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Q ABSORBANCE RATIO METHOD FOR SIMULTANEOUS ESTIMATION OF AMOXICILLIN AND PROBENECID IN TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, rapid, economical UV spectrophotometry method namely Absorption ratio, and RP-HPLC method have been developed and validated for estimation of Amoxicillin and Probenecid in combined tablet dosage form and can be used in routine analysis. In Absorbance ratio method absorbance measured 228nm and at Iso-absorptive point 237 and correlation coefficient was found to be 0.9971,0.9968 and 0.998,0.998 for Amoxicillin and Probenecid at 228nm and 237nm respectively. Mean assay was found to be 99.51251% and 98.93545% for Amoxicillin and Probenecid respectively by Absorption ratio method. Both the methods were found to be linear in the concentration range of 2-14µg/ml for Amoxicillin and 2-14µg/ml for Probenecid.

Keywords – UV spectrophotometry, Amoxicillin, Probenecid, Validation, Absorption ratio

1. INTRODUCTION

1.1 Amoxicillin[1-2]

Amoxicillin is amino penicillin and approved as antibiotic use. Amoxicillin binds to penicillin-binding protein 1A (PBP-1A) located inside the bacterial cell well. Penicillins acylate the penicillin-sensitive transpeptidase C-terminal domain by opening the lactam ring. This inactivation of the enzyme prevents the formation of a cross-link of two linear peptidoglycan strands, inhibiting the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that amoxicillin interferes with an autolysin inhibitor. Structure of the amoxicillin shown in figure no 1[5].

1.2 Probenecid[3-4]

Probenecid is an uricosuric drug that increases uric acid excretion in the urine. The prototypical uricosuric agent. It inhibits the renal excretion of organic anions and reduces tubular reabsorption of urate. Probenecid has also been used to treat patients with renal impairment, and, because it reduces the renal tubular excretion of other drugs, has been used as an adjunct to antibacterial therapy. Structure of the Probencid shown in figure no 2[6].

1.3 Use of combination[1]

Generally, drug combination of amoxicillin and probenecid use to treat infectious diseases.

1.4 Typical usage[2]

Respiratory, genito-urinary, skin and soft tissue, ENT infections due to susceptible strains of Gram negative organisms like *H.influenzae, E-coli, P.mirabilis* and *N. gonorrhea*. Both the drugs are official in IP, BP and USP[7-9]

2. MATERIALS AND METHODS

2.1 Selection of Solvent

14µg/ml each of Amoxicillin and 14µg/ml Probenecid was prepared in different solvent (methanol, 0.1 N HCl, 0.1NaOH). These prepared solutions were scanned between 200-400nm. From taken spectra and the solubility difference in different solvents, methanol was found to be the most suitable solvent. **(**Fig:3**)**

2.2 Preparation of standard stock solution (100 mg / ml)

Amoxicillin and Probenecid (100mg each) were weighed and transferred to separate 100ml volumetric flasks and dissolved in methanol. Sonicate it and volumes were made up to the mark with methanol to prepare standard stock solution containing 1000 µg/ml of Amoxicillin and Probenecid. The sufficient aliquot was pipette out and transferred to a 100ml volumetric flask and volume was adjusted to the mark with methanol to prepare working standards of 100µg/ml Amoxicillin and 100µg/ml Probenecid.

2.3 Selection of Analytical Wavelength

Solutions of Amoxicillin (2-14µg/ml) and Probenecid (2-14µg/ml) were prepared from working standard in methanol and spectrums were recorded between 200-400 nm and the isobestic point was found at 237 nm. Characteristic wavelength (Iso-absorptive point) was confirmed by varying the concentration of both drugs. The selected iso-absorptive point was considered as λ1 (237 nm) and the wavelength of the one drug (Amoxicillin) was considered as λ2 (228)

2.4 Calibration curve for Amoxicillin (2-14 mg/ml)

Appropriate volumes of aliquots from working standard of Amoxicillin were transferred to different volumetric flasks of 10 ml capacity and volume was adjusted upto the mark with methanol to obtain concentrations range of 2-14 µg/ml and absorbance measured at λ1 and λ2. The plot of absorbance vs. concentration was plotted for obtained absorbance at each selected wavelength. The regression equation and correlation coefficient were obtained.

