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UV- Spectrophotometric Third Order derivative Method for Simultaneous Estimation of Ambroxal hydrochloride and Salbutamol sulphate by in Combined pharmaceutical Dosage Form

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ABSTRACT

The objective of the study was to develop a economical , accurate, precise and rapid a UV spectrophotometric i.e. third order derivative method for the simultaneous determination of ambroxal hydrochloride and salbutamol sulphate in combined dosage form i.e. tablets by using distilled water 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 233.8 nm were selected for the estimation of ambroxal hydrochloride and salbutamol sulphate respectively. The linearity of the proposed method was found in the concentration range of 1 to 10 µg /ml (r^2= 0.9989) for ambroxal hydrochloride and 1 to 16 µg /ml (r^2= 0.9971) for salbutamol sulphate respectively. The percentage mean recovery was found to be 99.981 % for ambroxal hydrochloride and 100.02 % for salbutamol sulphate 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, Salbutamol sulphate.

INTRODUCTION

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 BP[1] and IP[2]. 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.

Salbutamol sulphate is chemically known as bis [(1RS)-2-[(1, 1- dimethylethyl) amino] -1-[4-hydroxy-3-(hydroxymethyl) phenyl] ethanol] sulphate, is beta-adrenoceptor agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease. It is official in IP [2]. It is used to increase the volume and reduce the viscosity of tenacious sputum and is used as expectorant for productive cough. In literature survey reveals UV spectrophotometric [3] and HPLC[4-6] for simultaneous determination of ambroxal hydrochloride and salbutamol sulphate in combined dosage form. Combination of ambroxal hydrochloride and salbutamol sulphate is used for the treatment of asthma and bronchitis

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 salbutamol sulphate were obtained from reputed firm with certificate of analysis.

Preparation of standard drug solutions

10 mg standard ambroxal hydrochloride was weighed accurately and transferred to a 10 ml volumetric flask and sonicated with 5 ml distilled water for 15 minutes. The volume was made up to the mark with distilled water 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 distilled water to give a working standard solution of concentration 100 µg/ml.

Similarly 10 mg standard salbutamol sulphate was weighed accurately and transferred to a 10 ml volumetric flask and sonicated with 5 ml of distilled water for 15 minutes. The volume was made up to the mark with distilled water to give a stock solution of distilled water 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 distilled water 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 2 mg of salbutamol sulphate was weighed and transferred in 100 ml of volumetric flask. A 30 ml of distilled water was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with distilled water to give concentration as 300 µg /ml of ambroxal hydrochloride and 20 µg/ml of salbutamol sulphate respectively. For working sample solution 1 ml of such solution was diluted to 100 ml and such solution was used for analysis.

Experimental

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 distilled water 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 third derivative spectrum was measured at 215 nm.

(b) For salbutamol sulphate

For the selection of analytical wavelength, 100 µg/ml solution of salbutamol sulphate was scanned in the spectrum mode from 350 nm to 200 nm by using distilled water 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 third derivative spectrum was measured at 233.8 nm.

Preparation of calibration curves

Series of solutions containing 1 – 10 µg/ ml of ambroxal hydrochloride and 1 -14 µg/ ml of salbutamol sulphate 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 spectra of ambroxal hydrochloride and salbutamol sulphate were given in Fig. 1(a), 1(b) respectively.

