

**Method development and validation for the analysis of Penciclovir and related impurity in bulk and pharmaceutical dosage forms by RP HPLC**Shiny Ganji\*<sup>1</sup> and D.Satyavati<sup>2</sup><sup>1</sup>Asst. Prof, St. Ann's College of Pharmacy, Vetapalem, Chirala, Prakasam Dist, Andhar Pradesh, India<sup>2</sup>Director, Brilliant Grammer School Educational Society's Group of Institutions, Integrated campus, Hayath Nagar, R.R.Dt, Hyderabad, India**\*Corresponding author e-mail:** [gshiny.d@gmail.com](mailto:gshiny.d@gmail.com)*Received on: 30-11-2015; Revised on: 15-12-2015; Accepted on: 07-01-2016***ABSTRACT**

The prime aim of the current work is to develop and validate a novel, specific, sensitive, precise, rapid and faster isocratic elution RP HPLC method for estimation of Penciclovir and its process related impurity in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved on Hypersil phenyl, 250 mm X4.6 mm, 5  $\mu$  with mobile phase composed of 0.1% orthophosphoric acid in 1000 ml of water and acetonitrile in the ratio of 60:40 using an isocratic mode. The temperature is maintained at 30°C, detection was made using UV detector and LC solution software at 254 nm and the flow rate was maintained at 1.0ml/min. The run rate was 20 min. The developed method was validated according to ICH guide lines. The linearity of calibration curve for each analyte in concentration range of 1200 $\mu$ g/ml – 3600 $\mu$ g/ml was good. The curve was linear for the impurity in concentration range of 8 - 24 $\mu$ g/ml. There exists a good correlation between peak area and analyte concentration. Relative standard deviation values for penciclovir is 0.111 and its process related impurity is 0.359. LOD for the active ingredient and its impurity was found to be 0.02 % and 0.5 % respectively. LOQ for active ingredient and its impurity was found to be 0.06 % and 0.15% respectively. Statistical analysis revealed that the proposed method was found to be highly sensitive, precise, accurate, robust and fast. The shorter retention time allows the analysis of large number of samples in short period of time and it is cost effective, so it can be successfully applied for routine analysis of active pharmaceutical ingredients and related impurities in bulk and pharmaceutical dosage forms.

**Keywords:** Penciclovir, Denavir, RP –HPLC method, mono alkyl impurities, validation, method development**INTRODUCTION**

Penciclovir is a guanine nucleoside analogue, active against herpes simplex virus type 1 and 2, Varicella zoster virus, Epstein virus and hepatitis B virus.<sup>[1,2]</sup> Its molecular formula is C<sub>10</sub>H<sub>15</sub>N<sub>5</sub>O<sub>3</sub> and its IUPAC name is 2-amino-9-[4-hydroxy-3-(hydroxymethyl)butyl]-6,9-dihydro-3H-purin-6-one.<sup>[3]</sup> But it has poor bioavailability when administered orally, so it was developed as a prodrug i.e famciclovir. It inhibits enzyme thymidine kinase and interferes with DNA synthesis.<sup>[4]</sup> It is official in USP and BP. It is mostly used as topical treatment under the brand name

Denavir. It is a synthetic acyclic guanine derivative and Fig 1 represents the structure of Penciclovir.

There are few HPLC methods available in the literature for the analysis of Penciclovir but no method is available for determination of penciclovir. These include 'activity of penciclovir in antiviral assays against herpes simplex virus,<sup>[5]</sup> a new validated RP HPLC method for determination of penciclovir in human plasma,<sup>[2]</sup> method development and validation of analysis of antiviral drug penciclovir<sup>[3]</sup> simple and reliable stability indicating RP HPLC method for determination of assay of

Famciclovir in drug substance<sup>[6]</sup> Extensive literature survey revealed that there were methods for validation of Penciclovir but no method was available for validation of Penciclovir and its related impurity, hence the present work aims at analysis of Penciclovir and its process related impurity in bulk and also pharmaceutical dosage forms.

## MATERIALS AND METHODS

**Chemicals and materials:** The active ingredient and its related impurity were procured from Mylan labs. HPLC grade double distilled water, analytical grade ortho phosphoric acid, acetonitrile were obtained from Qualigens Fine chemicals Ltd, Mumbai.

**Instrumentation:** The analytical separations were carried out on a liquid chromatograph equipped with variable wavelength UV detector and the output of signal was monitored and integrated using LC solution software. The analytical column used was Hypersil Phenyl, 250 mm X 4.6 mm, 5 μ. Mobile phase is composed of 0.1% orthophosphoric acid in 1000 ml of water, and acetonitrile (60:40). The flow rate was maintained at 1.0ml/min. and the run time was 20 min. The column was monitored at a temperature of 30°C and the volume of sample injected was 20 μl. Detection was made at 254 nm.

**Preparation of standard preparation:** Transfer 150.2 mg of Penciclovir and 1mg of mono alkyl impurity of working standard in to 50 ml volumetric flask and dilute to volume with diluent(mobile phase).