2.5 Calibration curve for Probenecid (2-14 mg/ml)

Appropriate volumes of aliquots from working standard of Probenecid were transferred to different volumetric flasks of 10 ml capacity and volume was adjusted upto the mark with methanol to obtain concentrations range of 2-14 µg/ml. Spectra of each solution recorded using methanol as a blank and absorbance measured at λ1 and λ2. The plot of absorbance vs. concentration was plotted for obtained absorbance at each selected wavelength. The regression equation and correlation coefficient were obtained

2.6 Sample Preparation

Twenty tablets were weighed and finely powdered. Powder equivalent to 10mg of Amoxicillin and 10mg of Probenecid was accurately weighed and transferred to 100ml volumetric flask and add methanol (25ml). Sonicate it for 15 min and make up volume with methanol. The above solution was Filtered. The aliquot 1ml was transferred to 10ml volumetric flask and make up final volume with methanol (solution-1).

Estimation of Amoxicillin and Probenecid: The response of solution-1 was measured at 228nm (λ2) and 237nm (λ1) for quantification of Amoxicillin and Probenecid respectively. The amounts of Amoxicillin and Probenecid present in the sample solution were determined by substituting the responses into the Absorption ratio equation for the simultaneous estimation.

2.7 Validation of Spectrophotometric Method

2.7.1 Linearity and range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity. Selected linearity range for Amoxicillin was 2-14 µg/ml and for Probenecid it was 2-14 µg/ml.

2.7.2 Accuracy

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the level of 80%, 100% and 120% to the pre-analyzed sample. Results of the recovery study were found to be within the acceptance criteria 100±10 %, indicating a good degree of sensitivity of the method towards detection of analytes in sample. In this method the known concentration of standard drug was added to the assay sample. The amount present was calculated and the assay amount was subtracted from it, which gives the amount recovered.

2.7.3 Precision

The intra-day and inter-day variation for determination of Amoxicillin and Probenecid were carried out three times in the same day and three consecutive days using three difference concentration of Amoxicillin (8, 10, 12 µg/ml) and Probenecid (8, 10, 12 µg/ml). % RSD was calculated. The method was found to be precise due to low values of the %RSD.

2.7.4 LOD and LOQ

The LOD and LOQ of developed method were studied as per ICH guidelines. Several approaches for determining the LOD & LOQ are possible, depending on the procedure i.e, a non-instrumental or instrumental. Among them here employed method was,

$$LOD = 3.3\sigma/S \text{ and}$$

$$LOQ = 10\sigma/S$$

Where, σ = the standard deviation of response

S = the slope of calibration curve.

3. RESULTS AND DISCUSSION

Among trialed solvent methanol was found to be best for further method development. For estimation of Amoxicillin and Probenecid, Absorption ratio method was employed. In this method two wavelength are required. One wavelength is selected at which Amoxicillin shows maximum absorbance with good linearity while other drug Probenecid shows considerable absorbance. The second wavelength is selected at iso-absorptive point were both drugs shows same absorbance with difference concentration.

Wavelength 228nm (λmax of one drug) and 237nm (iso-absorption point) were selected for the estimation of Amoxicillin and Probenecid respectively by Absorption ratio method.

Calibration data for Amoxicillin and Probenecid are shown in Table respectively. Calibration curves for Amoxicillin and Probenecid was plotted (absorbance vs concentration). The following regression equations for straight line were obtained for Amoxicillin and Probenecid. (Fig:4-7)

Linear equation for Amoxicillin: y = 0.050x + 0.031 and y = 0.040x + 0.020 at 228nm and 237nm respectively.

