INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

DEVELOPMENT AND VALIDATION OF DUAL WAVELENGTH UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF AMBROXOL HYDROCHLORIDE AND CEFPODOXIME PROXETILE IN THEIR COMBINED TABLET DOSAGE FORM

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

[1]Pharmaceutical Chemistry Department, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Petlad-Khambhat road, Anand, Gujarat, India

[2]Pharmacology Department, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Petlad-Khambhat road, Dharmaj, Anand, Gujarat, India

Article Received on: 12/02/12 Revised on: 23/03/12 Approved for publication: 10/04/12

*Email: jagdishkakadiya@yahoo.co.in

ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical dual wavelength spectrophotometric method for the simultaneous determination of Ambroxol Hydrochloride and Cefpodoxime Proxetile in combined tablet dosage form. The principle for dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". The method was based on determination of Cefpodoxime proxetile at the absorbance difference between 230 nm and 251.8 nm and Ambroxol Hydrochloride at the absorbance difference between 250.7 nm and 279 nm. The linearity was obtained in the concentration range of 6-42 µg/ml and 10-70 µg/ml for Ambroxol Hydrochloride and Cefpodoxime Proxetile respectively. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found. The suitability of these methods for the quantitative determination of Ambroxol Hydrochloride and Cefpodoxime Proxetile was proved by validation and recovery study. The proposed methods were found to be simple and sensitive for the routine quality control application of Ambroxol Hydrochloride and Cefpodoxime Proxetile in pharmaceutical tablet dosage form.

Keywords: Ambroxol Hydrochloride, Cefpodoxime Proxetile, dual wavelength UV spectrophotometric method.

INTRODUCTION

Ambroxol Hydrochloride (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 Proxetile (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 Dual Wavelength UV spectrophotometric method for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIALS AND METHODS

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.

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.

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.

Methodology

The working standard solutions of AMB and CEFPO were prepared separately in 25 ml volumetric flask using 0.1 N HCl as a solvent. They were scanned in the UV range of 200-400 nm. From the overlain spectra, four wavelengths 230 nm (λ_1), 251.8 nm (λ_2), 250.7 nm (λ_3) and 279 nm (λ_4) were selected for quantitation of both the drugs by proposed dual wavelength spectrophotometric method. The quantitative determination of CEFPO is carried out by measuring the absorbance difference value at between 230 nm and 251.8 nm where AMB have same absorbance at both the wavelengths. The absorbance difference between 230 nm and 251.8 nm is directly proportional to concentration of CEFPO. The quantitative determination of AMB is carried out by measuring the absorbance difference value at 250.7 nm and 279 nm where CEFPO has same absorbance at both the wavelengths. The absorbance difference between 250.7 nm and 279 nm is directly proportional to concentration of AMB.

