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[Research article]

Method Development and Validation of Related Substances by HPLC for Analysis of Valacyclovir Hcl in valacyclovir Hcl Tablet Formulations

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ABSTRACT

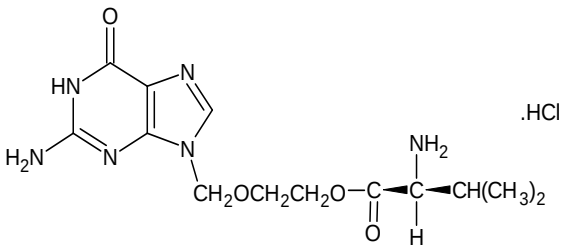
A simple, sensitive, and precise high performance liquid chromatographic method for the impurities profiling of Valacyclovir Hcl tablets has been developed, validated and used for the determination of impurities in commercial pharmaceutical products. The Impurities were well separated on a Zorbax SB Phenyl column (250 mm X 4.6 mm, 5µm) by the gradient program using Triflouro acetic acid, Acetonitrile, methanole Methanolic HCl at a flow rate of 1.0 mL min-1 with detection wavelength at 254 nm. The developed method was found to be specific, precise, linear, accurate. LOQ Values for all the known impurities were below reporting thresholds.

Keywords: Method Development, Validation, Valacyclovir Hcl, Impurities, HPLC.

INTRODUCTION

Valacyclovir is an antiviral drug used in the management of herpes simplex and herpes zoster (shingles). It is a prodrug, being converted in vivo to acyclovir(1). Chemically it is 2-[(2-amino- 6-oxo-3, 9-dihydropurin-9-yl) methoxy] ethyl-2-amino-3-methyl– butanoate [1], A number of analytical methods available to estimate the valacyclovir Hcl active ingredient in the pharmaceutical formulation. To the best of our knowledge, no single method which is available currently can separate and estimate all the known related compounds and degradation impurities of valacyclovir Hcl in pharmaceutical dosage form. (2-5)

Chemical structure:



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Related substances

Impurity –A: 2-amino –1,9-dihydro-6H-purine-6-one (guanine)

Impurity –B: 2-amino –9-[(2-Hydroxyethoxy) methyl]-1, 9-dihydro-6H-purine-6-one

Impurity –C: 2-[(2-amino-6-oxo-1, 6-dihydro-9H-purine-9-yl) methoxy] ehylN-methyl- L-valinate

Impurity–P: 2-[[2-[[[[9-[[2-[[(2S)-2-amino-3-methyl-1-methylenebutyl] oxy] ethoxy] methyl]-6-oxo-6,9-dihydro-]H-purin-2-yl]amino]methyl]amino]-6-oxo-1,6-dihydro-9H-purine-9yl]methoxy]ethyl L-valinate

EXPERIMENTAL SECTION

Chromatographic conditions

Column	: Zorbax SB Phenyl, 4.6 X 250mm, 5µm
Flow Rate	: 1.0 ml/min
Wave Length	: 254nm
Injection Volume	: 20µl
Column Temperature	: 30 ^{0}C
Run Type	: 55 min

Method for impurity testing (Related substance) was done by injecting standard, blank and Impurities- A, B, C and P into the chromatogram at conditions of Zorbax SB phenyl, 4.6 X 250mm, 5µm, flow rate at 1.0 ml/minutes, injection volume of 20µland maintaining the temperature at 30^{o}C for run time of 55 min. Detection was measured at 254 nm.

Preparation of standard Solutions

Accurately weigh and transfer about 58 mg of valacyclovir HCl W.S into a 100ml of V.F, add 2ml of diluent – A, sonicated. Add 60 ml of diluent – B and sonicated to dissolve. Dilute to volume with diluent – B and mix well. Diluted 5 ml of above solution to 100 ml with diluent – B. Further dilute 5ml of above solution to 25 ml with diluent – B.

