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Extraction-Spectrophotometric Determination of Certain-β-Lactam Antibiotics with Methylene Blue

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An extraction-spectrophotometric method was established for the determination of certain β-lactams, cefaclor, ampicillin and amoxycillin in pure and dosage forms using methylene blue (MB) as an analytical reagent. The method is based on the formation of a chloroform-extractable blue colored ion-association complex (MB cation-antibiotic anion) through the reaction of a β-lactam antibiotic with an excess of methylene blue at pH 9.5. Good agreement with Beer's law was found in the range of antibiotic concentration of 3.5-90.0 μg/ml with a detection limit of 3.0 μg/ml. The method is simple, precise and accurate for pure analyte with excellent recovery (97-102%); and also, this does not require any separation of soluble excipients in pharmaceutical preparations.

Cephalosporins and penicillins are β-lactam antibacterial agents, which are highly active against gram -positive and gram-negative bacteria. These antibiotics have been determined using several analytical methods, which include colorimetry[1], fluorimetry[2], fluorescence[3], chemiluminescence[4], polarography[5], high-pressure liquid chromatography[6] and spectrophotometry[7-18]. Of these, spectrophotometry is considered to be simple and economical for drug analysis. Spectrophotometric analysis of cephalosporins and penicillins using reagents[7-14] and through metal complexation with molybdate[17] and copper[18], have been reported in the literature.

Of various methods of spectrophotometric analysis, the method employing ionpair complexations[7,10,14] for the determination of cephalosporins and penicillins has received good attention. However, the incorporation of an extraction step in the procedure may impart better sensitivity to the spectrophotometric estimations. Thus, the aim of the present work was to develop an improved extraction and spectrophotometric method with greater precision, accuracy and sensitivity for the determination of some important antibiotics such as cefaclor, ampicillin and amoxycillin, using MB (Fig. 1), in bulk and dosage forms.

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MATERIALS AND METHODS

A CL-24 spectrophotometer (Elico Ltd. India) with 10-mm glass cell was used for the absorbance measurements. pH measurements were made using a Systronic 361 model pH-meter.

Cefaclor monohydrate (Lupin Laboratories Ltd., India), ampicillin and amoxycillin (Hi-Media Ltd., India) of 99.5% purity were used as standard samples. The pharmaceutical preparations were products of Ranbaxy Laboratories Ltd. New Delhi, Cadila Pharmaceutical Ltd., Ahmedabad. All other chemicals were of analytical reagent grade. Demineralized double- distilled water was used to prepare all solutions and in all experiments.

Preparation of solutions:

Stock solutions of pure antibiotics (0.4 mg/ml) and methylene blue (1.31 mM) were prepared in double-distilled water and stored at 4° till use. The 0.5 M ammonium chloride/ammonia buffer solutions covering the pH range from 7 to 10 were prepared by combining equal

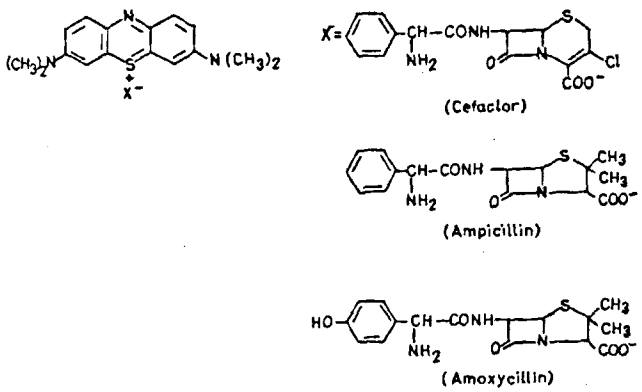

Fig. 1 : Structure of antibiotic-methylene blue ion-association complex

volume of 1.0 M ammonium chloride and 1.0 M ammonia solution and adjusting the pH with a few drops of 1.0 M sodium hydroxide or hydrochloric acid.

Assay procedure for pure antibiotics:

Aliquots of antibiotics having concentrations ranging between 3.0-90 μg/ml were prepared by the sequential dilutions of standard stock solution. In a series of 125 ml separating funnels, 4.0 ml of different aliquots, 5.0 ml of MB solution and 1.0 ml of ammonia buffer were mixed together. The ionic strength (μ) now produced by the buffer was 0.05 M. After adding 10 ml volume of chloroform to it, the entire contents (μ = 0.025 M) were shaken for 5 min. The separated chloroform layer was collected in a 25 ml volumetric flask. A new 10 ml volume of chloroform was added and shaken for 5 min. This second extract was combined with the first and then the volume was completed to the mark with chloroform. The absorbance of the organic phase was measured against a reagent blank. The reagent blank constitutes all reagents used in the above except analyte.

