.4 nm for ambroxol and cefixime. The amplitude in Vierordt's method at 285.0 nm and 244.4 nm for cefixime and ambroxol. Regression analysis of Beer's- Lambert plots showed a good correlation in the concentration ranges of 10 - 40μg/ml and 3-18μg/ml for both methods. The linearity, precision, detection and quantification limits were calculated. Applications of the procedure to the analysis of pharmaceutical preparations gave reproducible and accurate results. Further, the validity of the procedure was confirmed by applying the standard addition technique and the results were obtained in good agreement indicating its suitability in routine analysis.

KEYWORDS

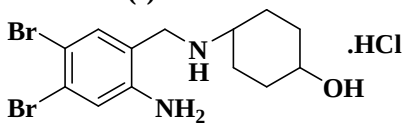
Cefixime trihydrate;
Ambroxol hydrochloride;
First order derivative;
Vierordt's method.

INTRODUCTION

Cefixime ((6R,7R)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino) acetamido]-8-oxo- 3-vinyl-5-thia-1-azabicyclo-[4,2,0]-oct-2-ene-2-carboxylic acid) (I, figure 1), is an orally absorbed third generation cephalosporin antibiotic. It has a broad antibacterial spectrum against various gram-positive bacteria and gram-negative bacteria, including Haemophilus influenzae, Neisseria gonorrhoeae, Escherichia coli, and Klebsiella pneumoniae resistant to ampicillin, cephalexin, cefaclor, and trimethoprim- ulfamethoxazole[1,2]. It was not hydrolyzed by the common plasmid or by chromosomal β-lactamases that inactivate the oral penicillins and cephalosporins[3]. These in vitro advantages may provide cefixime feasibility to treat some of the more difficult respiratory and urinary tract infections[4]. Until now, the determinations of cefixime in plasma have



(I) Cefixime



(II) - Ambroxol

Figure 1 : Structures of cefixime and ambroxol

mainly been focused on microbiological[5] and high performance liquid chromatographic (HPLC) techniques [6-12]. Due to poor selectivity, microbiological methods are only used for pharmacodynamic study now. Tokuma et al.[6] and Liu et al.[7] developed a sensitive HPLC-UV method to determine plasma and urine concentration of cefixime with a lower limit of quantification (LLOQ) of 0.05μg/ml by using a double column and double pump HPLC switching system, whereas the chromatographic run time of one sample was more than 15 min. Nowadays, liquid chromatography-tandem mass spectrometry (LC-MS-MS), due to its higher sensitivity and selectivity, has been applied to the quantification of cephalosporin antibiotic in biological samples[8].

Ambroxol, trans-4-(2-amino-3, 5-dibromo benzylamino) cyclohexanol hydrochloride (II, Figure 1), is a compound with potent mucolytic activity, for which it is used as an expectorant and bronchosecretolytic in therapeutics[9,10]. It is a pharmacologically active metabolite of bromhexine, *N*-cyclohexyl-*N* methyl-(2-amino-3, 5-dibromobenzyl) amine hydrochloride. Ambroxol stimulates the transportation of the viscous secretion in the respiratory organs and reduces the standstillness of the secretion. Ambroxol hydrochloride can be found in pharmaceutical preparations such as drops, granules, injections, syrups and tablets. Methods have been used for the individual determination of ambroxol hydrochloride in pharmaceutical solutions and tablets including TLC[11,12], spectrophotometry[10], HPLC[13], flow injection[14,15] and capillary electrophoresis[16]. More complex methods have been reported for ambroxol determination in biological fluids[9,10,16,17].

Extensive literature survey revealed no method for simultaneous determination of cefixime trihydrate and ambroxol hydrochloride in tablet dosage form. Aim of present work was to develop simple, economical, rapid, precise and accurate method for simultaneous determination of binary drug formulation using first order derivative spectrophotometry and Vierordt's method.

EXPERIMENTAL

Instrumentation

The instrument used in the present study was SHIMADZU double beam UV/visible spectrophotometer (Model 2450) with variable slit width.

