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# A NOVEL ISOCRATIC RP-HPLC FOR SIMULTANEOUS MULTICOMPONENT ANALYSIS OF AMOXICILLIN AND PROBENECID IN PHARMACEUTICAL FORMULATION

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**Keywords:**

HPLC, Amoxicillin, Probenecid

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## ABSTRACT

A rapid, precise, selective and sensitive RP-HPLC method for simultaneous determination of Amoxicillin (AM) and Probenecid (PR) in pharmaceutical dosage form was developed and validated in the present work. The chromatographic separation was performed by using 250×4.6mm, 5µm $C_{18}$(Hyperchrome ODS-BP) with Buffer (0.05 M Potassium dihydrogen phosphate): Methanol (30:70 v/v), at a flow rate of 1.0 ml/min. The effluent was monitored using a UV detector set at 237 nm. The retention time for Amoxicillin and Probenecid were observed at 5.433 and 3.190 min. Linearity for Amoxicillin and Probenecid were in the range of 5-15 µg/ml and 5-15 µg/ml respectively. Percent recovery was 99.93% and 99.81% for Amoxicillin and Probenecid respectively. The proposed method can be applied for the routine analysis of Amoxicillin and Probenecid in combination.

## INTRODUCTION

### Gonorrhea: [1]

Gonorrhea primarily affects the epithelium and mucous membranes of the lower genital tract; however, it may also involve the eyes, oropharynx, and anus. Humans appear to be the only natural host for this intracellular pathogen. Transmission of gonorrhea is almost always by sexual intercourse, although perinatal transmission of the disease may occur. Once the gonococci are attached to cell membranes by pili, one of the primary virulence factors, they are pinocytosed. Subsequently, polymorphonuclear leukocytes invade the tissue, leading to secretion of purulent exudates. The rate of male to female transmission of gonorrhea during sexual intercourse is reported to be higher than female to male transmission, probably because the cervix is a more accessible target.

### Amoxicillin [2-5]

This comes under category of ANTIBACTERIAL.

Amoxicillin(2*S*,5*R*,6*R*)-6-{[(2*R*)-2-amino-2-(4-hydroxyphenyl)-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid) interfere with the synthesis of the bacterial cell wall peptidoglycan. After attachment to binding sites on bacteria they inhibit transpeptidation enzyme that cross-link the peptide chains attached to the backbone of the peptidoglycan. At final inactivation of an inhibition of the autolytic enzyme in cell wall, this lead to lysis of bacterium.

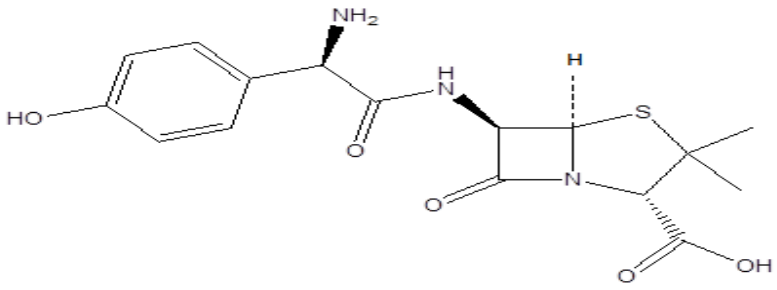



**Figure 1 : Chemical structure of Amoxicillin**

**Probenecid** (4-(dipropylsulfamoyl)benzoic acid) comes under category of ANTIGOUT.

Gout is associated with high level of the serum uric acid and poorly soluble substance that is major end product of purine metabolism. Uric acid is freely filtered at glomerulus .Uric acid is then completely reabsorbed in proximal convoluted tubule. Probenecid is highly lipid soluble organic acid.

Probenecid have uricosuric property which enhance the excretion of uric acid, thereby reducing the body pool of urate in patients suffering from Gout attacks.

