SIMULTANEOUS ESTIMATION OF NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CEFADROXIL AND PROBENECID BY RP-HPLC IN BULK AND MARKETED FORMULATION

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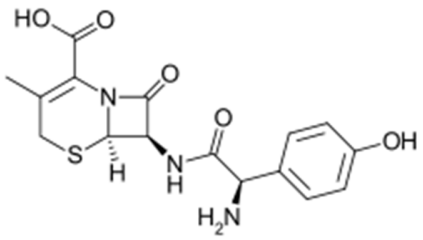
ABSTRACT

A rapid and accurate reverse phase high performance liquid chromatographic method has been developed for the validation of both Probenecid and Cefadroxil in tablet dosage form and in its pure form. Chromatography was performed on a Zorbax C18 (4.6 x 150mm, 5µm) column using a mixture of ACN: Water (55:45v/v) as the mobile phase at a flow rate of 1.0ml/min; the detection was performed at 255nm. Probenecid and Cefadroxil retention times were 2.061, 2.462 ±0.02min, respectively. The method produced linear responses in the concentration range of 10–50µg/ml of Probenecid and 5–25µg/ml of Cefadroxil. The method precision for the determination of assay was below 2.0%RSD.The method is useful in the quality control of bulk and pharmaceutical formulations..

Keywords: Probenecid, Cefadroxil, RP-HPLC, validation

INTRODUCTION

Drug : Cefadroxil
Synonym : Cefadroxil monohydrate, Cefadroxilum, D-Cefadroxil
Drug category: Anti-Bacterial Agent, Cephalosporins
Structure : :

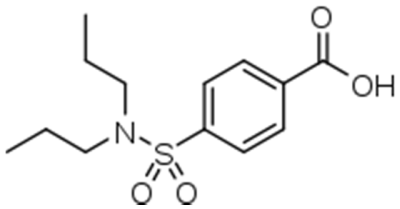


Chemical name/ UPAC Name: (6R,7R)-7-{[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
Molecular Formula : $C_{16}H_{17}N_3O_5S$
Molecular Weight : 363.389 g/mole
Official Pharmacopoeia : USP, BP, IP, EP

Drug : Probenecid

Synonym : Probenecida , Probenecidum , Probenecid acid

Drug category Antirheumatic Agents.
Structure : :



Chemical name/ Nomenclature / IUPAC Name : 4-(dipropylsulfamoyl)benzoic acid
Molecular Formula : C13H19NO4S

Molecular Weight : 285.36 gm/mole

Table: 1 INSTRUMENTS USED

S.No	Instruments And Glasswares	Model
1	HPLC	WATERS, Alliance 2695 separation module. Software: Empower 2,996 PDA detector.
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

7	Digital ultra sonicator	Labman

MATERIALS:

PURE SAMPLES: The pure standard samples of Probenecid and Cefadroxil were supplied as gift samples of Solitaire Pharma ltd

MARKETED SAMPLES: Distaclor -PD

SOLVENTS:: ACN : Water (55:45v/v)

All the reagents and chemicals used were of analytical grade and solvents were of spectroscopic grade.

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Probenecid and Cefadroxil working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol..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.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight 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

RESULTS AND DISCUSSIONS.

RP-HPLC Method Development Based on the drug solubility and pKa value the following chromatographic conditions have been selected to initiate the method development trials for determination of cefadroxil and probencid

Optimized Chromatogram (Standard)

Mobile phase : ACN: Water (55:45v/v)
Column : Zorbax C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 255 nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 8minutes

