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RESEARCH ARTICLE

Development and validation of analytical method for simultaneous estimation of Cephalexin and Probenecid in API and marketed formulation by RP-HPLC

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Abstract
A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Probenecid and Cephalexin, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Zorbax C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Phosphate Buffer pH 3.9 (55:45v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 255nm. The retention time of the Probenecid and Cephalexin was 2.061, 2.462 ±0.02min respectively. The method produce linear responses in the concentration range of 1-5µg/ml of Probenecid and 100-500µg/ml of Cephalexin. 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, Cephalexin, RP-HPLC, validation.

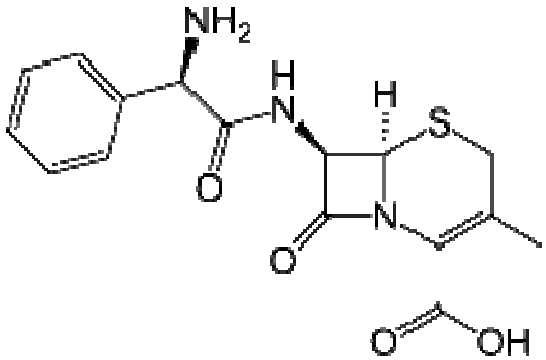
INTRODUCTION

DRUG PROFILE

Cephalexin It is chemically Céphalexine, Cepastar,Cefalexinum,Celexin,7-(D-alpha-Aminophenylacetamido)desacetoxycephalosporanic acid,

Drug category : Anti-Bacterial Agent, Cephalosporins

Structure :



Chemical name/ Nomenclature / IUPAC Name: (7R)-3-Methyl-7- (α- D -phenylglycylamino) -3-cephem-4-carboxylic acid monohydrate

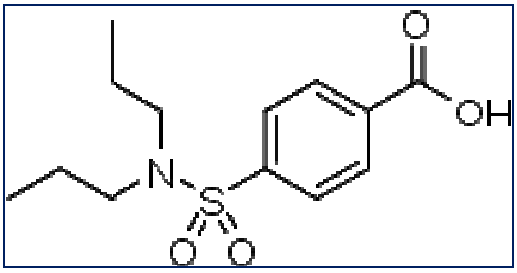
Molecular Formula : $C_{16}H_{17}N_3O_4S$

Molecular Weight : 347.39 g/mole.

Probenecid

Drug category : Antirheumatic Agents.

Structure



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

Molecular Formula : C13H19NO4S

Molecular Weight : 285.36 gm/mole.

MATERIALS AND METHODS

Cephalexin (purity 99.5, and 99.97 %) and Probencid were provided as a gift samples by Sura Pharma Pvt.Ltd.,Dilshuknagar,Hyderabad, India. All the other reagents used were of analytical grade. Methanol (HPLC grade), Acetonitrile (HPLC grade) were

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purchased from S.D fine chemical Pvt Limited, India.

Instrumentation

A Waters HPLC system equipped with a 2695 binary pump, an auto sampler and a 2996 photo diode array detector was employed for the study. The output signal was monitored and processed with Empower-2 software.

Preparation of Mobile Phase

Accurately measured 500ml (50%) of HPLC Acetonitrile and 500ml of HPLC Water (50%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of standard solution

Accurately weighed and transferred 10 mg of Velpatasvir and Sofosbuvir working standard into a 10mL of clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.15 & 0.6mL of the above Velpatasvir & Sofosbuvir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Optimized Chromatogram

Mobile phase : Methanol: Phosphate Buffer pH 3.9 (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

Method Validation

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Probenecid and Cephalexin in drug product.

Linearity

The linearity was observed for both the drugs 1-5µg/mL and 100-500 µg/mL for cephalexin and Probencid respectively. The resultant are shown in table 1 & 2. and Fig 3 & 4.

LINEARITY PLOT

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.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Accuracy

At each concentration, sample was injected thrice to check repeatability and from the %RSD values it was analyzed that the method was accurate as % recovery values found to be in the range of 99.25-100.90% for the Probenecid and 99.59-101.05% for Cephalexin at three different concentrations 50%, 100%, 150%. The results are given in Table 9, 10.

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.

