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Development and Validation of UV-Spectroscopy Method for the Determination of Cefpodoxime Proxetil and Ambroxol Hydrochloride in Pharmaceutical Formulation

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Abstract: An accurate, specific and précised two simple and sensitive spectrophotometric methods (A and B) for the simultaneous determination of Cefpodoxime proxetil (CEF) and Ambroxol hydrochloride (AMB) in tablet dosage form were described. The method A involves simultaneous equation, using methanol as common solvent showing absorption maxima at 235nm and 308nm.The linearity for both CEF and AMB hydrochloride in method A at the range of 5-30µg/ml and 3-18µg/ml respectively. The method B Derivative Spectrophotometric method, was based on the principle that both CEF and AMB hydrochloride spectra was derivatised into first order and the derivative spectra showed λ max at 279nm and 235 nm. Beer's law was observed in method B for both drugs at concentration range of 5-30µg/ml respectively. The correlation coefficient was found to be 0.9999, 0.9999 for CEF and 0.9999, 0.9998 for AMB in method A and 0.9996 for CEF, 0.9994 for AMB in method B. Thus the proposed method is accurate, precise and reproducible which can be suitably applied for the estimation of CEF & AMB hydrochloride in combined dosage forms.

Keywords: Cefpodoxime proxetil(CEF), Ambroxol hydrochloride(AMB), UV spectrophotometry, Simultaneous method, Derivative method.

INTRODUCTION[1-9]

Cefpodoxime proxetil (CEF) (fig.1a) is chemically [6R-[6α ,7β (z)]]-7-[[(2-amino-4-thiozolyl)(methoxyimino)acetyl]amino]-3-(methoxy-methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 1-[[(1-methyl ethoxy)carbonyl] oxy]ethyl ester. It is a orally administered THIRD generation cephalosporin's class of antibacterial agent more active against enterobacteriaceae,streptococcus aureus and β-lactamase producing H.influenzae, M.catarrhalis, N.gonorrhoeae & less active against gram +ve cocci. It binds to one or more of the penicillin binding proteins (PBPs) which inhibits the final transpeptidation step of peptidoglycon synthesis in bacterial cell wall, thus inhibiting biosynthesis and arresting cell wall assembly resulting in bacterial cell death.

Ambroxol hydrochloride (AMB) (fig. 1b) is chemically 4-[[(2-amino-3,5-dibromophenyl)-methyl] amino]cyclohexenol hydrochloride.It is a mucolytic expectorant that inhibits the release of arachidonic acid cell membrane phospholipids. Stimulates the release of surfactant by pneumocytes type II & act as a scavenger of hypochlorous and hydroxyl radicals, it blocks nitric oxide stimulated activation of guanylate cyclise.

However, most of the analytical methods developed for the quantization of CEF and AMB involve analysis of single component or combination with other. No methods were reported for the estimation of CEF and AMB in combined dosage form without prior separation.

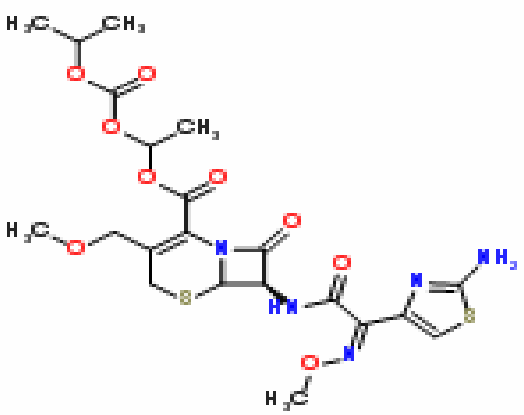
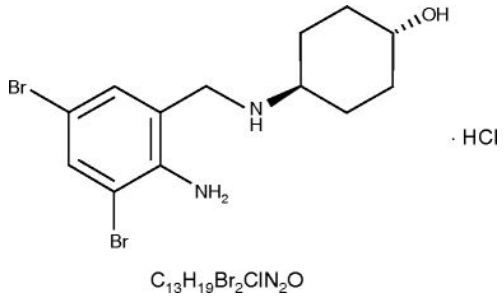
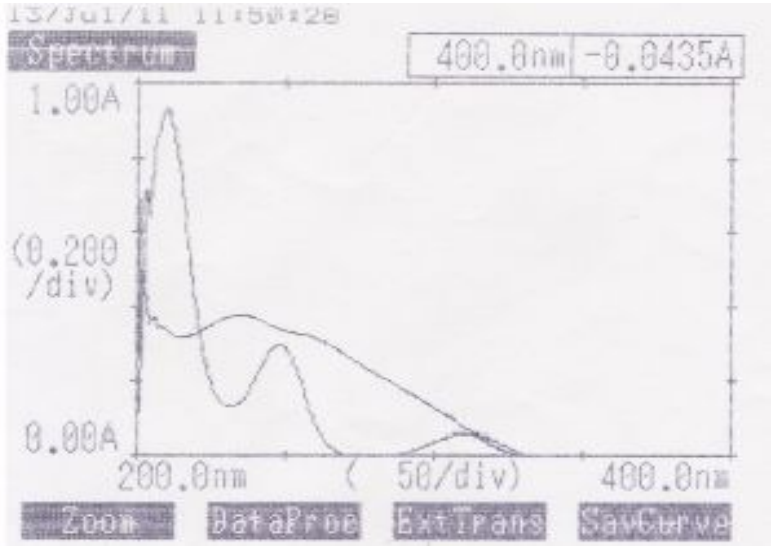


