



Assay of amoxicillin trihydrate and bromhexine hydrochloride by UV-spectrophotometric third order derivative method in combined dosage form

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Abstract

A new economical and sensitive derivative, UV spectrophotometric, third order method is proposed for the validation of amoxicillin and bromhexine hydrochloride. Tablet dosages. The ICH guidelines were used for validation. The validation involves the study of optical density of two component at zero crossing point of each others. Therefore 220 nm and 236.2 nm wavelengths were found to be suitable for the assay of bromhexine hydrochloride and amoxicillin tri-hydrate respectively. A concentration range of 1 to 14 µg/ml ($r^2 = 0.9991$) for bromhexine hydrochloride and 10 to 100 µg/ml ($r^2 = 0.9999$) for amoxicillin showed the linearity study of the proposed method respectively. The 100.2 % for bromhexine hydrochloride and 100.15 % for amoxicillin were found as RSD values respectively. A statistical studies was used for validation for its accuracy, linearity as well as for precision. A less percentage (%) of RSD values for study of intra and inter day variations showed higher degree of precision.

Keywords: Third order UV-spectroscopy, Amoxicillin trihydrate, Bromhexine hydrochloride.

Introduction

Amoxicillin tri-hydrate is given as 6 - (D - 4 hydroxy phenyl glycy 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 reported in USP¹, IP² and BP³.

A mucolytic agent, bromhexine, chemically 2-amino-3,5-dibromo-N-cyclohexyl-N-methyl benzenemethanamine. The drug is officially reported in IP¹ and BP². A High pressure liquid chromatography^{4,6}, capillary electrophoresis⁷ and spectrophotometry⁸⁻¹¹ methods for suggested in literature for assay of tablets.

In the new work, third order, UV derivative spectrophotometry validation is suggested for amoxicillin trihydrate and bromhexine hydrochloride in tablets as dosage form. Such method can easily apply for the routine analysis drugs.

Materials and methods

Instrument and reagents: UV-spectrophotometer, Shimadzu, model 1800, spectral band width of 0.5 nm was used. UV-Probe 2.42 software was used for all spectral measurements.

Reference standard of both drugs were used for analysis.

A 100 µg/ml as stock solution standard amoxicillin trihydrate and bromhexine hydrochloride were prepared accurately in 0.1 N hydrochloric acid.

For the determination average weight of each tablet twenty tablets was used. About 8 mg and 250 mg of bromhexine hydrochloride and of amoxicillin trihydrate powder blend was weighed exactly. Such powder blend was treated with small amount of solvent. The solvent used was 0.1 N hydrochloric acid. After dissolution, solution was filtered and diluted to give 80 µg/ml of bromhexine hydrochloride and 2500 µg/ml of amoxicillin respectively.

For amoxicillin: A 100 µg/ml of amoxicillin tri-hydrate, standard solution was scanned in UV region (blank solution is 0.1 N hydrochloric acid). A UV probe 2.42 software was used to record the third order derivative spectrum. From the spectrum, 236.2 nm wavelength was selected for recording the amplitude of the derivative spectrum.

For bromhexine hydrochloride: A 100 µg/ml of bromhexine hydrochloride, standard solution was scanned in UV region, (blank, 0.1 N hydrochloric acid). A UV probe 2.42 software was used to record The third order derivative spectrum. From the spectrum, 220 nm wave length was selected for recording the amplitude of the derivative spectrum.

Preparation of calibration curves: For linearity study, various aliquots of solutions of bromhexine hydrochloride (1 – 10 µg/ml) and amoxicillin tri-hydrates (10 -100 µg/ml) were used. The zero order absorbance spectra were recorded. With the help of UV-probe software derivative spectra .i.e. third was obtained. The Figure-1(a), (b) represents spectrum of bromhexine hydrochloride and amoxicillin tri-hydrate respectively.