Fig No1:Optimized Chromatogram

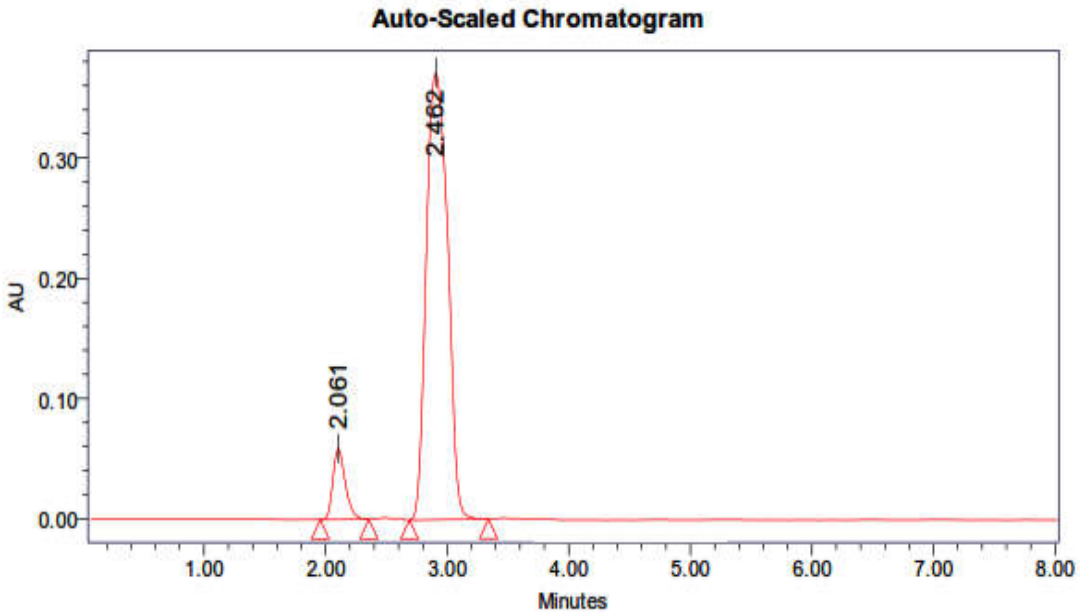


Table: 2- peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Probenecid	2.061	247392	58952	1.2	7243
2	Cefadroxil	2.462	3530866	371748	1.1	3389

METHOD VALIDATION:

The developed method was validated according to ICH Guideline Q2 (R1). The following parameters were evaluated
:

Specificity: It is the ability to assess the analyte unequivocally in the presence of other components which may be expected to be present. A blank (only diluentwithout drug) was injected into HPLC. No peaks were observed

Table:3 CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Probenecid:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	10	88442
66.6	20	165724
100	30	242754
133.3	40	315906
166.6	50	396371

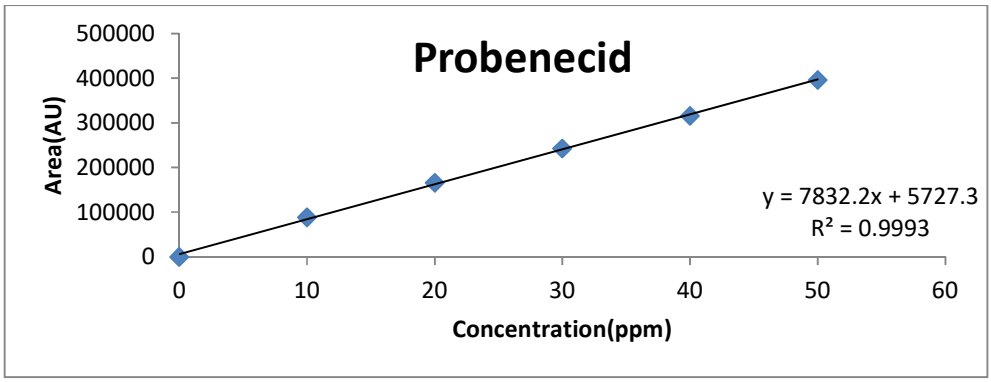


Fig No2: Calibration curve for Probeneci

Table: 4 CHROMATOGRAPHIC DATA FOR LINEARITY STUDY

Cefadroxi

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	5	1131032
66	10	2345302
100	15	3355282
133	20	4429382
166	25	5623754

