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Research Article

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Simultaneous UV-spectrophotometric estimation of bromhexine hydrochloride and salbutamol sulphate by third order derivative Method in combined pharmaceutical dosage form

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ABSTRACT

The objective of the study was to develop an accurate, precise and economical UV spectrophotometric, third order derivative, method for simultaneous estimation of bromhexine hydrochloride and salbutamol sulphate from combined pharmaceutical dosage form i.e. tablets. The validation was carried out by using ICH guidelines for the determination of bromhexine hydrochloride and salbutamol sulphate by using ethanol as the solvent in pharmaceutical dosage form. The proposed third order derivative method involves the measurement of absorbance of one drug at zero crossing point of other; hence wavelengths 220 nm and 233.8 nm were selected for the estimation of bromhexine hydrochloride and salbutamol sulphate respectively. The linearity of the proposed method was found in the concentration range of 2 to 14 µg /ml (r^2= 0.9991) for bromhexine hydrochloride and 2 to 16 µg /ml (r^2= 0.9997) for salbutamol sulphate respectively. The percentage mean recovery was found to be 100.02 % for bromhexine 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, bromhexine hydrochloride, salbutamol sulphate.

INTRODUCTION

In this communication the present work proposes UV spectrophotometric method, third order derivative method, for assay of bromhexine hydrochloride and salbutamol sulphate from combined pharmaceutical dosage form.

Bromhexine Hydrochloride is chemically named 2-amino-3,5- dibromo-N-cyclohexyl-N-methyl benzenemethanamine hydrochloride, is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus . The drug is official in IP [1] and BP [2].

Salbutamol sulphate is, chemically known as bis [(1RS)-2-[(1, 1-Di-methyl-ethyl) amino]-1-[4-hydroxy- 3-(hydroxyl methyl) phenyl] ethanol] sulphate. It is beta-adrenoceptor agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease. The drug is official in Indian pharmacopoeia [1]. Literature survey reveals HPLC [3], spectrophotometric [4,5] methods for simultaneous determination of bromhexine hydrochloride and salbutamol sulphate in combined dosage form. Combination of bromhexine hydrochloride and salbutamol sulphate is used for the treatment of asthma and bronchitis. 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.

EXPERIMENTAL SECTION

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 standards of bromhexine hydrochloride and Salbutamol sulphate were obtained from reputed firm with certificate analysis.

Preparation of standard drug solution

A 10 mg standard bromhexine hydrochloride was weighed accurately and transferred to a 10 ml volumetric flask and sonicated with 5 ml of ethanol for 15 minutes. The volume was made up to the mark with ethanol to give a stock solution of concentration 1000 µg /ml. From this solution, 1 ml of solution was pipetted out and transferred into 10 ml volumetric flask. The volume was made up to mark with ethanol to give a working standard solution of concentration 100 µg/ml.

A 10 mg standard Salbutamol sulphate was weighed accurately and transferred to a 10 ml volumetric flask and sonicated with 5 ml of ethanol for 15 minutes. The volume was made up to the mark with ethanol to give a stock solution of concentration 1000 µg /ml. From this solution, 1 ml of solution was pipetted out and transferred into 10 ml volumetric flask. The volume was made up to mark with ethanol to give a working standard solution of concentration 100 µg/ml.

Preparation of sample solution

Twenty tablets were weighed accurately and average weight of each tablet was determined. A powder equivalent to 8 mg of standard bromhexine hydrochloride and 2 mg of salbutamol sulphate were weighted accurately and transferred in 10 ml volumetric flask. To this flask 5 ml of ethanol was added and sonicated for 10 minutes. Such solution is diluted with ethanol to mark to give concentration as 800 µg /ml of bromhexine hydrochloride and 200 µg /ml. of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml of 800 µg /ml of bromhexine hydrochloride and 200 µg /ml of salbutamol sulphate solution to 10 ml with diluent to get concentration 80 µg /ml of bromhexine hydrochloride and 20 µg /ml. of salbutamol sulphate respectively. Such solution was used for further analysis.

Method: Third order derivative method

(a) For bromhexine hydrochloride

For the selection of analytical wavelength, 100 µg/ml solution of bromhexine hydrochloride was scanned in the spectrum mode from 400 nm to 200 nm by using ethanol 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 220 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 400 nm to 200 nm by using ethanol 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 233.8 nm.