From the derivative-3rd order spectroscopy of bromhexine hydrochloride and amoxicillin tri-hydrate, wave length selected was 220 nm for bromhexine hydrochloride 236.2 nm for amoxicillin. Figure-2 (a), (b) represents the linearity graph of amplitude against concentrations. Summary is represented in Table-1.

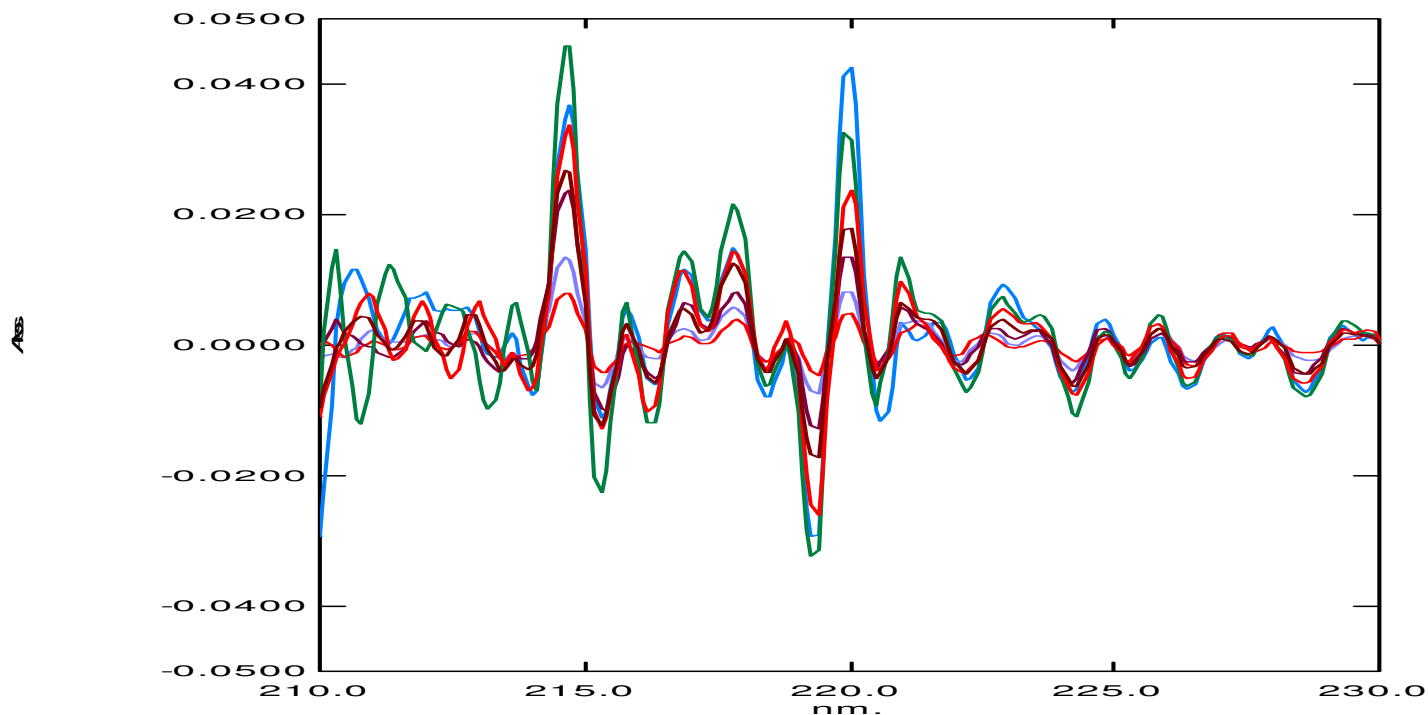


Figure-1(a): Bromhexine hydrochloride, derivative-3rd order spectra (2– 10 µg/ml).