Preparation of Sample Solution

Weigh and powder not less than 10 tablets. Accurately weigh transfer tablet powder equivalent to about 100 mg of valacyclovir in to a 100 ml V.F. Add about 2ml of diluent – A And sonicated. Add 60ml of diluent – B and sonicated for 30 min with intermittent shaking (maintain the sonicator temperature between 20 – 25 ^{0} C). Dilute to volume with diluent - B and mix well. Filter the solution through 0.45-µ

Preparation of impurities

Accurately weighed and transferred about 2.5 mg of impurities (A, B, C and P) into the 50ml of volumetric flask, add 10ml of diluent A, sonicated to dissolve. Dilute to volume with diluent B and mix well. Dilute 0.5ml of above solution to 25ml with 10ml of diluent A made up to volume with diluent B..

Preparation of mobile Phase A:

Mix 3 ml of Tri Flouro acetic acid with 1000 ml of water. Adjust the pH of the solution to 4.0 with Triethyl Amine and degas.

Preparation of mobile Phase B

Mix 3 ml of Tri Flouro acetic acid with 1000 ml solvent mixture. (Degased mixture of Acetonitrile and Methanol the ratio of 50:50 v/v.)

Diluent A: 2 % v/v of methanolic Hcl.

Diluent B: Mixture of Trifloro acetic acid, Water and Methanol in the ratio of 3.800:200 v/v/

Gradient Programme:

Separately injected equal volumes (20µl) of the blank, placebo preparation, standard preparation and sample preparation in to the chromatographic system

Table 1. Gradient Programme

Time (min)	Mobile phase – A (%V/V)	Mobile phase – B (%V/V)
0.01	100	0
20	80	20
40	20	80
42	100	0
55	100	0

Method Validation

Specificity Diluent as blank solution was injected into chromatographic system and founded no interference.(6-8). Placebo preparation was prepared by taking the placebo equivalent to about the weight in portion of test preparation and injected in to the HPLC system

Linearity

Linearity was performed by preparing minimum levels of all impurities (A, B, C, P) and drug substances into the range of 50 – 150 % of target concentration and injected into the chromatographic system in duplicate.

Accuracy

Accuracy of the test method was carried out by spiking known amounts of impurities at three concentration levels to the placebo in the range of 50 – 150% of target concentration in triplicate.(9-11)

RESULTS AND DISCUSSION

% W/W Impurity – A present in Valacyclovir HCl:

$$\frac{7316}{29424} \times \frac{50}{100} \times \frac{5}{100} \times \frac{5}{25} \times \frac{100}{284} \times \frac{705.2}{500} \times \frac{93.6}{100} \times \frac{324.3}{360.8} \times 100 = 0.0520\%$$

% W/W Impurity – B present in Valacyclovir HCl:

$$\frac{217463}{29424} \times \frac{31}{100} \times \frac{50}{100} \times \frac{5}{100} \times \frac{5}{25} \times \frac{100}{283.6} \times \frac{705.2}{500} \times \frac{93.6}{100} \times \frac{324.3}{360.8} \times 100 = 1.546\%$$

%W/W Impurity – C present in Valacyclovir HCl:

$$\frac{20230}{29424} \times \frac{50}{100} \times \frac{5}{100} \times \frac{5}{25} \times \frac{100}{283.6} \times \frac{705.2}{500} \times \frac{93.6}{100} \times \frac{324.3}{360.8} \times 100 = 0.143\%$$

%W/W Impurity – P present in Valacyclovir HCl:

$$\frac{8844}{29424} \times \frac{50}{100} \times \frac{5}{100} \times \frac{5}{25} \times \frac{100}{283.6} \times \frac{705.2}{500} \times \frac{93.6}{100} \times \frac{324.3}{360.8} \times 100 = 0.062\%$$

Specificity

Observation: No interference was observed.

