Simultaneous Spectrophotometric Determination of Amoxycillin and Probenecid in Tablet Dosage form

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Accepted 22 MAy 1998
Received 14 January 1998

Simple, rapid and economical Spectrophotometric methods, requiring no prior separation, have been developed and validated for the simultaneous estimation of amoxycillin and probenecid.These methods utilize spectrum mode of analysis of the recording spectrophotometer (Shimadzu 160 A). Absorption maxima of probenecid was found at 244 nm and that of amoxycillin at 247 nm. Both drugs show linearity in absorbance in the range of 0-50 µg/ml. Using derivative spectroscopy, estimation of probenecid was carried out in first order with N=6 at 272.2 nm and amoxycillin in third order with N=8 at 282 nm. For two wavelength method, absorbance difference was employed at 252.8 nm and 240.6 nm for probenecid and at 250.2 nm and 237.8 nm for amoxycillin. For both the methods the standard deviation values obtained on repeated analysis were below 1.2 and recovery of added standard drug was between 97-100.5%.

AMOXYCILLIN (AM) is a broad spectrum semisynthetic penicillin. It is effective against Gram positive and Gram negative bacteria and primarily used for urinary tract infection, respiratory tract infection and meningitis[1]. Probenecid (PB) inhibits the tubular secretion of amoxycillin being weakly acidic in nature and increases its plasma level and retention time in plasma, therefore reduces its frequency of administration[2].

The IP[3], BP[4] and USP[5] describe potentiometric, iodometric and spectrophotometric methods respectively for estimation of AM. Literature survey reveals a HPLC[6,7] spectrophotometric[8-10] and other methods for its estimation. Probenecid is official in IP[11], BP[12] and USP[13]. All describe alkalimetry method for its determination in powder form and BP[12] mentions spectrophotometric method for its estimation in tablets. Some chromatographic[14,15] and other methods are reported for its determination in dosage forms and biological fluids. No method has been reported for simultaneous estimation of AM and PB in combination. Present paper deals with two simple methods for the simultaneous analysis of two components.

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A shimadzu UV/Visible recording spectrophotometer (Model:160 A) was employed with spectral bandwidth of 3 nm, wavelength accuracy of 0.5 nm with automatic wavelength correction correction and a pair of 10 mm matched quartz cells.

Amoxycillin trihydrate IP (Cipla Ltd.), probenecid BP (Geno Pharmaceuticals), sodium hydroxide ExcelaR (Qualigens) and double distilled water were used in the present study. Tablet formulations of combined dosage forms were procured from the local market.

Stock solutions of 1000 µg/ml were prepared by dissolving 25 mg each of AM and PB in 25 ml volumetric flasks separately using 0.025 N NaOH. Finally Standard Drug Solutions of 50 µg/ml of AM and PB were prepared by diluting separately 5 ml of the above solutions to 100 ml with 0.025 N NaOH. Drug solutions of different strengths were further prepared from standard drug solutions. AM and PB, show linearity with absorbance at 247 nm and 244 nm respectively in the range of 0-50 µg/ml. By least square method, the slope, intercept and correlation coefficient for AM were found to be 0.0317, 0.0315 and 0.9994 and for PB 0.0358, 0.0222 and 0.9999 respectively.

Using derivative spectroscopy[16] AM and PB were

Table 1: Results of Analysis of commercial Tablets

Method	Label Claim (mg/tab)		Percent Found*		Standard Deviation		Percent Recovery**	
	AM	PB	AM	PB	AM	PB	AM	PB
D.S.	250	250	98.43	99.20	0.4542	0.5892	100.76	99.76
T.W.	250	250	98.92	99.24	1.205	0.9995	100.76	98.42

* - Mean of Six Readings
** - Mean of Four Readings.
D.S. - Derivative Spectroscopy.
T.W. - Two Wavelength Method.

estimated in different orders, as the condition of substantial absorbance at the zero crossing of the other, for each drug was not fulfilled in any single order. Am was estimated in III order (N=8) at 282 nm and PB in I order (N=6) at 272.2 nm.

Six mixed standards of the following concentrations 0, 10, 20, 30, 40, 50 µg/ml of AM and 50, 40, 30, 20, 10, 0 µg/ml of PB respectively were prepared by using appropriate volumes of standard drug solutions. The absorbances of these mixed standards at 272.2 nm and 282 nm in I and III order were used to plot the calibration curves for PB and AM respectively.