**Preparation of test solution:** Transfer 2420.3 mg of Denavir cream into 100 ml volumetric flask and dilute to volume with diluent. Take 5 ml to 50 ml volumetric flask and dilute with diluents. Take 10 ml of above solution to 20 ml volumetric flask and dilute with diluent.

**Assay:** 20 μl of standard and sample solutions were injected into chromatographic system and the areas of peaks for Penciclovir and its impurity were measured and the % assay was calculated using the formula

$$\frac{A_t \times W_s \times \text{avg. wt} \times P}{A_s \times W_t \times \text{claime wt}} = \%$$

As X Wt X claime wt

Where: At : Average area due to Denavir formulation peak in sample

As: Average area due to penciclovir & monoalkyl impurity peak in standard preparation

Ws: Weight of the working standard Penciclovir and mono alkyl impurity

Wt: Weight of sample Denavir formulation

P: Potency of working of the working standard

### Method validation:

Validation parameters like system suitability, linearity, accuracy, precision, specificity, limit of detection, limit of quantitation and robustness were performed as per ICH guidelines Q2 (R1) <sup>[7]</sup>

**Linearity and range:** Standard solutions of active pharmaceutical ingredient and mono alkyl impurity were diluted to 1201 μg/ml – 3604 μg/ml and 8 μg/ml – 24 μg/ml respectively. Set up the chromatographic system to equilibrate and inject the solutions, keeping the injection volume constant, 20 μl.

**System suitability:** Twenty microliters of the standard solution of active ingredient (0.3 μg/mL) and mono alkyl impurity (0.015mg/ml) were injected under optimized chromatographic conditions to evaluate the suitability of the system.

**Precision:** 0.3mg/ml of Penciclovir and 0.015mg/ml of mono alkyl impurity standard solutions were injected six times and record the response. Penciclovir sample was also injected six times to record the response.

**Accuracy:** Three known concentrations of Penciclovir and mono alkyl impurity were spiked at 80%, 100% and 120% and each solution was injected 3 times to evaluate accuracy.

**Specificity:** The diluent was injected as blank to get the steady line. 0.3 mg/ml of Penciclovir and 0.015 mg/ml of mono alkyl impurity of standard and sample were injected to record the response. Responses of the peaks were noted for any possible interferences of the excipient at the retention time of the drug.

**Limit of detection and limit of quantitation:** The limit of detection (LOD) is the lowest amount of analyte that can be detected in a sample, but not necessarily quantified, under the stated experimental conditions. The limit of quantification (LOQ) was identified as the lowest plasma concentration of the standard curve that could be quantified with acceptable accuracy, precision, and variability. They are determined by the signal-to-noise method. Starting with concentration of 20%, 10%, 5%, 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, 0.02%, 0.01%, 0.005%.

**Robustness:** Robustness studies were performed by altering column and variation of flow.

Different column: 0.3mg/ml of Penciclovir and 0.015 mg/ml of mono alkyl impurity standard solutions were injected in different column 3 times and response was recorded. Danavir sample was also injected 3 times and response was recorded.

Variation of flow: 0.3mg/ml and 0.15 mg/ml of Penciclovir and mono alkyl impurity respectively were injected 3 times and response was recorded and Danavir sample was also injected 3 times by increasing and decreasing the flow rate.

## RESULTS AND DISCUSSION

### Method development and optimization:

To optimize the chromatographic conditions, the effect of mobile phase is studied with various solvent system combinations for the determination of Penciclovir and its related impurity(mono alkyl impurity) in bulk and pharmaceutical dosage forms. A mixture of 0.1% orthophosphoric acid in 1000 ml of water and acetonitrile (60:40) was selected as it gave best resolution. The effect of flow rate was studied in the range of 0.9 to 1.2 ml/min and 1.0 ml/min was preferred to be effective. Under these conditions, the analyte peak obtained was well defined and free from tailing. The retention time (RT) was found to be 5.088 min for Penciclovir and 6.573 min for mono alkyl impurity. The optimized parameters were listed in Table 1. Chromatogram for standard solutions of Penciclovir and mono alkyl impurity were presented in Fig 2 and 3 respectively.

### System suitability studies:

These tests are an integral part of method development and are used to ensure adequate performance of chromatographic system. Retention time (RT), number of theoretical plates (N), tailing factor (T) and resolution were evaluated. The system suitability method acceptance criteria set in each validation run were capacity factor  $\geq 2.0$ , tailing factor  $\leq 2.0$  and theoretical plates  $> 2000$ . In all cases, the relative standard deviation for analyte peak area  $< 2.0\%$ . System suitability parameters were shown in Table 2. Chromatogram for system suitability studies is presented in Fig 4.

