**Hygeia:: journal for drugs and medicines**
October2012-March 2013
OPEN ACCESS
A half yearly scientific, international, open access journal for drugs and medicines
Research article section: Pharmaceutical Analysis

# Simultaneous Multicomponent Analysis of Ampicillin and Probenecid in Pharmaceutical formulation by Reverse Phase High Performance Liquid Chromatography

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*Article history: Received: 10 June 2012, revised: 14July, 2012, accepted:5 August 2012, Available online:10 October 2012*

## Abstract:

***Plan:*** *Reverse Phase High Performance Liquid Chromatographic method for Ampicillin and Probenecid described*
***Prologue:*** *Ampicillin trihydrate is an antibiotic active against mainly gram positive bacteria and some gram negative bacteria. Probenecid is a uricosuric agent used in gout therapy. The present study aims at the development of Reverse phase High Performance Liquid Chromatography method for simultaneous estimation of Ampicillin and Probenecid in combined dosage formulations.*
***Methodology:*** *The HPLC system used consists of Waters 501 pump and a Waters 486 tunable wave length UV-visible detector. The data station was computer controlled using base line 810 software which includes an integrator and a recorder. The mobile phase used was sodium acetate solution (50mm): acetonitrile (75:25). The UV response was measured at 235nm. The column used was Reverse phase(5 micron) 25cm x4.6mm.*
***Outcome:*** *The method provided adequate accuracy and precision .The developed RP-HPLC method can be used for the routine analysis of Ampicillin and Probenecid in combination.*
***Keywords:*** *Ampicillin, Probenecid, Reverse phase, High performance liquid chromatography.*

## 1. Introduction:

Ampicillin trihydrate[1] 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. Influenza. It is used in urinary tract infections and respiratory tract infections. It is also used in meningitis, biliary tract infections etc. Ampicillin[2] 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[3,4] in cold water (1 in 50). It is insoluble in alcohol and acetone. It is soluble in dilute solutions of acids and alkali hydroxides.

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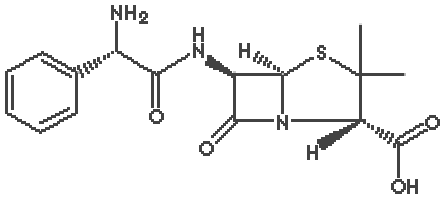
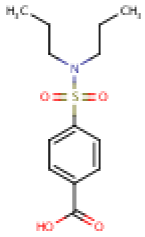
Rid.G-9465-2012
*Hygeia.J.D.Med. Vol.4 (2), Oct. 2012*

Probenecid[1]is a uricosuric 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. Chemically Probenecid[2] is 4-[(Dipropyl-amino) Sulphonyl] benzoic acid. Probenecid is soluble[3,4] in alcohol (1in25), acetone (1 in 12) and insoluble in water.




.3$H_2O$



*Ampicillin tri hydrate* *Probenecid*

Literature survey revealed that for Ampicillin and Probenecid combination, H.P.L.C methods are available for the determination in biological fluids[5,6], spectrophotometric method[7] and colorimetric methods[8,9] are reported for their determination in dosage forms. The United States Pharmacopeial method[10] involves iodometric method for Ampicillin and HPLC method for Probenecid. The present study aims at the development of simple, rapid, accurate and sensitive method for simultaneous estimation of Ampicillin and Probenecid in combined dosage formulations by reverse phase High Performance Liquid Chromatography method,

## 2. Experimental

Pure samples of Ampicillin trihydrate (99.54%W/W) was obtained as a gift sample GSK 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. Acetonitrile HPLC grade, Sodium acetate solution 50mM, Water HPLC grade from MilliQ system.

### *2.1. Chromatographic System*

The HPLC system used consisted of Waters 501 pump and Waters-406 tunable wavelength UV-Visible detector. The data station was computer controlled using Baseline 810 software, which includes an integrator and a recorder. The injector was Rheodyne 7125 with 20 micro liter fixed volume loop type. The mobile phase used was Sodium acetate solution 50mM: Acetonitrile (75:25).The mobile phase was filtered through 0.45µ Nylon66 membrane. Flow rate was kept 1ml/minute with an average operating pressure of 3000 psi and UV response was monitored at 235nm. The column used was Reverse phase(5 micron) 25cm x 4.6mm.

*2.2. Method development*

50mg each of Ampicillin and Probenecid was weighed separately and transferred to 100ml standard flask and was dissolved using Acetonitrile-Water ( 1:1) and was made up to mark. Further dilutions were made from the above solutions to obtain a concentration of 25 µg/ml of Ampicillin25 µg/ml of Probenecid. An aliquot of standard solution containing 25 µg/ml of Ampicillin25 µg/ml of Probenecid was injected into the column and the chromatogram was recorded (Fig 1.)