Acceptance criteria: Interference due to blank & placebo solution should not be more than 2.0%

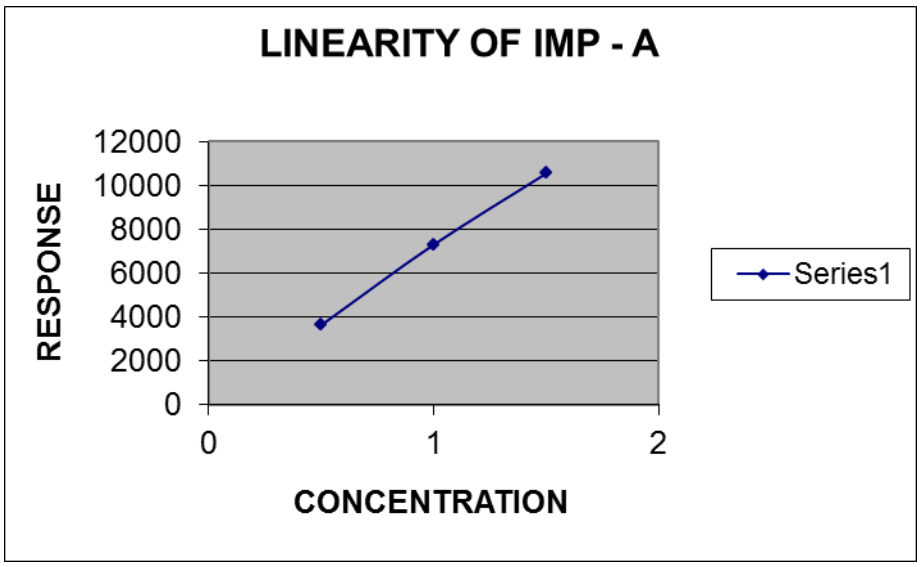
linearity:

Linearity of impurity – A:

Table 2. Linearity of impurity -A in Related substances method development

S.No	Linearity level	Concentration	Response
1	50	0.5	3645
2	100	1	7316
3	150	1.5	10564
Correlation coefficient = 0.999			

Graph -1 Linearity of impurity-A in Related substances method development

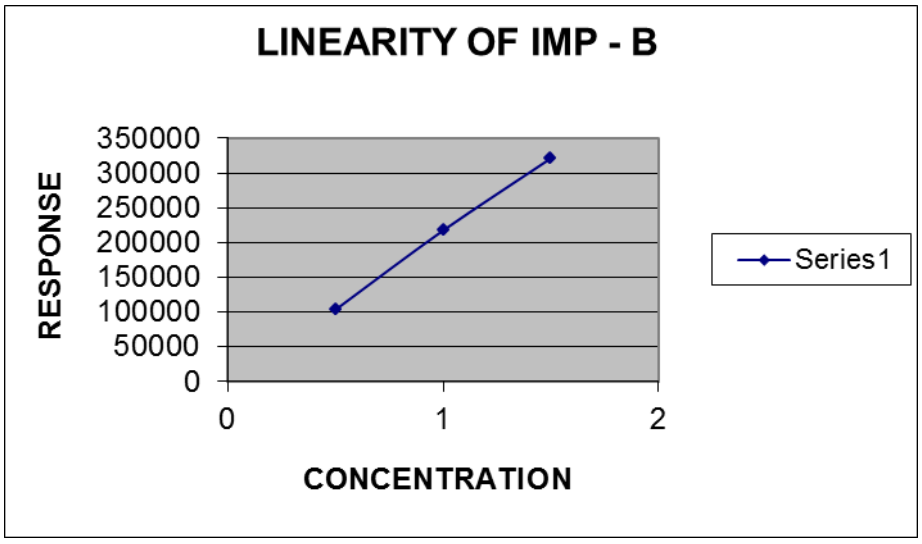


Linearity of impurity – B:

Table No–3 -Linearity of impurity - B in Related substances method development

Sl.No	Linearity level	Concentration	Response
1	50	0.5	103532
2	100	1	217463
3	150	1.5	321095
	Correlation coefficient =1.00		

