Research Article

Development and Validation of First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Ambroxol And Cefpodoxime in Combined Tablet Dosage Form

Jigar Goswami[1*], Jagdish Kakadiya[2] and Nehal Shah[1]

[1]Pharmaceutical Analysis and Quality assurance Department, Dharmaj degree Pharmacy College, Petlad-Khambhat road, Dharmaj, Anand, Gujarat, India.
[2]Pharmacology Department, Dharmaj degree Pharmacy College, Petlad-Khambhat road, Dharmaj, Anand, Gujarat, India.

ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical first derivative spectrophotometric method for the simultaneous determination of Ambroxol and cefpodoxime in combined tablet dosage form. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra was obtained in 0.1 N HCl and the determinations were made at 263 nm (ZCP of cefpodoxime) for Ambroxol and 307 nm (ZCP of Ambroxol) for cefpodoxime. The linearity was obtained in the concentration range of 6-72 µg/ml for Ambroxol and 10-90 µg/ml for cefpodoxime. The % mean recovery was 98.57 ± 1.6 % and 102.92 ± 1.32 % for Ambroxol and cefpodoxime, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of Ambroxol and cefpodoxime in pharmaceutical combined tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

Keywords: Ambroxol, Cefpodoxime, First order derivative spectrophotometric method.

1. INTRODUCTION

Ambroxol (AMB) is chemically trans–4–(2–Amino-3,5–dibromobenzylamino)-cyclohexanol[1] is a secretolytic agent used in the treatment of tracheobronchitis, emphysema with bronchitis pneumoconiosis, chronic inflammatory pulmonary conditions, bronchiectasis, bronchitis with bronchospasm asthma[2]. It is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP). IP[1] describes High Performance Liquid Chromatography (HPLC) method and BP[3] describes HPLC, Spectrophotometric and High Performance Thin Layer Chromatography (HPTLC) method. Literature survey also reveals Spectrophotometric[4-5], HPLC[6-7], Ultra Performance Liquid Chromatography (UPLC)[8] and HPTLC[9] methods for determination of AMB with other drugs. Cefpodoxime (CEFPO) is chemically 1-(isopropoxy carbonyloxy) ethyl (6R, 7R)-7-[2-(2-amino-4-thiazolyl)-(z)-2-methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate[10], is a third generation cephalosporin antibiotic. It is used for infections of the respiratory tract, urinary tract and skin and soft tissues. It has greater activity against staphylococcus aureus[11]. Cefpodoxime is official in IP and USP. IP[12] and USP[13] describe liquid chromatography method for its estimation. Literature survey reveals Spectrophotometric[14-15], RP-HPLC[16] and HPTLC[17] methods for determination of CEFPO with other drugs. The combined dosage forms of AMB and CEFPO are available in the market for the prophylaxis and treatment of chronic asthma and chronic bronchitis. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of AMB and CEFPO in their combined dosage forms. Literature survey does not reveal any simple Spectrophotometric or other method for simultaneous estimation of AMB and CEFPO in combined dosage forms. The present communication describes simple,

sensitive, rapid, accurate and economical spectrophotometric method based on First Order Derivative UV spectrophotometric method for simultaneous estimation of both drugs in their combined tablet dosage forms.

2. MATERIALS AND METHODS

2.1 Apparatus

A double beam UV/Visible spectrophotometer (shimadzu model 1800, Japan) with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. An analytical balance (K.ROY instruments Pvt. Ltd., Varanasi, India), an ultrasonic bath (Janki Impex Pvt. Ltd., Ahmedabad, Gujarat, India) was used in the study.

2.2 Reagents and Materials

AMB and CEFPO bulk powder was kindly gifted by Cadila Pharmaceuticals Ltd. Ahmedabad, Gujarat, India and Baroque Pharmaceutical Ltd., Khambhat, Anand, Gujarat, India respectively. The commercial fixed dose combination product FINECEF- AM (AMB – 60 mg, CEFPO – 100 mg) was procured from the local market which is manufactured by Abott Healthcare Private Limited (AHPL). 0.1 N Hydrochloride (HCl) solution is used as solvent for the preparation of different concentration of both drugs AMB and CEFPO.