It is competitively blocks active transport of organic acid by OATP (Organic anion transporting polypeptide) at all sites**.**

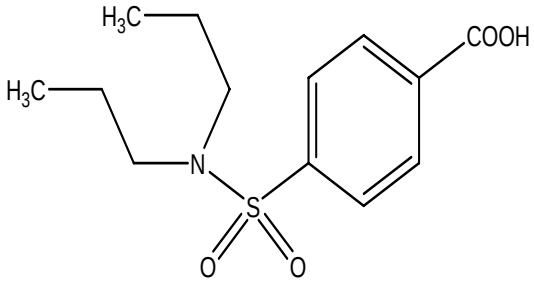



**Figure 2: Structure of Probenecid**

Both Amoxicillin and Probenecid have been analyzed by various techniques either alone or in combination with other drugs. Several methods have been applied to determine Amoxicillin involving UV Method [6-10], RP-HPLC [11-14], LC-MS/MS [15], HPTLC[16].

Literature review revealed different analytical methods such as Spectrophotometric method [17-19], HPLC [20-22] for the quantitative determination of Probenecid.

**EXPERIMENTAL**

**Standards and reagents**

Amoxicillin (AM) and Probenecid (PR) was provided by Altra laboratory, Ahmedabad,wasused as a working standard. Methanol, HPLC grade, Potassium dihydrogen phosphate and phosphoric acid were obtained from Chemdyes Corporation, Rajkot.

**Instrumentation**

The HPLC system wasS 1122 Analytical Technologies,UV detector. The mobile phase contained 0.05M potassium dihydrogen orthophosphate – acetonitrile (30:70) and flow rate was maintained at 1.0 ml/min and monitored at 237 nm. Chromatographic separations were performed at ambient temperature on250×4.6mm, 5µm C18(Hyperchrome ODS-BP) , and the injection volume was 20 µl.

**Standard stock solution**

About 10 mg of each reference standard PR and AM was weighed accurately and transferred to 100 ml volumetric flask. Both drugs were dissolved in 25 ml methanol, sonicated for 15 min and volume was made up to the mark with methanol to obtain 100µg/ml of PR and AM respectively.Further pipette out 1 ml of this solution transferred in to 100 ml volumetric flask and diluted up to 100 ml with mobile phase to get the concentration 10 µg/ml of PR and AM.

**Preparation of sample solution of PR and AM**

Weigh and powder 20 tablets. Weigh accurately a quantity of powder containing about 100mg of Amoxicillin and Probenecid transferred in to 100ml volumetric flask. Add 60 ml of Methanol and sonicated for 15 min make up to 100ml with HPLC grade Methanol and filtered through Whatman filter paper no.41 and first few drops of filtrate was discarded.Thisproduce 1000µg/ml solution of PR and AM. From this filtrate take 1 ml of solution and make up to 10 ml with HPLC grade Methanol to obtain (100µg/ml) of PB and AM. From above solution pipette out 1.0ml of solution,transferred in 10ml volumetric flask and make up to 10 ml with mobile phase to get 10µg/ml of PR and AM.

**Method validation**

As per ICH guidelines Q2R1, the method validation parameters studied were linearity, range, accuracy, precision, limit of detection and limit of quantification.

**Linearity and Range**

Mixed working standard solutions (0.5, 0.75, 1, 1.25 and 1.5 ml equivalent to 5, 7.5, 10, 12.5 and 15µg/ml of PR and to 5, 7.5, 10, 12.5 and 15µg/ml AM) were transferred in a series of 10 ml volumetric flasks and diluted to the mark with mobile phase.

An aliquot (20µL) of each solution was injected under the operating chromatographic conditions as described earlier. Chromatograms were recorded.

Calibration curves were constructed by plotting peak areas versus concentrations and the regression equations were calculated. Each response was average of five determinations.

Range**:** Range is the interval between upper and lower concentration of analyte in sample for which it has been demonstrated that the analytical method has suitable level of precision accuracy and linearity.

The linear response was observed over a range of 5-15µg/ml for PR and AM.

**Accuracy (% Recovery)**

It was determined by calculating the recovery of PR and AM from formulation by standard addition method. To a fixed amount of test 80%, 100% and 120% amount of standard was added and the amount of standard added was calculated using regression equation.

