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CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION STABILITY-INDICATING FIVE IMPURITIES AND ITS DEGRADATION PRODUCTS IN VALGANCICLOVIR HYDROCHLORIDE POWDER FOR ORAL SOLUTION, 50 MG/ML

***[†]Naresh Chandra Reddy Male and [††]Kavitha Male**

[†]Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur, India.
[††]Richer Pharmaceuticals, IDA, Prasanthi Nagar, Kukatpally, Hyderabad, (A.P) India – 500072.
[†††]Department of Chemistry, College of Engineering, JNT University, Kukatpally, Hyderabad 500085, India.

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***Corresponding Author**
Naresh Chandra Reddy Male
Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur, India.

ABSTRACT

A Novel RPHPLC Quantification method was developed for estimation of Valganciclovir HCl known impurities like its Ganciclovir, Guanine, Bis Valine Ester, Methoxymethylguanine and Monoacetoxyganciclovir which, were separated on Zorbax SB C18, 150 mm x 4.6 mm, 3.5 µm. Using a mixture of Ammonium Phosphate Monobasic buffer and Methanol as a gradient mobile phase with a flow rate of 1.0 ml/min; λ max at 254 nm. The developed method was validated all the parameters like linearity, specificity, LOD, LOQ, accuracy, robustness, ruggedness , precision, filter variation, solution stability and forced degradation studies.

KEYWORDS: Method development and validation, Valganciclovir HCl, Related substances, Stability-indicating, Powder for oral suspension.

INTRODUCTION

Valganciclovir hydrochloride is an antiviral prescription medicine approved by the U.S. Food and Drug Administration (FDA) for the treatment of cytomegalovirus retinitis (CMV retinitis) in adults with AIDS. Valganciclovir hydrochloride is also FDA-approved for the

prevention of CMV disease in recipients of organ transplants who are at risk for CMV diseases.

CMV diseases, including CMV retinitis, can be opportunistic infections (OIs) of HIV. An OI is an infection that occurs more frequently or is more severe in people with weakened immune systems—such as people with HIV—than in people with healthy immune systems. Many techniques have been reported quantitave estimation including Spectrophotometric.[3-4] liquid chromatographic,[5-6] UPLC,[7] LC/MS method for human plasma.[8]

Since no method has been developed for the separation and estimation of impurities in Valganciclovir HCl powder for oral suspension and the drug is being marketed in domestic and international market the present study by the author describes a rapid, accurate and precise RP – HPLC method for the estimation of known related impurities, i.e., Ganciclovir, Guanine, Bis Valine Ester, Methoxymethylguanine and Monoacetoxyganciclovir and degrading products under stress conditions present in Valganciclovir HCl powder for oral suspension. The method was validated as per ICH guidelines.[9]

EXPERIMENTAL

MATERIAL AND METHODS

Chromatographic Conditions

Waters HPLC with 2489 and 2998 with Empower software connected with a Zorbax SB C18, 150 mm x 4.6 mm, 3.5 µm.

Chemicals and reagents

Valganciclovir HCl pure drug and impurities, Ammonium Phosphate Monobasic, Acetonitrile (HPLC Grade), water (HPLC Grade), Methanol(HPLC Grade), orthophosphoric acid 85% pure were AR grade from SD Fine Chem., was used in the present study. The tablet formulations purchased from local market Hyderabad, India.

Mobile phase

Weigh and transfer about 11.50 g of Ammonium Phosphate Monobasic into 900 mL of water and dissolve completely. Adjusted the pH to 2.8 ± 0.05 with Phosphoric Acid. Dilute with water to obtain 1000 mL of solution. Filter the solution through a 0.45 µm nylon membrane filter and degas at least for 15 minutes. Label as 0.1 M Ammonium Phosphate Buffer Solution.

Preparation of Mobile Phase A

Use 0.1M Ammonium Phosphate Buffer Solution as Mobile Phase A.

Preparation of Mobile Phase B

Use Methanol as Mobile Phase B.

Diluent

Pipet 1.0 mL of 1N Hydrochloric Acid Solution into 1000 mL of water, mix well and label as Diluent.

Standard solution preparation

Accurately weigh and transfer about 22.0 mg of Valganciclovir Hydrochloride standard into a 200-mL volumetric flask. Add about 100 mL of diluent and sonicate (if necessary) to dissolve. Dilute to the volume with diluent, mix well and label. The concentration is about 100 µg/mL of Valganciclovir.

Preparation of Standard Solution

Pipet 4.0 mL of Standard Stock Solution into a 100-mL volumetric flask, dilute to the volume with diluent, mix well and label. The concentration is about 4 µg/mL of Valganciclovir.

