ABSTRACT
A simple and precise, reliable, rapid, reproducible reverse phase HPLC method has been developed and validated for simultaneous estimation of Probenecid & Cefadroxil chromatography was carried out on zorbaxC18 (4.6*150mm, 5um) column using mixture of ACN: WATER (55:45v/v) as the mobile phase at flow rate of 1.0ml for min the detection wavelength carried out at255nm the retention time of probenecid & cefadroxil was 2.061&2.462respectively. The method produce linear response in the concentration range of 10-50µg/ml of probencid&5-25µg/ml of cefadroxil the method precision for the determination of assay was below 2.0%RSD. The proposed method can be useful in quality control of bulk& pharmaceutical dosage form

Keywords: Probenecid, Cefadroxli, Rp-hplc validation

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INTRODUCTION

Probenecid:
4-(dipropylsulfamoyl)benzoic acid is uricosuric Drug that increasing uric acid excretion in the urine. it is uricosuric Drug that increasing uric acid excretion in the urine. it is primarily used in treating gout and hyperuricemia it inhibits the renal excretion of organic anions and reduces tubular reabsorption of urate, probencid has also been used to treat patients with renal impairment and because it reduces the renal tubular excretion of other Drugs. Mechanism of action: Probenecid inhibits the tubular reabsorption of urate, thus increasing the urinary excretion of uric acid and decreasing serum urate levels. Probenecid may also reduce plasma binding of urate and inhibit renal secretion of uric acid at sub therapeutic concentrations. The mechanism by which probenecid inhibits renal tubular transport is not known, but the Drug may inhibit transport enzymes that require a source of high energy phosphate bonds and/or non-specifically interfere with substrate access to protein receptor sites on the kidney tubules.

Cefadroxil:
(6R, 7R)-7-[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid[1],is first generation antibiotic, effective in gram positive and gram negative bacterial infections. It is a bacterial antibiotic it is used to treat urinary tract infections, skin and pharyngitis and tonsillitis. Mechanism of action: Cefadroxil binds to specific penicillin –binding proteins located inside the bacterial cell wall causing 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 Cefadroxil interferes with an autolysin inhibitor. Cefadroxil can be used for treating infected wounds on animals. Cefadroxil and probenecid are official in IP [2], BP [3], and USP [4].

MATERIALS AND METHOD

Chemicals and reagents:
Probenecide and cefadroxil were obtained as gift samples from SURA labs pvt. Ltd, Hyderabad. We used HPLC grade, Acetonitril, water.
**Instrumentation:**
Method development and validation carried out using a water alliance 2695 quaternary pump hplc equipped with waters 996 PDA detector. Zorbax C18 (4.6*150mm, 5µ) waters empower 2softwere was used for processing data.

**Preparation of mobile phase:**
Accurately measured 550ml of ACN and 450ml of water (55:45%) v/v. were mixed and degassed in digital ultra sonicator for 10min and then filtered through 0.45µ filter under vacuum filtration.

**Diluent Preparation:** The Mobil phase was used as the diluents.

**Preparation of Standard Solution:**
Accurately weigh and transfer 10mg of Probenecid and 10mg of Cefadroxil working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the mark with the same solvent. Further pipette out 0.3ml of Probenecid and 0.15ml of Cefadroxil from above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

**Preparation of Sample Solution:**
Accurately weighed amount of powder equivalent to 10mg of Probenecid and Cefadroxil sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.3ml of Probenecid and 0.15ml of Cefadroxil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

\[
\% \text{ASSAY} = \frac{\text{Sample Area}}{\text{Standard Area}} \times \frac{\text{Weight of Standard}}{\text{Dilution of Standard}} \times \frac{\text{Weight of Tablet}}{100} \times \frac{\text{Weight of Sample}}{\text{Purity}} \times \frac{\text{Dilution of Sample}}{\text{Purity}} \times \frac{\text{Label Claim}}{100}
\]

**Method Development:**
The optimized chromatographic conditions (fig-1) the best peak shape and maximum separation was achieved with mobile phase composition of Acetonitril: Water (55:45) using, peak symmetry and reproducibility wear obtained on Zorbox C18 (4.6*150mm, 5µ) column. The optimum wavelength for detecting the analysts was found to be 255nm, a flow rate of 1ml/min yielded optimum separation and peak symmetry. The optimized chromatographic conditions were shown Table.1
To saturate the column, the mobile phase was pumped for about 30 minutes thereby to get the base line corrected. The separate standard calibration lines were constructed for each Drug. A series of aliquots were prepared from the above stock solutions using diluents to get the concentrations 10-50 µg/ml for Probenecid and 5-25 µg/ml Cefadroxil each concentration 6 times was injected in to chromatographic system. Each time peak area and retention time were recorded separately for all the Drugs. Calibration curves were constructed as by taking average peak area on Y-axis and concentration on X-axis separately for both Drugs. From the calibration curves regression equations were calculated, these regression equations were used to calculate Drug content in formulation.

![Auto-Scaled Chromatogram](image)

**Figure 3: Optimized Chromatogram of Probenecid & Cefadroxil**

**Table 1: Optimized Chromatographic conditions**

| Column       | Zorbax C18(4.6*150mm, 5µ) |
|--------------|---------------------------|
| Mobile phase | Acetonitril: Water (55:45) |
| Flow rate    | 1ml/min                   |
| Column temp. | Ambient                   |
| Injection vol.| 10µl                      |
| Detection wavelength | 255nm            |
| Run time     | 8 mins                    |
| Retention time | 2.061, 2.462              |
| Remarks      | This method is suitable for validation |

**Analytical Method Validation:**

**Specificity:**

The specificity of developed method was examined by injecting solutions of standard, sample & placebo separately. The absence of interfering peak of additives in a pharmaceuticals dosage form at the retention time of probenecid and cefadroxil proved the specificity of method.

