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SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF AMOXICILLIN TRIHYDRATE AND BROMHEXINE HYDROCHLORIDE BY SECOND ORDER DERIVATIVE METHOD IN COMBINED DOSAGE FORM

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ABSTRACT

The object of the study was to develop a simple, accurate, precise and rapid UV spectrophotometric, second order derivative method for validation of amoxicillin trihydrate and bromhexine hydrochloride from its combined pharmaceutical dosage form. The validation was carried out by using ICH guidelines for the determination of amoxicillin trihydrate and bromhexine hydrochloride by using 0.1N hydrochloric acid as the solvent in combined dosage form. The proposed second order derivative method involves the measurement of absorbance of one drug at zero crossing point of other; hence wavelengths 241.8 nm and 223.7 nm were selected for the estimation of amoxicillin trihydrate and bromhexine hydrochloride, respectively. The linearity of the proposed method was found in the concentration range of 10 to 100 µg/mL (r^2= 0.9998) for amoxicillin trihydrate and 1 to 14 µg/mL (r^2 = 0.9997) for bromhexine hydrochloride, respectively. The percentage mean recovery was found to be 100.129 % for amoxicillin and 100.178 % for bromhexine hydrochloride, 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.

Key words: UV-Spectrophotometric second order derivative estimation, Amoxicillin trihydrate, Bromhexine hydrochloride, Validation.

INTRODUCTION

In this communication a new UV spectrophotometric, second order derivative method is developed for assay of amoxicillin trihydrate and bromhexine hydrochloride in their combined dosage form. Amoxicillin trihydrate described chemically as 6- (D-4 hydroxy phenyl glycyl amino) penicillinic acid trihydrate. It is semi-synthetic penicillin that belongs to the class of β-lactam antibiotics. It is generally used as antibacterial. Bromhexine

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hydrochloride is 2,4-dibromo-6-{(cyclohexyl (methyl amino) methyl)} aniline hydrochloride. It is broncho-secretolytic and mucolytic. Bromhexine hydrochloride is used with antibiotics to enhance their efficiency in the treatment of respiratory infections.

Amoxicillin trihydrate is official in USP[1], IP[2] and BP[3]. Bromhexine hydrochloride is also official in IP[2] and BP[3]. Literature survey reveals HPLC[4], capillary electrophoresis[5] and spectrophotometric[6] methods for assay of combined dosage form. In this communication a new simple, UV spectrophotometric, second order derivative method is reported for simultaneous determination of amoxicillin trihydrate and bromhexine 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.

EXPERIMENTAL

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 standards of amoxicillin trihydrate and bromhexine hydrochloride were obtained from reputed firm with certificate analysis.

Preparation of standard drug solution

A 100 mg standard amoxicillin tri-hydrate was weighed accurately and transferred to a 100 mL volumetric flask and sonicated with 30 mL of methanol for 15 minutes. The volume was made up to the mark with methanol to give a stock solution 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 HCl to give a working standard solution of concentration 100 μg/mL.

A 100 mg standard bromhexine hydrochloride was weighed accurately and transferred to a 100 mL volumetric flask and sonicated with 30 mL of methanol for 15 minutes. The volume was made up to the mark with methanol to give a stock solution 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 HCl to give a working standard solution of concentration 100 μg/mL.

Preparation of sample solution

Powdered from twenty capsules were collected and weighed accurately and average weight of powder from each capsule was determined. Powder equivalent to 250 mg of amoxicillin trihydrate and 8 mg of bromhexine hydrochloride was weighed and transferred in 1000 mL of volumetric flask. A 100 mL of methanol was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with methanol to give concentration as 250 µg/mL of amoxicillin and 8 µg/mL of bromhexine hydrochloride, respectively. Such solution was used for further analysis.

Method: Second order derivative method

(a) For amoxicillin: For the selection of analytical wavelength, 100 µg/mL solution of amoxicillin was scanned in the spectrum mode from 400 nm to 200 nm by using 0.1 N hydrochloric acid as blank. The second 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 241.8 nm.

(b) 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 0.1 N hydrochloric acid as blank. The second 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 223.7 nm.

Preparation of calibration curves

Series of solutions containing 10-100 µg/mL of amoxicillin trihydrate and 1-14 µg/mL of bromhexine hydrochloride were used to determine linearity of the proposed method, respectively. Solutions were scanned in the spectrum mode and absorbance spectra were converted to second order derivative spectra. The overlain spectrum of amoxicillin and bromhexine hydrochloride was given in Fig. 1(a), 1(b), respectively.