Graph No 2 -Linearity of impurity - B in Related substances method development

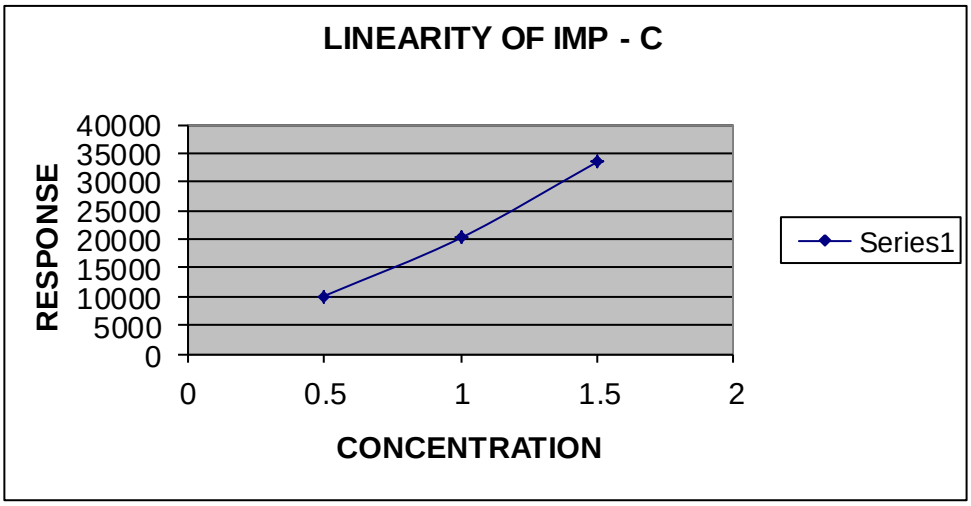


Linearity of impurity – C:

Table No –4 - Linearity of impurity - C in Related substances method development

S.No	Linearity level	Concentration	Response
1	50	0.5	12150
2	100	1	20230
3	150	1.5	33490
Correlation coefficient =0.99			

Graph No –3 - Linearity of impurity - C in Related substances

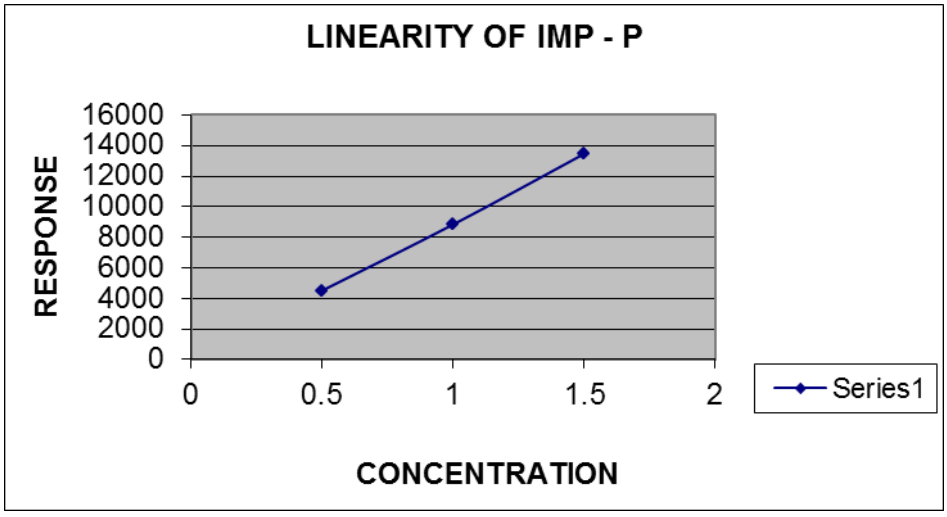


Linearity of Impurity –P

Table No– 5-Linearity of impurity-P in Related substances method development

S.No	Linearity level	Concentration	Response
1	50	0.5	4522
2	100	1	8844
3	150	1.5	13476
Correlation coefficient =0.999			

Graph No–4 -Linearity of impurity -P in Related substances method development



Accuracy:

Accuracy of impurity – A

Table No –6 - Accuracy of impurity - A in Related substances

Level	Replicate	% Recovery	Mean	Total Mean
50%	1	90.1	90.6	
	2	90.5		
	3	91.3		
100%	1	92.3	92.3	91.8%
	2	93.2		
	3	91.5		
150%	1	92.7	92.5	
	2	93.2		
	3	91.8		

