Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (19):191-196
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Spectrophotometric Simultaneous Estimation of Ambroxal hydrochloride and Amoxicillin by Third Order derivative Method in Combined Dosage Form

Rajan V. Rele

Central research laboratory, D.G. Ruparel College, Matunga, Mumbai 400016

ABSTRACT

The objective of the study was to develop a economical, a, precise and rapid a UV spectrophotometric i.e. Third order derivative method for the determination of ambroxal hydrochloride and amoxicillin in combined dosage form i.e. tablets by using 0.1 N hydrochloric acid as a solvent. The method was further validated by ICH guidelines. The proposed third order derivative method involves the measurement of absorbance of one drug at zero crossing point of other; hence wavelengths 215 nm and 236.2 nm were selected for the estimation of ambroxal hydrochloride and amoxicillin respectively. The linearity of the proposed method was found in the concentration range of 1 to 10 µg /ml (r^2= 0.9987) for ambroxal hydrochloride and 10 to 100 µg /ml (r^2= 0.9999) for amoxicillin respectively. The percentage mean recovery was found to be 99.981 % for ambroxal hydrochloride and 100.15 % for amoxicillin respectively. The method was also statistically validated for its linearity, accuracy and precision. Both intra and inter day variations showed less percentage (%) RSD values indicating high grade of precision of this method.

Keywords: UV spectrophotometric estimation, Third order derivative method, Ambroxal hydrochloride, Amoxicillin

---

INTRODUCTION

Amoxicillin tri-hydrate is described chemically as 6 - (D - 4 hydroxy phenyl glycyl amino) penicillin acid tri-hydrate. It is semi- synthetic penicillin that belongs to the class of β – lactam antibiotics. It is generally used as antibacterial. Amoxicillin tri-hydrate is official in USP [1], IP [2] and BP [3].

Ambroxal Hydrochloride is trans-4-[(2Amino-3,5-dibromobenzyl)amino] cyclohexanol. It shows molecular formula as $C_{13}H_{18}Br_2N_2O.HCl$ with molecular weight 414.57. It is official in IP [2] and BP [3]. Ambroxal is a metabolite of bromhexine. It is an expectoration improver and mucolytic agent used in the treatment of acute and chronic disorders characterized by the production of excess or thick mucus.

Literature survey reveals HPLC [4], HPTLC[5, 6] and spectrophotometric [7, 8] methods for assay of combined dosage form. In this communication a new simple, UV spectrophotometric, third order derivative method is reported for simultaneous determination of amoxicillin trihydrate and ambroxal hydrochloride in combination dosage form. This simple method can also be used for the routine analysis of this combination formulation. In the proposed work development, optimization and validation of the method are presented.

MATERIALS AND METHODS

Instrument and reagents

Spectral scan was made on a Shimadzu UV-spectrophotometer, model 1800 (Shimadzu, Japan) with spectral band width of 0.5 nm with automatic wavelength corrections by using a pair of 10 mm quartz cells. All spectral measurements were done by using UV-Probe 2.42 software.

Reference standard of ambroxal hydrochloride and amoxicillin were obtained from reputed firm with certificate of analysis.

Preparation of standard drug solutions

A 100 mg standard ambroxal hydrochloride was weighed accurately and transferred to a 100 ml volumetric flask and sonicated with 30 ml 0.1 N hydrochloric acid for 15 minutes. The volume was made up to the mark with 0.1 N hydrochloric acid to give a stock solution of ambroxal hydrochloride of concentration 1000 µg /ml. From this solution, 10 ml of solution was pipetted out and transferred into 100 ml volumetric flask. The volume was made up to mark with 0.1 N hydrochloric acid to give a working standard solution of concentration 100 µg/ml.

Similarly 100 mg standard amoxicillin was weighed accurately and transferred to a 100 ml volumetric flask and sonicated with 30 ml of 0.1 N hydrochloric acid for 15 minutes. The volume was made up to the mark with 0.1 N hydrochloric acid to give a stock solution of 0.1 N hydrochloric acid of concentration 1000 µg /ml. From this solution, 10 ml of solution was pipetted out and transferred into 100 ml volumetric flask. The volume was made up to mark with 0.1 N hydrochloric acid to give a working standard solution of concentration 100 µg/ml.