Probenecid: LOD =3.3 × 1760.8/78322 =0.07µg/ml
Cephalexin: LOD =3.3 × 61155/11150 =18.0µg/ml

Limit of quantization

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

Probenecid: LOQ =10×1760.8/78322 = 0.2µg/ml
Cephalexin: LOQ**=** 10 × 61155/11150 = 54.8µg/ml

ROBUSTNESS

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Probenecid and Cephalexin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Probenecid and Cephalexin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 1. Linearity data of Probenecid

Concentration Level (%)	Concentration (µg/ml)	Average Peak Area
33.3	1	88442
66.6	2	165724
100	3	242754
133.3	4	315906
166.6	5	396371

Table 2. Linearity data of Cephalexin

Concentration Level (%)	Concentration (µg/ml)	Average Peak Area
33	100	1131032
66	200	2345302
100	300	3355282
133	400	4429382
166	500	5623754

Table 3. Data of Probenecid

S No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Probenecid	2.065	249684	12079	5343	1.0
2	Probenecid	2.064	249696	12068	5473	1.2
3	Probenecid	2.064	246325	11949	5473	1.1
4	Probenecid	2.065	249816	11811	5389	1.1
5	Probenecid	2.067	249892	11735	5180	1.0
Mean			249082.6			
Std. Dev			1543.964			
% RSD			0.61986			

Table 4. Precession Data of Cephalexin

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Cephalexin	2.486	3233700	59095	6654	1.2
2	Cephalexin	2.484	3241323	57552	6524	1.3
3	Cephalexin	2.482	3245927	57213	6440	1.3
4	Cephalexin	2.483	3245927	57096	6411	1.4
5	Cephalexin	2.483	3222194	54363	6260	1.4
Mean			3237814			
Std. Dev			10060.62			
% RSD			0.310722			

Table 5.The accuracy results for Probenecid

%Concentration (at specification Level)	Peak 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 6.The accuracy results for Cephalexin

%Concentration (at specification Level)	Peak 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%	

Table 7. Robustness data of 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