After observing the overlain second order derivative spectra of amoxicillin trihydrate and bromhexine hydrochloride, the first wave length selected was 241.8 nm, where bromhexine hydrochloride has zero absorbance but amoxicillin showed considerable absorbance. The second wavelength was 223.7 nm, where amoxicillin showed zero absorbance but bromhexine hydrochloride showed considerable absorbance. The calibration curves were plotted of amplitude of second order derivative against concentrations [Fig. 2 (a), 2(b)].

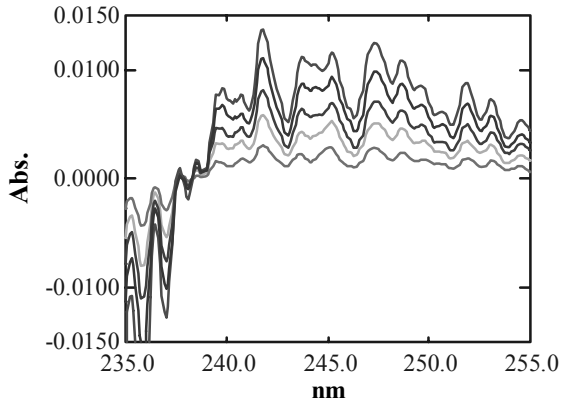

Fig. 1(a): Overlay spectra of second order derivative of amoxicillin in the concentration range of 20-100 µg/ mL

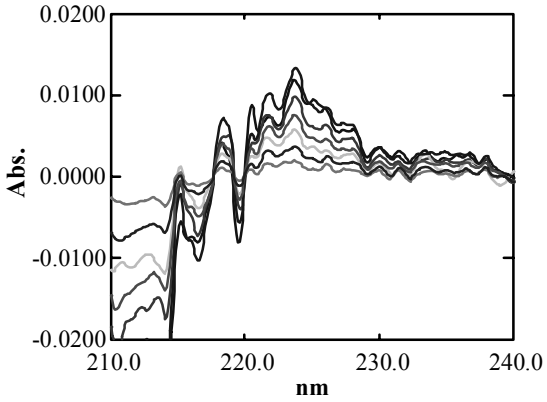

Fig. 1(b): Overlay spectra of second order derivative of bromhexine hydrochloride in the concentration range of 2-14 µg/ mL

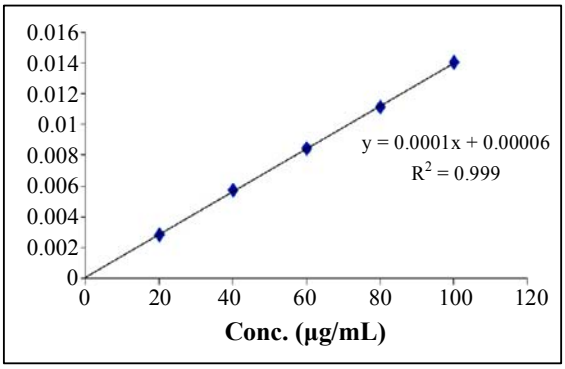

Fig. 2 (a): Calibration curve of amoxicillin trihydrate in the conc. range of 10-100 µg/mL

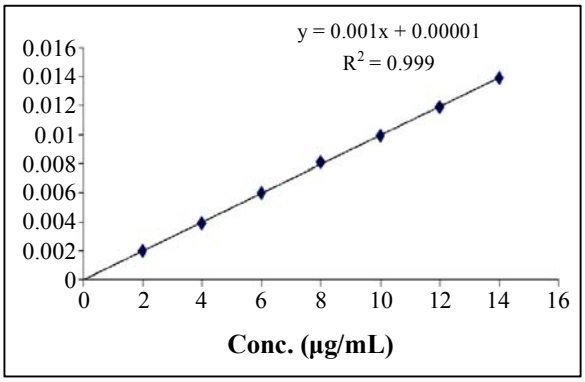

Fig. 2 (b): Calibration curve of bromhexine hydrochloride in the concentration range of 1-14 µg/mL

Results of the analysis are given in Table 1.