*2.3. Analysis of formulation*

Twenty tablets each containing 250mg of Ampicillin and Probenecid was 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 transferred to 100 ml volumetric flask dissolved using Acetonitrile-Water (1:1) and was made up to mark, filtered through whatmann filter paper. The solution was further diluted to get 25µg/ml of Ampicillin and 25µg/ml of Probenecid. An aliquot of the sample solution was injected into the column and the chromatogram was recorded (figure 2). The area of the curves of standard and samples were compared and and the concentration of drugs and the amount of Ampicillin and Probenecid was calculated. Results are shown in Table 1.

*2.4. 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 25µg/ml of Ampicillin and 25µg/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.1. The chromatogram of recovery study was given in figure 3**.**

Table 1. Data for analysis of formulations of Ampicillin and Probenecid

| *Drug* | *Concentration mcg/ml* | *Amount (mg/tablet)** | | | |
|---|---|---|---|---|---|
| | | *Labelled* | *Found Mean* | *% label Claim* | *% Recovery* |
| Ampicillin | 24.87 | 250 | 248.7 | 99.2 | 98.82 |
| Probenecid | 25.09 | 250 | 250.9 | 100.36 | 100.7 |

## 3. Results and Discussion

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.

## 4. Conclusion

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

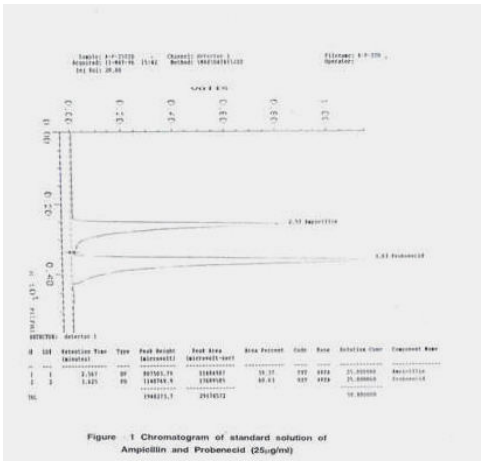


Figure 1 Chromatogram of standard solution of Ampicillin and Probenecid (25µg/ml)

Fig.1 Chromatogram of the standard solution of Ampicillin and Probenecid (25mcg/ml)

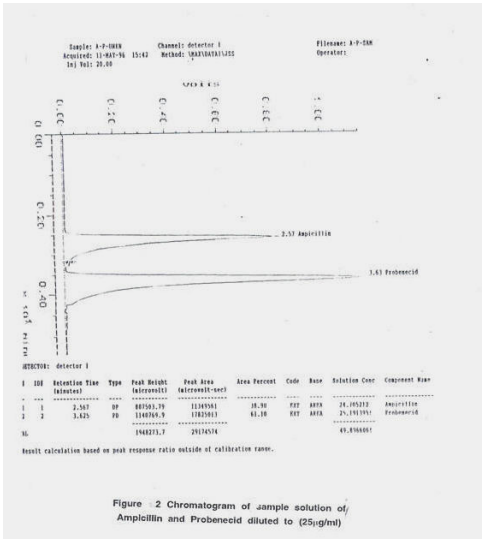


Figure 2 Chromatogram of sample solution of Ampicillin and Probenecid diluted to (25µg/ml)

Fig.2 Chromatogram of the Sample solutions of Ampicillin and Probenecid (25mcg/ml)

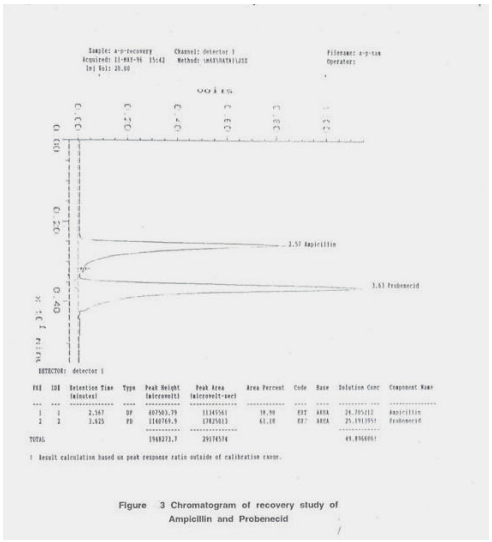


Figure 3 Chromatogram of recovery study of Ampicillin and Probenecid

Fig.3. Chromatogram of the recovery study of Ampicillin and Probenecid

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