Linear equation for Probenecid: y = 0.052x + 0.043 and y = 0.059x - 0.028 at 228nm and 237nm respectively.

3.1 Linearity

Linear correlation was obtained between absorbance Vs concentrations of AMOX and PROB in the ranges of 2 – 14 µg/ml respectively. Regression parameters are mentioned in Table 1 and 2. The calibration curves of these two drugs at 228 nm and 237 nm are shown in Fig. 8 & Fig.9.

3.2 Accuracy

The recovery experiment was performed by the standard addition method. The recovery mean were 99.51 ± 1.20 and 98.98 ± 1.34 % for AMOX and PROB, respectively. The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 3

3.3 Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as %RSD.

3.4 Repeatability

The RSD values for Amoxicillin and Probenecid were found to be 0.54 ± 0.36 % and 0.51 ± 0.53%, respectively at 228 nm and at 237 nm (Table 4). Values of %RSD were found to be <2 %, which indicates that proposed method is repeatable

3.5 Intermediate Precision

The low RSD values of interday (0..44 – 0.604% and 0.616 – 0.79 %) and intraday (0.61 – 0.79% and 0.64 - 0.78 %) variations for AMOXICILLIN and PROB, respectively at 237 nm and interday (0.2 – 0.85 %) and intraday (0.683 – 0.98 %) variations at 237 nm, revealed that the proposed method is precise .

3.6 LOD and LOQ

LOD values for AMOX and PROB were found to be 0.02 and 0.022 µg/ml, respectively at 228 nm 0.184 and 0.138 µg/ml at 255.6 nm. LOQ values for AMOX and PROB were found to be 0.0618 and 0.126 µg/ml, respectively at 228 nm 0.561and 0.41 µg/ml at 237 nm. Table-5. These data show that this method is sensitive for the determination of AMOX and PROB.

3.7 Application of Developed Method to Pharmaceutical Dosage Form

The proposed validated method was successfully applied to determine AMOX and PROB in their combined dosage form. The result obtained for AMOX and PROB was comparable with the corresponding labeled amount Table 6. No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine estimation of AMOX and PROB in pharmaceutical combined dosage for.