### Linearity:

Aliquots of standard solutions of drug and also impurity were taken and diluted to 40%, 60%, 80%, 100%, 120%

Each of these drug solutions was injected in to the column and peak areas and retention times were recorded. A calibration graph was obtained by plotting graph between peak area versus concentration. Excellent correlation was obtained between peak area and concentration with  $R^2 = 0.999$  for Penciclovir and 0.999 for mono alkyl impurity.

Results are shown in Table 3 and 4 and the linearity curves are shown in Fig 5 and 6.

### Accuracy:

Accuracy studies were done by standard addition method. Accuracy is expressed as % recovery of the standard spiked to previously analysed test sample of tablet. It was measured in drug products by spiking known amounts (80%, 100% and 120%) of the analyte into the analyzed tablet powder and calculating the percent recovered. The closeness of obtained value to true value indicates that the proposed method is accurate. The recovery data for accuracy studies was shown in Table 5. The accuracy chromatograms for the respective concentrations were shown in Fig 7, 8, 9

### Precision:

It is the agreement between replicate measurements of the same sample. It is expressed as relative standard deviation of replicate measurements. The standard solution was injected six times and area of peak is measured. The % RSD for the peaks was found within the limits,  $RSD \leq 1$ . Results were reported in Table 6 and 7 and chromatograms were presented in Fig 10 and 11

### Limit of Detection (LOD) and Limit of Quantitation(LOQ):

LOD and LOQ were determined based on the standard deviation of response and slope (S) of regression as per ICH guide lines according to the formula. The LOD of an analytical method is the lowest amount of analyte in a sample which can be detected but not necessarily quantified. It can be determined from the equation,

$$LOD = \frac{3.3 \times SD}{S}$$

$$LOQ = \frac{10 \times SD}{S}$$

Where S is the slope of calibration curve and SD is standard deviation. It was determined starting with the concentration of 20%, 10%, 5%, 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, 0.02%, 0.01%.

LOD and LOQ for Penciclovir was found to be 0.62 and 1.89  $\mu\text{g/ml}$  respectively

LOD and LOQ for mono alkyl impurity was found to be 0.06 and 0.18  $\mu\text{g/ml}$  respectively

### Robustness:

The drug solution was subjected to small, deliberate changes like flow rate, wave length and change in mobile phase ratio. The results obtained were not affected by varying the conditions and were in accordance with the results in original conditions. Results were reported in Table 8 and 9

**Different column:**

Robustness for different column can be evaluated by injecting 0.3 mg/ml of Penciclovir and 0.015 mg/ml of mono alkyl impurity standard solution 3 times and record the response. Inject Denavir sample 3 times and record the response. Chromatograms for standard and test samples are shown in Fig 12 and 13

**Variation in flow:**

It can be evaluated by injecting 0.3mg/ml of Penciclovir and 0.015 mg/ml of mono alkyl impurity standard solution 3 times and record the response. Inject Denavir sample for 3 times and record the response by changing the flow rate. Chromatograms are depicted in Fig 14, 15,16 and 17

**CONCLUSION**

The method proposed for the analysis of Penciclovir and its related impurity i.e mono alkyl impurity in

bulk and pharmaceutical dosage forms was found to be specific, precise, accurate, rapid and economical. The developed method was validated in terms of accuracy, linearity, robustness and precision in accordance with ICH guide lines. The method is cost effective due to short retention time which enabled analysis of Penciclovir and mono alkyl impurity with small amount of mobile phase. The method was found to be precise and accurate from precise studies. The method is sensitive due to low detection and quantitation limits. Robustness data indicate that the method is not susceptible to small changes in chromatographic conditions. This method can be applied successfully in estimation of active pharmaceutical ingredient and its related impurities in bulk and pharmaceutical dosage forms.

**Table 1 Optimised chromatographic conditions**

<b>Elution</b>	Isocratic
<b>Mobile phase</b>	0.1 % orthophosphoric acid in 1000 ml water and acetonitrile (60:40)
<b>Column</b>	Hypersil phenyl (250 mm X 4.6 mm, 5 µ)
<b>Flow rate</b>	1.0 ml/min
<b>Detection</b>	254 nm
<b>Injection volume</b>	20 µl
<b>Temperature</b>	30 °C
<b>Retention time</b>	5.088 min for active drug and 6.573 for impurity
<b>Run time</b>	20 min

**Table 2 System suitability parameters**

S.No	Parameters	Penciclovir	Mono alkyl impurity
1	Retention time	5.088 min	6.573 min
2	Theoretical plates	2723.392	3252.024
3	Tailing factor	1.564	1.168
4	Resolution	0.000	3.901
5	Peak area	15711099	79890

**Table 3. Linearity results for Penciclovir**

Concentration ( $\mu\text{g/ml}$ )	peak area
1201.6	6993363
1802.4	9649137
2403.2	13254794
3004.0	15759134
3604.8	19015896

**Table 4. Linearity results for mono alkyl impurity**

Concentration ( $\mu\text{g/ml}$ )	Peak area
8	40808
12	56664
16	69892
20	82599
24	95449