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

Parameter	Amoxicillin	Bromhexine hydrochloride
Detection wavelength (nm)	241.8	223.7
Beer law limits (µg/mL)	20-100	1-14
Correlation coefficient (r^2)	0.9998	0.9997
Regression equation (y = b + ac)		
Slope (a)	0.0001	0.001
Intercept (b)	0.00004	0.00001

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 250 mg of amoxicillin and 8 mg of bromhexine hydrochloride was weighed and transferred in 1000 mL of volumetric flask. A 100 mL of methanol was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with methanol to give concentration as 250 µg/mL of amoxicillin and 8 µg/mL of bromhexine hydrochloride, respectively. Such solutions were scanned in the range of 200-400 nm against 0.1 N hydrochloric acid as blank. The absorbance spectra were converted to second order derivative spectra. Calculations were done as per the equations. The concentrations of

amoxicillin and bromhexine hydrochloride present in capsules were calculated by substituting the values of absorbance in linearity equations.

(a) For amoxicillin Y = 0.0001x + 0.00006

(b) For bromhexine hydrochloride Y = 0.001x + 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 amoxicillin trihydrate and bromhexine hydrochloride was found in the range of 100.12% and 100.16% (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	
	Amox.	Brom.	Amox.	Brom.	Amox.	Brom.	Amox.	Brom.	Amox.	Brom.
80%	25	8.0	20	6.4	45.067	14.425	100.15	100.17	100.06	100.34
	25	8.0	20	6.4	45.040	14.498	100.09	100.68		
	25	8.0	20	6.4	44.973	14.428	99.94	100.19		
100%	25	8.0	25	8.0	50.025	16.081	100.05	100.50	100.03	100.20
	25	8.0	25	8.0	50.140	16.051	100.28	100.31		
	25	8.0	25	8.0	49.840	15.967	99.68	99.79		
120%	25	8.0	30	9.6	55.126	17.685	100.23	100.48	100.29	99.846
	25	8.0	30	9.6	55.258	17.553	100.47	99.73		
	25	8.0	30	9.6	55.104	17.483	100.19	99.33		
Mean									100.12	100.16

Amox. = Amoxicillin trihydrate, Brom. = Bromhexine hydrochloride

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solutions of amoxicillin trihydrate and bromhexine hydrochloride. For both the drugs concentration range was found to be 10-100 µg/mL for amoxicillin and 1-14 µg/mL for bromhexine hydrochloride.

Precision

The method precision was established by carrying out the analysis of powder blend from capsules containing 250 mg of amoxicillin trihydrate and 8 mg of bromhexine hydrochloride. The assay was carried out for the drugs by using proposed analytical method in six replicates. The values of relative standard deviation were 0.1193% for amoxicillin trihydrate and 0.2383% for bromhexine hydrochloride, 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

S. No.	Sample No.	% Assay	
		Amoxicillin	Bromhexine hydrochloride
1	1	100.15	100.50
2	2	100.09	100.31
3	3	99.94	99.79
4	4	100.27	100.22
5	5	100.24	100.18
6	6	100.09	100.07
Mean % assay		100.129	100.178
% R.S.D.		0.1193	0.2383

Intra-day precision was estimated by assaying tablets powder blend containing 250 mg of amoxicillin trihydrate and 8 mg of bromhexine hydrochloride. 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 250 mg of amoxicillin trihydrate and 8 mg of bromhexine hydrochloride 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

S. No.	Parameters	Amoxicillin	Bromhexine hydrochloride
1	Intra-day precision	100.15%	100.31%
	(N = 3) amount found ± % R.S.D.	0.1192	0.2383
2	Inter-day precision	99.484	99.792%
	(N = 3) amount found ± % R.S.D.	0.1360	0.2386

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 second order derivative spectrophotometric method for simultaneous determination of amoxicillin trihydrate and bromhexine hydrochloride in formulation was found to be simple and convenient for the routine analysis of two drugs. The method is used to eliminate the spectral interference of one drug with other drug. Reason for not using simultaneous equation and absorbance ratio methods were not used as there is maximum spectral overlap and more difference in the absorbance. 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 amoxicillin and bromhexine hydrochloride in different concentration range. The correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity [Fig. 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 amoxicillin trihydrate and bromhexine hydrochloride in combined dosage form. The method does not require any ratio of second order derivatives. The amplitude of second order

derivative can be directly used to assay of formulation. 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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