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Simultaneous Multicomponent Spectrophotometric analysis of Ampicillin and Probenecid in Pharmaceutical formulation by Derivative spectroscopy

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Abstract:

A simple, fast and precise simultaneous multicomponent derivative spectrophotometric method has been developed for simultaneous determination of Ampicillin and Probenecid in pharmaceutical formulation. The first derivative spectra has absorption maxima at 222.2nm for Ampicillin and 288 nm for Probenecid in 0.1N NaOH used as solvent. The peak with good absorption at different concentrations, obeyed Beer Lambert law only was chosen. Both Ampicillin and Probenecid showed linearity in the range of 10 to 50 µg/ml concentration.

Keywords: *Ampicillin, Probenecid, simultaneous, multicomponent, derivative spectrophotometric method*

1. Introduction

Ampicillin[2-4] is chemically 6-[(Amino phenyl acetyl) amino]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid. It is sparingly soluble in cold water (1 in 50). It is insoluble in alcohol and acetone. It is soluble in dilute solutions of acids and alkali hydroxides. Ampicillin trihydrate is an antibiotic active against mainly gram positive bacteria and some gram negative bacteria. It is used for the treatment of infections due to streptococci and *H.influenzae.* It is used in urinary tract infections and respiratory tract infections. It is also used in meningitis, biliary tract infections etc.

Probenecid is a uricosoric agent used in gout therapy. When Ampicillin is co-administered with Probenecid, the renal excretion of Ampicillin is inhibited. The combination is used in gastrointestinal tract and respiratory tract infections.

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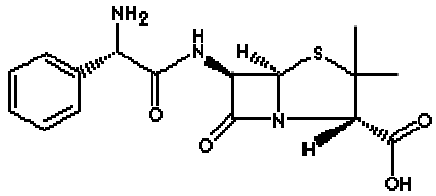
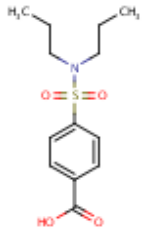
Literature survey revealed that for Ampicillin and Probenecid combination, the extractive spectrophotometric method[6, 9, 10] and estimation in biological fluids[7, 8] are available.



Ampicillin



Probenecid

The United States Pharmacopoeial method[11] involves iodometric method for Ampicillin and HPLC method for Probenecid. The present study aims in the development of simple, rapid, accurate and sensitive method for simultaneous estimation of Ampicillin and Probenecid in combined dosage forms using 0.1N Sodium hydroxide as solvent using derivative spectroscopy.

2. Experimental

Pure samples of Ampicillin trihydrate (99.54%w/w) was obtained as a gift sample Smith Kline Beecham Pharmaceuticals, Bangalore. Probenecid B.P. (99.91%w/w) was obtained as a gift sample from American Remedies, Chennai. Marketed formulations were taken for study, which contained Ampicillin and Probenecid 250mg each. Shimadzu 160A UV-VISIBLE recording spectrophotometer was used for analysis.

3. Method development

Ampicillin 1mg/ml and Probenecid 1mg/ml stock solution in 0.1N sodium hydroxide were prepared. Further dilutions were done to get a concentration of 10µg/ml of Ampicillin and 10µg/ml of Probenecid. The solutions were scanned and the first derative spectrum for Ampicillin was determined at 222.2nm and that of Probenecid was determined at 288 nm against 0.1N sodium hydroxide as blank. The absorbance values are recorded in table 1. Both Ampicillin and Probenecid showed linearity in the range of 10µg/ml to 50µg/ml.The first derivative spectra of Ampicillin, Probenecid and the mixture of Ampicillin and Probenecid are shown in figure 1, 2 and 3.

4. Analysis of formulation

Twenty tablets each containing 250mg of Ampicillin and Probenecid were weighed and the average weight was calculated. The tablets were crushed together to a fine powder and a quantity of powder equivalent to 50 mg each of Ampicillin and Probenecid was dissolved in 0.1N Sodium hydroxide, filtered through Whatman filter paper.