Known amount of standard solutions of PR (4, 5 and 6 µg/ml) and AM (4, 5 and 6µg/ml) were added to a pre-quantified sample solution of PR and AM (5 and 5 µg/ml, respectively). Each solution was injected in triplicate and the percentage recovery was calculated by measuring the responses and fitting these values into the regression equations of the respective calibration curves**.**

**Precision**

Repeatability: The repeatability of the method was determined by measuring the responses 6 times for 100% concentration of PR (10 µg/ml) and AM (10 µg/ml) each**.** Intraday: Intraday precision of the method was determined by measuring the responses 3 times on the same day at interval of 1 hr for 3 different concentration of PR (5,10 and 15 µg/ml) and AM (5,10 and 15µg/ml) each. The results were reported in terms of relative standard deviation**.** Inter-day: Inter-day precisions of the method was determined by measuring the responses 3 times on 3 different consecutive days for 3 different concentration of PR (5,10 and 15 µg/ml) and AM (5,10 and 15 µg/ml) each. The results were reported in terms of relative standard deviation.

**Limit of Detection (LOD)**

The LOD is estimated from the set of 5 calibration curves used to determine method linearity.
The LOD may be calculated as,
LOD = 3.3 x (SD / Slope)
Where, SD = Standard deviation of Y- intercept of 5 calibration curves.
Slope = Mean slope of the 5 calibration curves.

**Limit of Quantification (LOQ)**

The LOQ is estimated from the set of 5 calibration curves used to determine method linearity.
The LOQ may be calculated as,
LOD = 10 x (SD / Slope)
Where, SD = the standard deviation of Y- intercept of 5 calibration curves.
Slope = the mean slope of the 5 calibration curves.

**Robustness:**

This parameter was performed by taking AM (10µg/ml) and PB (10µg/ml) by changing pH (±0.2), Mobile phase (±2ml), and Flow Rate(±2ml/min) by measuring the corresponding responses 3 times. %RSD for area was calculated which should be less than 2%.

**RESULTS**

**Selection of Wave length**

Both the components show reasonably good response at 237 nm.

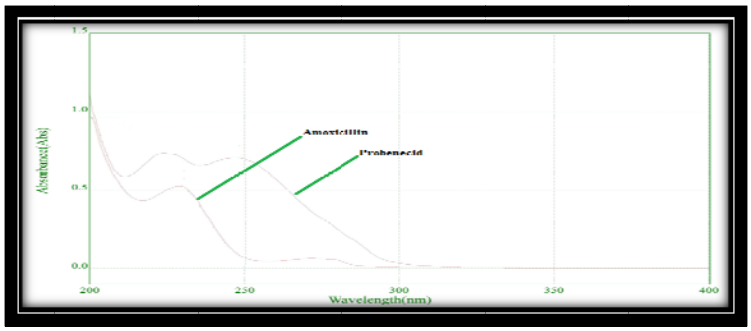



**Figure: 3 UV spectra of standard PR (10µg/ml) and standard AM (10µg/ml)**

**Table 1: System suitability parameters**

| SYSTEM SUITABILITY PARAMETERS | DRUG | |
|---|---|---|
| | Ketorolac | Fluorometholone |
| **Retention time (min )** | 3.173 ± 0.00286 | 5.364 ± 0.0041 |
| **Tailing factor (T)** | 1.545 ± 0.007 | 1.370 ± 0.0308 |
| **Number of theoretical plates (N)** | 5579 ± 9.791 | 7194± 144.692 |
| **Resolution ( R )** | 10.360 | |

**Linearity:**

Linearity of AM and PR were found to be less than 2% RSD.