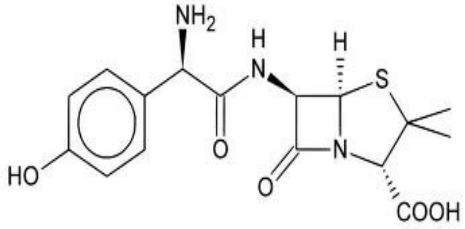

Fig. 1: Structure of the amoxicillin

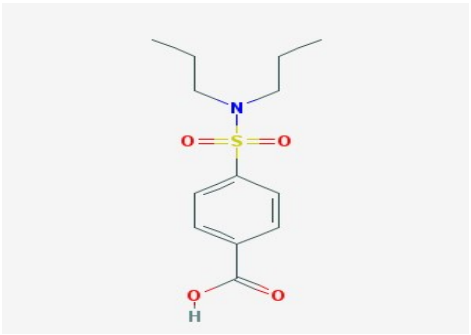

Fig. 2: Structure of the amoxicillin

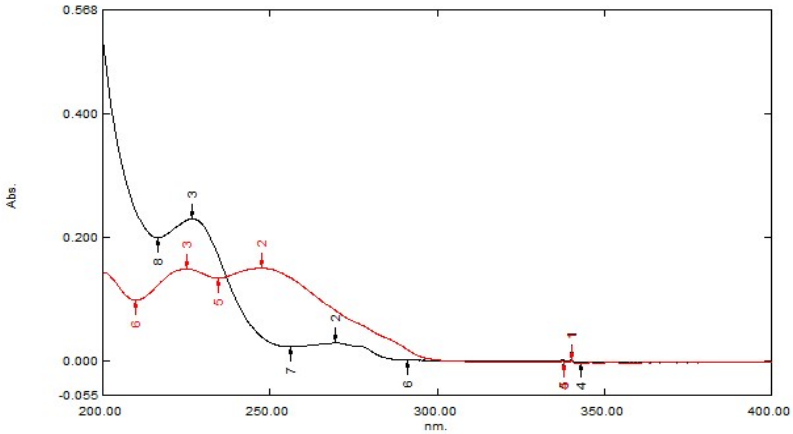

Fig. 3: Overlain spectra of AMOX and PROB

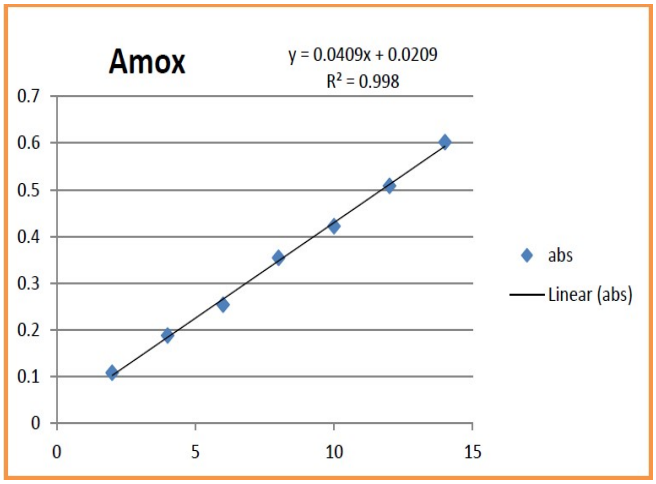

Fig.4: Calibration Curve of AMOX at 228 nm

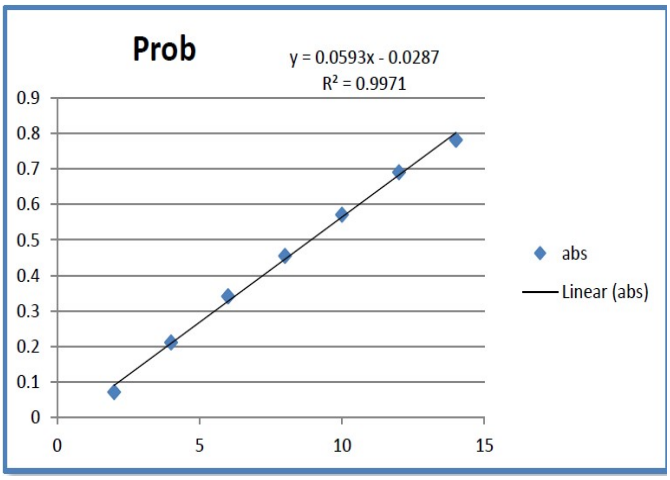

Fig. 5: Calibration Curve of PROB at 228nm

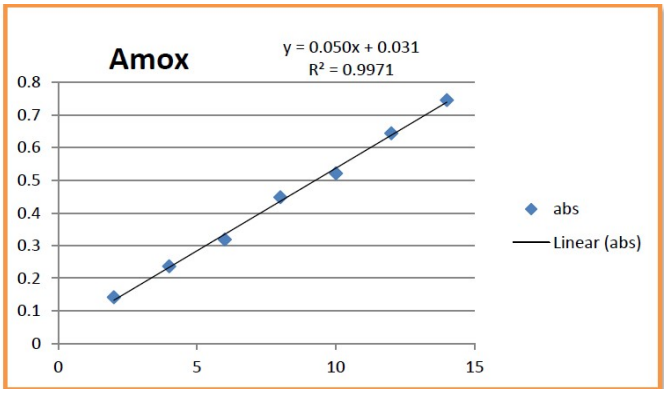

Fig 6: Calibration Curve of AMOX at 237 nm