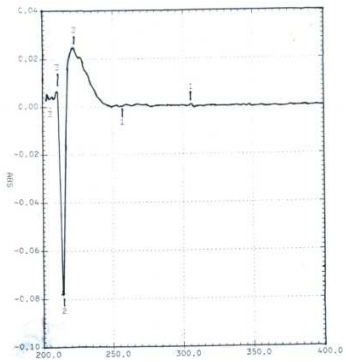


Fig. 1 First derivative spectra of Ampicillin

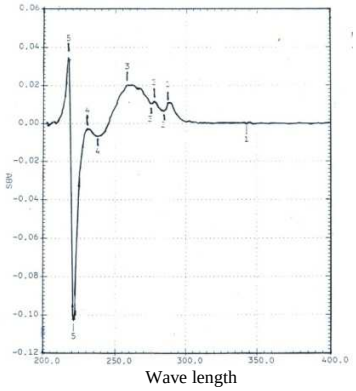


Fig. 2 First Derivative spectra of Probenecid

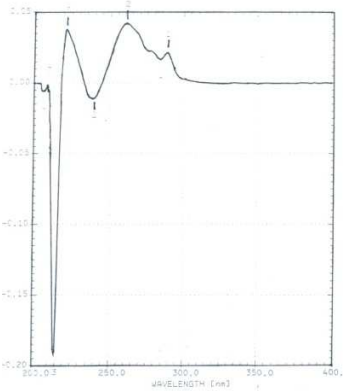


Fig.3 First derivative spectra of Ampicillin Probenecid combination

More amount of solvent was passed and the volume was finally made to 50ml with 0.1N Sodium hydroxide. The solution was further diluted to get 20µg/ml of Ampicillin and 20µg/ml of Probenecid. The first derivative spectrum of the solution was obtained against 0.1 Normal sodium hydroxide as blank, figure 3. The absorbance of the solution was observed near 222.2nm for Ampicillin and at 288 nm for Probenecid. The amount of Ampicillin and Probenecid was then calculated by extrapolation using calibration curve of standard solutions.

5. Recovery Experiments

In order to confirm the suitability and reliability of the proposed method, a known quantity of Ampicillin and Probenecid were added to previously analysed samples and the mixtures were analysed by the proposed method. 5ml aliquot of the pre- analysed sample was transferred to a 10 ml standard flask and 5ml of standard solution containing 10µg /ml of Ampicillin and 10µg /ml of Probenecid were added.

Then the procedure described under preparation of standard curve was followed. The results of the recovery studies are shown in the table.2

Table 1. Data for Beer's law Plot for Ampicillin

Concentration (µg/ml)	λmax (nm)	Absorbance*
10	222.2	0.025
20	222.2	0.039
30	222.2	0.056
40	222.2	0.070
50	222.2	0.088

Conc. =635.95× Absorbance -5.306 * Average of 3 determinations

Table 2. Data for Beer's law Plot for Probenecid

Concentration (µg/ml)	λmax (nm)	Absorbance*
10	288.0	0.011
20	288.0	0.021
30	288.0	0.032
40	288.0	0.042
50	288.0	0.051

Conc. = 990.60× Absorbance -1.0772 * Average of 3 determinations

Table 3. Data for analysis of formulations of Ampicillin and Probenecid

Drug	Absorbance		Amount (mg/tablet)*		% label Claim	% Recovery
			Labelled	Found Mean ± SD		Mean ± SD*
Ampicillin	222.2	0.038	250	235.74 ± 1.25	94.2 ± 0.356	98.6 ± 0.45
Probenecid	288	0.022	250	258.9 ± 0.98	103.5 ± 0.364	100.2 ± 0.75

* Average of 5 determinations

6. Results and Discussion

The Ultraviolet first derivative spectra of Ampicillin and Probenecid in 0.1N sodium hydroxide showed absorption maxima at 222.2 nm and 288 nm. Both Ampicillin and Probenecid showed linearity at 10 to 50 µg/ml concentration. The results of the method were is good agreement with the label claim of the Formulations. The recovery studies were done and it also showed good results.

7. Conclusion

The developed method can be used for the routine analysis of Ampicillin and Probenecid in combination. The method provided adequate accuracy and precision.

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