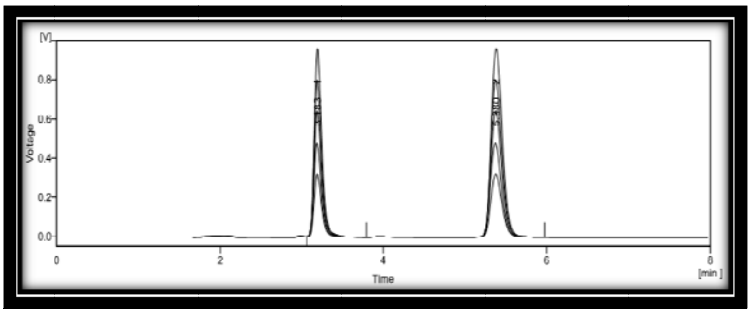
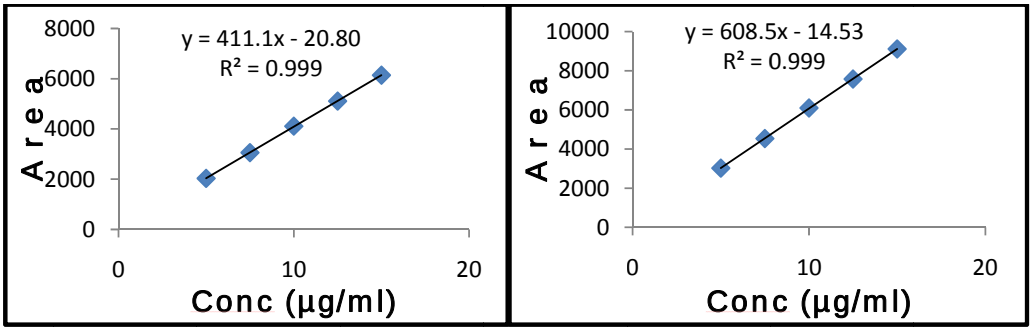



**Figure: 4 Overlain chromatogram of PR (5.0-15 µg/ml )& AM (5-15 µg/ml )**




**(A)** **(B)**

**Figure: 5 (A) Calibration plot of PR, (B) Calibration plot of AM**

**Table 2: Lenearity for PR (5-15µg/ml) and AM (5-15µg/ml)**

| Conc. (µg/ml) | | Mean Area ± SD | | % RSD | |
|---|---|---|---|---|---|
| **PR** | **AM** | **PR** | **AM** | **PR** | **AM** |
| 5 | 5 | 2031.143 ± 3.904 | 3026.667 ± 2.577 | 0.91 | 0.085 |
| 7.5 | 7.5 | 3056.177 ± 1.390 | 4536.782 ± 2.939 | 0.0454 | 0.0647 |
| 10 | 10 | 4111.003 ± 5.009 | 6099.036 ± 4.096 | 0.121 | 0.0671 |
| 12.5 | 12.5 | 5111.53 ± 1.186 | 7578.116 ± 3.509 | 0.232 | 0.0463 |
| 15 | 15 | 6142.581 ± 1.870 | 9112.429 ± 2.132 | 0.0304 | 0.023 |

**Accuracy**

80%, 100% and 120% selected for accuracy.**(Table 3, 4)**

**Table 3: Accuracy for PR**

| AMOUNT OF PR (µg/ml) | % OF STD PR ADDED | TOTAL AMOUNT ADDED | AMOUNT FOUND (µg/ml) | % RECOVERY | % RECOVERY (MEAN ± SD) | %RSD |
|---|---|---|---|---|---|---|
| 5 | 80 | 9 | 8.97 | 99.75 | 99.86 ± 0.9788 | 0.98 |
| | | | 8.88 | 98.72 | | |
| | | | 9.1 | 101.11 | | |
| | 100 | 10 | 10.16 | 101.68 | 100.57 ± 0.8424 | 0.83 |
| | | | 10.03 | 100.39 | | |
| | | | 9.96 | 99.64 | | |
| | 120 | 11 | 11.03 | 100.31 | 99.386 ± 0.7110 | 0.71 |
| | | | 10.92 | 99.27 | | |
| | | | 10.84 | 98.58 | | |

**Table 4: Accuracy for AM**

| AMOUNT OF AM (µg/ml) | % OF STD AM ADDED | TOTAL AMOUNT ADDED | AMOUNT FOUND (µg/ml) | % RECOVERY | % RECOVERY (MEAN ± SD) | %RSD |
|---|---|---|---|---|---|---|
| 5 | 80 | 9 | 8.95 | 99.58 | 99.63 ± 0.8134 | 0.81 |
| | | | 8.87 | 98.67 | | |
| | | | 9.05 | 100.66 | | |
| | 100 | 10 | 10.03 | 100.32 | 100.22 ± 1.0595 | 1.05 |
| | | | 10.14 | 101.47 | | |
| | | | 9.89 | 98.88 | | |
| | 120 | 11 | 11.14 | 101.33 | 99.59 ± 1.3183 | 1.32 |
| | | | 10.92 | 99.30 | | |
| | | | 10.79 | 98.14 | | |