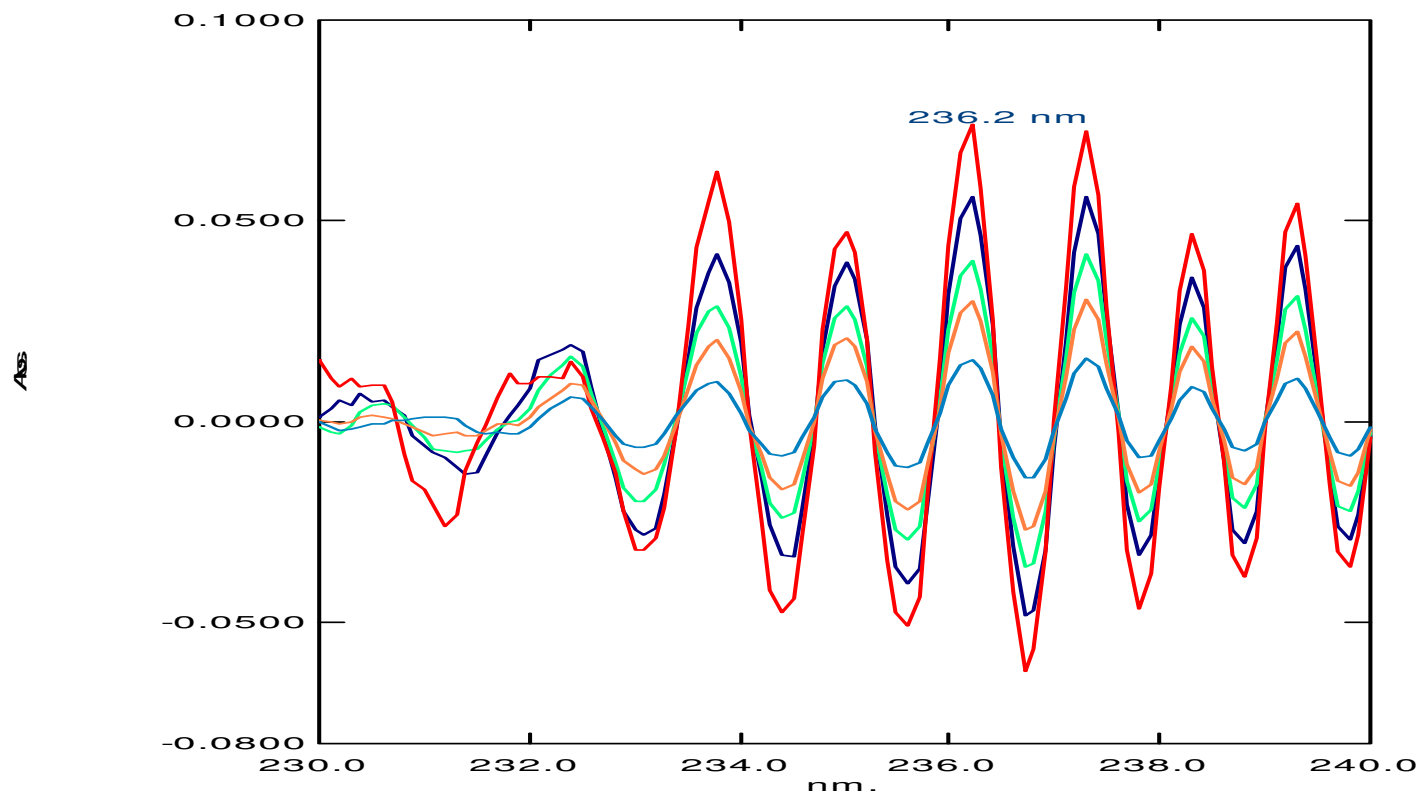


Figure-1(b): Amoxicillin tri-hydrate, derivative-3rd order spectra (20 – 100 µg/ml.)