Assay procedure for pharmaceutical preparations:

The average content weight of a capsule, a tablet and a unit dosage of injection and suspension (available as a granule packet) were determined. An amount of powder of these preparations equivalent to 20 mg of active constituent was weighed accurately, thoroughly dissolved in water by magnetic stirring and finally filtered through a filter paper (Whatman No. 40) to remove residual insoluble matter, if any. The solution was transferred into a 25 ml volumetric flask and the volume was made up with double-distilled water. The two different aliquots of this solution giving analyte concentrations about 15 and 33 μg/ml were obtained by sequential dilution method. Each aliquot (4.0 ml) was mixed with 5.0 ml of MB solution and 1.0 ml of ammonia buffer (pH 9.5) and then subjected to the extraction-spectrophotometric measurement in the similar manner as has been adopted for the assay of pure antibiotics.

RESULTS AND DISCUSSION

In aqueous alkaline medium, cephalosporins and penicillins form a blue colored ion-association complex with methylene blue. This complex was extractable in chloroform. The absorption spectra of these complexes along with their respective reagent blank are shown in Fig. 2. The analytical wavelength for measurements was absorption maximum of cefaclor-MB complex observed at 640 nm, whereas the same for ampicillin-MB and amoxycillin-MB complexes has been observed at 635 nm against the reagent blank. An absorption maximum

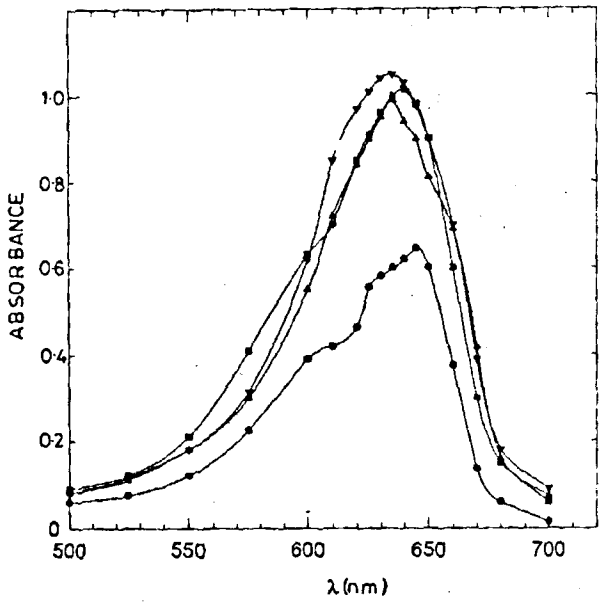

Fig. 2 : The absorption spectra of blank reagent (●), cefaclor-MB (■), ampicillin-MB (▲) and amoxycillin-MB (▼) complex formed in 0.05 M NH_4^+/NH_3 buffer containing 1.3 x 10^{-3} M MB and extracted with chloroform

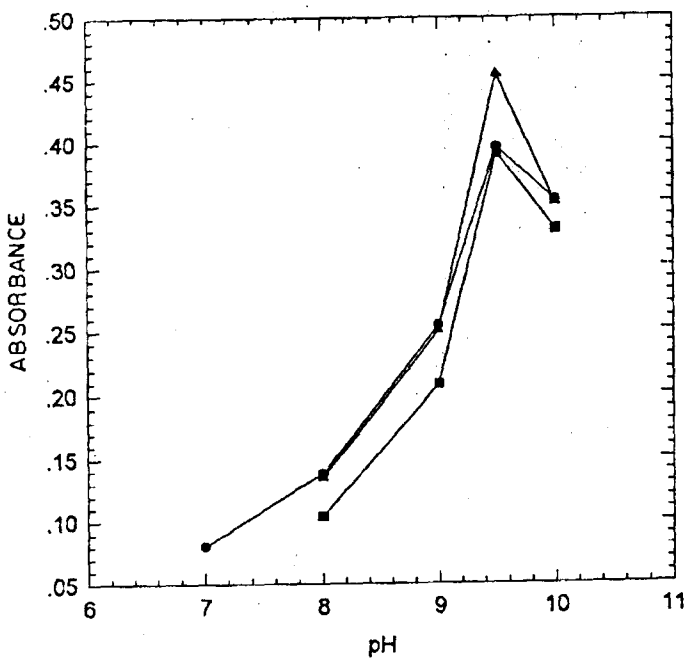

Fig. 3 : Variation in absorbance of the antibiotic-MB complex formed in aqueous 0.05 M NH^+_4/NH_3 buffer containing $1.3x10^{-3}$ M MB at different pH values. [Cefaclor-MB (●, λ_{max} = 640 nm), ampicillin-MB (■ λ_{max}= 635 nm), amoxycillin-MB (▲, λ_{max} = 635 nm)]