Estimation from tablets

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 30 mg of ambroxal hydrochloride and 250 mg of amoxicillin was weighed and transferred in 100 ml of volumetric flask. A 30 ml of 0.1 N hydrochloric acid was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with 0.1 N hydrochloric acid to give concentration as 300 µg /ml of ambroxal hydrochloride and 2500 µg/ml of amoxicillin respectively. For working sample solution 1 ml of such solution was diluted to 100 ml and such solution was used for analysis.

Method: Third order derivative method

(a) For ambroxal hydrochloride

For the selection of analytical wavelength, 100 µg/ml solution of ambroxal hydrochloride was scanned in the spectrum mode from 350 nm to 200 nm by using 0.1 N hydrochloric acid as blank. The third order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured at 215 nm.

(b) For amoxicillin

For the selection of analytical wavelength, 100 µg/ml solution of amoxicillin was scanned in the spectrum mode from 350 nm to 200 nm by using 0.1 N hydrochloric acid as blank. The third order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured at 236.2 nm.

Preparation of calibration curves

Series of solutions containing 1 – 10 µg/ ml of ambroxal hydrochloride and 10 -100 µg/ ml of amoxicillin were used to determine linearity of the proposed method respectively. Solutions were scanned in the spectrum mode and absorbance spectra were converted to third order derivative spectra. The overlain spectrum of ambroxal hydrochloride and amoxicillin were given in Fig. 1(a), 1(b) respectively.

Fig. 1(a): Overlain spectra of third order derivative of ambroxal hydrochloride in the concentration range of 2– 10 µg/ ml

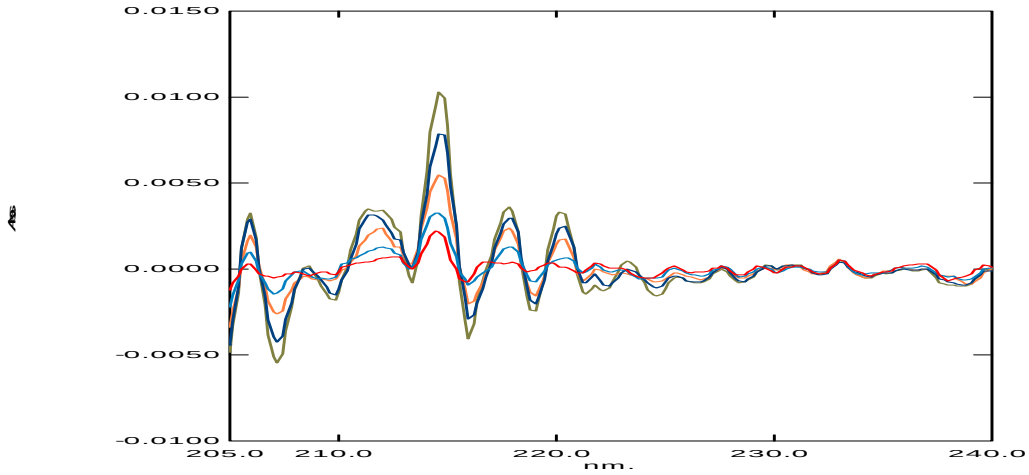
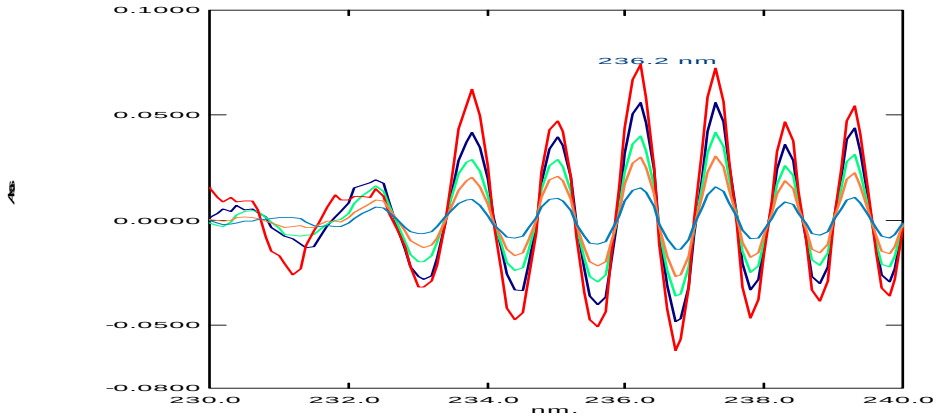