2.3 Preparation of standard stock solutions

An accurately weighed quantity of AMB (100 mg) and CEFPO (100 mg) were transferred to a separate 100 ml volumetric flask and 50 ml 0.1 N HCl is added to both volumetric flask and sonicated for 5 minutes. Volume was adjusted up to the mark with 0.1 N HCl to obtain standard solution having concentration of AMB (1000 µg/ml) and CEFPO (1000 µg/ml). 10 ml solutions of AMB (1000 µg/ml) and CEFPO (1000 µg/ml) were transferred to a separate 100 ml volumetric flask and diluted up to concentration of AMB (100 µg/ml) and CEFPO (100 µg/ml) with 0.1 N HCl.

2.4 Methodology

The standard solutions of AMB (18 µg/ml) and CEFPO (30 µg/ml) were scanned separately in the UV range of 200-400 nm. The zero-order spectra thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 0.1 nm. The two spectra were overlain and it appeared that AMB showed zero crossing at 244.2 nm, 276 nm, 307 nm, while CEFPO showed zero crossing at 263 nm. At the zero crossing point (ZCP) of AMB (307 nm), CEFPO showed a significance first-derivative absorbance, whereas at the ZCP of CEFPO (263 nm), AMB showed a significance first-derivative absorbance. Hence 263 and 307 nm was selected as analytical wavelengths for determination of AMB and CEFPO, respectively. These two wavelengths can be employed for the determination of AMB and CEFPO without any interference from the other drug in their combined dosage formulations.

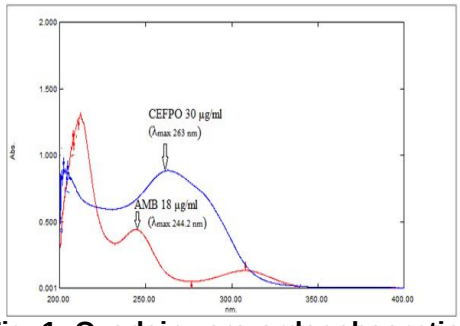

Fig. 1: Overlain zero-order absorption spectra of AMB and CEFPO in 0.1NHCl

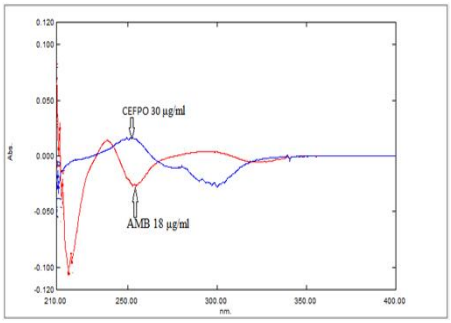

Fig. 2: Overlain first-order derivative spectra of AMB and CEFPO in 0.1 N HCl

3. VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[18].

3.1 Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 6-72 µg/ml for AMB and 10-90 µg/ml for CEFPO. Accurately measured standard solutions of AMB (0.6, 1.2,1.8,2.4,3.0,3.6,4.2,4.8,5.4,6.0,6.6 and 7.2 ml) and CEFPO (1.0,2.0,3.0,4.0,5.0,6.0,7.0,8.0, and 9.0 ml) were transferred to a series of 10 ml of volumetric flasks from the standard stock solution of AMB (100 µg/ml) and CEFPO (100 µg/ml) respectively and diluted to the mark with 0.1 N HCl. First-derivative absorbance (D1) was measured at 263 nm for AMB and 307 nm for CEFPO. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

3.2 Method precision (repeatability)

The precision of this method was checked by repeated scanning and measurement of absorbance of solution (n = 6) for AMB (42 µg/ml) and CEFPO (70 µg/ml) without changing the parameter of the first-derivative spectrophotometry method.

3.3 Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of AMB and CEFPO (54,60,66 µg/ml for AMB and 50,60,70 µg/ml for CEFPO). The result was reported in terms of relative standard deviation (% RSD).

3.4 Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of AMB and CEFPO by the standard addition method. Known amounts of standard solutions of AMB and CEFPO were added at 50, 100 and 150 % level to prequantified sample solutions of AMB and CEFPO (18 µg/ml and 30 µg/ml for AMB and CEFPO, respectively). The amounts of AMB and CEFPO were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times.

4. Analysis of Amb and Cefpo In Combined Tablet Dosage Form

Twenty Tablets were weighed and powdered. The powder equivalent to 60 mg of AMB and 100 mg of CEFPO was transferred to a 100 ml volumetric flask. 0.1 N HCl (50 ml) was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with 0.1 N HCl. This solution is expected to contain 600 µg/ml of AMB and 1000 µg/ml of CEFPO. This solution (10 ml) was taken in to a 100 ml volumetric flask and the volume was adjusted up to mark with 0.1 N HCl to get a concentration of AMB (60 µg/ml) and CEFPO (100 µg/ml). This solution (3 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with 0.1 N HCl to get a concentration of AMB (18 µg/ml) and CEFPO (30 µg/ml). The responses of the sample solution were measured at 263 nm and 307 nm for quantification of AMB and CEFPO, respectively. The amounts of the AMB and CEFPO present in the sample solution were calculated by fitting the responses into the regression equation for AMB and CEFPO in the proposed method.