Accuracy of impurity – B

Table No –7- Accuracy of impurity- B in Related substances method development

Level	Replicate	% Recovery	Mean	Total Mean
50%	1	91.8	91.9	
	2	92.6		
	3	91.3		
100%	1	93.1	92.4	92.2%
	2	92.6		
	3	91.5		
150%	1	93.2	92.3	
	2	92.6		
	3	91.2		

Accuracy of impurity – C

Table No– 8-Accuracy of impurity-C in Related substances method development

Level	Replicate	% Recovery	Mean	Total Mean
50%	1	93.2		
	2	92.1	92.5	
	3	92.3		
100%	1	92.5		
	2	91.5	92.0	92.0%
	3	92.2		
150%	1	91.3		
	2	92.5	91.7	
	3	91.5		

Accuracy of impurity – P

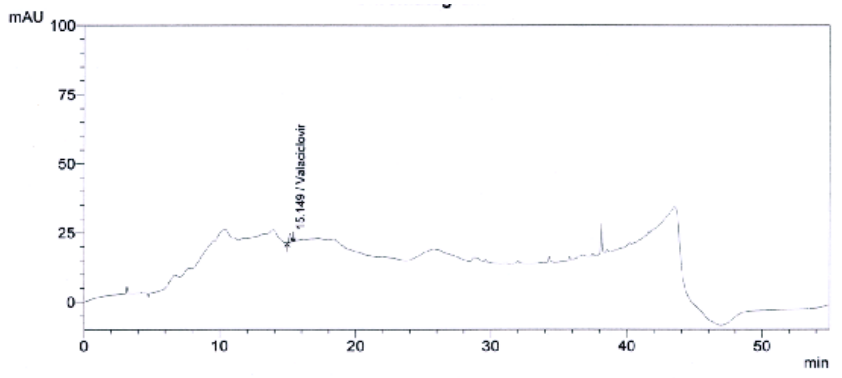
Table No– 9-Accuracy of impurity-P in Related substances method development

Level	Replicate	% Recovery	Mean	Total Mean
50%	1	92.5	92.1	91.9%
	2	91.3		
	3	92.5		
100%	1	91.3	92.3	
	2	92.5		
	3	93.1		
150%	1	91.6	91.4	
	2	91.5		
	3	91.3		

Acceptance criteria

The % recovery should be between 80.0%-102.0

Fig.No –1 Chromatogram of standard in Related substances method development



Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Name	Area	Area %
1	15.149	Valaciclovir	29424	100.00
Total			29424	100.00