Twenty tablets were weighed and ground to fine powder. An accurately weighed powder sample equivalent to 5 mg each of AM and PB was transferred to a 100ml volumetric flask dissolved in 0.025 NNaOH and volume was made upto the mark. The solution was then filtered through Whatman filter paper No.41 and diluted to get final concentrations of 15µg/ml, 20µg/ml and 25µg/ml of Am and PB each and absorbances were noted at the respective derivative order at specified wavelengths. Concentration of each component in the sample solution was obtained from the calibration curves prepared. The results obtained by replicate analysis from all the methods are reported in table 1. Recovery study was conducted by addition of different amounts of pure drugs to a reanalyzed tablet sample. The result are recorded in Table 1.

Overlain spectra show that, at the absorbance maxima both drug's show high degree of interface with each other. Hence for two wavelength method, two such wavelengths were selected where the other drug (interfering compound) has the same absorbance, irrespective of the absorbance maxima. AM was estimated at 250.2 nm and 237.8nm and PB at 252.8 nm and 240.6 nm.

Six mixed standards as mentioned above were used to prepare the calibration curve. Solutions were scanned at the selected wavelengths in the quantitative mode of the instrument and the absorbance differences were used to plot the calibration curves.

Tablet samples of 15 µg/ml, 20 µg/ml and 25 µg/ml of each drug were prepared similarly as mentioned in the previous method. The samples were scanned at selected wavelength for AM and PB and from the absorbance difference values, the concentration of each component was obtained by using the respective calibration curves. Results of multiple analysis are recorded in Table 1. Recovery studies conducted in the same manner as per the above method gave satisfactory recoveries. (Table 1).

The individual ultraviolet spectra of AM and PB in 0.025 NNaOH show substantial absorbances over the wavelength range 200-320 nm. Hence normal ultraviolet spectroscopy cannot be used for individual quantification if both the compounds are present in the pharmaceutical formulation. Hence it was though necessary to develop a derivative method of analysis for AM and PB, as the derivative method of analysis overcomes the interference due to spectral overlap by selecting a suitable order of derivatization and corresponding derivative interval. The Guassian spectra of both the drugs were derivatized in all orders (first to

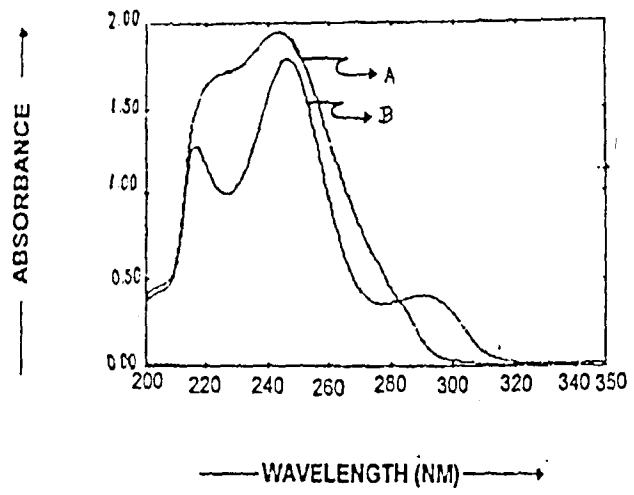

Fig. 1: Overlain Spectra of Amoxycillin (B) and Probenecid (A)

fourth) with various derivative intervals and zero crossing, peaks and valleys of both the drugs were keenly observed. It was found that the analysis of PB is possible in first order derivative with N=6 at 272.2 nm while analysis of Am is possible in third order derivative with N=8 at 282.2 nm for their simultaneous estimation respectively. By this method the percent mean label claim and standard deviation (Table 1) were found to be 98.432% and 0.4542 for AM and 99.202% and 0.5892 for PB, respectively.

Dual wavelength method of analysis was also developed for AM and PB. As the λmax of both the drugs lie in close proximity to each other and a substantial interference was observed as shown in Figure 1. Added to this, when λmax of one component is selected as one of the sampling wavelengths then no other wavelength is observed where interfering component has same absorbance corresponding to the λmax of the first component. These aforecited reasons compelled us to select two wavelengths other than the respective λmax of the drugs. Probenecid was estimated at 252.8 nm and 240.6 nm and amoxycillin at 250.2 nm and 237.8 nm. Percent mean label claim and standard deviation were found to be 98.92% and 1.205 for AM and 99.242% and 0.9995 for PB, respectively.

The statistical validation and recovery studies conclude that all the methods are satisfactory but derivative spectroscopy shows high degree of accuracy, as is understood due to shorter difference (3 nm) in the absorbance maxima of the drugs.

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