Derivative Sepctrophotometric Method for Simultaneous Estimation of Cephalexin and Probenecid in Two component Solid Dosage form

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Simple and accurate procedure, requiring no prior separation, has been developed for the estimation of cephalexin and probenecid in two component tablet formulation by derivative ultraviolet spectrophotometry. Both the drugs obey Beer's law in the concentration ranges employed for the method. The method has been validated statistically and by recovery studies.

CEPHALEXIN[1] (CP) is a first - generation cephalosporin antibiotic and is effective against respiratory tract infections, urinary tract infections, skin and other soft tissue infections. Probenecid[2] (PB) inhibits the tubular secretion and therefore increases the plasma level of cephalexin and other weakly acidic drugs. The IP[3] and BP[4] describe iodimetric titration and USP[5] describes microbial assay method for estimation of CP. Literature survey reveals a HPLC[6], polarimetry[7] and other methods for its determination. PB is official in IP[8], BP[9] and USP[10]. All describe alkalimetry method for its determination and spectrophotometric method for its estimation in tablets. Some chromatographic[11-13] and other methods are reported for its determination in dosage forms and biological fluids. No method is available for simultaneous estimation of both the drugs. A successfull attempt was made to estimate them simultaneously by third order derivative spectrophotometry. Derivative spectrophotometry[14-16] has its application for quantitative determination of an analyte in the presence of interference and for qualitative identification of species where the enhanced detail of a derivative spectrum makes it possible to distinguish among compounds having overlapping spectra.

A Shimadzu UV/Vis recording spectrophotometer (Model : 160 A) was employed with spectral band width (resolution) of 3 nm, wavelength accuracy of 0.5 nm with automatic wavelength correction and a pair of 10 mm quartz cells. Cephalexin (IP), probenecid (USP), methanol A. R. (Ranbaxy), potassium dihydrogen phosphate Excelar (Qualigens), sodium hydroxide Excelar (Qualigens) and double distilled water were used in the study. Freshly prepared 5% methanolic phosphate buffer (pH 7.0) was used as solvent. Tablet formulations of combined dosage forms were procured from the local market.

The standard stock solution of CP and PB were prepared by dissolving 20 mg of each in 10 ml volumetric flask separately using methanol. Finally standard stock solution of 50 μg/ml of CP and PB were prepared by diluting 2.5 ml of the above solution to 100 ml with phosphate buffer of pH 7.0.

Both the drugs show linearity with absorbance in the range 0 to 50 μg/ml concentration which is validated by least square method.

$$\text{Equation for CP} \Rightarrow -4.151 = 7a + 210b.$$
$$180.29 = 210a + 9100b$$

$$\text{and for PB} \Rightarrow 7.677 = 7a + 210b$$
$$334.45 = 210a + 9100b$$

where coefficient of correlation was 0.9995 and 0.9997 respectively, for CP and PB.