Filter the solution through a 0.45 µm Nylon or PVDF syringe filter, discard the first 5 mL of filtrate prior to collecting the standard solution into a HPLC vial for analysis.

Sample preparation

Pipet 1.0 mL of the Constituted Oral Solution Sample into a 50-mL volumetric flask. Dilute to the volume with diluent, mix well and label as Sample Stock Solution. The concentration is about 1000 µg/mL of Valganciclovir.

Pipet 5.0 mL of Sample Stock Solution into a 25-mL volumetric flask. Dilute to the volume with diluent, mix well and label as Sample Solution. The concentration is about 200 µg/mL of Valganciclovir.

Filter the solution through a 0.45 µm Nylon or PVDF syringe filter, discard the first 5 mL of filtrate prior to collecting the sample solution into a HPLC vial for analysis.

Impurities Calculation

$$\% \text{ of Impurity} = \frac{\text{Impurity Area x Standard weight x 1 x 100 x 1 x standard Potency}}{\text{Average Standard Area x 50 x 100 x sample weight x Label amount}} x\ RF$$

% of Total Impurities = Sum of % Individual impurities,

RF – Response Factor

RESULTS AND DISCUSSION

System Suitability

System suitability was evaluated from the standard solution preparation by injecting six times into the HPLC. The parameters measured were Theoretical plates, asymmetry, %RSD, the observed results asymmetry is about 1.8, theoretical plates about 65000, % RSD is 0.18 and the resolution between two peaks greater than 2.0 indicates the method suitable for related substances estimation.

Placebo and impurities interference

Interference from placebo and impurities was carried out by preparing the following specificity samples. Performed related substances on Placebo equivalent to the amount present in test preparation and injected into the chromatography. By preparing and inject impurities at 1.0 % of test concentration, by preparing active sample as per test concentration, by spiking the active sample with individual known impurities at 1.0% of test concentration. The above samples were injected and observed for any interference from blank and placebo at the retention time of analyte and known impurity peaks. This was further demonstrated by determining the peak purity of analyte and known impurity peaks. Since no interference of blank, placebo and known impurities was observed at the retention time of analyte. Individual impurity peaks are separated from the analyte peak. Peak purity of analyte peak and known impurity peaks are greater than 0.99, so the method is specific for Valganciclovir HCl powder for oral suspension.

Limit of Quantitation and Detection

The limit of quantitation (LOQ) and detection (LOD) were conducted on the basis of signal to noise ratio method. Different concentrations of impurities with sample solution were injected, LOQ established the values which give the signal to noise ratio about 10.0, for LOD of impurities were established which give the signal noise ratio about 3.0; the results of both LOQ & LOD values were tabulated in Table-2.

Linearity and Detector Response

The linearity of detector response for impurities was demonstrated by prepared solutions of Lacosamide and its impurities over the range of LOQ to 200% level and the detector response was found to be linear and the correlation coefficient was more than 0.998, proves Valganciclovir HCl and its impurities are linear, the results were tabulated in Table-3 and the chromatogram shown in (fig. 3).

Establishment of RRT's and RF Values for Impurities

The RRT's and RF values were calculated from the linearity levels of LOQ, 50%, 75%, 100%, 150% and 200%. The RRT's and RF values were calculated and the results were tabulated in Table-4.

Precision

Six sample preparations representing a single batch were injected, the each impurity area were determined and the precision was evaluated, the %RSD of each impurity results was less than 10.0 indicates the method is precise, the results are tabulated in Table-4.

Intermediate Precision

The ruggedness of the method was injected six preparations of a single batch sample by different analyst (analyst-2), different column (column-2) and different instrument (instrument-2). The %RSD of each impurity was calculated; the results were less than 10.0. consider the precision results for analyst-1, column-1 and system-1, the mean %RSD values of both precision and intermediate calculated, the results were less than 15.0 shows the method is rugged and the results were tabulated in Table-4.

Accuracy

The accuracy of the test method was prepared recovery samples (i.e. test sample with known quantities of known impurities) at the level of LOQ, 100% and 200% of target concentration, as the recovery results were found between 90 to 110% the method is accurate for the estimation of Valganciclovir HCl powder for oral suspension and its impurities over the range of LOQ to 200% level of target concentration and the results were tabulated in Table-5.

Table 1: HPLC Gradient Program.

Time (min)	Mobile phase A	Mobile phase B
0	92	8
5	92	8
15	80	20
30	30	70
42	92	8
57	92	8

Table 2: LOD & LOQ results.

