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Concurrent Spectrophotometric Assay of Amoxycillin and Ambroxol from Combination Tablets

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Two simple and economical UV spectrophotometric methods for simultaneous estimation of amoxycillin and ambroxol in multi-component formulations have been developed. Method I is based on Vierdot's method (simultaneous equations) in which the respective absorbance maximas, *i.e.*, 272 and 308 nm are utilized, whereas, in method II the absorbance differences between 272 and 327 nm and between 308 and 328 nm were utilized for quantification of amoxycillin and ambroxol, respectively. Proper selection of wavelengths eliminates complex interference raised in estimation of one drug by other. The results of analysis have been validated statistically at a 95 per cent confidence level with Student's t-test and by recovery studies. Accurate and reproducible results were obtained in samples containing 100–500 μg/mL of amoxycillin trihydrate and 10–60 μg/mL of ambroxol hydrochloride.

Key Words: Vierdot's Method, Absorbance difference, Amoxycillin, Ambroxol.

INTRODUCTION

Amoxycillin trihydrate (AMX), an extended spectrum penicillin is often an effective therapy for sinusitis, otitis media, acute excerbations of chronic bronchitis and epiglottis, upper and lower respiratory infections[1]. Ambroxol hydrochloride (BXL), a metabolite of bromhexine, is a mucolytic expectorant particularly useful in bronchitis with chronic obstructive bronchitis and more effective in silicosis as secretolytic and surfactant stimulant, asthma, COPD, sinusitis, secretory otitis media, smokers cough if mucus plugs are present[2]. Antimicrobial and mucolytic combination containing 250/500 mg of AMX and 30 mg of BXL are often used for upper and lower respiratory tract infections in patients with bronchial asthma or COPD. UV spectrophotometric (IP), titration with mercuric nitrate (IP, BP) and HPLC (USP) methods are specified for estimation of AMX in bulk and various formulations[3–5]. Apart from a number of

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HPLC methods two spectrophotometric methods[6, 7] are reported for the determination of amoxycillin in bulk and pharmaceutical formulations. BXL is not official in any pharmacopoeia and the reported methods for its analysis in various formulations include derivative spectrophotometry[8], voltametry[9] and HPLC methods[10, 11]. The combined dosage forms of AMX and BXL are non-official and only one reported HPLC method[12] specifies their simultaneous analysis. The paper presents two simple, accurate and economical methods for simultaneous estimation of both drugs in multicomponent formulations.

EXPERIMENTAL

AMX (IP), BXL and double distilled water were used in the present investigation. A Thermo Spectronic Genesys 2 split beam dual detector UV/Vis recording spectrophotometer with spectral band width of 2 nm was employed for all spectroscopic measurements using a pair of 10 mm matched quartz cells. Standard stock solutions of AMX and BXL were prepared separately in distilled water and suitably diluted to different concentrations. The linearity was studied at respective absorbance maximas, *i.e.*, 272 and 308 nm respectively by least square method. Beer's law holds good in the range 0–500 μg/mL for AMX and 0–100 μg/mL for BXL.

Method I: The method is based on Vierdot's method[13] and utilizes corresponding absorbance maximas, *i.e.*, 272 and 308 nm respectively for quantification. The mean absorptivity coefficients of both drugs at each wavelength were determined from different dilutions (eight independent determinations) of corresponding drugs within Beer's law concentration range limit. Using these a set of two simultaneous equations was framed:

$$A_{272} = 3.0882C_{AMX} + 2.833C_{BXL} \quad (1)$$

$$A_{308} = 0.1989C_{AMX} + 7.2522C_{BXL} \quad (2)$$

where A_{272} and A_{308} are absorbances of samples containing AMX and BXL at 272 and 308 nm respectively. C_{AMX} and C_{BXL} are concentrations of AMX and BXL in g/L.

Solving both equations and converting units of concentration to working units in μg/mL, the concentration of AMX and BXL can be readily found out as:

$$C_{AMX} = 332.1696A_{272} - 129.758996A_{308} \quad (3)$$

$$C_{BXL} = 141.4467A_{308} - 9.1091A_{272} \quad (4)$$

Method II: BXL shows valley at absorbance maxima of AMX, *i.e.*, 272 nm and shows absorbance of same magnitude at 327 nm (Fig. 1). The absorbance difference between these two wavelengths for BXL is zero whereas for AMX is maximum. Hence, AMX was quantified from the absorbance difference between 272 and 327 nm. AMX shows valley at absorbance maxima of BXL, *i.e.*, 308 nm and shows absorbance of apparently same magnitude at 328 nm (Fig. 1). The absorbance difference values for different concentrations of AMX between these two wavelengths are found almost negligible, whereas the same for BXL is

considerably high. This enables utilization of the absorbance difference between 308 and 328 nm for quantification of BXL.