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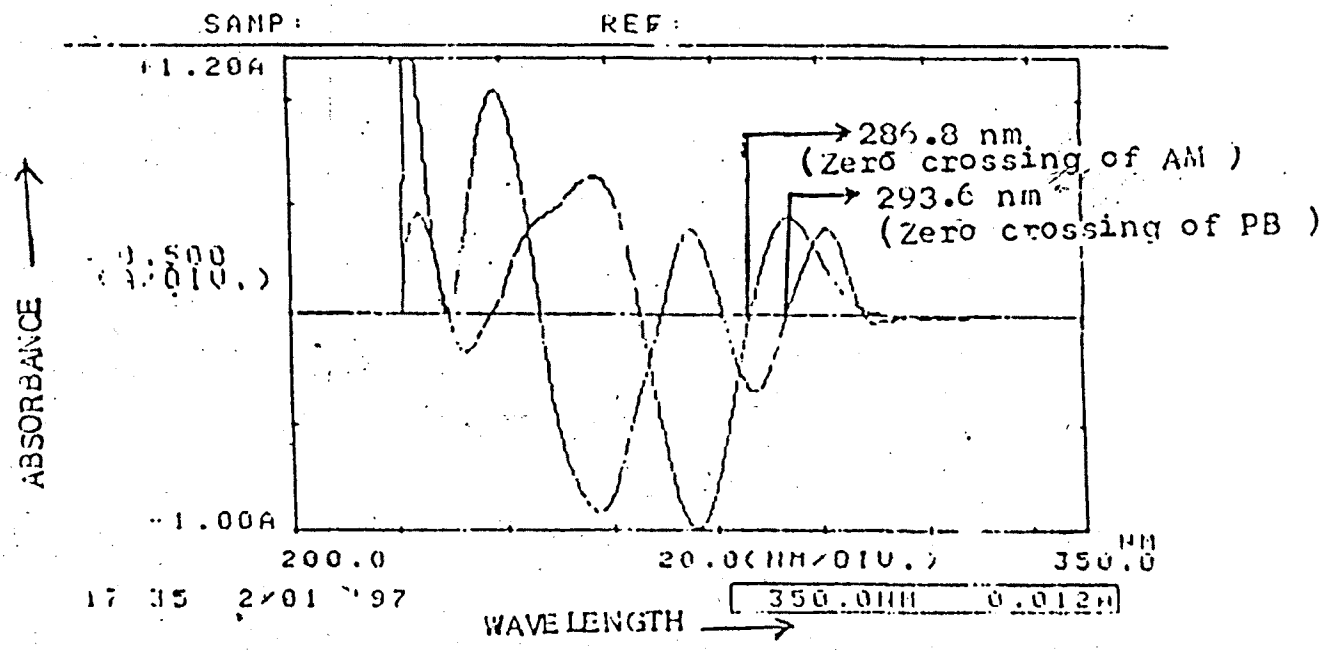

Fig. 1: Overlain Third Derivative Order (n = 9) Spectra of Cephalexin and Probenecid

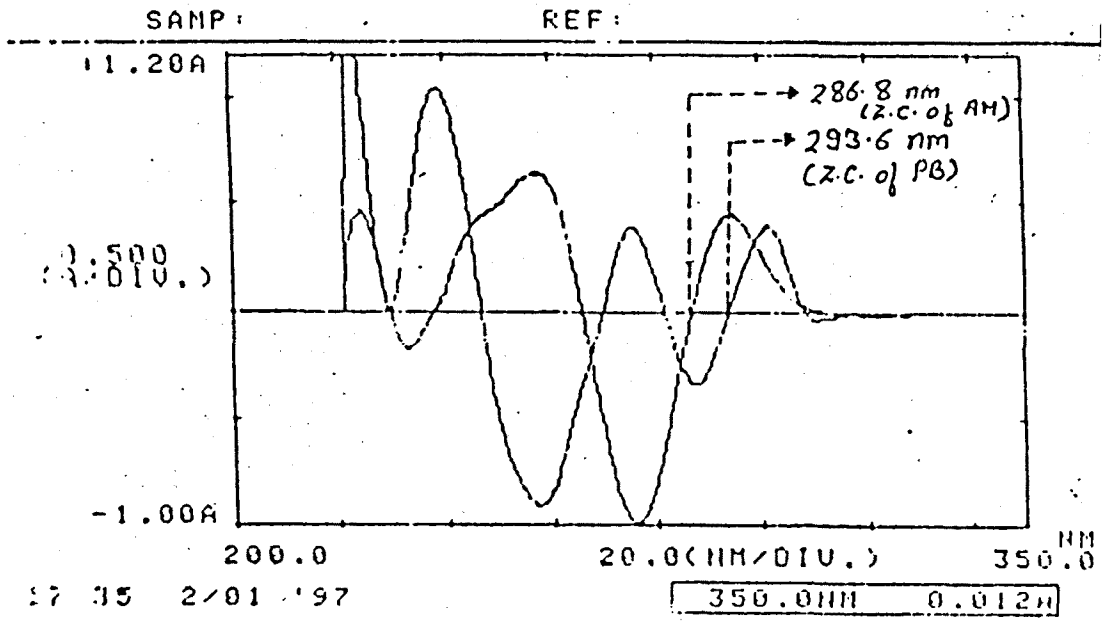

Fig. 2: Overlain Derivative Order (n=9) Spectra of Cephalexin and Probenecid

Six mixed standards having concentrations 0, 10, 20, 30, 40, 50 µg/ml of CP and 50, 40, 30, 20, 10, 0 µg/ml of PB, respectively, were prepared by diluting appropriate volumes of the standard stock solution. The solutions were scanned between 400 - 200 nm against blank in third derivative spectra with d(N) = 9, and absorbances were noted at 293.6 nm and 286.9 nm., for CP and PB respectively (Fig. 1). Calibration curves were plotted from the absorbance values recorded from the derivative spectra.