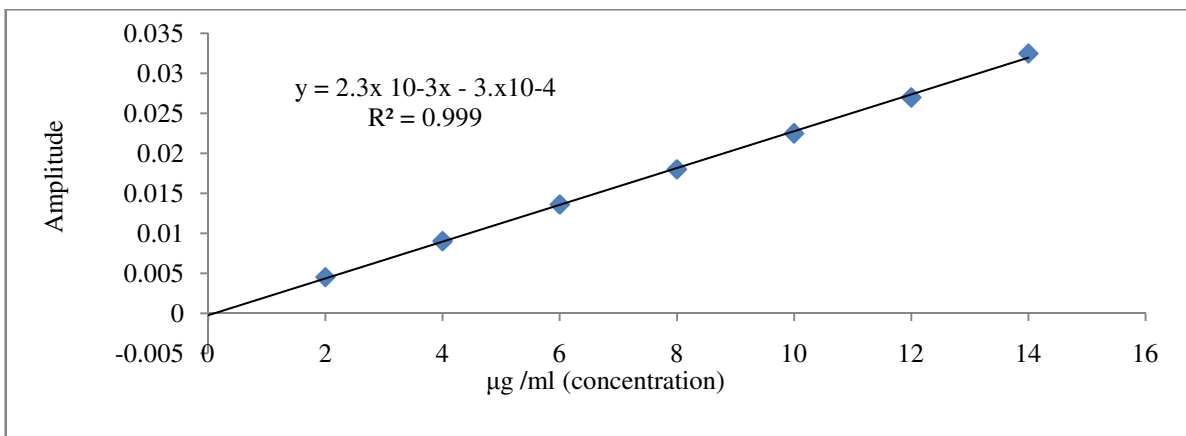


Figure-2(a): Linearity graph of bromhexine hydrochloride.

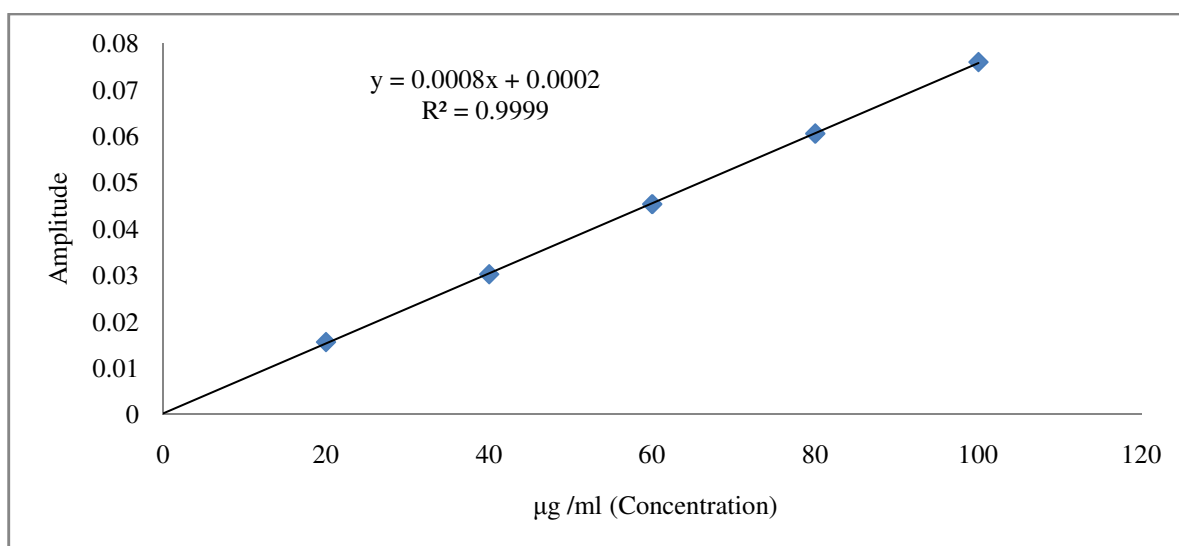


Figure-2(b): Linearity graph of amoxicillin.

Table-1: Values of linearity.

Terms	Bromhexine hydrochloride	Amoxicillin tri-hydrate
Spectral values	220 nm	236.2 nm
Validity of Beer Law	1-10 (µg/ml)	10-100 (µg/ml)
Correlation coefficient(r^2)	0.9991	0.9999
Slope	0.0023	0.0008
Intercept	-0.0003	0.0002

Estimation from dosage form: For the determination average weight of each tablet twenty tablets was used. About 8 mg and

250 mg of bromhexine hydrochloride and of amoxicillin trihydrate powder blend was weighed exactly. Such powder blend was treated with small amount of solvent. It was further diluted to give volume using solvent. It gave as 80 µg/ml and 2500 µg/ml of bromhexine hydrochloride and amoxicillin respectively.