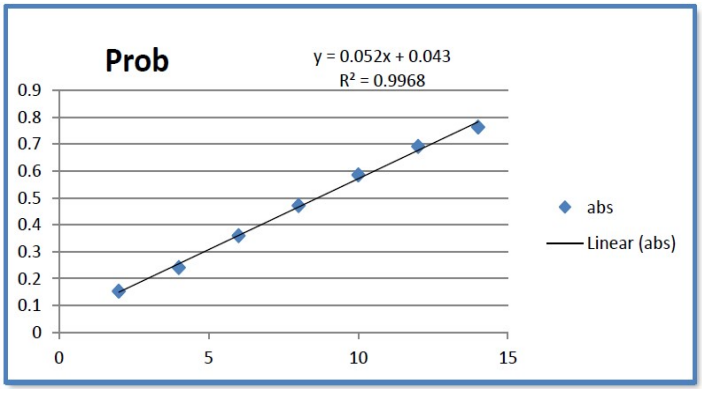

Fig 7: Calibration Curve of PROB at 237 nm

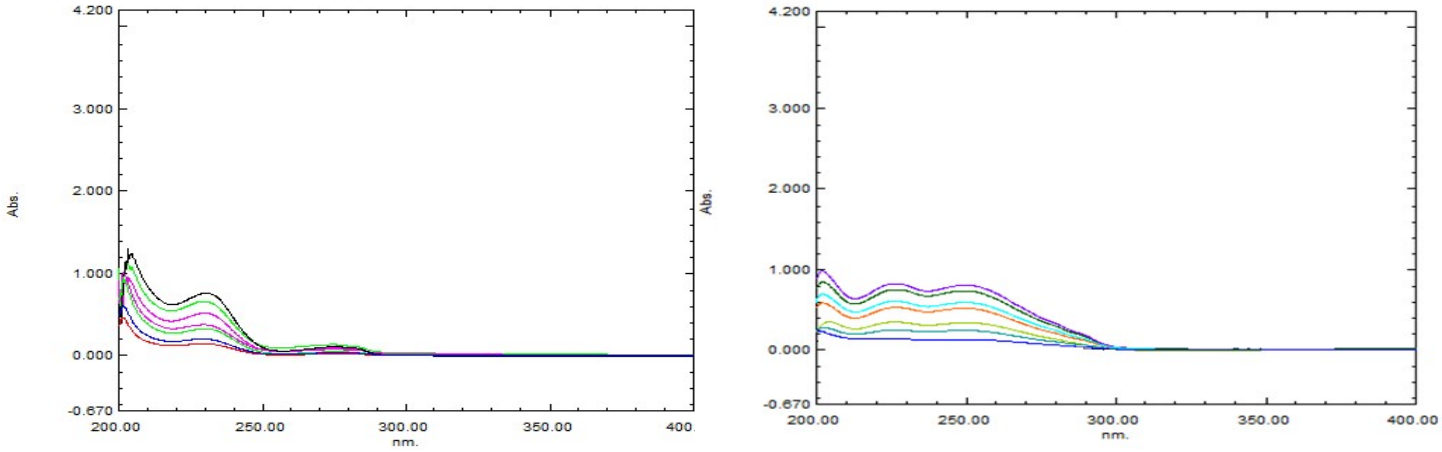

Fig. 8: Spectral data for Amoxicillin (A) and Probenecid (B)

Table 1: Calibration data of Amoxicillin and Probenecid At 228nm

Conc. (mcg/ml)	Abs (Amoxicillin)	Abs (Probenecid)
2	0.141	0.153
4	0.263	0.241
6	0.318	0.359
8	0.448	0.471
10	0.521	0.585
12	0.644	0.691
14	0.746	0.763
Corelation Coefficient	0.9971	0.9968
SD	0.002	0.00258
Slope	0.0506	0.0528
LOD	0.0201	0.22
LOQ	0.0618	0.126
%RSD	0.61955	0.6803