**Table 5 Accuracy studies of Penciclovir and mono alkyl impurity**

Name	spiked level	peak area	percent recovery
Penciclovir	80%	14424731	86.9
	100%	17520754	116.71
	120%	19959153	101
Monoalkyl impurity	80%	89484	105.48
	100%	118378	115.2
	120%	137428	130.06

**Table 6. Precision results for standard and test sample of Penciclovir**

S.No	Retention time (min)		Peak area (AU)	
	Standard	test sample	standard	test sample
1	5.075	5.061	15805617	15852172
2	5.082	5.063	15809955	15844420
3	5.095	5.065	15814326	15847963
4	5.095	5.064	15804874	15844376
5	5.091	5.068	15773528	15843308
6	5.077	5.070	15777603	15837013
AVERAGE	5.086	5.065	15797650	15844875
%RSD	0.175	0.068	0.111	0.032
SD	0.009	0.003	17486	5050

**Table 7 . Precision results for standard and test sample of mono alkyl impurity**

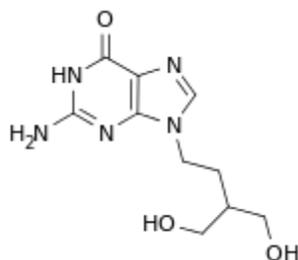
S.No	Retention time(min)		peak area (AU)	
	Standard	test sample	standard	test sample
1	6.759	6.716	93404	93046
2	6.778	6.721	93031	93921
3	6.818	6.726	93716	93864
4	6.817	6.731	93196	93721
5	6.807	6.739	93135	93174
6	6.761	6.746	93904	93834
Average	6.790	6.730	93408	93594
%RSD	0.402	0.163	0.359	0.408
Standard deviation	0.027	0.011	335	382

**Table 8 Robustness study for Penciclovir**

Parameters studied	Retention time		peak area		% RSD	
	Standard	sample	standard	sample	standard	sample
Different column	5.073	5.076	15776876	15926815	0.048	0.096
Flow decrease (0.9ml/min)	5.639	5.639	17664796	17711740	0.063	0.03
Flow increase (1.1 ml/min)	4.611	4.609	14450403	14546880	0.025	0.026

**Table 9 Robustness study for mono alkyl impurity**

Parameters studied	Retention time		peak area		% RSD	
	Standard	sample	standard	sample	standard	sample
Different column	6.775	6.784	104704	105791	0.111	0.050
Flow decrease (0.9ml/min)	7.536	7.536	117551	117833	0.104	0.024
Flow increase (1.1 ml/min)	6.163	6.160	95410	96351	0.045	0.045