**Table 5: Summary of validation parameter**

| PARAMETER | | Probenecid | Amoxicillin |
|---|---|---|---|
| Linearity(n=5) | | 0.72-1.00 | 075-1.09 |
| Precision (% RSD) | | | |
| Intraday (n=3) | | 0.81-0.98 | 0.76-0.82 |
| Interday (n=3) | | 0.98-1.23 | 0.82-1.03 |
| Repeatability (n=6) | | 0.94 | 0.79 |
| Accuracy (%RSD) | | 0.71-0.98 | 0.81-1.32 |
| Robustness (%RSD) | Flow rate | 0.57 | 0.34 |
| | pH | 0.67 | 0.43 |
| | Mobile phase | 0.61 | 0.40 |
| LOD (µg/ml) | | 0.1188 | 0.0733 |
| LOQ (µg/ml) | | 0.3602 | 0.222 |

**Assay**

The amount of AM and PR was found to be 99.44 and 98.933%.**(Table 8)**

**Table 6: Results from analysis of Amoxicillin and Probenecid in the combined dosage form**

| Formulation | Label cliam (mg) | | Amount found | | % of label claim RSD | |
|---|---|---|---|---|---|---|
| | PR | AM | PR | AM | PR | AM |
| **Moxilong** | 10 | 10 | 10.11 | 10.10 | 100.26 ± 0.63 | 100.04± 0.61 |

**DISSCUSION**

The newly developed method for the simultaneous estimation of Amoxicillin and Probenecid was found to be precise and accurate with low values of coefficient of variation(Table No.1 & 5). Hence it can be conveniently adopted for the routine quality control analysis.

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**REFERENCES**

1. Heafindal ET, QuanDJ(2006) Textbook of Therapeutics. Lippincott Williams and Wilkins
2. Barar FSK Essentials of Pharmacotherapeutics. S chand and company Limited, Ram Nagar, New Delhi
3. Rang HP, Dale (2003) Pharmacology. Churchill Livingstone, Elsevier Science Limited
4. Satoskar RS,Bhandarkar SD, Nirmala NR(2005) Pharmacology and pharmacotherapeutics. Popular Prakashan Private Limited, Mumbai
5. Seth SD, Seth V Pharmacology. Elsevier India private Limited, New Delhi
6. Patel SA, Patel NJ(2011)Int Res J Pharm 9: 48-51
7. Dhoka MV,Gawande VT, Joshi PP(2011)Int Res J Pharm 2(3):197-201
8. Jain AK, Lariya N, Agarwal A, Kharya AK, Agrawal GP(2010)Int J Chem Res1(1): 41-45
9. Jambulingam M, Aswini M, Bittu PK, BarakathRifaya AH, Saketh N, Kamlakannan D, Ananda TS(2013)Pharm Ana Qua Ass1
10. Gupta YA, Rawat VS(2011)Drug Invention Today3(4):33-34
11. Brooks MA, Hackman MR, Mazzo DJ(1981)J Chroma210: 531-535
12. Matar KM(2006)Chromatographia64(5-6): 225-260
13. Tavakoli N(2007)J Pharma Biomed Ana43: 325–329
14. Liu C, Wang H, Jiang Y, Du Z(2011)J Chroma 879: 533–540
15. PingaleSG,Badgujar LM, Mangaon KV, Mastorakis NE(2009) Wseas Transactions on Biology and Biomedicine9: 1-13

16. Chitlange SS, Tawargeri SR, BholeRP(2013)PharmaAnaActa4: 1-5
17. TillsonEK,Schuchardt GS, Fishman JK, Beyer KH(1954) PharmaAnaActa111: 385-394
18. Chaudhari SV, Karnik A, Adhikary A,Tandale RS,VaviaPR (2006)PharmaSci Div68: 53-63
19. Kurian T, Kurien J(2011)H.J.D.Med3(2): 57-61.
20. Falco PC,Herraez-Hernandez R, Sevillano-Cabeza A(1993)Chromatographia35: 317-320
21. Kurian T, Kurien J, William H, Bijumol C(2012)H J D Med2: 65-69
22. ShindeVM, Desai BS, Tendolkar NM(1994) Ind JPharmaSci56: 58-60.