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Analytical method validation for tablet of phenoxymethyl penicillin potassium by RP-HPLC method

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Abstract

The present work described a simple, precise, accurate and linear reverse phase HPLC method for determination of Phenoxymethy penicillin potassium from tablet formulations. The determination was carried out on a Hypersil, ODS, C-18 (30cm   4mm) (3 to 10mm) 5µ column using a mobile phase of water: acetonitrile: glacial acetic acid (500:500:5.75).The flow rate and run time were 1ml/min and 10 min respectively. The method was reproducible with theoretical plates and % RSD.

Keywords: PENVK (Penoxymethy penicillin potassium), RP-HPLC, ICH guideline and USP.

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Introduction

Phenoxymethylpenicillin potassium is potassium (6R)-6-(2-phenoxyacetamide) penicillinate produced by the growth of certain strains of penicillium notatum or related organisms on a culture medium containing an appropriate precursor[1]. Phenoxymethylpenicillin potassium is agent which is indicated for the treatment of bacterial infection . Chemically, It is the potassium salt of (2S,5R,6R)-3,3dimethyl-7-oxo-6[(phenoxyacetyl)anino]-4-thia-1-abicyclo[3.2.0]heptane-2-carboxylic acid[2].

A survey of literature has not revealed any RP-HPLC analytical method validation for determination of phenoxymethyl-penicillin potassium in tablet whereas reports are available for the plasma by RP-HPLC[3], capillary electrophoresis[4] and spectrometric methods[5].The present paper describes the modified RP-HPLC method using isocratic mobile phase concentration water:actonitrile:glacial acetic acid (500:500:5.75) that offers

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certain advantage in its simplicity, stability and time saving. The result of analysis was calculated by statistical method and recover studies as per ICH guideline and U.S.P. [6,7].

Materials and methods

Materials: Phenoxymethylpenicillin potassium BP reference substance was obtained from Micro Labs Ltd.(Banglore.India)Tablets of brand Phenoxymethylpenicillin 250mg,B.P.(Batch No.PKTB0001 Micro Labs Ltd.) Containing 250mg of PENVK were procured from a local pharmacy.HPLC Grade (Millipore filtered) acetonitrile , water and ARGrade Glacial acetic acid was used as the mobile phase.
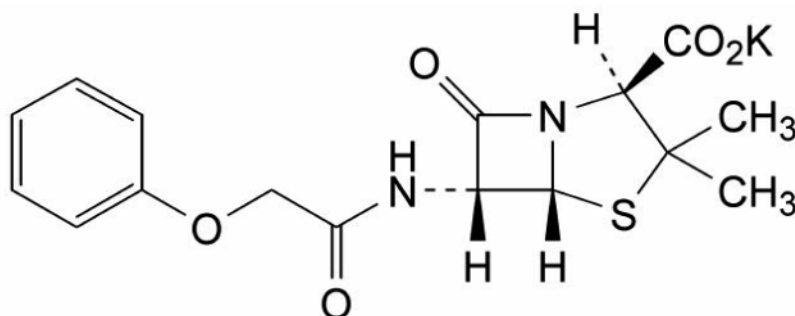
Fig:-1 Phenoxy methyl penicillin potassium

Instrument: A HPLC (LC-2010HT, shimadzu, Japan) connected to computer loaded with class VP chromatographic software. System was coupled with SPD 10A UV detector. All weights were taken on semi microbalance (sartorious CP 2250)

Standard stock solution
The standard solution of Phenoxymethylpenicillin potassium was prepared by dissolving accurately weighed quantity of 125mg PENVK working standard in 30ml of mobile phase than make up to 50ml with mobile phase and filtered.

Chromatographic system
The determination was carried out on a hypercil ODS,C-18(25cm × 4mm,5 micron) column using a mobile phase of water ,acetonitrile and glacial acetic acid in ratio of 500:500:5.75respetively.The flow rate and runtime were 1ml/min and 10min respectively. The eluent was monitored at 254nm.The injection volume was 10µl.

Estimation of Phenoxymethyl penicillin potassium
20 Tablets was weighed and powdered. Transferred an accurately weighed portion of the powder ,equivalent to about 400.000USP Penicillin V unit to a 100ml volumetric flask, diluted with mobile phase to volume and shake for about 5minutes.Filtered a portion of this solution through a suitable filter of 0.5µm of finer porosity and used the filtrate for assay.

Result and discussion

This method was validated for statistical parameters i.e. system suitability, linearity, precision (reputability), specificity, accuracy, ruggedness and robustness criteria. Results of the method validation experiments are given in table 1

The accuracy of the method was established at three levels in the range of 90% to 110% of the working concentration of sample. Calculated amount of PENVK was added in placebo to attain 90%, 100% and 110% of working concentration. Preparation was prepared as described in estimation of PENVK and each preparation was injected in duplicate % recovery and mean of %recovery was calculated at each level and recorded in table 1.

Table 1: Result of Method Validation Experiments of Phenoxymethyl Penicillin potassium.