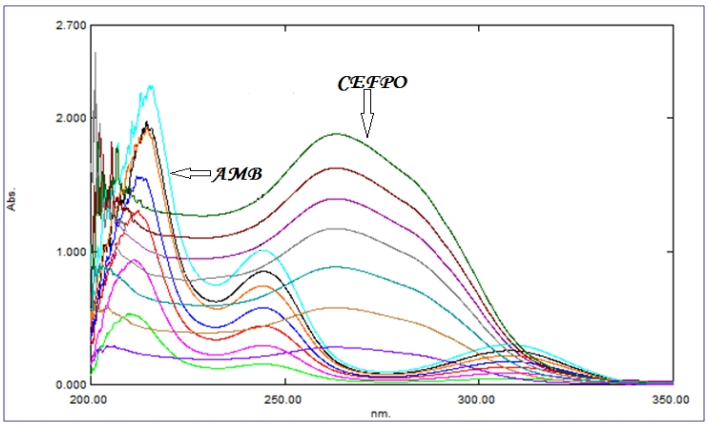

Figure 1: Overlain zero-order absorption spectra of AMB and CEFPO in 0.1NHCl

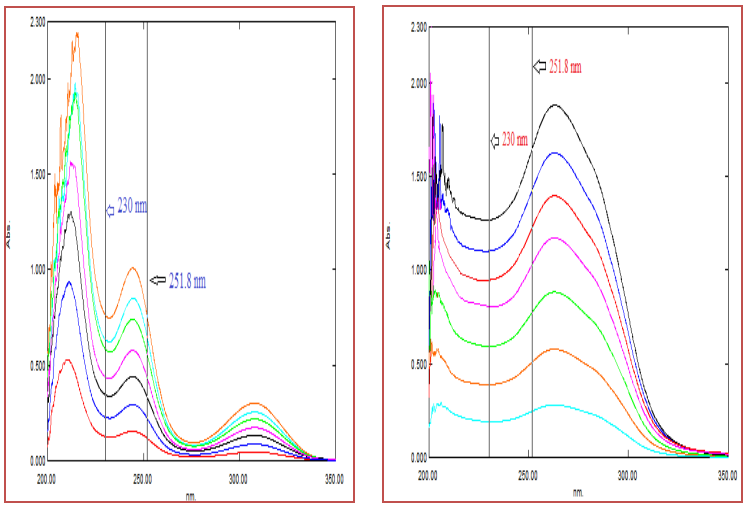

Figure 2: Spectra of Ambroxol and Cefpodoxime for different conc. at 230nm and 251.8 nm where AMB has same Absorbance and CEFPO has different Absorbance

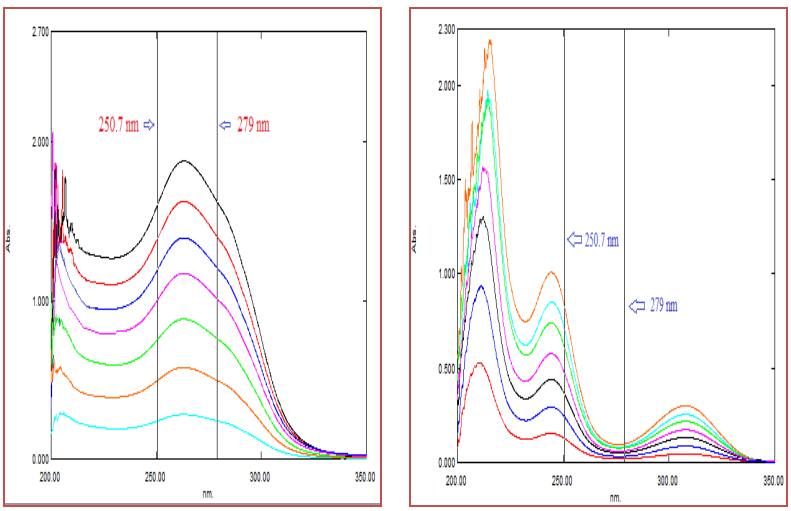

Figure 3: Spectra of Ambroxol and Cefpodoxime for different concentration at 250.7 nm and 279 nm where CEFPO has same Absorbance and AMB has different Absorbance

VALIDATION OF THE PROPOSED METHOD

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

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 6-42 µg/ml for AMB and 10-70 µg/ml for CEFPO. Appropriate volume of aliquot from standard stock solution AMB (100 µg/ml) and CEFPO (100 µg/ml) was transferred to different volumetric flasks of 25 ml capacity. The volume was adjusted to the mark with the 0.1 N HCl to obtain concentration of 6, 12, 18, 24, 30, 36 and 42 µg/ml AMB and 10, 20, 30, 40, 50, 60 and 70 µg/ml. These solutions scanned separately in the UV range of 200-400 nm. The absorbances of the solutions were measured at 230 nm (λ_1), 251.8 nm ($\lambda 2$), 250.7 nm ($\lambda 3$) and 279 nm ($\lambda 4$). The difference in absorbance between 230 nm (λ_1) and 251.8 nm (λ_2) is due to the CEFPO and was plotted against CEFPO concentration (µg/ml). The difference in absorbance between 250.7 nm (λ_3) and 279 nm (λ_4) is due to the AMB and was plotted against AMB concentration (µg/ml) and two different regression equations were obtained.

Method precision (repeatability)

The precision of this method was checked by repeated scanning and measurement of absorbance of solution (n = 6) for AMB (6, 12, 18, 24, 30, 36 and 42 µg/ml) and CEFPO (10, 20, 30, 40, 50, 60 and 70 µg/ml) without changing the parameter of the proposed spectrophotometry method.