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

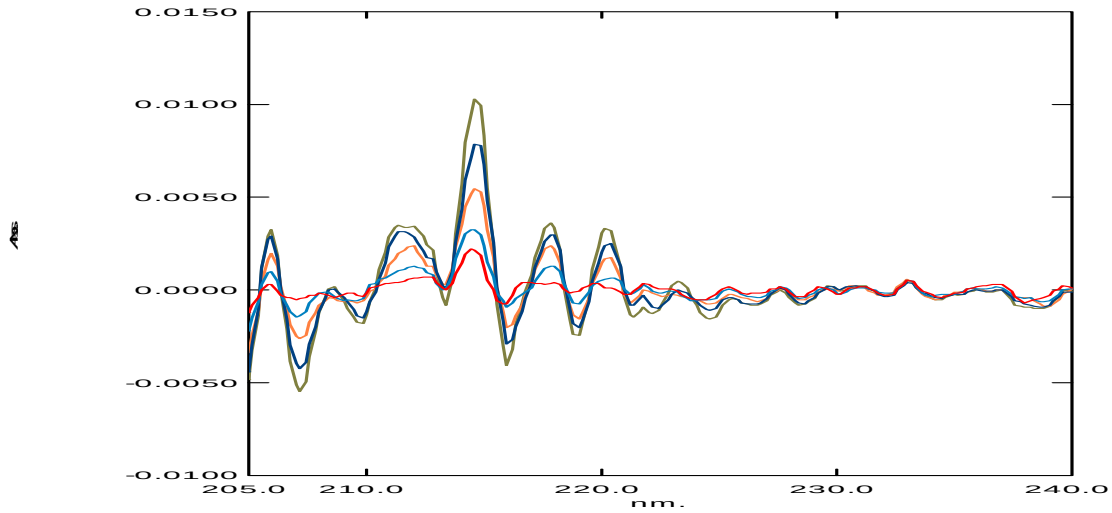
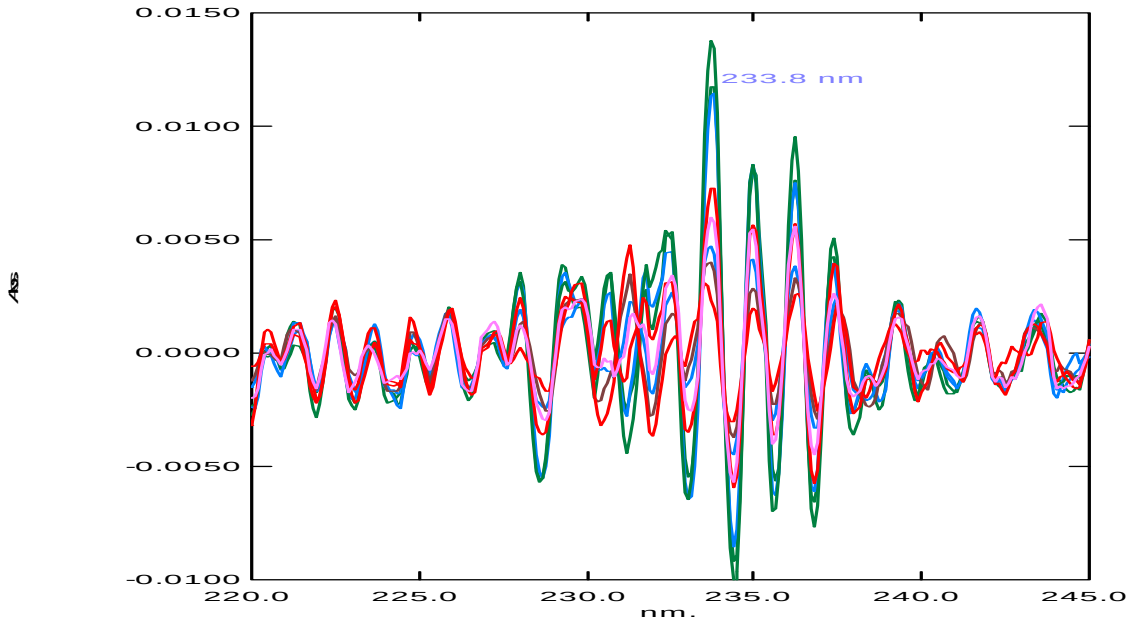


Fig. 1(b): Overlay spectra of third order derivative of salbutamol sulphate in the concentration range of 2 and 14 µg/ ml



After observing the overlain third order derivative spectra of ambroxal hydrochloride and salbutamol sulphate, the zero crossing points of both drugs were selected for analysis of other drug. The wave length selected was 215 nm, the zero crossing point of salbutamol sulphate where ambroxal hydrochloride showed considerable absorbance. The third wavelength was 233.8 nm, the zero crossing point of ambroxal hydrochloride, where salbutamol sulphate 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 2-12 µg/ml