Fig. 1(b): Overlain spectra of third order derivative of amoxicillin in the concentration range of 20 – 100 µg/ ml



After observing the overlain third order derivative spectra of ambroxal hydrochloride and amoxicillin, the zero crossing points of both drugs were selected for analysis of other drug. The third wave length selected was 215 nm, the zero crossing point of amoxicillin where ambroxal hydrochloride showed considerable absorbance. The second wavelength was 236.2 nm, the zero crossing point of ambroxal hydrochloride, where amoxicillin showed considerable absorbance. The calibration curves were plotted of amplitude against concentrations [Fig. 2 (a), 2(b)].

Fig.2 (a): Calibration curve of ambroxal hydrochloride in the concentration range of 2-10 µg/ml

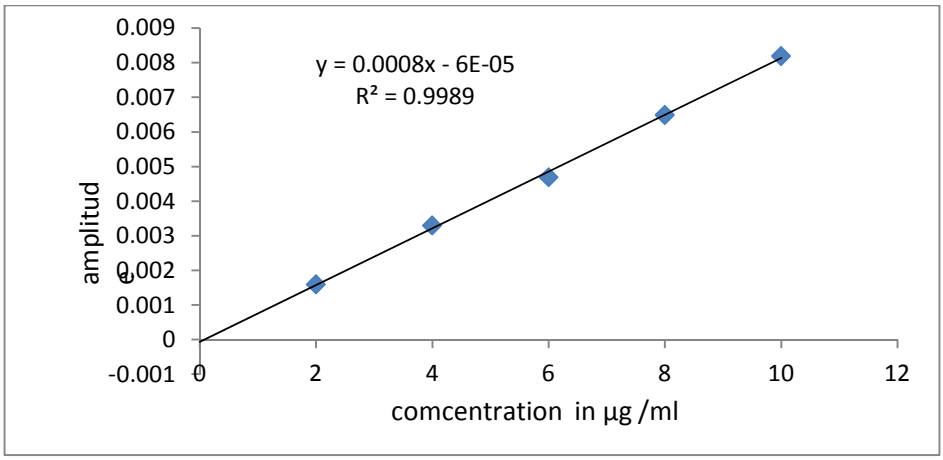
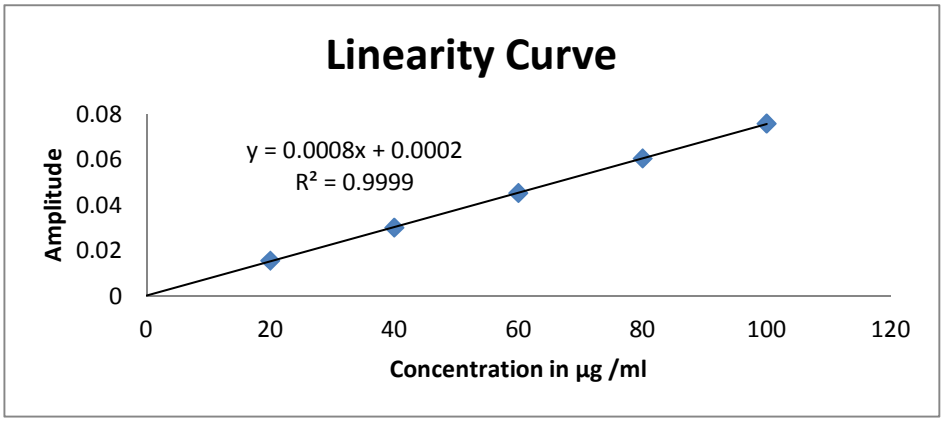


Fig.2 (b): **Calibration curve of amoxicillin in the concentration range of 10-100 µg/ml**



Results of the analysis are given in table 1.