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 (24,30,36 µg/ml for AMB and 50,60,70 µg/ml for CEFPO). The result was reported in terms of relative standard deviation (% RSD).

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 (18µg/ml and 30µg/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.

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). From this solution 7.5 ml was taken in to a 25 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 230 nm (λ_1), 251.8 nm (λ_2), 250.7 nm (λ_3) and 279 nm (λ_4) for quantification of AMB and CEFPO. 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.

RESULTS AND DISCUSSION

The standard solutions of AMB and CEFPO were scanned separately in the UV range 200 – 400 nm. From the overlain spectra of both drugs, four specific wavelengths are selected. The absorbance at 230 nm (λ_1) and 251.8 nm (λ_2) wavelengths was found to be with same absorbance for AMB. The difference in absorbance at these two wavelengths ($A_{251.8} - A_{230}$) cancels out the contribution of absorbance of AMB. These two selected wavelengths were employed to determine the concentration of CEFPO. Similarly, the absorbance at 250.7 nm (λ_3) and 279 nm (λ_4) wavelengths was found to be with same absorbance for CEFPO. The difference in absorbance at these two wavelengths ($A_{250.7} - A_{279}$) cancels out the contribution of absorbance of CEFPO. These two selected wavelengths were employed to determine the concentration of AMB.

The proposed method was found to be simple, sensitive, rapid, accurate, precise and economic for the routine simultaneous estimation of two drugs. The linearity range for AMB and CEFPO were found to be 6-42 µg/ml and 10-70 µg/ml respectively. Regression analysis data and summary of all validation parameters is given in Table 1. Precision was calculated as repeatability (% RSD) and intra and inter day variation (% RSD) for both the drugs. Accuracy was determined by calculating the recovery and the mean was determined. The LOD and LOQ were found to be 1.06 and 3.23µg/ml respectively for AMB and 2.95 and 8.94 µg/ml respectively for CEFPO indicates sensitivity of the proposed method. The method was successfully used to determine the amounts of AMB and CEFPO present in tablets. The results obtained are in good agreement with the corresponding labelled amount. By observing the validation parameters, the method was found to be sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these drugs in combinations.

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

Parameters	Dual wavelength Spectroscopy method	
	AMB	CEFPO
Concentration Range (µg/ml)	6-42	10-70
Slope (m)	0.01715	0.00526
Intercept (c)	0.00476	0.01067
Correlation Coefficient (r^2)	0.99927	0.99800
Accuracy (% recovery) (n = 3)	98.75 – 99.97 %	99.76 – 100.4%
Repeatability (%RSD) (n = 6)	0.63 %	0.74 %
Interday (n = 3) (%RSD)	0.25 – 0.64 %	0.30 – 0.67 %
Intraday(n = 3) (%RSD)	0.16 – 0.48 %	0.82 – 1.80 %
LOD (µg/ml)	1.06 µg/ml	2.95 µg/ml
LOQ (µg/ml)	3.23 µg/ml	8.94 µg/ml

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	17.90	99.44 ± 0.65
	50 %	18	9	26.98	99.95 ± 0.28
	100 %	18	18	35.55	98.75 ± 0.12
	150 %	18	27	44.98	99.97 ± 0.52
CEFPO	0 %	30	0	30.12	100.4 ± 0.29
	50 %	30	15	44.92	99.82 ± 0.35
	100 %	30	30	59.87	99.78 ± 0.19
	150 %	30	45	74.82	99.76 ± 0.49

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	18.13	30.36	100.72	101.2

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 6-42 µg/ml and 10-70 µg/ml for AMB and CEFPO, respectively with co-efficient of correlation, (r^2)=0.99928 and (r^2) = 0.99800 for AMB and CEFPO, respectively. The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of AMB and CEFPO in tablet dosage form. The method utilizes easily available and cheap solvent for analysis of AMB and CEFPO hence the method was also economic for estimation of AMB and CEFPO from tablet dosage form. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of AMB and CEFPO in method, hence it can be conveniently adopted for routine quality control.

ACKNOWLEDGEMENT

The authors are thankful to Cadila Pharmaceuticals Ltd. Ahmedabad, Gujarat, India and Baroque Pharmaceutical Ltd., Khambhat, Anand, Gujarat, India for providing gift sample of AMB and CEFPO, respectively for research. The authors are highly thankful to Indubhai Patel college of Pharmacy and Research centre, Dharmaj, Gujarat, India for providing all the facilities to carry out the work.