S. No	Name of the Component	LOD RESULTS		LOQ RESULTS	
		S/N Ratio	% level of component w.r.t to sample concentration	S/N Ratio	% level of component w.r.t to sample concentration
1	Valganciclovir HCl	3.25	0.0051	10.52	0.1027
2	Ganciclovir	2.95	0.0020	11.22	0.0403
3	Guanine	3.10	0.0020	13.25	0.0398
4	Bis Valine Ester	3.56	0.0086	12.45	0.0855
5	Methoxymethylguanine	3.45	0.0019	10.65	0.0382
6	Monoacetoxyganciclovir	3.85	0.0039	10.78	0.0393

Table 3: Linearity Results.

Compound Name	Correlation coefficient	Slope	Y-Intercept	Residual sum square	Residual standard deviation
Valganciclovir HCl	1.0000	42717	1.20%	875865.3383	15124
Ganciclovir imppurity	1.0000	59839	0.02%	875865.3383	14582
Guanine impurity	1.0000	75171	-0.26%	600703.473	15842
Bis Valine Ester	0.9999	23345	-1.34%	187245.9927	16548
Methoxymethylguanine	0.9998	53137	-1.26%	366197.5139	15875
Monoacetoxyganciclovir	0.9999	43609	-0.26%	20191.49832	16854

Table 4: Precision, Intermediate Precision, RF and RRT Results.

Parameter	Ganciclovir	Guanine	BisValine Ester	Methoxymethylguanine	Monoacetoxyganciclovir
Precision	1.25	1.56	1.56	1.28	2.54
Intermediate precision	1.24	1.45	1.48	1.24	2.04
Mean	1.52	1.52	1.50	1.20	2.08
RRT	0.471	0.319	1.781	0.903	1.521
RF	1.37	1.70	0.52	1.16	0.98

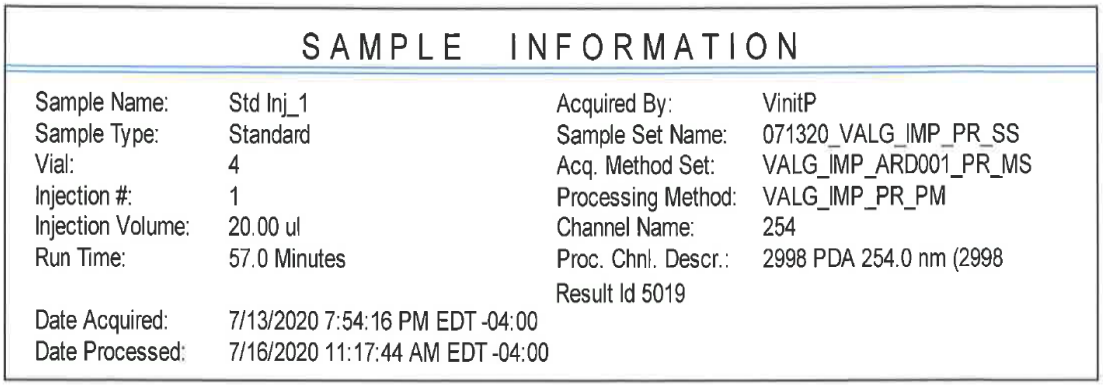
Table 5: Accuracy results.

Spike Level	***Amount added(ppm)***	***Mean Amount recovered(ppm)***	***% Mean Recovery***	***%RSD***
Recovery of Valganciclovir				
LOQ level	0.0999	0.1082	108.3	1.3
100%	3.9951	4.0679	101.8	0.2
200%	7.9902	8.0409	100.6	0.5
Recovery of Ganciclovir				
LOQ level	0.0401	0.0373	93.0	0.7
100%	4.0109	4.0582	101.2	0.1
200%	8.0218	8.0210	100.4	0.4
Recovery of Guanine				
LOQ level	0.0402	0.0402	100.0	0.8
100%	2.0114	2.0331	101.1	0.1
200%	4.0228	4.0624	101.0	0.3
Recovery of Bis Valine Ester				
LOQ level	0.0813	0.0837	103.0	1.5
100%	0.2034	0.2159	106.1	0.8
200%	0.4067	0.4355	107.1	0.7
Recovery of Methoxymethylguanine				
LOQ level	0.0376	0.0407	108.2	1.9
100%	0.6008	0.6583	109.6	0.8
200%	1.2017	1.3111	109.1	0.8
Recovery of Monoacetoxyganciclovir				
LOQ level	0.0389	0.0410	105.1	1.5
100%	0.3115	0.3158	101.2	0.2
200%	0.6230	0.6217	100.5	0.1

Table-6: Degradation Results.