Fig: 1a

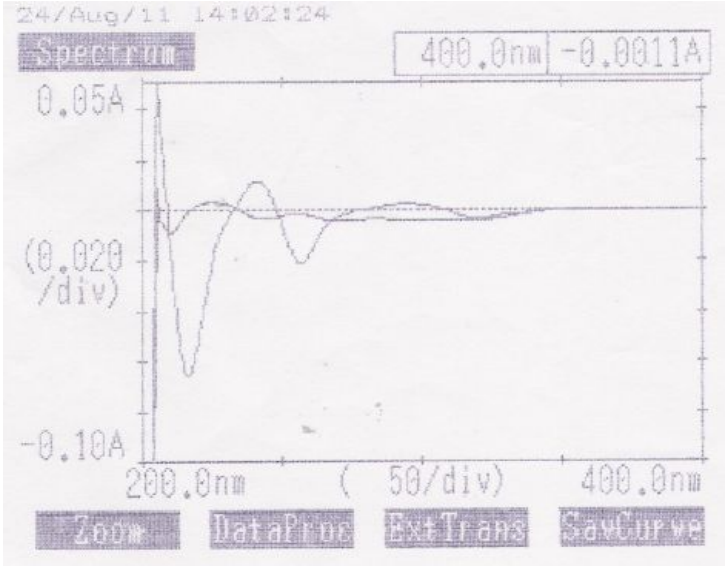


$C_{13}H_{19}Br_2ClN_2O$

Fig: 1b



OVERLAID SPECTRA OF CEFPODOXIME PROXETIL + AMBROXOL



OVERLAID SPECTRA OF FIRST ORDER DERIVETISED SPECTRA OF CEFPODOXIME AND AMBROXOL

EXPERIMENTAL[1-18]

INSTRUMENTATION

A Shimadzu UV spectrophotometer (LAMDA 25, Perkin Elmer) with 1cm matched quartz cells was used for the estimation.

CHEMICAL AND REAGENTS

CEF and AMB were kindly supplied by Madras pharmaceuticals, Chennai as gift samples. Tablets containing CEF and AMB were procured from local pharmacy. All the reagents were of analytical grade. Methanol was used throughout the experiment.

STANDARD PREPARATION

Accurately weighed quantities (10 mg each) of CEF and AMB were dissolved separately in sufficient quantity of methanol in a 100ml volumetric flask. The stock solutions of CEF and AMB were separately diluted in methanol, to get concentrations of 10µg/ml each, and scanned in the wavelength range of 200-400nm. From the overlaid spectra of both drugs wavelengths 235nm and 308nm (λ max of CEF & AMB) were selected for the formation of simultaneous equation and from the derived spectra of first order derivative method, zero crossing points of both drugs were selected at 235nm and 279nm for CEF and AMB.

ANALYSIS IN TABLET FORMULATION

For the estimation of drugs from the commercial formulations, twenty tablets of FINECEF-AM (Abbott health care ltd. Mumbai, India) containing 100mg of CEF and 60mg of AMB were weighed, and finely powdered. Quantity of powder equivalent to 30mg of AMB was transferred to 50ml volumetric flask, dissolved in sufficient quantity of methanol, sonicated and the volume was adjusted up to the mark with distilled water to obtained a stock solution of 1000µg/ml of CEF and 600µg/ml of AMB. The solution was then filtered through Whatman filter paper No. 41 and the filtrate was appropriately diluted to obtain final concentrations 10µg/ml and 6µg/ml of AMB. Absorbance of this solution was measured at appropriate wavelengths and values were substituted in the respective formulae to obtain concentrations by Simultaneous equation method and Derivative method.