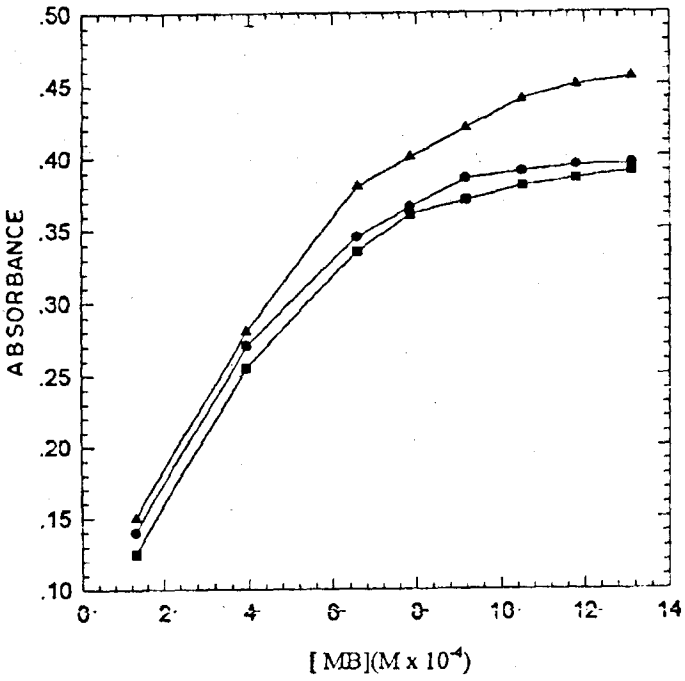

Fig. 4 : Variation in absorbance of the antibiotic-MB complex formed in aqueous 0.05 M NH^+_4/NH_3 buffer containing different concentration of MB [(cefaclor-MB (●, λ_{max}=640 nm), ampicillin-MB (■ λ_{max} = 635 nm), amoxycillin-MB (▲, λ_{max} = 635 nm)]

at 645 nm was observed for the reagent blank when identical experimental conditions were used. The reagent blank shows an enhancement in absorption with increase of pH revealing a significant contribution due to formation of some extractable X-MB (X^- = OH^-) species in chloroform.

The optimum pH, where the ion-association complex shows maximum absorbance, was found to be 9.5 in an experiment where the antibiotic was mixed with MB in aqueous solutions of varying pH 7-10 Fig. 3. It should be borne in mind that the pH of the medium is an important factor in the formation of antibiotic-MB complex. The alkaline medium facilitates the formation of anionic form of antibiotic that can readily be exchanged with anion of methylene blue. The decrease in absorbance beyond pH 9.5 is presumably due to the new ion-association complex formation between methylene blue and hydroxyl anion in alkaline medium since experimentally the reagent blank absorbance increases. As a matter of fact, the absorbance of the reagent blank does not show any fall in alkaline media. Hence, a pH of 9.5 was used in all the subsequent experimental work.

Apparently, the extent of formation of ion-association antibiotic-MB complexes is governed by MB concentration. To determine the optimum MB concentration, the solution absorbances were plotted as a function of MB concentration. Figure 4 shows that the absorbance of the complexes initially increased in the range of (1.3-10.1) x 10^{-4} M concentration MB and then attained practically a constant value in the concentration range of (10-13) x 10^{-4} M MB. Thus, a concentration 13.0 x 10^{-4}M MB was used as the optimum concentration.

For quantitative recovery of the antibiotic-MB complex, two extractions were necessary. Absorbance spectra of complex in separated extracts after 5 min shaking were stable for at least 30 min.