5. RESULTS AND DISCUSSION

The standard solutions of AMB and CEFPO were scanned separately in the UV range, and zero-order spectra (Figure 1) thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 0.1 nm. The first derivative spectra showed significance absorbance at 263 nm (ZCP of CEFPO) for AMB and 307 nm (ZCP of AMB) for CEFPO. So, first-derivative absorbances (D1) were recorded 263 nm for AMB and 307 nm for CEFPO (Figure 2). First derivative spectra give good quantitative

determination of both the drugs at their respective without any interference from the other drug in their combined dosage formulations.

Linear correlation was obtained between absorbance and concentration of AMB and CEFPO in the concentration ranges of 6-72 µg/ml and 10-90 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table 1). The RSD values for AMB and CEFPO for repeatability were found to be 1.71 and 0.97 %, respectively (Table 1). The relative standard deviation (less than 2 %) indicates that the proposed method is repeatable. The RSD values of interday (1.63-1.96 % and 1.39-1.9 %) and intraday (1.61-1.94 % and 1.38-1.88 %) for AMB and CEFPO, respectively, reveal that the proposed method is precise (Table 1). LOD values for AMB and CEFPO were found to be 1.931 and 2.249 µg/ml, respectively and LOQ values for AMB and CEFPO were found to be 5.85 and 6.816 µg/ml, respectively (Table 1). These data show that proposed method is sensitive for the determination of AMB and CEFPO.

The recovery experiment was performed by the standard addition method. The % mean recoveries were 98.57 ± 1.6 % and 102.92 ± 1.32% for AMB and CEFPO, respectively (Table 2). The results of recovery studies indicate that the proposed method is accurate. The proposed validated method was successfully applied to determine AMB and CEFPO in their combined dosage form. The results obtained for AMB and CEFPO were comparable with the corresponding labelled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of AMB and CEFPO in pharmaceutical dosage forms.

Table 1: Regression analysis data and summary of validation parameters for the proposed method

Parameters	First order Derivative UV Spectroscopy	
	AMB	CEFPO
Concentration Range (µg/ml)	6-72	10-90
Slope (m) Intercept (c)	0.000533 0.000955	0.0006 0.000444
Correlation Coefficient (R^2)	0.9982	0.999
Accuracy (% recovery) (n = 3)	98.57 ± 1.6	102.92 ± 1.32
Repeatability (%RSD) (n = 6)	1.71 %	0.97 %
Interday (n = 3) (%RSD)	1.63-1.96 %	1.39-1.9 %
Intraday(n = 3) (%RSD)	1.61-1.94 %	1.38-1.88 %
LOD (µg/ml)	1.931	2.249
LOQ (µg/ml)	5.85	6.816

Table 2: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Amount Recovered (µg/ml) (n=3)	% Recovery (n=3)
AMB	0 %	18	0	17.33	96.29
	50 %	18	9	26.66	98.76
	100 %	18	18	36.66	100
	150 %	18	27	44.66	99.25
CEFPO	0 %	30	0	30.44	101.48
	50 %	30	15	45.44	102.22
	100 %	30	30	62.11	104.44
	150 %	30	45	78.22	103.55

Table 3: Analysis of AMB and CEFPO by proposed method

	Label claim (mg)		Amount taken (µg/ml)		Amount Recovered (µg/ml) (n=3)		% Label claim	
Tablet	AMB	CEFPO	AMB	CEFPO	AMB	CEFPO	AMB	CEFPO
I	60	100	18	30	17.33	30.44	96.27	101.46
II	60	100	18	30	18	31	100	103.33

6. CONCLUSION

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 6-72 µg/ml and 10-90 µg/ml for AMB and CEFPO, respectively with co-efficient of correlation, (R^2)=0.9982 and (R^2) = 0.999 for AMB and CEFPO, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. This is also a cost effective method. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of AMB and CEFPO. The method can be used for the routine analysis of the AMB and CEFPO in combined dosage form without any interference of excipients.

7. ACKNOWLEDGEMENT

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