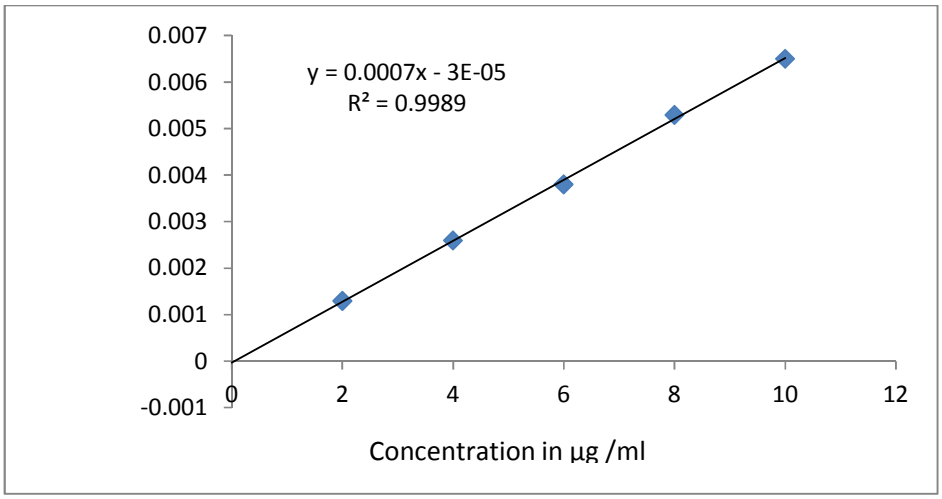
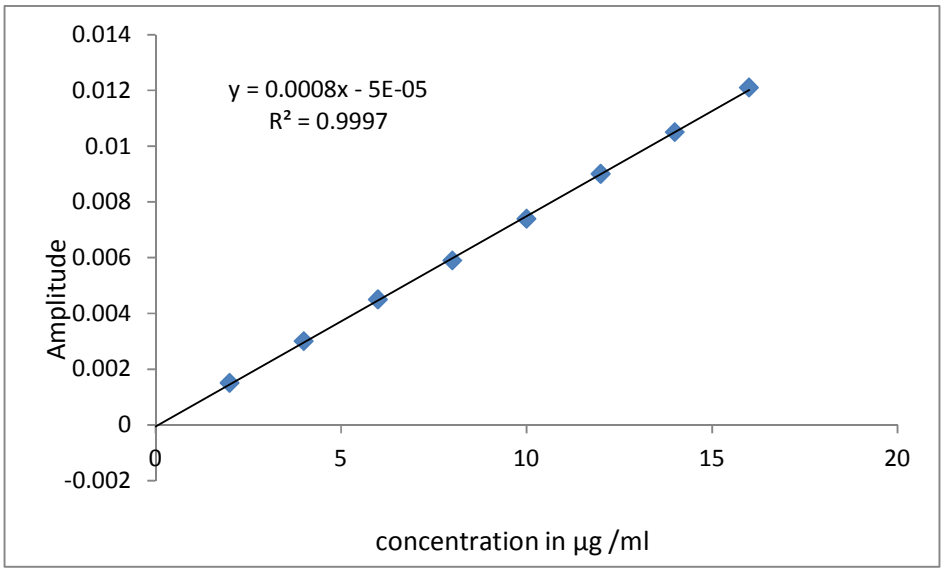


Fig.2 (b): Calibration curve of salbutamol sulphate in the concentration range of 2-14 µg/ml



Results of the analysis are given in table 1.

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

Parameter	Ambroxal hydrochloride	Salbutamol sulphate
Detection Wavelength (nm)	215	233.8
Beer Law Limits (µg/ml)	1-10	2-16
Correlation coefficient(r^2)	0.9989	0.9997
Regression equation (y=b+ac)		
Slope (a)	0.0007	0.0008
Intercept (b)	-0.00003	-0.00005

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 2 mg of salbutamol sulphate was weighed and transferred in 100 ml of volumetric flask. A 30 ml of distilled water was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with distilled water to give

concentration as 300 µg /ml of ambroxal hydrochloride and 20 µg /ml of salbutamol sulphate respectively. A 10 ml of such solutions was diluted to 100 ml. It was scanned in the range of 200-350 nm against distilled water 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 salbutamol sulphate present in capsules were calculated by substituting the values of absorbance in linearity equations.