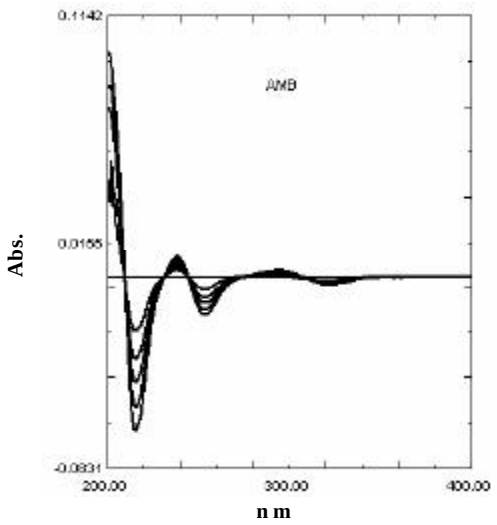


Figure 2 : Spectra of AMB (Method A, First derivative): Zero crossing wavelength of AMB-275.4 nm (3 - 9μg/ml)

Reagents and chemicals

An analytically pure sample of CFX was kindly supplied by Macleods Pharmaceuticals Ltd. (Daman, India) and AMB was kindly supplied by Glenmark pharmaceuticals Ltd. (Nashik, India) used as such without further purification. The pharmaceutical dosage form used in this study was Cembol-100 tablets labeled to contain 100 mg cefixime trihydrate and 30 mg of ambroxol hydrochloride.

Theory

Derivative UV/visible spectrophometry have been widely used over the last few years in the analysis of multicomponent mixtures. This transformation shows two principal advantages on derivative spectrophotometry. Firstly, an even order spectrum is of narrower spectral band width than its fundamental spectrum. A derivative spectrum therefore shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of the λmax of the individual bands. Secondly, derivative spectrophotometry discriminates in favour of substances of narrow spectral bandwidth against broad bandwidth substances. This is because the derivative amplitude (D), that is the distance from a maximum to a minimum, is inversely proportional to the fundamental spectral bandwidth (w) raised to the power (n) of the derivative order.[18] Thus,

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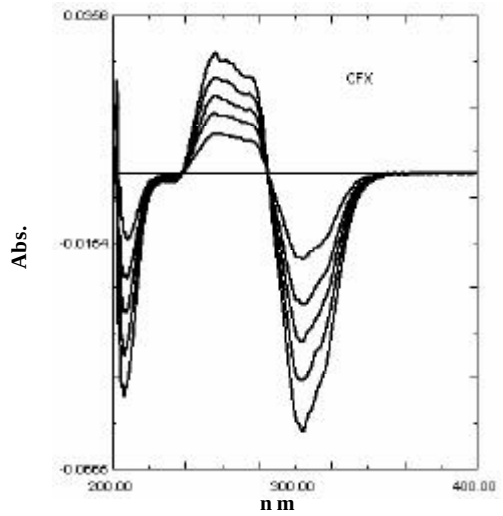

Figure 3: Spectra of CFX (Method A, First derivative): Zero-crossing wavelength of CFX-238.0 nm (10-30µg/ml)

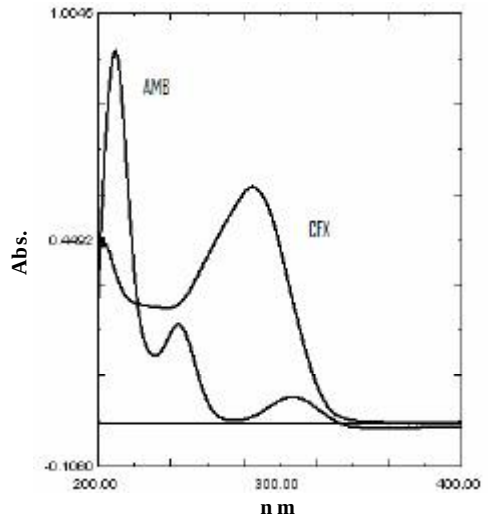

Figure 4: Overlain spectra of CFX and AMB (10 and 3µg/ml respectively) by Method B (Veirdot's method)

D α (1/W)^n

For this reason, diverse procedures for the resolution of overlapped derivative peaks have been applied. Thus, the zero-crossing method has been used for the first- and second-derivative spectra in diverse mixtures[19]. Using appropriate dilutions of standard stock solution, the dilutions were scanned and the first derivatives of them were traced with the aid of computer using scaling factor 1 (Figure 2 and 3). The zero-crossing wavelength of CFX was 238.0 nm and that of AMB was 275.4 nm.