Fig.No- 2– Chromatogram of blank in Related substances method development

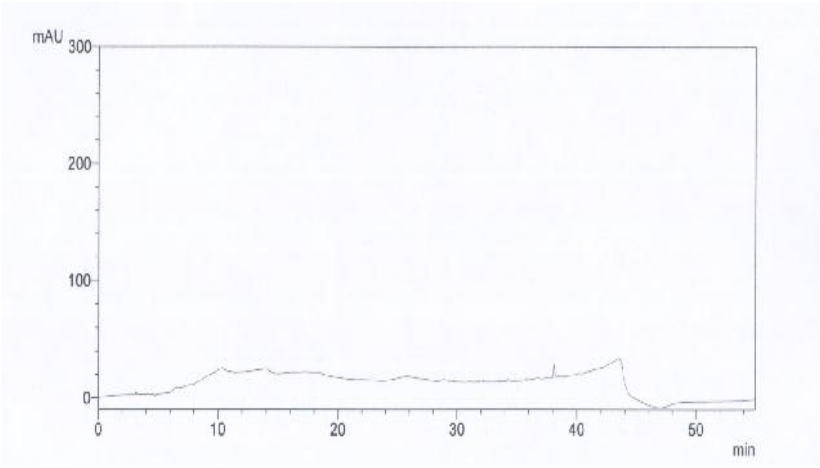


Fig.No-3– Chromatogram of placebo in Related substances method development

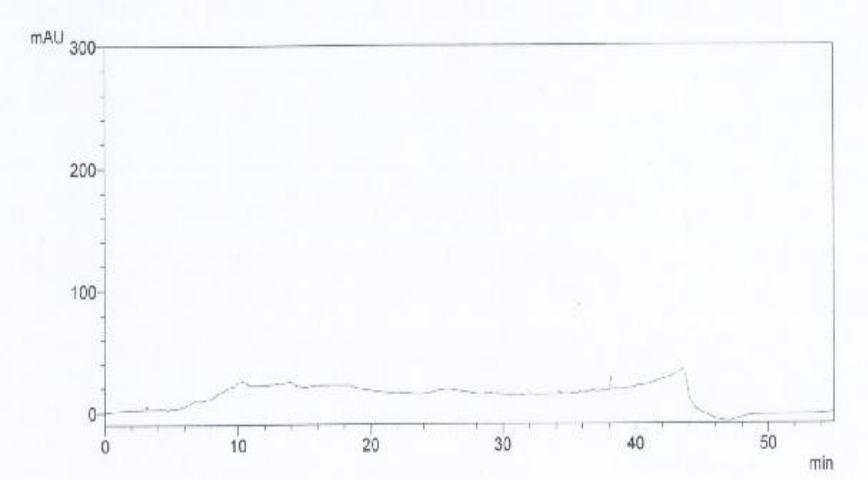
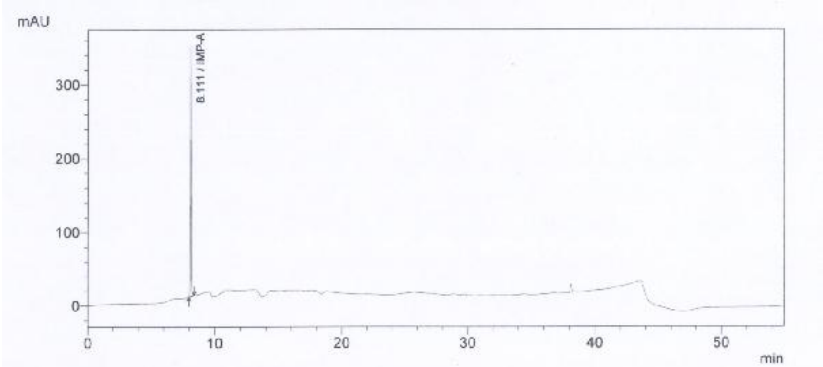


Fig.No- 4–Chromatogram of impurity-A in Related substances method development

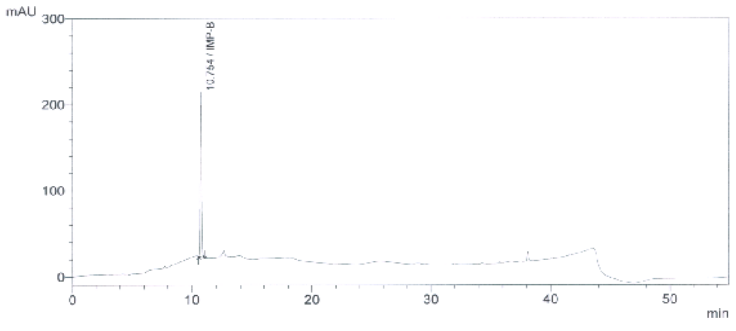


Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Name	Area	Area %
1	8.111	IMP-A	2033810	100.00
Total			2033810	100.00

Fig.No -5 Chromatogram of impurity - B in Related substances method development

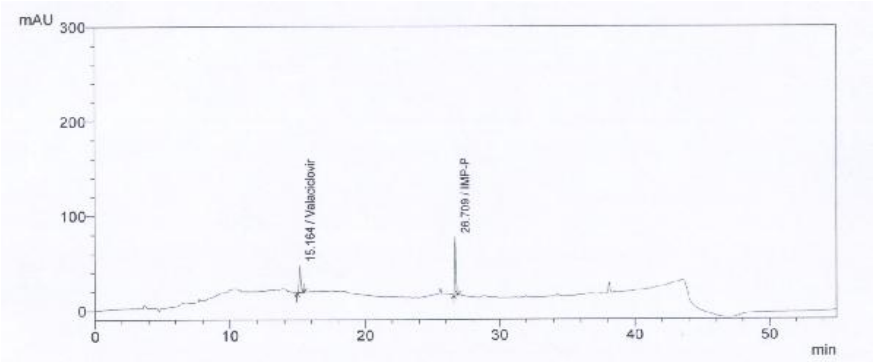


Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Name	Area	Area %
1	10.754	IMP-B	1510859	100.00
Total			1510859	100.00