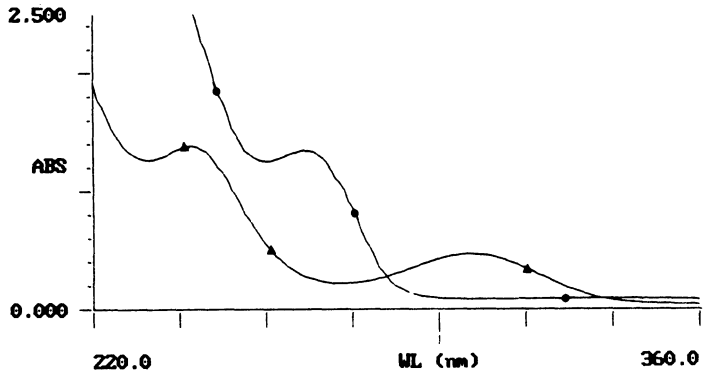

Fig. 1. Overlain spectra of AMX (•) and BXL (▲)

For AMX absorbance differences between 272 and 327 nm (DA_x) and for BXL absorbance differences between 308 and 328 nm (DA_y) were recorded from different dilutions of individual drugs. Working calibration curves were plotted with linearity validation by least squares method.

$$C_{AMX} = 346.25 \times DA_x - 0.6234 \qquad (n = 10, r = 0.9999) \qquad (5)$$

$$C_{BXL} = 217.97 \times DA_y + 0.144 \qquad (n = 12, r = 0.9998) \qquad (6)$$

Preparation of tablet sample solutions: Commercial tablets procured from local market were used for analysis. Twenty tablets were weighed and ground to a fine powder. An accurately weighed powder sample equivalent to 500 mg of AMX as per label claim was transferred to a 100 mL volumetric flask. The powder was dissolved and the volume was made up with distilled water. The solution was then filtered through Whatmann filter paper No. 41. The filtrate was suitably diluted to a concentration containing AMX equivalent to 250 μg/mL. The absorbances at selected wavelengths were recorded. The concentrations of both drugs were worked out utilizing both proposed methods.

To study the recovery of both drugs, preanalyzed samples were taken to which different quantities of pure drugs (reference standards) were added within the analytical concentration range limitations in proposed methods. The added quantities of individual drugs were estimated by both methods.

RESULTS AND DISCUSSION

The modalities adopted in experimentation were successfully validated as per standard analytical procedures. Prior to analysis of commercial formulations both methods were validated by preliminary analysis of mixed standards containing both drugs in different ratio and authentic laboratory samples. The results of replicate determinations (n = 8) by the proposed method was validated statistically with the true value at each instance (Table-1). Precise and accurate results

were obtained with samples containing 50–500 μg/mL of AMX and 10–60 μg/mL of BXL (Table-1).

TABLE-1
ANALYSIS OF AUTHENTIC SAMPLES (n = 8)

Analyte	Method–I			Method–II		
	C.I.	SD	%SE	C.I.	SD	%SE
AMX	100.82 ± 1.123	1.620	0.573	98.21 ± 0.587	0.847	0.299
BXL	103.30 ± 2.220	2.205	1.033	97.14 ± 5.628	2.232	0.813

SD: Standard deviation; %SE: per cent standard error; C.I. (confidence interval within which true value may be found at 95% confidence level) $= R \pm ts/\sqrt{n}$ where R is mean per cent result of analysis; t: theoretical 't' values at 95% confidence level for $(n_1 + n_2 - 2)$ degrees of freedom is $t(0.05, 14) = 2.145$.

Recovery studies were found satisfactory in the range of 99 to 102%. Both analyses of authentic samples and recovery study showed that there was no interference from common adjuvants used in the formulation, indicating accuracy and reliability of both methods. The results of analysis of authentic samples obtained in each instance were compared with theoretical value of 100 per cent by means of Student's 't' test at a 95 per cent confidence level. The results of analysis of commercial formulations are found to be satisfactory (Table-2).

TABLE-2
ANALYSIS OF COMMERCIAL TABLETS (n = 4)

Formulation	Analyte	Label claim (mg/tab)	Method–I		Method–II	
			Mean amount found (mg/tab)	% SD	Mean amount found (mg/tab)	% SD
T1	AMX	250	252.531	1.253	246.52	1.249
	BXL	30	30.761	2.108	29.13	2.308
T2	AMX	500	504.154	1.429	491.73	1.092
	BXL	30	30.692	2.093	29.43	2.438

Both the proposed methods have been found to be accurate, simple, convenient and are suitable for routine analysis in laboratory.

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