**Linearity:**

Linearity was evaluated by analyzing a series of varies concentrations of probenecid and cefadroxil six concentrations (10, 20, 30, 40, 50µg/ml) of Probenecid and (5, 10, 15, 20, 25µg/ml) of Cefadroxil.
were injected in triplicate linear response were obtained between the concentration of the analyte and the peak areas, which was confirmed by a high correlation coefficient (r²=0.999)

**Precision:**

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. It was verified by five replicate injections of standard solution containing probenecide & cefadroxil. The method precision was carried out the analyte five times using the proposed method. Repeatability was measured by multiple injections of homogeneous sample of probenecid & cefadroxil.

**Accuracy:**

Accuracy was carried out by % recovery studies at three different concentrations levels. To the pre analysed sample solution of probenecid and cefadroxil a known amount of standard Drug powder of Probenecid and Cefadroxil were added at 50%, 100%, 150% levels.

**Robustness:**

Robustness was evaluated by making deliberate variations in method parameters such as variation of wavelength, flow rate, and change in mobile phase composition. The Robustness of the method was studied for probenecid and cefadroxil.

**Limit of detection and Limit of quantitation:**

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 × ASD/S and LOQ = 10 × ASD/S, Where, 'ASD' is the average standard deviation and 'S' is the slope of the line.

**RESULTS AND DISCUSSION:**

![Graph showing the linear relationship between concentration and area](image-url)
Figure 4: Linearity Graphs of Probenecid and Cefadroxil

Table 2: Linearity results of Probenecid and Cefadroxil

| S.No. | Concentration Of Probenecid | Concentration of Cefadroxil | Area of Probenecid | Area of Cefadroxil |
|-------|-----------------------------|----------------------------|-------------------|-------------------|
| 1     | 10                          | 05                         | 88442             | 1131032           |
| 2     | 20                          | 10                         | 165724            | 2345302           |
| 3     | 30                          | 15                         | 242754            | 3355252           |
| 4     | 40                          | 20                         | 315906            | 4429382           |
| 5     | 50                          | 25                         | 396371            | 5623754           |

Correlation Coefficient

0.999

Table 3: Repeatability for Probenecid and Cefadroxil

| S.No. | Injections  | Area of Probenecid | Area of Cefadroxil |
|-------|-------------|--------------------|--------------------|
| 1     | Injection-1 | 249684             | 3233700            |
| 2     | Injection-2 | 249696             | 3241323            |
| 3     | Injection-3 | 246325             | 3245927            |
| 4     | Injection-4 | 249816             | 3245927            |
| 5     | Injection-5 | 249892             | 3222194            |
| Mean  | -           | 249082.6           | 3237814            |
| Std. Dev | -         | 1543.96           | 10060.62           |
| %RSD  | -           | 0.6198             | 0.3107             |

Table 4: Intermediate Precision Result for Probenecid and Cefadroxil

| S.no  | Injections  | Area of Probenecid | Area of Cefadroxil |
|-------|-------------|--------------------|--------------------|
| 1     | Injection-1 | 242721             | 325309             |
| 2     | Injection-2 | 240155             | 3323780            |
| 3     | Injection-3 | 240945             | 3328190            |
| 4     | Injection-4 | 240385             | 3329035            |
| 5     | Injection-5 | 249920             | 3325968            |
| 6     | Injection-6 | 240820             | 3327725            |
| Mean  | -           | 343991             | 3326668            |
| Std.Dev | -         | 4641.97            | 1985.64            |
| %RSD  | -           | 1.5                | 0.05969            |
Table 5: Accuracy result for Probenecid and Cefadroxil

| Drug Name | Spike Level | Area (µg/ml) | Amount added(µg/ml) | Amount found(µg/ml) | % Recovery | Mean Recovery (%) |
|-----------|-------------|--------------|---------------------|--------------------|------------|-------------------|
| Probenecid | 50%         | 124675.6     | 15                  | 15.1               | 101%       | 100.4%            |
|           | 100%        | 242006.3     | 30                  | 30.1               | 100.5%     |                   |
|           | 150%        | 354115.6     | 45                  | 44.9               | 99.7%      |                   |
| Cefadroxil | 50%         | 1696259      | 18.75               | 18.71              | 99.8%      | 99.2%             |
|           | 100%        | 3351661      | 37.5                | 37.2               | 99.4%      |                   |
|           | 150%        | 4975094      | 56.25               | 55.47              | 98.6%      |                   |

Table 6: Result for LOD & LOQ

| S. No | Drug Name | Std. Dev | Slope | Lod | Loq |
|-------|-----------|----------|-------|-----|-----|
| 1     | Probenecid| 1760.8   | 78322 | 0.07| 0.2 |
| 2     | Cefadroxil| 61155    | 11150 | 18.0| 54.8|

CONCLUSION:

The Proposed RP-HPLC method Developed and Validated for Probenecid and Cefadroxil in pharmaceutical dosage form and assured the satisfactory precision, accuracy and also determining lower concentration of Drug in its solid dosage form by hplc. The method was found to be simple, accurate, economical, and rapid they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials.

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