Preparation of calibration curves

Series of solutions containing 2 – 14 µg/ ml of bromhexine hydrochloride and 2 -16 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 bromhexine hydrochloride and salbutamol sulphate were given in Fig. 1(a), 1(b) respectively.

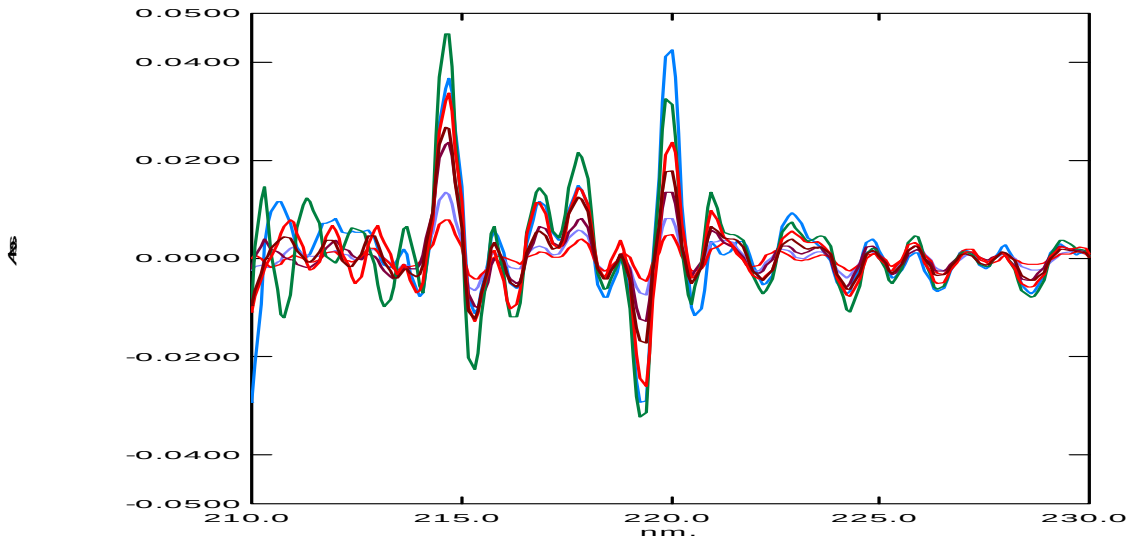


Fig. 1(a): Overlay spectra of third order derivative of bromhexine hydrochloride in the concentration range of 2 – 14 µg/ ml at 220 nm