METHOD-A: SIMULTANEOUS EQUATION METHOD

From the standard preparation, various dilutions were made at concentration range from 5-30µg/ml and 3-18µg/ml. It was observed that it obey's the beer's law.

The simultaneous equations formed were,

At λ_1 $A_1 = a\,x_1bc_x + ay_1bc_y$ ---(1)

$A1 = 331.83\,C_X + 182.29\,C_Y$--- (2)

At λ_2 $A_2 = a\,x_2bc_x + ay_2bc_y$----(3)

$A2 = 91.78\,C_X + 90.24\,C_Y$ ------ (4)

Where A_1 and A_2 are the absorbance of sample solution at 235 and 308 nm respectively. Cx and C_Y are the concentration of CEF and AMB respectively (µg /ml) in sample solution.

The absorbances ($A_{1\&}$ A_2) of the sample solution were recorded at 235 and 308nm respectively and concentration of both the drugs were calculated using above mentioned equation (2&4). Precision of the method was determined by carrying out Intra-Day (n = 3) and Inter Day (n = 3) studies.

METHOD -B: DERIVATIVE SPECTROSCOPY DETERMINATION :

UV spectra of both the drugs (CEF and AMB) were derivatised to first order with $\Delta\lambda$ = 1 for the entire spectra. Zero crossing points for CEF and AMB was found to be 235 and 279 nm respectively (Fig 2). From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 10-60 µg/ml of CEF and 6-36 µg/ml of AMB and the readings were taken in the first order mode at the selected wavelengths. Optical and regression data were calculated. Accuracy of the method was checked by preparing five mixed standards containing different concentration, absorbance was measured at respective zero crossing points in first order UV spectra and amount present in the sample was calculated from their respective calibration curve. Precision of the method was determined by performing Intra Day (n = 3) and Inter Day (n = 3).

TABLE.1: Optical characteristics of Cefpodoxime proxetil and Ambroxol HCl

Parameters	METHOD A				METHOD B	
	CEF	CEF	AMB	AMB	CEF	AMB
λ max (nm)	235nm	308nm	235nm	308nm	308nm	235nm
Beer's law limit(µg/ml)	5-30	5-30	3-18	3-18	10-60	6-36
Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U)	0.0299481	0.1088326	0.0565370	0.1158274	2.7990782	1.7888693
Molar absorptivity (L/mol/cm)	18470.067	51116.304	7499.1844	3693.7898	194.56606	242.87007
Correlation coefficient (r)	0.99988	0.99980	0.99958	0.99976	0.99964	0.99949
Regression equation (Y=mx+C)	Y=0.03340x+.002972000	Y=0.00922x+0.0004470000	Y=0.0179728x+0.001149405	Y=0.00876428x+0.00145	Y=0.0003573x+0.0000839	Y=0.000593x+0.0000458333
Slope(m)	0.0033420	0.0092201	0.0179728	0.0087642	0.0003573	0.0005903
Intercept (c)	-0.0029720	0.0004470	0.0011494	0.00145	0.0000839	0.0000458
LOD (µg/ml)	0.3230845	0.4745232	0.5375612	0.3544688	0.0469131	1.3573344
LOQ (µg/ml)	0.9790439	1.4379492	1.6289733	1.0741479	0.1421611	4.1131346
Standard error	0.0056158	0.0021277	0.0035634	0.0012939	0.0002026	0.0002527
Absorbtivity (A1%,1cm)	331.8334	91.78546	182.29397	90.249228	3.5348329	5.8657408

TABLE 2: Recovery of Cefpodoxime proxetil and Ambroxol HCl by simultaneous equation and derivative spectroscopic method (n=3)

Drug		Percentage	% recovered	S.D	% R.S.D	S.E
Method A	CEF	80 100 120	99.62 98.91 99.34	0.357563	0.360112	0.039729
	AMB	80 100 120	100.26 100.49 100.37	0.118004	0.117559	0.013112
Method B	CEF	80 100 120	101.18 100.86 101.23	0.202884	0.20069	0.022543
	AMB	80 100 120	99.93 99.84 100.29	0.237929	0.237876	0.026437

TABLE:3 Quantification, precision and ruggedness values of Cefpodoxime proxetil and Ambroxol HCl (n=6).