**Fig 1 Chemical structure of Penciclovir**

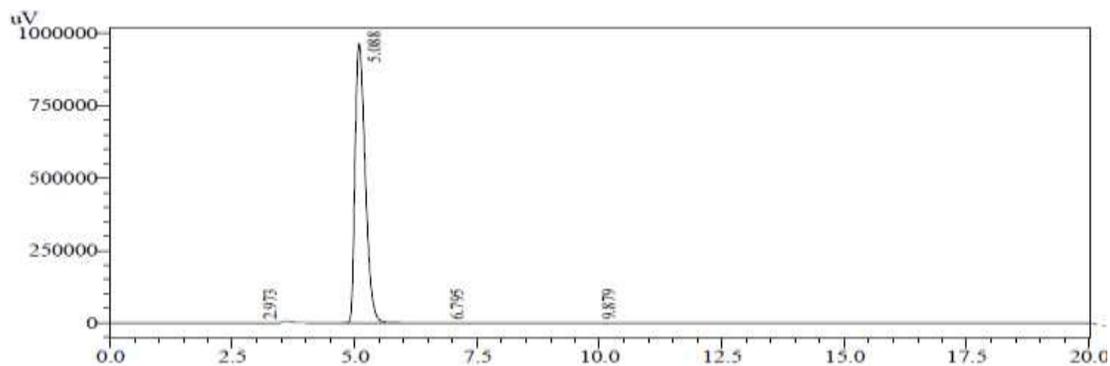


Fig 2 Chromatogram for penciclovir

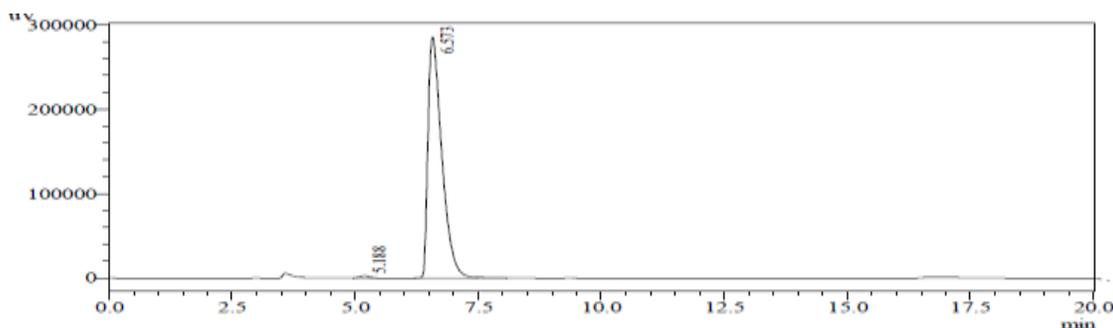


Fig 3 Chromatogram for mono alkyl impurity

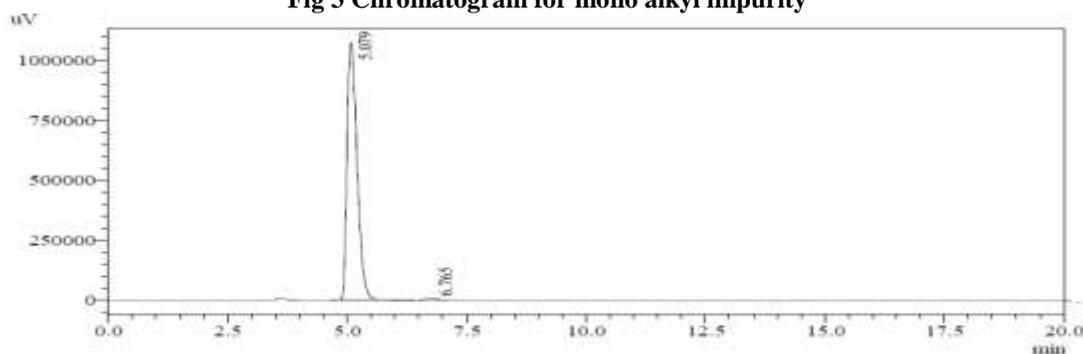


Fig 4 Chromatogram representing system suitability parameters

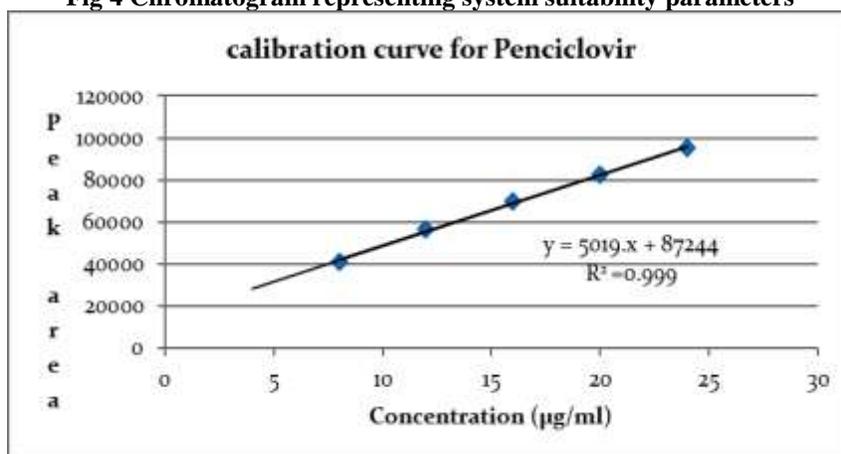


Fig 5 Calibration curve for Penciclovir