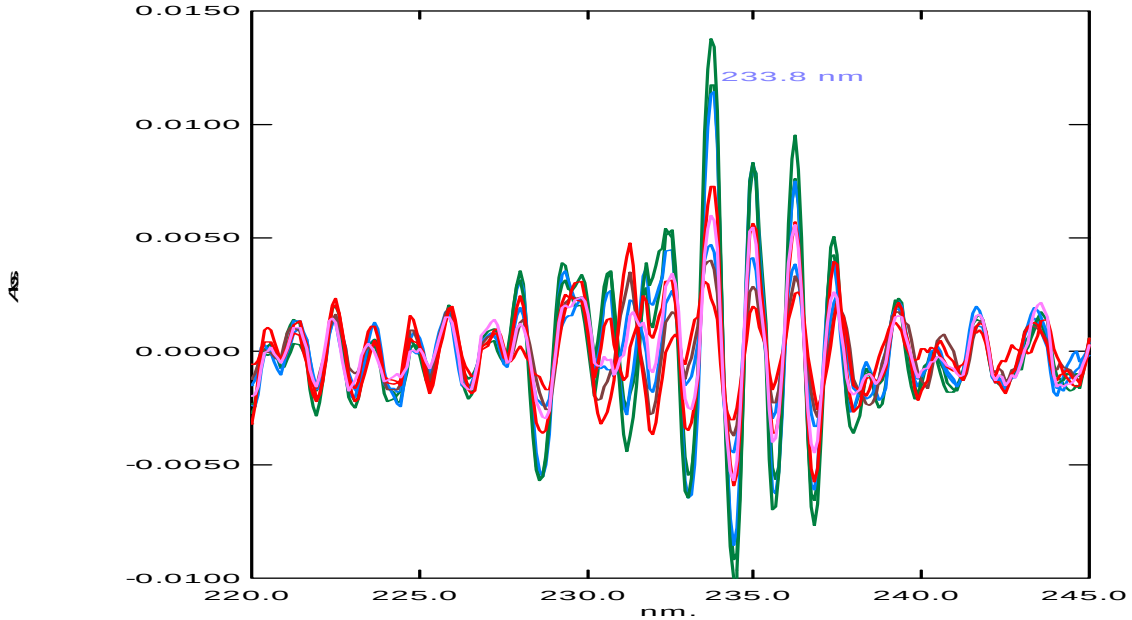


Fig. 1(b): Overlay spectra of third order derivative of salbutamol sulphate in the concentration range of 2 – 16 µg/ ml at 233.8 nm

After observing the overlain third order derivative spectra of bromhexine hydrochloride and salbutamol sulphate, the zero crossing points of both drugs were selected for analysis of other drug. The wave length selected was 220 nm, the zero crossing point of salbutamol sulphate where bromhexine hydrochloride showed considerable absorbance. The third wavelength was 233.8 nm, the zero crossing point of Bromhexine 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 bromhexine hydrochloride in the concentration range of 2-14 µg/ml

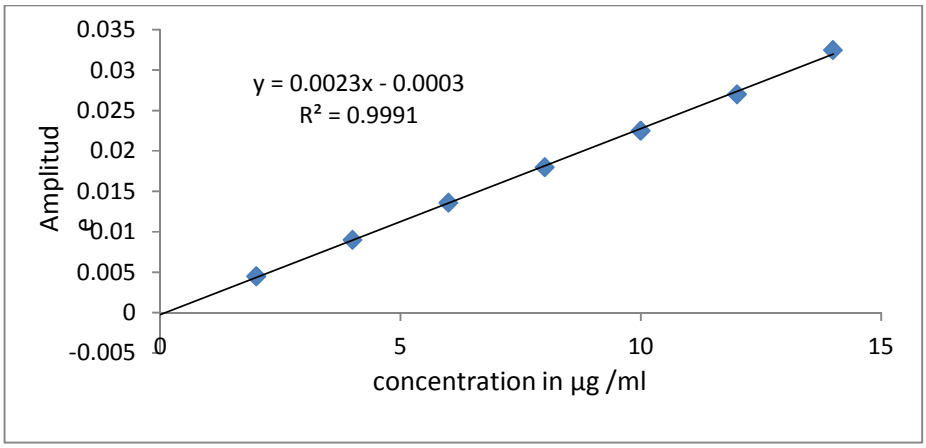
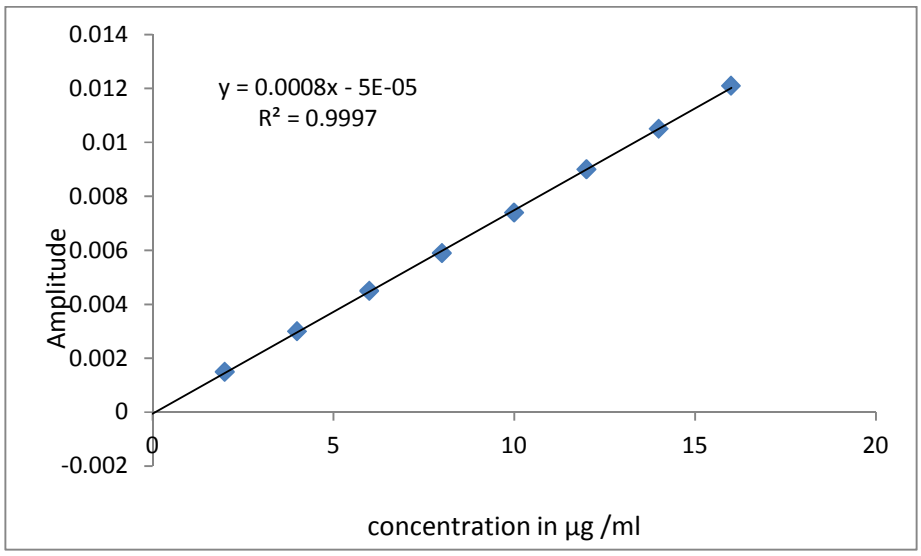


Fig.2 (b): Calibration curve of salbutamol sulphate in the concentration range of 2-16 µg/ml



Results of the analysis are given in table 1.