Fig.No-6– Chromatogram of impurity - C in Related substances method development

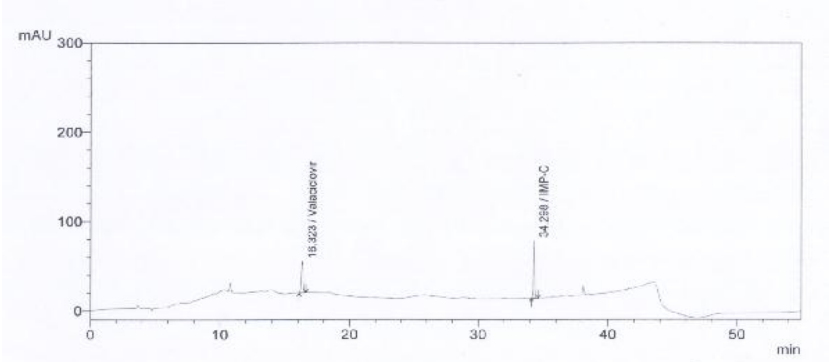


Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Name	Area	Area %
1	15.164	Valaciclovir	277486	43.65
2	26.709	IMP-P	358164	56.35
Total			635650	100.00

Fig.No- 7– Chromatogram of impurity - P in Related substances method development

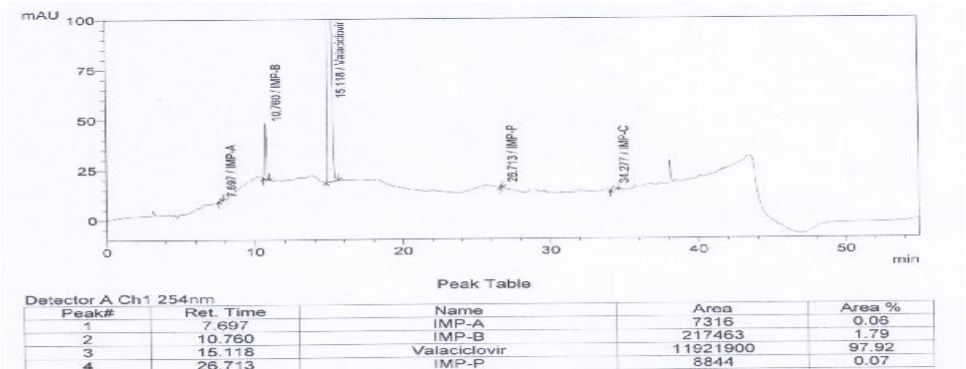


Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Name	Area	Area %
1	16.323	Valaciclovir	340742	44.56
2	34.298	IMP-C	424000	55.44
Total			764742	100.00

Fig.No–8 Chromatogram of spiked sample in Related substances method development



Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Name	Area	Area %
1	7.697	IMP-A	7316	0.06
2	10.760	IMP-B	217463	1.79
3	15.118	Valaciclovir	11921900	97.92
4	26.713	IMP-P	8844	0.07
5	34.277	IMP-C	20230	0.17
Total			12175753	100.00

The percent of impurity – A present in valacyclovir HCl is 0.0520 %, impurity B is 1.546 %, impurity – C is 0.143 % and impurity – P is 0.062 % calculated . According to Pharma Europa 2006 the

percent of impurity present in valacyclovir HCl is within the limits. The result of specificity shows that there is no interference due to blank and placebo and it is not more than 2.0%.

Conclusion

By taking into account of the factors such as economy and rapidity the present method can also be a good choice for the analysis of Valacyclovir Hcl. The developed method was found to be specific, precise, linear, accurate. LOQ values for all the known impurities were below reporting thresholds.

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