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SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF AMOXICILLIN AND CARBOCISTEINE BY SECOND ORDER DERIVATIVE SPECTROSCOPY METHOD IN COMBINED DOSAGE FORM

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ABSTRACT

The objective of the study was to develop a simple, accurate, precise and rapid UV spectrophotometric, second order derivative method for validation of amoxicillin and carbocisteine. The validation was carried out by using ICH guidelines for the determination of amoxicillin and carbocisteine by using 0.1 N 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 208 nm were selected for the estimation of amoxicillin and carbocisteine, respectively. The linearity of the proposed method was found in the concentration range of 20 to 100 µg/mL (r^2 = 0.9998) for amoxicillin and 10 to 100 µg/mL (r^2 = 0.9988) for carbocisteine, respectively. The percentage mean recovery was found to be 100.129% for amoxicillin and 100.163% for carbocisteine, 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, Carbocisteine, Validation.

INTRODUCTION

In this communication, the present work proposes UV spectrophotometric, second order derivative method for assay of amoxicillin and carbocisteine from combined pharmaceutical dosage form i.e. tablet.

Amoxicillin trihydrate is described chemically as 6 - (D - 4 hydroxy phenyl glycyl amino) penicillin acid trihydrate. It is semi-synthetic penicillin that belongs to the class of

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β-lactam antibiotics. It is generally used as antibacterial. Amoxicillin trihydrate is official in USP[1], IP [2] and BP[3].

Carbocisteine is described chemically as (2R)-2-amino-3-[(carboxy-methyl) sulphanyl] propanoic acid. Carbocisteine is a mucolytic drug, which breaks down mucus in the body so that it can be more easily cleared from the body. In chronic obstructive pulmonary disease (COPD), symptoms involve the over secretion of mucus. Mucolytic have great potential for treatment of this disease. Additional characteristics of COPD include airflow limitation oxidative, stress and airway inflammation. Carbocisteine is official in British Pharmacopoeia[3] and European Pharmacopoeia[4]. In literature survey, HPLC[5-7] methods were reported for validation of combined 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 and carbocisteine were obtained from reputed firm with certificate of analysis.

Preparation of standard drug solution

A 100 mg standard amoxicillin trihydrate was weighed accurately and transferred to a 100 mL volumetric flask and sonicated with 30 mL of 0.1 N hydrochloric acid for 15 min. The volume was made up to the mark with 0.1 N HCl 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 carbocisteine was weighed accurately, transferred to a 100 mL volumetric flask and sonicated with 30 mL of 0.1 N HCl for 15 min. The volume was made up to the mark with 0.1 N HCl 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 25 mg of amoxicillin and 15 mg of carbocisteine 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 min and filtered. The filtrate and washing were diluted up to the mark with 0.1 N hydrochloric acid to give concentration as 250 µg/mL of amoxicillin and 150 µg/mL of carbocisteine, respectively. Such solution was used for further analysis.

Second order derivative method

(a) Amoxicillin

For the selection of analytical wavelength, 100 µg/mL solution of amoxicillin was scanned in the spectrum mode from 400 nm to 190 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) Carbocisteine

For the selection of analytical wavelength, 100 µg/mL solution of carbocisteine was scanned in the spectrum mode from 400 nm to 190 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 208 nm.

Preparation of calibration curves

Series of solutions containing 20-100 µg/mL of amoxicillin and 10-100 µg/mL of carbocisteine 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 carbocisteine are given in Fig. 1(a) and 1(b), respectively.

After observing the overlain second order derivative spectra of amoxicillin and carbocisteine, the first wave length selected was 241.8 nm, where carbocisteine has minimum absorbance but amoxicillin showed considerable absorbance. The second wavelength was 208 nm, where amoxicillin showed minimum absorbance but carbocisteine showed considerable absorbance. The calibration curves were plotted in amplitude of second order derivative against concentrations [Fig. 2 (a) and 2(b)].