Under optimum conditions (pH 9.5, ionic strength 0.025 M, 1.3 x 10^{-3} M MB), the calibration curves depicting absorbance (A) versus antibiotic concentration (C) of twelve aliquots resulted in linear regression equation

TABLE 1 : ABSORPTION CHARACTERISTICS OF METHYLENE BLUE ION-ASSOCIATION COMPLEX OF CEFACLOR, AMPICILLIN AND AMOXYCILLIN

Sample	λmax (nm)	(Molar absorptivity) x 10^3 (M^{-1} cm^{-1})	Linearity range (μg/ml)	Detection limit[a] (μg/ml)	Sandell Sensitivity (S)[b] (μg/ml/cm^2)	Linear regression equation[c]
Cefaclor	640	5.3	4.0-85	3.0	0.015	A=(1.149±0.114)C+ (0.0559±0.0057)
Ampicillin	635	4.4	3.5-90	3.0	0.014	A=(1.080±0.108)C+ (0.0587±0.0059)
Amoxycillin	635	6.0	3.5-85	3.0	0.008	A=(1.260±0.126)C+ (0.1050±0.0104)

[a] The detection limit was set for the minimum measurable concentration

[b] Sandell sensitivity (S) = 10^{-3}/a; S=number of micrograms of the determinand per ml of a solution having a cross section of 1 cm^2 and absorbance of 0.001 and a = absorbance of 1-μg/ml solution determined in a cuvette with an optical path length of 1 cm.

[c] Concentration (C) in μg/ml.

TABLE 2 : ANALYSIS OF SAMPLES OF CEFACLOR, AMPICILLIN AND AMOXYCILLIN

Sample	S.No.	Concentration taken (μg/ml)	Concentration found (μg/ml)	Standard deviation (n=5) (μg/ml)	Recovery±Standard deviation (%)
Cefaclor	1	28.6	29.3	1.0	101.0±3.5
	2	21.4	20.8	0.9	97.3±4.2
	3	14.3	14.3	0.7	100.0±4.9
Ampicillin	1	30.6	29.7	1.4	97.0±4.6
	2	23.0	22.8	1.0	99.1±4.3
	3	15.3	15.4	0.7	100.6±4.6
Amoxycillin	1	27.5	28.0	1.2	101.8±4.4
	2	20.6	20.8	0.9	101.0±4.4
	3	13.7	13.9	0.6	101.4±4.4

Pure authentic bulk samples obtained for Lupin Laboratories Ltd. (cefaclor) and Hi-Media Ltd. (ampicillin and amoxycillin) were analysed.

Table 1. The correlation coefficients (γ) were close to unity (n=5) for all analytes, indicating excellent linearity. The molar absorptivity was ranged from (4.4-6.0) x 10^3 M^{-1} cm^{-1} and the Sandell sensitivity was between 0.008-0.015 μg/ml/cm^2 with a detection limit of 3.0 μg/ml for all the three antibiotics.

To confirm the validity of the method, pure authentic bulk samples of cefaclor, ampicillin and amoxycillin were analyzed and results are presented in Table 2. Excellent recovery (amount found/amount taken) in these analyses shows that the method is accurate and precise. The results of analysis for commercially available dosage

TABLE 3 : ANALYSIS OF FORMULATED ANTIBIOTICS IN DIFFERENT CONCENTRATIONS

Sample	Specification	Concentration taken µg/ml	Mean Concentration determined µg/ml	Standard Deviation (n=5) µg/ml
Cefaclor	Capsule, Ranbaxy	29.6	30.4	1.0
		14.8	14.4	0.8
		(0.250)	(0.248)	
	Tablet, Ranbaxy	30.2	30.8	1.2
		15.1	15.2	0.7
		(0.250)	(0.255)	
	Syrup, Ranbaxy	32.0	31.7	1.4
		16.0	15.6	0.7
		(0.125)	(0.126)	
Ampicillin	Capsule, Ranbaxy	33.1	33.0	1.1
		16.6	16.8	0.7
		(0.250)	(0.250)	
	Injection, Ranbaxy	32.3	32.5	1.5
		16.2	16.0	1.0
		(0.500)	(0.498)	
	Syrup, Ranbaxy	32.0	31.6	1.1
		16.0	15.9	0.7
		(0.125)	(0.124)	
Amoxycillin	Capsule, Ranbaxy	33.6	32.9	1.2
		16.8	16.8	1.0
		(0.250)	(0.248)	
	Injection, Cadila	32.3	32.2	1.2
		16.2	15.6	1.1
		(0.500)	(0.493)	
	Syrup, Ranbaxy	32.0	32.2	1.2
		16.0	16.0	0.7
		(0.125)	(0.126)	

Values in parentheses indicate labelled/certified amount (g) with standard deviation ± 0.004 to 0.037g for five determinations.

forms are shown in Table 3. The proposed method is economical, simple and precise with higher sensitivity than the other similar method[14]. There is virtually no need for the separation of soluble excipients present in various marketed preparations as the results were always reproducible and equivalent to the labeled contents of the preparations.

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