(a) For ambroxal hydrochloride Y = 0.0007x – 0.00003
(b) For salbutamol sulphate Y = 0.0008x - 0.00001

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 salbutamol sulphate was found in the range of 99.651 % to 100.403% and 100.332% to 100.750 % 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	SAL	AMB	SAL	AMB	SAL	AMB	SAL	AMB	SAL
80%	10	2	8	1.6	18.025	3.605	**100.14**	100.15	100.09	100.15
	10	2	8	1.6	18.016	3.603	100.09	100.11		
	10	2	8	1.6	18.030	3.606	100.17	100.19		
100%	10	2	10	2	20.010	4.006	100.05	100.17	99.883	100.093
	10	2	10	2	19.970	4.008	99.85	100.22		
	10	2	10	2	19.950	3.995	99.75	99.89		
120%	10	2	12	2.4	22.008	4.407	100.04	100.18	100.03	100.143
	10	2	12	2.4	22.033	4.408	100.15	100.19		
	10	2	12	2.4	21.980	4.402	99.91	100.06		

AMB = Ambroxal hydrochloride, SAL=Salbutamol sulphate

Linearity
The linearity of measurement was evaluated by analyzing different concentration of the standard solutions of ambroxal hydrochloride and salbutamol sulphate. For both the drugs concentration range was found to be 1-10 µg/ml for ambroxal hydrochloride and 1-14 µg/ml for salbutamol sulphate.

Precision
The method precision was established by carrying out the analysis of powder blend from capsules containing 30 mg of ambroxal hydrochloride and 2 mg of salbutamol sulphate. The assay was carried out for the drugs by using proposed analytical method in six replicates. The values of relative standard deviation were 0.1573 % for ambroxal hydrochloride and 0.1433% for salbutamol sulphate in 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	Salbutamol sulphate
1	1	99.87	100.04
2	2	100.11	100.19
3	3	100.15	100.07
4	4	99.75	100.12
5	5	100.14	99.89
6	6	99.87	99.81
Mean % assay		99.9816	100.02
%R.S.D.		0.1573	0.1433

Intra-day precision was estimated by assaying tablets powder blend containing 30 mg of ambroxal hydrochloride and 2 mg of salbutamol sulphate. 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 2 mg of salbutamol sulphate 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	salbutamol sulphate
1	Intra-day precision (N=3)amount found ±% R.S.D.	99.75% 0.1912	99.75% 0.1514
2	Inter-day precision (N=3)amount found ±% R.S.D.	98.774 0.1142	98.925% 0.1142

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

RESULT AND DISCUSSION

The developed third order derivative spectrophotometric method for simultaneous determination of ambroxal hydrochloride and salbutamol sulphate 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 salbutamol sulphate 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 salbutamol sulphate 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.

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REFERENCES

[1] British pharmacopoeia. Licensing division HMSO ,Norwich. **2003.**
[2] Indian Pharmacopeia, Controller of Publication, Delhi, **2007**, Vol- 1, II, III.
[3] P.A. Patel, M.N. Dole, P.S. Shedpure, S.D. Sawant, *Asian Journal of Pharmaceutical and Clinical Research***, 2011**, 4(3), 42-45.
[4] Avinash V. Deosarkar, Shruti D. Deshpande, Sanjay G.Walode, Deepali S.Tuljapure, Sainath G. Tekale, Vijay M. Waghmode, *International Journal of Pharmacy and Pharmaceutical Sciences*, **2012** , 4 (l4), 307-311.
[5] V. SriKalyani, D. Meena Bharathi, M. Anusha, B. Chandra Priyanka and Buchi N. Nalluri. *Journal of chemical an pharmaceutical research*, **2013** , 5(11), 443-449.
[6] Parag G Bhortake , Rama S Lokhande. *International journal of pharma research and health sciences* **2014** , 2 (6), 408-412.