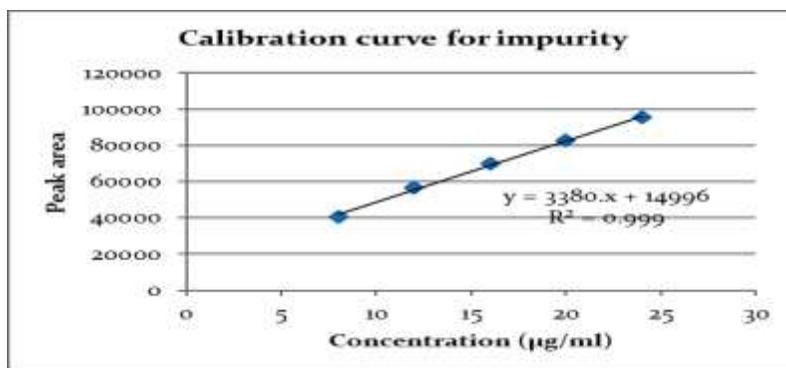


Fig 6 Calibration curve for mono alkyl impurity

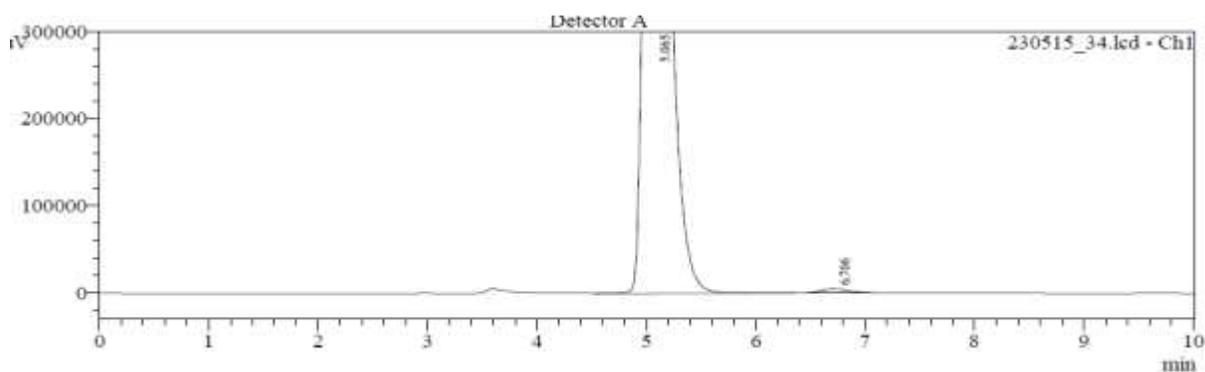


Fig 7 Accuracy studies

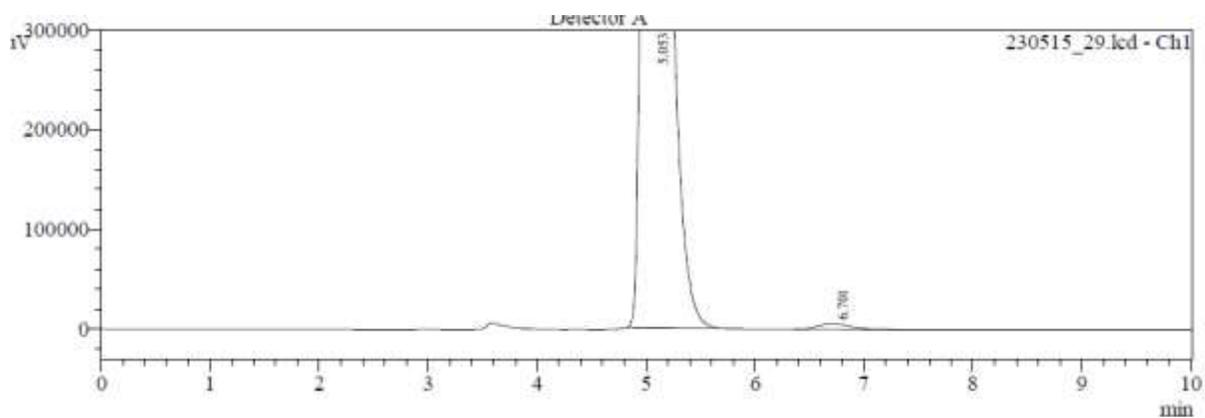


Fig 8

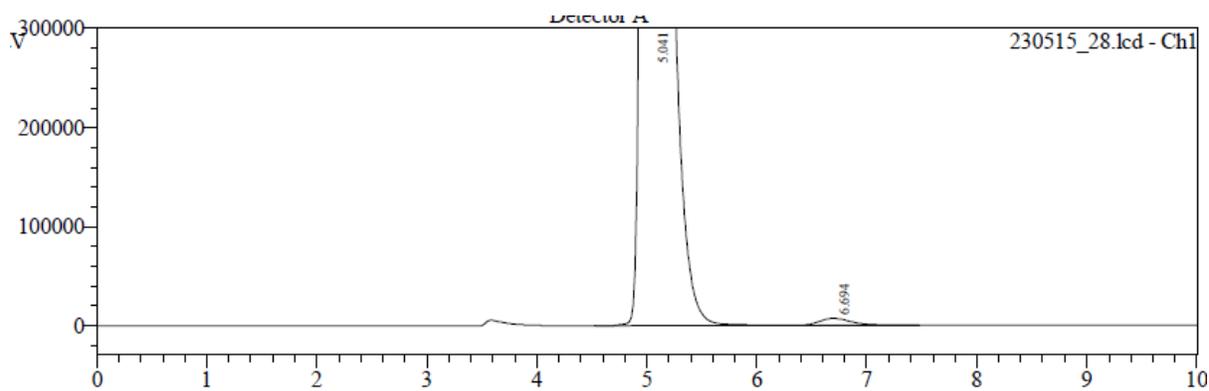


Fig 9

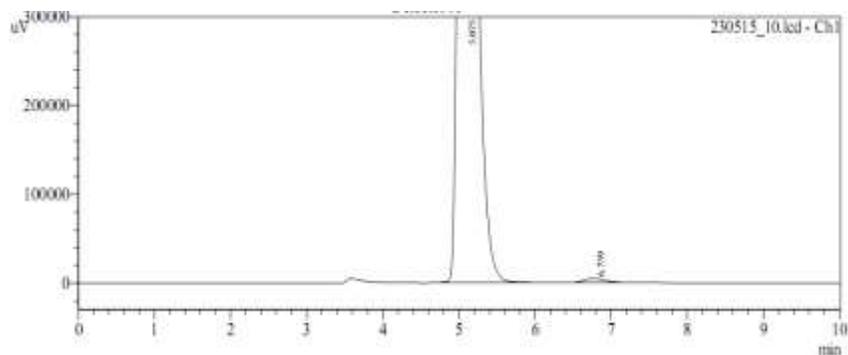


Fig 10 Chromatogram representing precision for Penciclovir

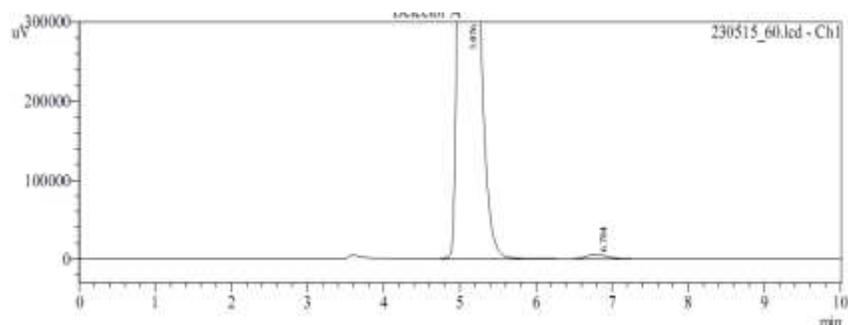