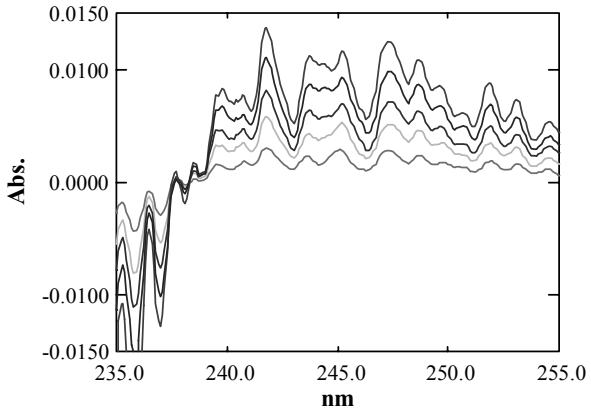

Fig. 1(a): Overlay spectra of second order derivative of amoxicillin in the concentration range of 20-100 µg/mL

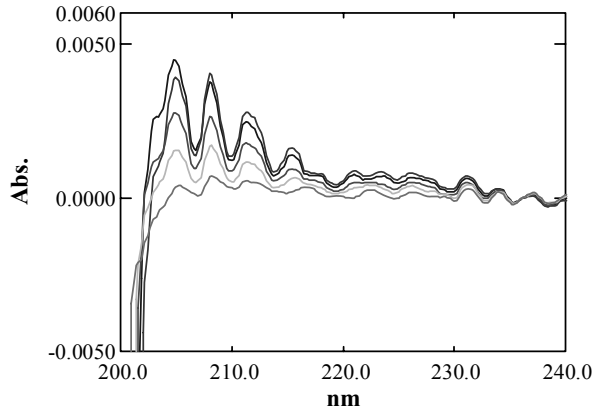

Fig. 1(b): Overlay spectra of first order derivative of carbocisteine in the concentration range of 10-100 µg/mL

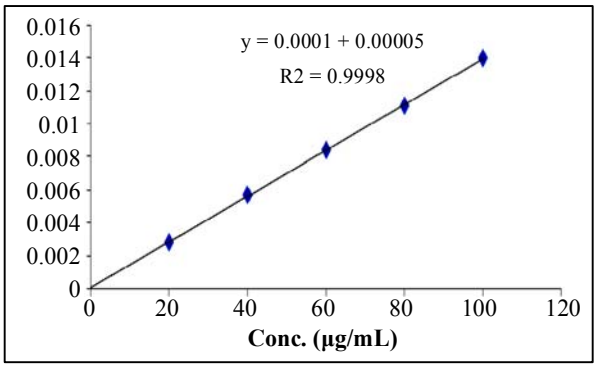

Fig. 2 (a): Calibration curve of amoxicillin in the concentration range of 20-100 µg/mL

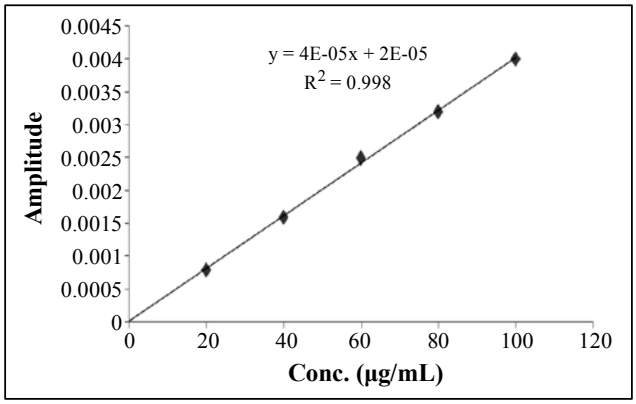

Fig. 2 (b): Calibration curve of carbocisteine in the concentration range of 10-100 µg/mL

Results of the analysis are given in Table 1.

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

Parameter	Amoxicillin	Carbocisteine
Detection wavelength (nm)	241.8	208
Beer law limits (µg/mL)	20-100	10-100
Correlation coefficient (r^2)	0.9998	0.9988
Regression equation (y = b + ac)		
Slope (a)	0.0001	0.00005
Intercept (b)	0.00004	0.00002

Estimation from capsules

Powder from twenty capsules were collected, weighed accurately and average weight of powder from each capsule was determined. Powder equivalent to 25 mg of amoxicillin and 15 mg of carbocisteine 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 min and filtered. The filtrate and washing were diluted up to the mark with 0.1 N hydrochloric acid to give concentration as 250 µg/mL of amoxicillin and 150 µg/mL of carbocisteine, respectively. Such solutions were scanned in the range of 190-400 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 carbocisteine present in capsules were calculated by substituting the values of absorbance in linearity equations.