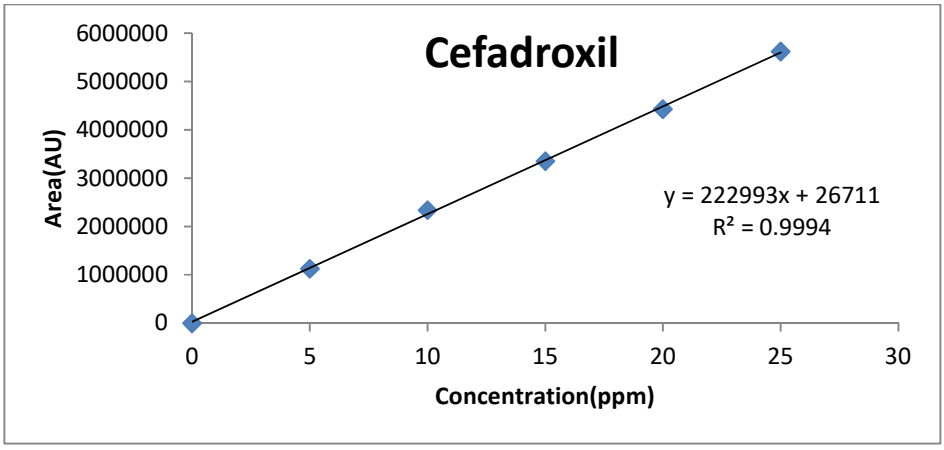


Fig No3: Calibration curve for Cefadroxil

Table:5 Results of Intermediate precision for Probenecid

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Probenecid	2.066	242721	11323	5272	1.21
2	Probenecid	2.066	240155	11564	5168	1.16
3	Probenecid	2.066	240945	11887	5310	1.14
4	Probenecid	2.065	240385	11938	5275	1.19
5	Probenecid	2.069	249920	11652	5078	1.10
6	Probenecid	2.067	240820	11750	5225	1.17
Mean	-	-	243991			
Std. Dev	-	-	4641.97			
RSD	-	-	1.5			

Table:6 Results of Intermediate precision for Cefadroxil

S no	Name	\ Rt	Area	Height	USP plate count	USP Tailing
1	Cefadroxil	2.477	3325309	54143	6149	1.25
2	Cefadroxil	2.478	3323780	53740	6127	1.21
3	Cefadroxil	2.483	3328190	54791	6607	1.28
4	Cefadroxil	2.486	3329035	55098	6769	1.28
5	Cefadroxil	2.489	3325968	52379	6709	1.30
6	Cefadroxil	2.483	3327725	54779	6756	1.36
Mean			3326668			
Std.Dev			1985.641			
% RSD			0.059689			

Table:7 The accuracy results for Probenecid

Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	124675.7	15	15.1	101%	100.4%
100%	242006.3	30	30.1	100.5%	
150%	357449	45	44.9	99.7%	

Table:8 The accuracy results for Cefadroxil

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
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%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Probenecid:

=3.3 × 1760.8/78322

=0.07µg/ml

Cefadroxil:

=3.3 × 61155/11150

=18.0µg/ml

LIMIT OF QUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Probenecid:

=10×1760.8/78322

= 0.2µg/ml

Cefadroxil:

=10 × 61155/11150

TABLE:9 RESULTS FOR ROBUSTNESS

PROBENECID:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	247392	2.061	7243	1.2
Less Flow rate of 0.9 mL/min	69214	2.267	4713	1.3
More Flow rate of 1.1 mL/min	388838	1.864	4740	1.2
Less organic phase	445628	2.165	4709	1.2
More organic phase	69404	1.967	5590	1.4

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

TABLE: 10 RESULTS FOR ROBUSTNESS

CEFADROXIL:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3530866	2.462	3389	1.1
Less Flow rate of 0.9 mL/min	527373	2.690	5275	1.0
More Flow rate of 1.1 mL/min	4363129	2.284	5611	1.0
Less organic phase	3965572	2.590	5550	1.0
More organic phase	527708	2.390	6273	1.0

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Probenecid and Cefadroxil in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Probenecid and Cefadroxil was freely soluble in ethanol, methanol and sparingly soluble in water. ACN: Water (55:45v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Probenecid and Cefadroxil in bulk drug and in Pharmaceutical dosage forms.

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