REFERENCES

1. Indian pharmacopoeia, Govt. of India, "ministry of health and family welfare", controller and publication, Delhi, 2007, Vol. 2, 78-79.
2. http://www.drugs.com/international/ambroxol.html
3. British Pharmacopoeia, Government British Pharmacopoeial Commission, 2011, volume I and II, monograph 1489.
4. Prasanthi N. L., Mohan Ch. Krishna, Manikiran S.S., Rao N. Rama, "Estimation of Ambroxol hydrochloride and Guiaphensin in tablet dosage form by simultaneous equation method", International Journal of Research in Ayurveda & Pharmacy. 2010; 1(1): 140-146.
5. Makarand Avhad, Dr. C.G. Bonde, "Development and validation of simultaneous UV spectrophotometric Method for the determination of Levofloxacin and Ambroxol in tablets", International Journal of ChemTech Research. 2009; 1(4): 873-878.
6. Mukesh Maithani, Richa Raturi, Vertika Gautam, Dharmendra Kumar, Anand Gaurav and Ranjit Singh, "Simultaneous estimation of Ambroxol hydrochloride and Cetirizine hydrochloride in tablet dosage form by RP-HPLC method", International Journal of Comprehensive Pharmacy.2010;1(2): 1-3.
7. Maarit Heinanen, Coral Barbas, "Validation of an HPLC method for the quantification of Ambroxol hydrochloride and Benzoic acid in a syrup as pharmaceutical form stress test for stability evaluation", Journal of Pharmaceutical and Biomedical Analysis. 2001; 24:1005-10.
8. Rakshit Kanubhai Trivedi, Mukesh C. Patel, Sushant B. Jadhav, "A Rapid, Stability Indicating RP-UPLC method for simultaneous determination of Ambroxol hydrochloride, Cetirizine hydrochloride and antimicrobial preservatives in liquid pharmaceutical formulation", Scientific Pharma.2011;79: 525–43.
9. Shashikant B. Bagade, Narendra M. Gowekar And Avinash V. Kasture, "Simultaneous HPTLC Estimation of Ambroxol HCl and Cetirizine HCl in Their Combined Dose Tablet", Asian Journal of Chemistry. 2007; 19(2): 1487-1493.
10. http://www.drugbank.ca/drugs/DB01416
11. Sweetman SC, Eds. In The Martindale: The Complete Drug Reference, 35th edition. London, Pharmaceutical Press, 2007; 207.
12. Indian pharmacopoeia, Govt. of India, "ministry of health and family welfare", controller and publication, Delhi, 2010; Vol. III: 1018.
13. United State Pharmacopoeia; Government of United States "Department Of Health and Human Service" USP30-NF25; 31(04):1673.
14. Patel Sanket A, Patel Satish A. " Dual Wavelength Spectrophotometric Method for Simultaneous Estimation of Ofloxacin and Cefpodoxime Proxetil in Tablet Dosage Form"Asian Journal of Pharmacy and Life Science 2001; 1(3): 201.
15. Gandhi SV, Patil UP, Patil NG."Simultaneous spectrophotometric determination of Cefpodoxime proxetil and Potassium clavulanate" Hindustan Antibiot Bull. 2009; 51(1-4):24-8.
16. Malathi S, Dubey R. N, and Venkatnarayanan R. "Simultaneous RP-HPLC Estimation of Cefpodoxime Proxetil and Clavulanic Acid in Tablets" Indian J Pharm Sci. 2009; 71(1): 102–105.
17. Thomas A. B., Dighe S. B, Nanda R K., Kothapalli l. P, Jagdale S. N. & Deshpande A. D. "A validated stability indicating HPTLC method for simultaneous estimation of cefpodoxime proxetil and potassium clavulanate in bulk and tablet dosage form" Journal of liquid chromatography & related technologies.2010;33(18):1689-1703.
18. ICH Harmonized Tripartile Guideline. Validation of Analytical Procedures: Text and Methodology Q2 (R2), International Conference on Harmonization, Geneva, Switzerland Nov. 2005.

Source of support: Nil, Conflict of interest: None Declared