Fig 11 Chromatogram representing precision studies for mono alkyl impurity

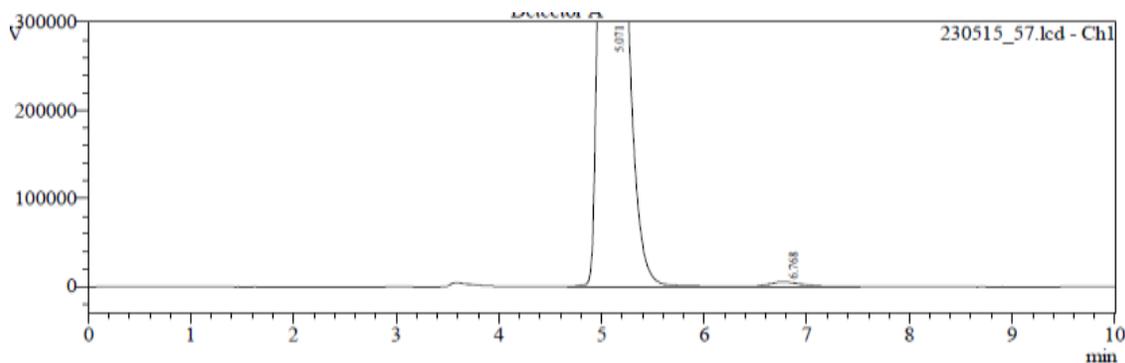


Fig 12 chromatogram representing standard solutions with different column

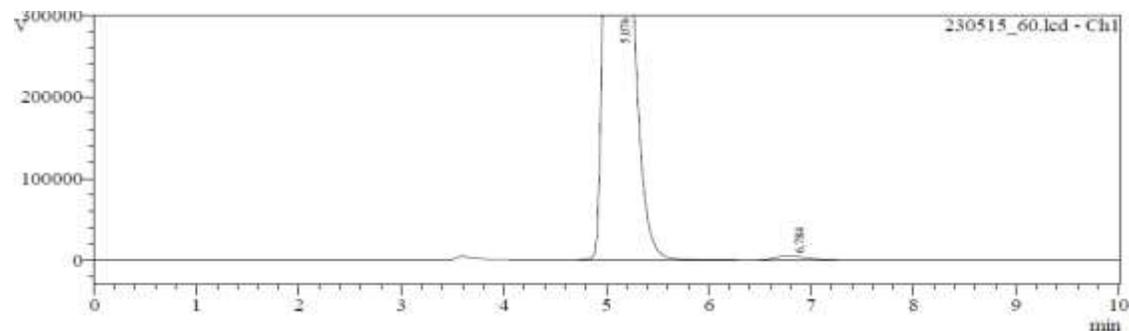


Fig 13 Chromatogram representing test solutions with different column

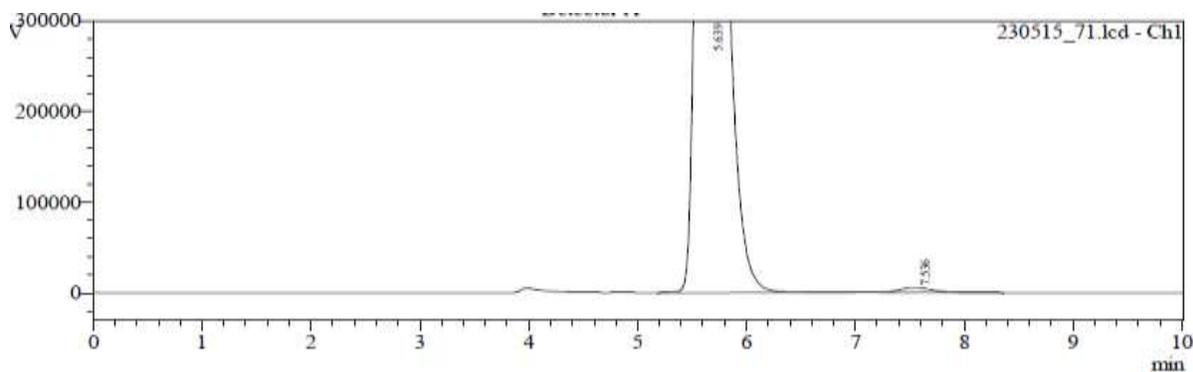


Fig 14 Chromatogram for standard solutions with flow decrease