Parameters	Percentage obtained	S.D	%R.S.D
QUANTIFICATION			
CEF in SEM	101.6467 %	0.08165	0.080327
AMB in SEM	99.5883 %	0.30096	0.302204
CEF in DSM	99.9423 %	0.406252	0.406486
AMB in DSM	101.7700 %	0.606432	0.595885
PRECISION			
INTRA-DAY			
CEF in SEM	101.6300 %	0.017321	0.017043
AMB in SEM	99.6300 %	0.091652	0.091992
CEF in DSM	99.4733 %	0.366652	0.368593
AMB in DSM	100.2467 %	0.636579	0.635013
INTER-DAY			
CEF in SEM	101.0567 %	0.958871	0.948845
AMB in SEM	99.6666 %	0.16773	0.168291
CEF in DSM	99.5833 %	0.197315	0.198141
AMB in DSM	100.9067 %	.0670249	0.664226
RUGGEDNESS			
ANALYST – I			
CEF in SEM	101.6467 %	0.08165	0.080327
AMB in SEM	99.5883 %	0.30096	0.302204
CEF in DSM	99.9423 %	0.406252	0.406486
AMB in DSM	101.7700 %	0.0606432	0.595885
ANALYST – II			
CEF in SEM	101.4833 %	0.08165	0.080456
AMB in SEM	99.9366 %	0.302104	0.302295
CEF in DSM	99.2966 %	0.228619	0.230238
AMB in DSM	100.5800 %	0.898532	0.893351

SEM-simultaneous equation method, DSM-derivative spectroscopic method

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

The developed method discussed in the present work provides a convenient, accurate and time consuming. The wavelength selected for the simultaneous analysis of CEF and AMB was validated with respect to stability, linearity, sensitivity, precision, accuracy, specificity, robustness and ruggedness.

Validation of developed method

Linearity and precision

In quantitative analysis the calibration curve was constructed for both CEF and AMB after analysis of consecutively increased concentrations. Six samples of the same concentration (n=6) of CEF and AMB were prepared and analysed. The low % RSD values of 0.0803 and 0.3022 in method A, 0.4064 and 0.5958 in method B had high precision and reproducibility. The regression equation, slope, intercept, correlation coefficient, precision and linearity range are given in **table 1.**

Accuracy (Recovery studies)

The recovery experiment was done by adding known concentrations of Cefpodoxime proxetil and Ambroxol HCl raw material to the 50% preanalyzed formulation. Standard CEF and AMB in the range of 80 %, 100 % and 120% to the 50% preanalyzed formulation into a series of 10 ml volumetric flasks and dissolved. The amount of each drug recovered. The procedure was repeated for three times for each percentage recovery and % RSD was 0.36011 and 0.1175 in method A, 0.2009 and 0.2378 in method B respectively. (**Table 2).**

Repeatability

Ruggedness of the method was confirmed by the analysis of formulation was done by the different analysts, using similar operational and environmental conditions. Intra-day precision and accuracy were evaluated by analysing three samples of two different concentrations, prepared on the same day, the % RSD were 0.0170 and 0.0919 in method A, 0.3685 and 0.6350 in method B. Inter-day variability was assessed by analysing two concentrations on three different days, over a period of one week, the % RSD were 0.9488 and 0.1682 in method A, 0.1981 and 0.6642 in method B respectively indicates the accuracy and reproducibility of the assays. (**Table 3)**

CONCLUSION

In this study a simple ,precise, accurate and sensitive UV-spectroscopy methods were developed for the simultaneous estimation of Cefpodoxime proxetil and Ambroxol HCl in pure and in tablet dosage form. As these proposed methods have the lowest LOD values 0.32308, 0.47452 for CEF and 0.5375, 0.35446 for AMB in method A and 0.04691 for CEF, 1.35733 for AMB in method B and wider linearity range, time consuming and less solvent was consumed so it is more sensitive method which was not yet published in this combination. From the results obtained, we conclude that the suggested methods showed high sensitivity, accuracy, reproducibility and specificity. Moreover these methods were simple and this can be employed for the routine quality control analysis of Cefpodoxime proxetil and Ambroxol HCl in pure and in tablet dosage form.

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