Table 2: Calibration data of Amoxicillin and Probenecid At 237nm

Conc. (mcg/ml)	Abs (Amoxicillin)	Abs (Probenecid)
2	0.108	0.071
4	0.188	0.211
6	0.254	0.341
8	0.354	0.455
10	0.422	0.571
12	0.508	0.691
14	0.602	0.782
Corelation Coefficient	0.998	0.9971
SD	0.00278	0.00289
Slope	0.0405	0.0593
LOD	0.1842	0.1381
LOQ	0.5610	0.4183
%RSD	0.7991	0.7383

Table 3: Accuracy data of AMOX and PROB

Drug	Level	Amt. Taken (µg/ml)	Amt. Added (µg/ml)	Total amount (µg/ml)	%Mean Recovery*±SD
Amoxicillin	80%	5	4	9	99.51±1.71
	100%	5	5	10	100.75±0.71
	120%	5	6	11	98.75±0.65
Probenecid	80%	3	4	9	99.78±1.64
	100%	3	5	10	98.93±0.95
	120%	3	6	11	98.68±0.50

Table 4: Repeatability data of AMOX and PROB at both the wave length

Sr. No.	Absorbance of AMOX at 228 nm	Absorbance of PROB at 228 nm	Absorbance at isoabsorptive point (237 nm) AMOX	Absorbance at isoabsorptive point (237 nm) PROB
1	0.524	0.591	0.422	0.57
2	0.521	0.592	0.423	0.572
3	0.523	0.596	0.42	0.571
4	0.525	0.594	0.425	0.575
5	0.527	0.593	0.426	0.568
6	0.529	0.59	0.422	0.576
mean	0.524	0.592	0.423	0.572
SD	0.0028	0.0021	0.0021	0.0030
% RSD	0.544	0.0364	0.5179	0.530

Table 5: LOD data for AMOX and PROB

Parameter	AMOX		PROB	
	At 228	At 237	At 228	At 237
LOD	0.0201	0.18425	0.02204	0.13811
LOQ	0.0618	0.561028	0.126	0.41863

Table 6: Analysis of Pharmaceutical Dosage form

Sample No.	Label Claim (mg/tablet)		Amount found (mg/tablet)		% Assay	
	AMOX	PROB	AMOX	PROB	AMOX	PROB
1	250	250	247.1344	247.1344	98.85374	98.85374
2	250	250	245.5545	249.825	98.22178	99.92999
3	250	250	253.655	245.0565	101.462	98.02262
Mean			247.1344	247.1344	99.51251	98.93545
SD			0.30		1.71763	0.956305

Table 7: Optical regression characteristics and Validation parameters

Parameter	AMOX		PROB	
	At 228	At 237	At 228	At 237
Calibration range (µg/ml)	2-14	2-14	2-14	2-14
Regression Equation	y = 0.050x + 0.031	y = 0.040x + 0.020	y = 0.052x + 0.043	y = 0.059x - 0.028
Slope (m)	0.050	0.040	0.052	0.059
Intercept (c)	0.031	0.020	0.043	0.028
Correlation co-efficient (r)	R^2 = 0.9971	R^2 = 0.998	R^2 = 0.9968	R^2 = 0.9968
Intraday (%RSD, n=3)	0.641	0.621	0.469	0.455
Interday (%RSD, n=3)	0.733	0.901	0.685	0.782
Detection limit (µg/ml)	0.0201	0.1842	0.02204	0.1384
Quantitation limit (µg/ml)	0.016	0.5610	0.126	0.41863

4. CONCLUSION

In this proposed method, the linearity is observed in the concentration range of 2 – 14 µg/ml with co-efficient of correlation, (r2) = 0.997 and (r2) = 0.9968 for AMOXICILLIN and PROB, respectively at 228 and (r2) = 0.998 at 237 nm and and (r2) = 0.9971 at 237 nm for AMOX and PROB.

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the AMOX and PROB in combined dosage form without any interference of the excipients.

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