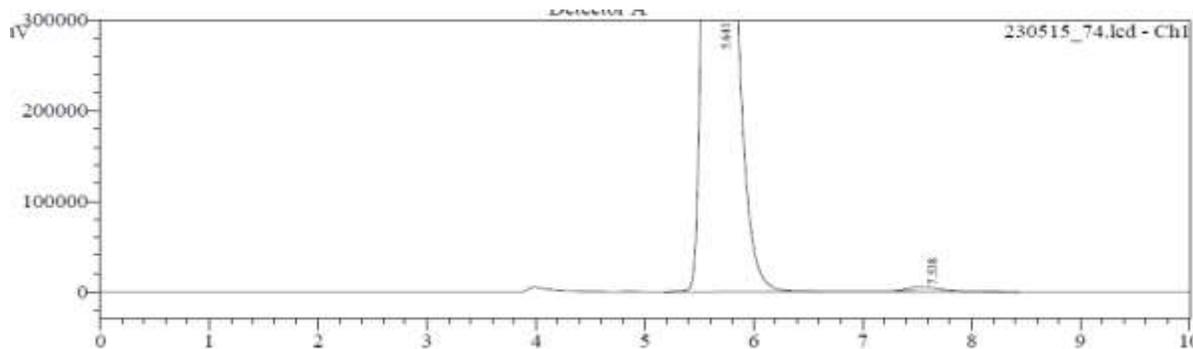


Fig 15 Chromatogram for test solutions for flow decrease

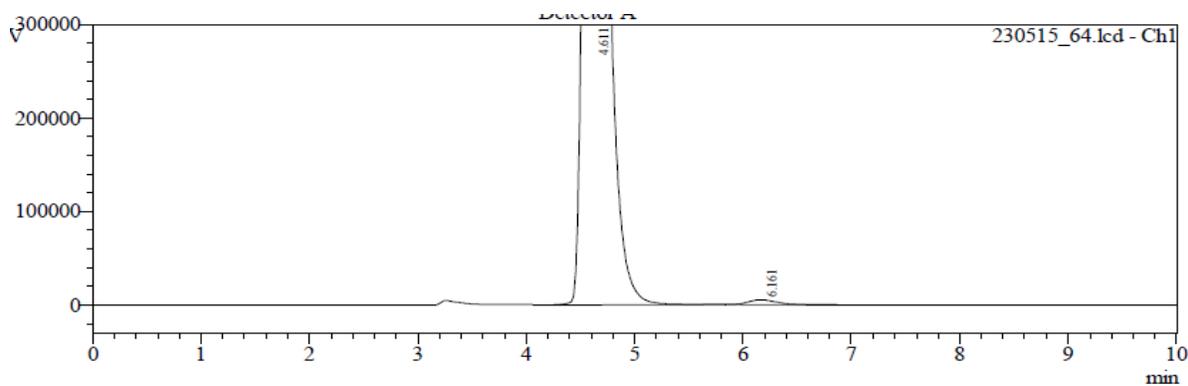


Fig 16 Chromatogram representing standard solutions for flow increase

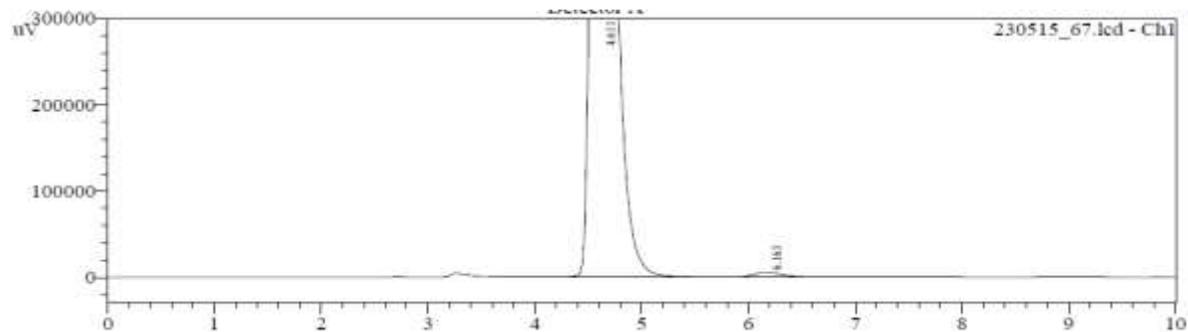


Fig 17 Chromatogram representing test solutions for flow increase

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