Table 1: Values of results of optical and regression of drugs

Parameter	Ambroxal hydrochloride	Amoxicillin
Detection Wavelength (nm)	215	236.2
Beer Law Limits (µg/ml)	1-10	10-100
Correlation coefficient(r^2)	0.9989	0.9999
Regression equation (y=b+ac)		
Slope (a)	0.0008	0.0008
Intercept (b)	0.00006	0.0002

Estimation from capsules

Powdered from twenty capsules were collected and weighed accurately and average weight of powder from each capsule was determined. Powder equivalent to 30 mg of ambroxal hydrochloride and 100 mg of amoxicillin was weighed and transferred in 100 ml of volumetric flask. A 30 ml of 0.1 N hydrochloric acid was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with 0.1 N hydrochloric acid to give concentration as 30 µg /ml of ambroxal hydrochloride and 100 µg /ml of amoxicillin respectively. A 10 ml of such solutions was diluted to 100 ml. It was scanned in the range of 200-350 nm against 0.1 N hydrochloric acid as blank. The absorbance spectra were converted to third order derivative spectra. Calculations were done as per the equations. The concentrations of ambroxal hydrochloride and amoxicillin present in capsules were calculated by

substituting the values of absorbance in linearity equations.

(a) For ambroxal hydrochloride Y = 0.0008x - 0.00006
(b) For amoxicillin Y = 0.0008x + 0.0002

Method Validation
These methods were validated according to ICH guidelines.

Accuracy
To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percentage recovery for ambroxal hydrochloride and amoxicillin was found in the range of 99.630 % to 99.95% and 99.736% to 100.11 % respectively. (Table2).

Table 2: Statistical evaluation of the data subjected to accuracy

Level of % recovery	Amount present in µg/ml		Amount added in µg/ml		Amount found in µg/ml		% Recovery		Mean % recovery	
	AMB	AMO	AMB	AMO	AMB	AMO	AMB	AMO	AMB	AMO
80%	3	25	2.4	20	5.406	45.019	100.12	100.19	99.91	100.12
	3	25	2.4	20	5.039	45.036	99.86	100.08		
	3	25	2.4	20	5.387	45.049	99.76	100.11		
100%	3	25	3.0	25	5.993	50.080	99.89	100.16	100.05	100.02
	3	25	3.0	25	6.007	49.920	100.12	99.84		
	3	25	3.0	25	6.004	50.035	100.08	100.07		
120%	3	25	3.6	30	6.611	55.082	100.18	100.15	100.01	99.975
	3	25	3.6	30	6.594	54.890	99.91	99.80		
	3	25	3.6	30	6.605	55.121	99.94	100. 22		

AMB = Ambroxal hydrochloride, AMOX = Amoxicillin

Linearity
The linearity of measurement was evaluated by analyzing different concentration of the standard solutions of ambroxal hydrochloride and amoxicillin. For both the drugs concentration range was found to be 1-10 µg/ml for ambroxal hydrochloride and 10-100 µg/ml for amoxicillin.