(a) For amoxicillin

$$y = 0.0001x + 0.00005 \quad \ldots(1)$$

(b) For carbocisteine

$$y = 0.00004x + 0.00002 \quad \ldots(2)$$

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 and carbocisteine was found in the range of 100.06% to 100.74% (Table 2).

Table 2: Statistical evaluation of the data subjected to accuracy

Level of % Recovery	Amount present (µg/mL)		Amount added (µg/mL)		Amount found (µg/mL)		% Recovery		Mean % recovery	
	Amox	Carbo	Amox	Carbo	Amox	Carbo	Amox	Carbo	Amox	Carbo
80	25	15	20	12	45.067	17.025	100.15	100.16	100.06	100.303
	25	15	20	12	45.040	17.098	100.09	100.58		
	25	15	20	12	44.973	17.028	99.94	100.17		
100	25	15	25	15	50.025	30.081	100.05	100.27	100.033	100.11
	25	15	25	15	50.140	30.051	100.28	100.17		
	25	15	25	15	49.840	29.967	99.68	99.89		
120	25	15	30	18	55.126	33.085	100.23	100.26	100.296	100.0766
	25	15	30	18	55.258	32.953	100.47	99.86		
	25	15	30	18	55.104	33.036	100.19	100.11		
Mean									100.129	100.163

Amox = Amoxicillin, Carbo = Carbocisteine

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solutions of amoxicillin and carbocisteine. For both the drugs, concentration range was found to be 20-100 µg/mL for amoxicillin and 10-100 µg/mL for carbocisteine.

Precision

The method precision was established by carrying out the analysis of powder blend from capsules containing 250 mg of amoxicillin and 150 mg of carbocisteine. 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 and 0.1456% for carbocisteine, 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

Sample No.	% Assay	
	Amoxicillin	**Carbocisteine**
1	100.15	100.17
2	100.09	100.11
3	99.94	100.29
4	100.27	100.12
5	100.24	99.88
6	100.09	99.97
Mean % assay	100.13	100.09
% R.S.D.	0.1193	0.1456

Intra-day precision was estimated by assaying tablets powder blend containing 250 mg of amoxicillin and 150 mg of carbocisteine. 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 and 150 mg of carbocisteine 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

Parameters	Amoxicillin	carbocisteine
Intra-day precision	100.15%	100.17%
(N = 3) amount found ± % R.S.D.	0.1192	0.1456
Inter-day precision	99.484	99.762%
(N = 3) amount found ± % R.S.D.	0.1360	0.1486

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 and carbocisteine 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 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 carbocisteine 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 and these 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 validated as per ICH guidelines.

CONCLUSION

The proposed method is simple, precise, accurate and rapid for the determination of amoxicillin and carbocisteine 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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REFERENCES

1. The United States Pharmacopeia, United States Pharmacopeia Convention Inc, Rockville, USP 29 NF 24, **30(4)**, 1208 (2008).
2. Indian Pharmacopeia, Controller of Publication, Delhi, 1, II, III (2007).
3. British Pharmacopoeia. Licensing Division HMSO, Norwich, 357 (2003).
4. European Pharmacopoeia 5.0. Volume-2 (2005).
5. A. Nadiminti, A. Gunda, K. Reddy Tupally, A. P. Lakshmi, K. Jakka and A. Sai Nagubandi, J. Pharm. Res., **5(4)**, 1889-1895 (2012).
6. Sonali K. Sarode, Sujata T. Pise, P. Dhore, D. R. Mundhada and S. Bhaskaran, Bio. Med. Rx, **1(4)**, 360-362 (2013).
7. V. A. Patil, A. C. Vispute, S. B. Bari and D. A. Patil, Trade Science Inc., **13(5)** (2013).

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