Vierordt's method the absorbance for the standard working solutions of CFX (λ_1) and AMB (λ_2) at 285.0 nm and 244.4 nm , CFX and AMB at 244.4 nm and 285.0 nm and also was measured(Figure 4). The absorbance A (1%, 1cm) for each drug at the two analytical wavelengths was calculated and the mean values determined. Similarly the absorbance of the mixed sample solutions was measured; and the concentration of each compound calculated from the following simultaneous equations:

$A1 = \alpha 1 \cdot C1 + \beta 1 \cdot C2$,

$A2 = \alpha 2 \cdot C1 + \beta 2 \cdot C2$

Where A1 and A2 denote the absorbances of a mixture solutions of CFX and AMB and α and β represent the values of A1 1 (1%, 1 cm) values calculated for CFX and AMB, respectively, at λ_1 and λ_2. C1 and C2 are the concentrations of CFX and AMB, respectively. The subscripts 1 and 2 refer to λ_1 (285.0 nm) and λ_2 (244.4 nm), respectively.

2.4 Preparation of standard stock solution

Standard stock solutions were prepared by dissolving separately 10 mg of each drug in 10 ml of methanol. To get concentration of 1000µg/ml. from that 200µg/ml. Beer's law was obeyed in the concentration range of 10-40µg/ml for CFX and 3-18µg/ml for AMB.

Preparation of sample stock solution

Contents of 20 tablets were weighed accurately and powdered. Powder equivalent to 100 mg of CFX and 30 mg of AMB was weighed and dissolved in 10: 90 ml 0f methanol:0.1 M HCl with aid of sonication for 5 min. The solution was filtered through whatman filter paper no.41 to 100 ml volumetric flask. Filter paper was washed with 0.1 M HCl, adding washings to the volumetric flask. From this stock solution further dilutions were made of required concentration.

Recovery studies

The accuracy of the proposed methods were checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels within the range of linearity for both the drugs.

RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve (Figures 5- 8), assay of tablets and recovery studies were performed. Critical evaluations of proposed methods were performed by statistical analysis