Precision
The method precision was established by carrying out the analysis of powder blend from tablet containing 30 mg of ambroxal hydrochloride and 100 mg of amoxicillin. The assay was carried out for the drugs by using proposed analytical method in six replicates. The values of relative standard deviation were 0.1605 % for ambroxal hydrochloride and 0.07227 % for amoxicillin respectively indicating the sample repeatability of the method. The results obtained are tabulated in table 3**.**

Table 3: Statistical evaluation of the data subjected to method of precision

Sr. No.	Sample No.	% Assay	
		Ambroxal hydrochloride	Amoxicillin
1	1	99.87	100.17
2	2	100.11	100.21
3	3	100.15	100.07
4	4	99.75	100.12
5	5	100.14	100.25
6	6	99.87	100.08
Mean % assay		99.9816	100.15
% R.S.D.		0.1573	0.072279

Intra-day precision was estimated by assaying tablets powder blend containing 30 mg of ambroxal hydrochloride and 100 mg of amoxicillin. The assay was carried out for the drugs by using proposed analytical method in six replicates. The results were average for statistical evaluation.

Inter-day precision was estimated by assaying tablets powder blend containing 30 mg of ambroxal hydrochloride

and 100 mg of amoxicillin for three consecutive days (i.e. 1st, 3rd and 5th days). The statistical validation data for intra and inter day precision is summarized in table 4.

Table 4: Summary of validation parameter for intra-day and inter-day

Sr. No.	Parameters	Ambroxal hydrochloride	amoxicillin
1	Intra-day precision (N=3)amount found ± % R.S.D.	99.83% 0.1574	100.14% 0.07335
2	Inter-day precision (N=3)amount found ± % R.S.D.	98.884 0.1559	988.561% 0.05448

Both intra- day and inter-day precision variation found to be less in % RSD values. It indicates high degree of precision of the method.

RESULTS AND DISCUSSION

The developed third order derivative spectrophotometric method for simultaneous determination of ambroxal hydrochloride and amoxicillin in tablet formulation was found to be simple and convenient for the routine analysis of two drugs. The method is used to eliminate the spectral interference from one of the two drugs while estimating the other drug by selecting the zero crossing point on the derivative spectra of each drug as the selected wavelength. The proposed method is accurate, precise and reproducible. It is confirmed from validation data as given in tables 1 to 4. The % RSD was found to be less than 1, which indicates validity of method. Linearity was observed by linear regression equation method for ambroxal hydrochloride and amoxicillin in different concentration range. The correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity figure 2 (a) and 2 (b).

The assay results obtained by proposed method is shown in table 2 are in good agreement. Hence proposed method can be used for routine analysis of these two drugs in combined dosage form. Method is simple, accurate, precise, reliable, rapid, sensitive, reproducible and economical. It is validate as per ICH guidelines.

CONCLUSION

The proposed method is simple, precise, accurate and rapid for the determination of ambroxal hydrochloride and amoxicillin in combined dosage form. This method can be adopted as an alternative to the existing methods. It can be easily and conveniently adopted for routine quality control analysis.

Acknowledgement
Authors express sincere thanks to the principal of D.G. Ruparel College, Dr. Tushar Desai, for encouragement and providing laboratory facilities.

REFERENCES

[1] The United States Pharmacopeia. United States Pharmacopeia convention Inc, Rockville, USP 29 NF 24, **2008**,Vol no.30 (4), ,1208.
[2] Indian Pharmacopeia, Controller of Publication, Delhi, , **2007**, Vol- 1, II, III.
[3] British pharmacopoeia. Licensing division HMSO, Norwich, , **2003,** 357.
[4] 4.Chitlange SohanS.,Tawarengi SnehaR. Chaturevadi KaushalendraK.,*Asian journal of research in chemistry***, 2011**, 4(6),
[5] Sunil Rajaram Dhaneshwar, Madhura Vishal Dhoka, Shakuntal Chopade, Vidhya Bhusari, *Asian J.Pharm. Biol. Res.***,2011**,1(2),129-135.
[6] Chitlange SohanS.,Tawarengi SnehaR, Ritesh P Bhole, *Pharmaceutica Analytical Acta*,**2013**, 4,(7),1000261
[7] Madhura Dhoka, Shakuntala Sandage and Snehal Dumbre, *Asian Journal of Biochemical and pharmaceutical research*, **2011**,2 (1) ,585-592,
[8] Rele Rajan V., *Research J. Pharm. and Tech*.**2014,** 7(12),1441-1445.