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Research Article

NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ANALYSIS OF ANTI VIRAL DRUG PENCICLOVIR

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ABSTRACT

A simple, rapid and precise reverse phase high performance liquid chromatography method was developed for the analysis of Penciclovir in tablet and serum. Chromatographic separation of Penciclovir was performed by using a kromosil C_{18} column (250 x 4.6mm, 5 µm) as stationary phase with a mobile phase comprising of 0.01 M Sodium di hydrogen phosphate: acetonitrile:water 40:40:20 (v/v) at a flow rate of 1.0ml/min and UV detection at 286nm. The linearity of Penciclovir is in the range of 10µ g/ml to 50 µg/ml. The limit of detection for Penciclovir was found to be 5 nano grams. The recovery was calculated by standard addition method. The proposed method was found to be accurate, precise and rapid for the analysis of Penciclovir.

Keywords: Penciclovir, accurate, precise, recovery, linearity.

INTRODUCTION

Penciclovir Molecular formula $C_{10}H_{15}N_5O_3$.Molecular Weight is 253.258 grm/mol. IUPAC Name 2-amino-9-[4-hydroxy-3-(hydroxymethyl) butyl]-6,9-dihydro-3*H*-purin-6-one . This activated form inhibits viral DNA polymerase, thus impairing the ability of the virus to replicate within the cell[1-4].

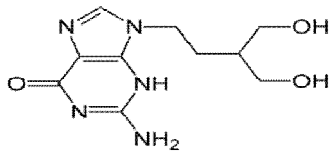

Fig.1: Structure of Peniciclovir

MATERIALS AND METHODS

Methanol, Acetonitrile, Ortho phosphoric acid used was analytical grade. Chromatographic separation was performed with PEAK high performance liquid chromatography having LC-P7000 isocratic pump, equipped with PEAK LC-UV7000 variable wavelength detector. Chromatograms and data were recorded by means of PEAK Chromatographic Software version 1.06.

Preparation of standard Solution

15 mg of Penciclovir was taken in a 10ml volumetric flask and 10ml of mobile phase was added to obtain 1.5 mg/ml of Penciclovir standard solution.

Chromatographic Conditions

Mobile phase : Acetonitrile (40%), 0.01M Sodium di hydrogen phosphate(40%) and Water (20%)

PH : 4.2

Analytical Column : Kromosil C_{18} column (250mm x 4.6mm) 5µ

UV detection	:	286nm
Flow rate	:	1.0ml/min.
Injection Volume	:	20µl
Temperature	:	Ambient
Run time	:	10 min.
Retention time	:	5.3 min.

Linearity

In order to check the linearity for the developed method, solutions of five different concentrations ranging from 10µg -50 µg were prepared. The chromatograms were recorded and the peak areas were given in table-1.A linear relationship between areas versus concentrations was observed in about linearity range. This range was selected as linear range for analytical method development of Penciclovir.

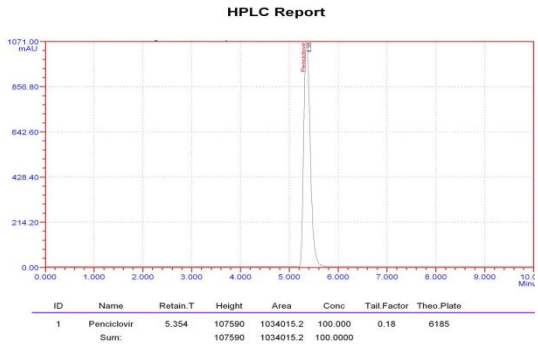

Table 1: Results of Linearity study

Linearity level	Concentration (µg/ml)	Area
1	10	48743.2
2	20	92765.5
3	30	145878.3
4	40	190565.5
5	50	235043.2

Precision (Repeatability)

1.5 mg/ml standard solution was prepared to calculate the precision for the developed method. The prepared solution was injected into injector at same concentrations and same chromatographic conditions. The chromatograms were recorded.

R.S.D for the values calculated is 0.225. So, the developed method shows precision.

Table 2: Precision study

Day	Precision Mean area	R.S.D.
Day - 1	997066.6	0.178
Day - 2	995612.38	0.271
Day - 3	995164.5	0.227

Limit of Quantification (LOQ) and Limit of Detection (LOD)

The LOQ and LOD were established at a signal to noise ratio. The LOD of Penciclovir is 5ng/ml. The LOQ of Penciclovir is 35ng/ml.

RESULTS AND DISCUSSIONS

The Reverse Phase High Performance Liquid Chromatography method was developed a stability indicating assay method. Pure drugs chromatogram was run in different mobile phases containing methanol, acetonitrile, THF, and different buffers like potassium dihydrogen phosphate, sodium dihydrogen phosphate,Ortho phosphoric acid in different volumes ratios. Different columns like C_8, C_{18}, phenyl, cyano with different dimensions were used. Then retention time and tailing factor were calculated. Finally 0.01 M Sodium di hydrogen phosphate: acetonitrile:water 40:40:20 V/V and Kromosil C_{18} analytical column was selected which gave a sharp and symmetrical peak with 0.18 tailing. Calibration graph was found to be linear at range 10 µg/ml to 50 µg/ml. five different concentrations of Penciclovir in range given above were prepared and 20µl of each concentration injected in HPLC. The slope (m) and intercept (c) obtained were found to be 4704 and -0.284.The correlation of coefficient (r^2) obtained was found to be 0.999. It was observed that the concentration

range showed a good relationship. The limit of detection for Penciclovir was found to be 5ng/ml and the limit of quantification was found to be 35ng/ml. It proves the sensitivity of method. The low values of standard deviation and coefficient of variation at each level of the recovery experiment indicate high precision of the method.

CONCLUSION

The RP-high performance liquid chromatographic method for the analysis of Penciclovir from their formulations was found to be accurate and precise. Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of Penciclovir formulations.

REFERENCES

1. Farmaceutiska Specialiteter i Sverige - the Swedish official drug catalog. [http://www.fass.se Fass.se --> Vectavir. Retrieved on August 12, 2009. Translated from "Tiden för läkning, smärta och påvisbart virus förkortas med upp till ett dygn."
2. Pan Y, Gao X, Guo N, Li J and Guo X**.** HPLC method for the determination of penciclovir in human plasma and application to a bioequivalence study. J Chromatogr Sci. 2008;46(9):819-822.
3. Yongyu Gao, Xinxing Guo, Na Li, Junming Guo and Xingjie. HPLC Method for the Determination of Penciclovir in Human Plasma and Application to a Bioequivalence Study. Journal of Chromatographic Science. 2008;46(4):819-822.
4. Rp-HPLC Ion Pair Method Development, Validation and Stability Indicating Assay For Famciclovir. PharamInfo.net. 2007;5.