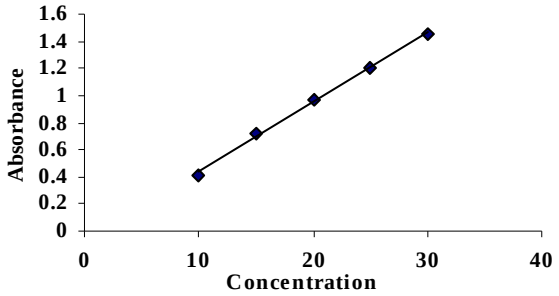

Figure 5: Calibration curve of cefixime trihydrate by method B

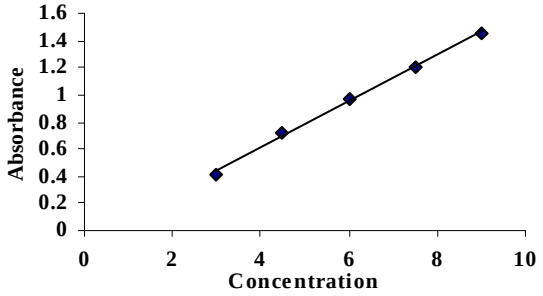

Figure 6: Calibration curve of Ambroxol hydeochloride by method B

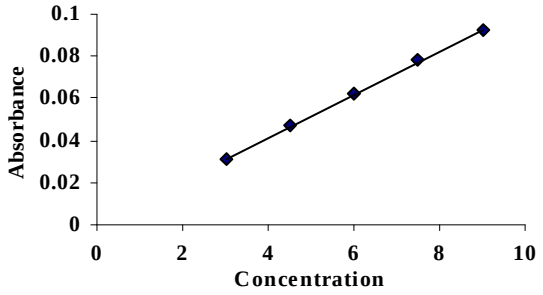

Figure 7: Calibration curve of Ambroxol hydrochloride by method A

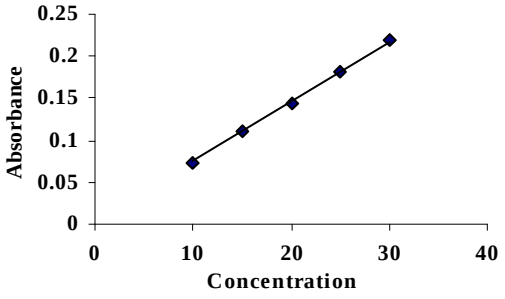

Figure 8: Calibration curve of cefixime trihydrate by method A

of data where slopes, intercepts, correlation coefficients, the detection limit (LOD) for the proposed methods were calculated using the following equation[20].

LOD = 3.3s/k

Where s is the standard deviation of replicate determination values under the same conditions as for sample analysis in the absence of the analyte and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance's were calculated and listed in TABLE 1.The limits of quantitation, LOQ, defined as[20].

LOQ = 10s/k

According to this equation, the limit of quantitation were calculated and listed in TABLE 1. The proposed methods were also evaluated by the assay (n=6) of commercially available tablets containing CFX and AMB. The % assays were found to be 101.07 % and 99.19 % using first order derivative method (TABLE 2) and 101.3% and 100.6 % for CFX and AMB using Vierordt's method (TABLE 3) respectively.

The results of recovery studies are shown in TABLES 4 and 5. For CFX the recovery study results ranged from 98.78 to 101.3% and 98.60 to 101.2 %

TABLE 1 : Optical characters of proposed methods

Sr. no.	Parameter	Method A		Method B	
		CFX	AMB	CFX	AMB
1	λmax (nm)	275.4 (zero-crossing of AMB)	238.0 (zero-crossing of CFX)	285.0	244.4
2	Beer's law limit (μg/ml)	10-40	3-18	10-40	3-18
3	Slope	0.00716	0.0102	0.06049	0.006582
4	Intercept	0.00288	0.00048	-0.00042	0.01228
5	Correlation coefficient	0.9994	0.9991	0.9998	0.9998
6	LOD (μg/ml)	0.511	0.727	0.137	0.432
7	LOQ (μg/ml)	1.548	2.203	0.415	1.310
8	Molar absortivity (L mol^{-1} cm^{-1}) × 10^4	3.74	1.76	2.45	4.12

TABLE 2 : Results of commercial formulation analysis by method

Drug	Label claim (mg/tablet)	% Label claim estimated	Standard deviation	% R.S.D.
CFX	100	101.07	0.011	0.56
AMB	30	99.18	0.012	0.28

Average of six determinations

TABLE 3 : Results of commercial formulation analysis by method B

Drug	Label claim (mg/tablet)	% Label claim estimated	Standard deviation	% R.S.D.
CFX	100	101.3	0.311	0.33
AMB	30	100.6	0.172	0.57

Average of six determinations

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TABLE 4 : Results of recovery study by method A

Drug	Level of % recovery	% Mean recovery	Standard deviation	% R.S.D.
CFX	50	98.78	0.04	0.29
	100	99.27	0.35	1.71
	150	101.3	0.62	2.00
AMB	50	98.04	0.02	0.65
	100	98.10	0.02	0.35
	150	99.01	0.09	1.2

Mean of three determinations

TABLE 5: Results of recovery study by method B

Drug	Level of % recovery	% Mean recovery	Standard deviation	% R.S.D.
CFX	50	101.2	0.04	0.28
	100	99.46	0.52	1.92
	150	98.60	0.28	1.13
AMB	50	100.5	0.03	0.71
	100	100.8	0.09	1.62
	150	98.66	0.09	1.23

Mean of three determinations

for both the methods respectively. Also the results of recovery studies for AMB ranged from 98.04 to 99.01% and 98.66 to 100.5 % for both the methods respectively. The accuracy and reproducibility is evident from the data as results are close to 100 % and low standard deviation. The proposed methods are simple, economical, rapid, precise and accurate. Hence these can be used for routine analysis of CFX and AMB in tablet formulation.

CONCLUSION

The validated spectrophotometric method employed here proved to be simple, economical, rapid, precise and accurate. Thus it can be used for routine simultaneous determination of CFX and AMB in tablet dosage form instead of processing analyzing each drug separately

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