Table 8. Robustness data of Cephalexin

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

Fig 1.Chromatogram of blank

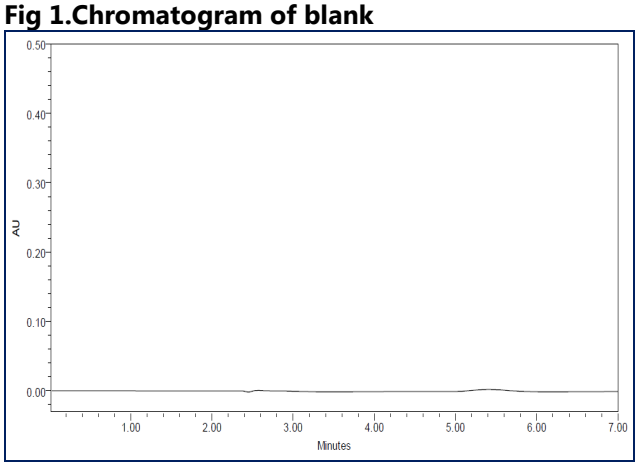


Fig 2.Typical Chromatogram of Mixed working standard solution

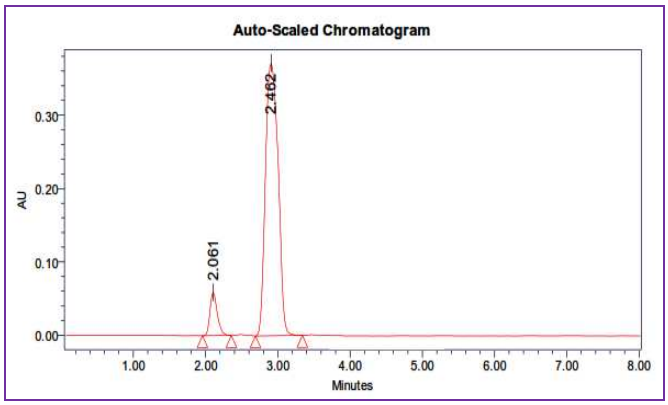


Fig 3. Calibration graph for Probenecid

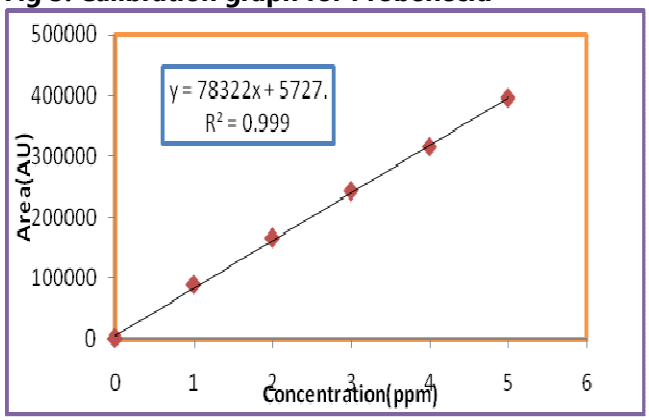
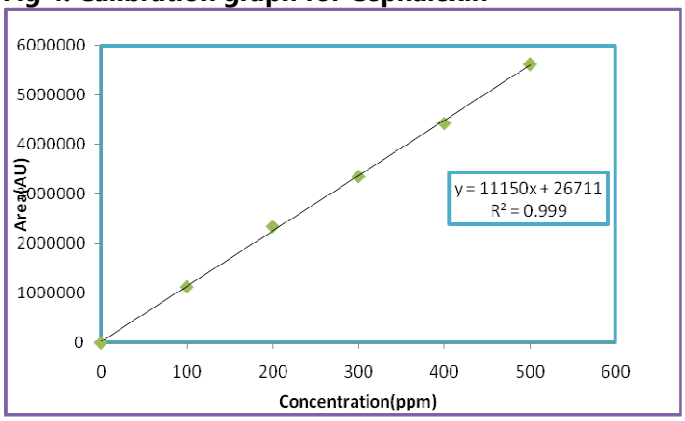


Fig 4. Calibration graph for Cephalexin



SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 255nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The

column used for study was Zorbax C_{18} because it was giving good peak. 35ºC temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Phosphate Buffer pH 3.9 (55:45v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 8min because analyze gave peak around 2.061, 2.462 ±0.02min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range 1-5µg/ml of Probenecid and 100-500µg/ml of Cephalexin of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Probenecid and Cephalexin 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 Cephalexin was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Phosphate Buffer pH 3.9 (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 Cephalexin in bulk drug and in Pharmaceutical dosage forms.

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CONFLICT OF INTERESET

Nil

REFERENCES

1. Shetti PD. High Performance Liquid Chromatography. 2001, 116.
2. The Merck Index. An encyclopedia of chemicals, drugs and biological. 14th ed., USA; 2006.
3. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry, 4th Ed., C.B.S. Publications, 1.
4. Malik V. Drugs and Cosmetics Act 1940, 16th Edn., Eastern Book Company, Lucknow, 5.
5. Willard HH, Merit LL, Dean FA, Settle FA. Instrumental methods of analysis, 7th ed., C.B.S. Publishers, New Delhi, 2002.
6. Mendham J, Denney RC, Barnes V, Thomas MJK. Vogel's Text book of Qualitative Chemical Analysis, 6th ed., 261-287.
7. Skoog DA, West DM. Fundamentals of Analytical Chemistry, 7th edn.
8. Sharma BK. Instrumental methods of Chemical analysis, 19th ed., 2000.
9. Willard, HH, Merrit LL, Dean JA. Instrumental methods of analysis, 7th ed., CBS Publishers, New Delhi.
10. ICH, Q2A Text on validation of analytical procedures, Oct, 1994. www.ich.org.
11. ICH, Q3B Validation of analytical procedures: methodology, Nov, 1996.
12. Jeswani RM, Sinha PK, Topagi KS, Damle MC. A Validated Stability Indicating HPTLC Method for Determination of Cephalexin in Bulk and Pharmaceutical Formulation. *International Journal of PharmTech Research*, 1(3), 2009, 527-536.
13. Halkar UP, Rane SH, Bhandari NP. Simultaneous Determination Of Cephalexin And Probenecid In Pharmaceutical Preparations By RP-HPLC. *Indian Drugs,* 34(9), 1997 , 539-541.
14. Shinde VM, Desai BS, Tendolkar NM. Simultaneous Determination of Cephalexin And Probenecid For Tablets By RP-HPLC. *Indian Journal of Pharmaceutical Sciences*, 1994, 56(2), 58-60.