Table 1 : Analysis results of commercial tablets

Tablet	Label Claim (mg/tab)		Found* (mg/tab)		Percent Found	
	CP	PB	CP	PB	CP	PB
I	250	250	244.79	253.77	97.92	101.51
II	250	250	490.57	500.25	98.15	100.05

* Mean of five readings, CP - Cephalexin, PB - Probenecid

For analyzing tablet formulations, twenty tablets weighed and ground to a fine powder. An accurately weighed powder sample equivalent to 5 mg of PB and CP was transferred to a 100 ml volumetric flask and dissolved in 5% methanolic phosphate buffer (pH 7.0) and volume was made up to the mark. The solution was then filtered through Whatman filter paper No.42 and diluted to get a final concentration of 25 μg/ml of CP and PB each. Concentration of each component in the sample solution was determined from calibration curve. The results obtained by replicate analysis are shown in Table 1. The recovery studies conducted by addition of different amounts of pure drugs to a pre-analyzed tablet sample solution gave satisfactory recovery data.

The proposed method is simple and rapid for the simultaneous estimation of CP and PB from combined dosage forms. The estimation of CP and PB was performed in the third derivative at zero-crossing of PB (293.6 nm) and zero-crossing of CP (286.9 nm) respectively. The wavelengths were selected at the zero-crossing point of the drug where other drug shows substantial absorbance in the third order derivative spectra. Methanolic phosphate buffer of pH 7.0 was used as the solvent after considering the solubility and stability factor. At higher pH values probenecid is soluble but cephelexin is unstable, and at lower pH values probenecid is insoluble in low concentration of methanol.

The results of tablet analysis obtained by the proposed method are validated by statistical data, where standard deviation for CP and PB were found to be 1.051 and 1.167 respectively for one batch of tablets and 0.888 and 1.351 respectively for another batch of tablets. Recovery study which lies between 97-101% indicates the reproducibility of the method. Low values of relative standard deviation and coefficient of variation indicate high precision of the method. The developed method supports the reproducibility and accuracy of the derivative spectroscopic method[14,15] where the wavelength difference is smaller between the peaks of the two components (8 nm between probenecid and cephalexin).

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Spectro Photometric Determination of Promethazine using Sodium Nitroprusside

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A simple, accurate and rapid method for the quantitative determination of promethazine in either pure form or in pharmaceutical formulations is proposed. The method is based on the formation of red product with sodium nitroprusside in sulphuric acid medium having maximum absorption at 515 nm.

PROMETHAZINE hydrochloride, 10-[2-(dimethylamino) Propyl] phenothiazine hydrochloride, is a drug used as an anthistamine. It has also some anticholinergic, antiserotoninergic and marked local anaesthetic properties[1].

The determination of promethazine has been achieved by different procedures, e.g., by forming ion-association complex[2], and spectrophotometry[3] using N-bromophthalimide, fluorimetry[4], partition column chromatography[5] and ion-exchange chromatography[6].

Gas Chromatography has been used to separate promethazine from other phenothiazines[7] and for its determination in the presence of paracetamol[8]. Titrimetric procedures including thermometric titrimetry[9] have been widely used. Low levels of promethazine have been determined by anodic stripping voltammetry[10] and flow-injection analysis (FIA) procedures[11,12].

In the present work, a simple, accurate and rapid spectrophotometric method for the determination of promethazine using sodium nitroprusside in sulphuric acid medium is proposed.

Absorbance measurements were carried out using an Elico model CL- 27 Digital spectrophotometer, provided with 1cm matching cells.