For sample solution was further diluted to give working solution. Solution was scanned. The zero order absorbance spectra were recorded. With the help of UV-probe software derivative spectra was obtained. For calculations of concentrations of drugs present in tablet, linearity equations were used.

Linearity equation: i. $Y = 2.3 \times 10^{-3}x - 3 \times 10^{-4}$, For bromhexine hydrochloride, ii. $Y = 8 \times 10^{-4}x + 2 \times 10^{-4}$, For amoxicillin trihydrate.

Method validation: The ICH guidelines were used for validation.

Accuracy: By standard addition method, the recovery study was carried out. Table-2 represents percentage recovery of bromhexine hydrochloride and amoxicillin.

bromhexine hydrochloride and amoxicillin was selected It was finely powdered and replicates analysis were performed. The repeatability of the method was confirmed. It is given in Table-3.

Linearity: The linearity aliquots of different standard solutions of bromhexine hydrochloride and amoxicillin were used.

Day (intra and inter) study was carried out from tablets powder blend. The statistical evaluation id given in Table-4.

Precision: For study, tablet containing 8 mg and 250 mg of

Table-2: Data for accuracy.

% recovery	Initial (µg/ml)		Sample addition in µg/ml		Recovery of drug (µg/ml)		Percentage of Recovery		Mean Recovery (%)	
	BRH	AMO	BRH	AMO	BRH	AMO	BRH	AMO	BRH	AMO
80%	8.0	25	6.4	20	14.423	45.019	100.16	100.19	100.04	100.12
	8.0	25	6.4	20	14.388	45.036	99.92	100.08		
	8.0	25	6.4	20	14.472	45.049	100.05	100.11		
100%	8.0	25	8.0	25	16.027	50.080	100.17	100.16	100.06	100.02
	8.0	25	8.0	25	16.022	49.920	100.14	99.84		
	8.0	25	8.0	25	15.979	50.035	99.87	100.07		
120%	8.0	25	9.6	30	17.621	55.082	100.12	100.15	100.13	99.975
	8.0	25	9.6	30	17.629	54.890	100.17	99.80		
	8.0	25	9.6	30	17.61	55.121	100.11	100.22		

BRH = Bromhexine hydrochloride, AMOX = Amoxicillin tri-hydrate.

Table-3: Data of precision.

Replicate	% Assay	
	BRH	AMOX
1	100.16	100.17
2	100.08	100.21
3	100.05	100.07
4	99.82	100.12
5	100.09	100.25
6	100.17	100.08
Mean % assay	100.06	100.15
% Relative Standard Deviation.	0.1273	0.072279

Table-4: Values for intra-day and inter-day validation.

Terms	BRH	AMOX
In % R.S.D., precision values for amount found for intra day.	100.10% 0.1273	100.14% 0.07335
In % R.S.D, precision values for amount found for inter day	98.15% 0.1314	98.561% 0.05448

The study of % relative standard deviation values of intra-day and inter-day precision indicate high degree of precision.

Results and discussion

A proposed spectrophotometric 3rd order derivative study for validation of bromhexine hydrochloride and amoxicillin in drug formulation is suitable for the routine analysis. The advantage of method is that it does not show a spectral interference. The Tables-1 to 4 gives validation data, it of method. The % relative standard deviation was found to be less than 1 Indicates precision of method. Linear regression equation is useful for Linearity. The coefficient of correlation of these drugs was nearly 1.00.

Conclusion

The newly developed method is rapid and precise for the validation of both drugs in combined formulation. Such method is used as a substitute to the literature methods. Hence it is conveniently useful for assay of both drugs separately and in combined dosage also.

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