S.No	Parameters	Acceptance criteria	Result obtained
1	System suitability		
	% RSD	% RSD of standard NMT 2.0	0. 686
	Peak Asymmetry	Not more than 2	1.41
	Theoretical plates	Not less than 1800	4070.2
2	Linearity	r^2 = 0.995 to 1.0	0.999
3	Precision (Repeatability)	RSD NMT 2.0%	0.424
4	Specificity	No interference with placebo	Passes
5	Accuracy	Recovery : 98.0 to 102.0%	100.2%
6	Ruggedness	RSD NMT 2.0%	0.431
7	Robustness (Flow rate)	NMT $\pm$ 1	0.8%

The linearity was determined at 5 levels over the range of 80% to 120% of test concentrations. A standard linearity solution was prepared to attain concentration of 80%, 90%, 100%, 110% and 120% of the test concentration. Each linearity solution of sample was injected in duplicate. The mean area at each level is calculated and graph of mean area versus concentration is plotted. The correlation co-efficient was calculated and recorded.

The precision of the method was determined by knowing percentage RSD of mean of three replicate solutions of the entire three independent samples. The text solution was prepared by using sample weight 280.1mg, 351mg, and 422.1mg of sample for 80%, 100% and 120% respectively attending the level of concentration. The specificity was determined by performing two time assay, first with sample and again with placebo.

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The Ruggedness was determined by analysis of aliquots from homogeneous lot by different analyst it was assayed by performing eight determination i.e. two concentration and two replicates of each concentration by two analyst. Weight taken by first analyst 279.4 mg and 351.2mg for the level of concentration of 80 and 100 percentages respectively similarly weight taken by next analyst 278.2 mg and 249.1mg for the level of concentration of 80 and 100 percentages respectively. The percentage RSD of above consecutive concentration was calculated

The robustness of method was established by making deliberate minor variation in the flow rate. Method was performed twice first by same procedure and second time by changing the flow rate1.2ml/min and the percentage deviation was calculated.
A typical chromatogram obtained in the present investigation is shown in fig 1.The result obtained were summarize in table.1. Prior to the analysis method was subjected to system suitability tests. It was determined by using the five working standard injections. The percentage RSD, peak symmetry and theoretical plates was calculated.

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Method Name     :C:\CLASS-VP\Methods\Pen v k tab.met
Sequence Name   :C:\CLASS-VP\Sequence\2007\MAY\02'05'07 Pen V K Tab.seq
Data Name       :C:\CLASS-VP\Data\2007\FEB\02'05'07  002
User            :System
Run Time        :5/2/2007 11:25:58 AM
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Sample ID:Penicillin V Potassium Std for System Suitability
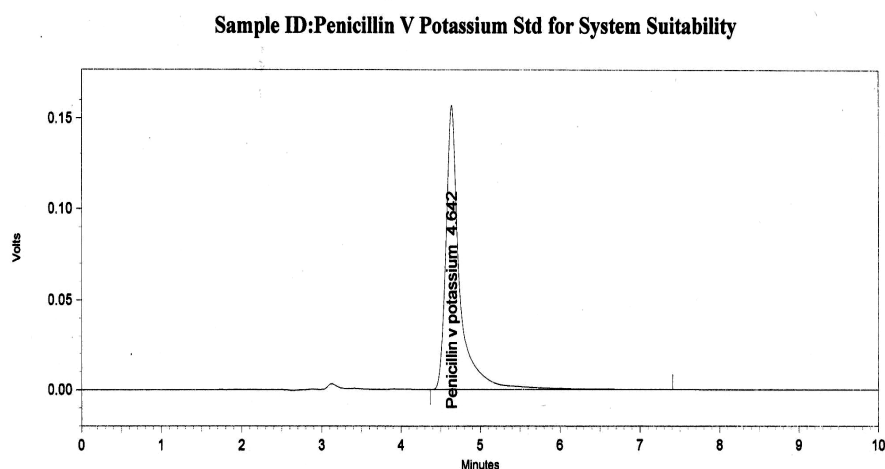
Fig.:- 2 Standard chromatogram of PENVK

The statistical parameters in method validation studies for linearity, precision, (repeatability), specificity, accuracy, ruggedness and robustness were justified the validity of the modified method. The result of the method validation studies given in table 1 has shown that the method is simple, accurate, and non –interference from table excipients.

Conclusion

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This method is simple, specific, and easy to perform and requires short time to analyze the samples. Low RSD value of precision makes this method suitable for use in quality control. This method enables determination of Phenoxymethylpenicillin potassium because of good separation of the chromatographic peak. The method was found to be linear, precise, accurate, rugged and robust.

Acknowledgement

References

1. Indian pharmacopoeia, volume-II, the controller of publication, Delhi, **1996**, 583
2. British pharmacopoeia, volume IV, the British pharmacopoeia commission, London, **2005**. A-167
3. Lindberg RL; Huupponen RK; Huovinen P. *Antimicrob Agents Chemother*, **1984**, 26(3); 300–302.
4. Genowefa P; katarzyna M; Stefan T. *Journal of chromatography A*, **2005**, 1087(1-2); 197-202
5**.** Churilova NS. *Antibiotic Medicinal Biotechnol*, **1985**, 30(2); 90-110
6**.** Text on validation of Analytical Procedures, International conference on Harmonization (ICH) of Technical requirement for registration of pharmaceuticals for Human use, ICH steering committee,**1994**
7. The United States Pharmacopoeia, 29/NF 24. The official compendia of standard Asian Edition, **2006**, 3050-3053