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

Parameter	Bromhexine hydrochloride	Salbutamol sulphate
Detection Wavelength (nm)	220	233.8
Beer Law Limits (µg/ml)	2-14	2-16
Correlation coefficient(r^2)	0.9991	0.9997
Regression equation (y=b+ac)		
Slope (a)	0.0023	0.0008
Intercept (b)	-0.0003	-0.00005

Estimation from tablets

Twenty tablets were weighed accurately and average weight of each tablet was determined. A powder equivalent to 8 mg of standard bromhexine hydrochloride and 2 mg of salbutamol sulphate were weighted accurately and transferred in 10 ml volumetric flask. To this flask 5 ml of ethanol was added and sonicated for 10 minutes. Such solution is diluted with ethanol to mark to give concentration as 800 µg /ml of bromhexine hydrochloride and 200 µg /ml. of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml of 800 µg /ml of bromhexine hydrochloride and 200 µg /ml of salbutamol sulphate solution to 10 ml with diluent to get concentration 80 µg /ml of bromhexine hydrochloride and 20 µg /ml. of salbutamol sulphate respectively. Such

solutions were scanned in the range of 350-200 nm against ethanol as blank. The absorbance spectra were converted to third order derivative spectra. Calculations were done as per the equations. The concentrations of amoxicillin and carbocisteine present in capsules were calculated by substituting the values of absorbance in linearity equations.

(a) For bromhexine hydrochloride, Y = 0.0023x - 0.0003
(b) For salbutamol sulphate, Y = 0.0008x - 0.00005

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 bromhexine hydrochloride and salbutamol sulphate was found in the range of 100.11 % to 100.23 %. (Table 2).

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	
	Sal	Brom	Sal	Brom	Sal	Brom	Sal	Brom	Sal	Brom
80%	2.0	8.0	1.6	6.4	3.606	14.423	100.17	100.16	100.11	100.04
	2.0	8.0	1.6	6.4	3.602	14.388	100.08	99.92		
	2.0	8.0	1.6	6.4	3.610	14.472	100.09	100.05		
100%	2.0	8.0	2.0	8.0	4.004	16.027	100.11	100.17	100.15	100.06
	2.0	8.0	2.0	8.0	4.008	16.022	100.22	100.14		
	2.0	8.0	2.0	8.0	4.004	15.979	100.12	99.87		
120%	2.0	8.0	2.4	9.6	4.407	17.621	100.17	100.12	99.97	100.13
	2.0	8.0	2.4	9.6	4.392	17.629	99.84	100.17		
	2.0	8.0	2.4	9.6	4.396	17.619	99.91	100.11		

Brom = Bromhexine hydrochloride, Sal = Salbutamol sulphate

Linearity
The linearity of measurement was evaluated by analyzing different concentration of the standard solutions of bromhexine hydrochloride and salbutamol sulphate. For both the drugs concentration range was found to be 1- 14 µg/ml for bromhexine hydrochloride and 2-16 µg/ml for salbutamol sulphate respectively.

Precision
The method precision was established by carrying out the analysis of tablets powder blend containing 8 mg of bromhexine 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 100.02 % for bromhexine hydrochloride and 100.06 % for salbutamol sulphate 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	
		Salbutamol sulphate	Bromhexine hydrochloride
1	1	100.04	100.16
2	2	100.19	100.08
3	3	100.07	100.05
4	4	100.12	99.82
5	5	99.89	100.09
6	6	99.81	100.17
Mean % assay		**100.02**	**100.06**
%R.S.D.		**0.1433**	**0.1273**

Intra-day precision was estimated by assaying powder blend of tablet containing 8 mg of bromhexine 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 powder blend containing 8 mg of bromhexine 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	Bromhexine hydrochloride	Salbutamol sulphate
1	Intra-day precision (N=3)amount found ± % R.S.D.	100.10% 0.1273	100.09% 0.1263
2	Inter-day precision (N=3)amount found ± % R.S.D.	98.15% 0.1314	98.93% 0.1245

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 UV, third order derivative spectrophotometric method for simultaneous determination of bromhexine hydrochloride and Salbutamol sulphate in pharmaceutical formulation was found to be simple and economical for the routine analysis of two drugs in combined dosage form. 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 bromhexine 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. It is validate as per ICH guidelines.

CONCLUSION

The proposed method is simple, precise, accurate and rapid for the determination of bromhexine 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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