VALGANCICLOVIR HCL POWDER FOR ORAL SUSPENSION						
Sample Name	**Condition**	**% Rec Based on Control**	**Total Impurities (%)**	**Mass Balance**	**Purity Angle**	**Purity Threshold**
Control	Not Stressed	100.0	0.614	100.6	0.105	0.248
					0.097	0.244
Acid Hydrolysis	5.0 mL of 0.5 N HCl, 80°C for 1 hour	92.7	5.967	98.7	0.097	0.253
					0.090	0.247
Base Hydrolysis	5.0 mL of 0.01N NaOH, 60°C for 1 hour	100.5	1.271	101.8	0.111	0.249
					0.097	0.242
Water Hydrolysis	5.0 mL of water, 60°C for 5 hours	101.2	0.763	102.0	0.118	0.255
					0.125	0.254
Oxidation	5.0 mL of 5% H_2O_2, Room Temperature for 6 hours	101.9	0.586	102.5	0.128	0.254
					0.128	0.250
UV/White	SUNTEST CPS+,	100.5	0.862	101.4	0.128	0.252

Light	Room Temperature for 8 hours*				0.115	0.250
Elevated Temperature	105°C for 3 days	100.1	0.584	100.7	0.117	0.253
					0.103	0.247



SAMPLE INFORMATION

Sample Name:	Std Inj_1	Acquired By:	VinitP
Sample Type:	Standard	Sample Set Name:	071320_VALG_IMP_PR_SS
Vial:	4	Acq. Method Set:	VALG_IMP_ARD001_PR_MS
Injection #:	1	Processing Method:	VALG_IMP_PR_PM
Injection Volume:	20.00 ul	Channel Name:	254
Run Time:	57.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 254.0 nm (2998
		Result Id 5019	
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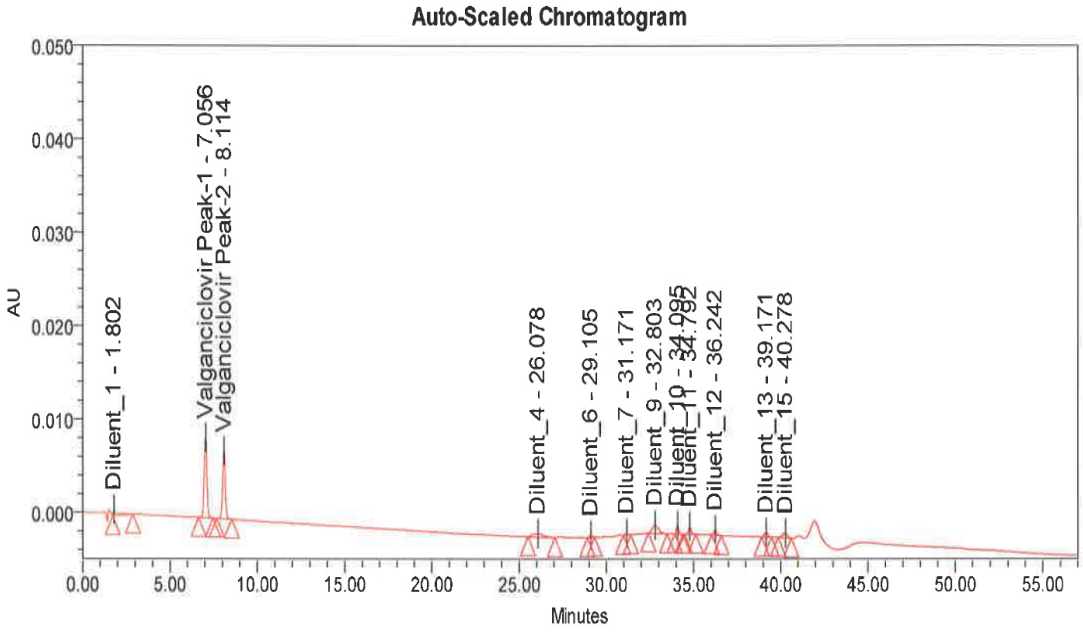
Date Processed:	7/16/2020 11:17:44 AM EDT -04:00		



Peak Results

	Name	RT	Area	Int Type	USP Plate Count	USP Tailing
1	Diluent_1	1.802	11896	BB	4235	9.2
2	Valganciclovir Peak-1	7.056	92081	BB	9961	1.1
3	Valganciclovir Peak-2	8.114	77968	BB	13185	1.1
4	Diluent_4	26.078	17560	BB	8869	1.4
5	Diluent_6	29.105	2282	BB	147178	1.0
6	Diluent_7	31.171	1789	BB	177967	1.2
7	Diluent_9	32.803	21616	BB	32846	1.3
8	Diluent_10	34.095	7949	BB	359393	1.3
9	Diluent_11	34.792	8791	BB	243991	1.2
10	Diluent_12	36.242	5810	BB	271740	1.5

Fig. 1: Representative Chromatogram of Standard Solution.

SAMPLE INFORMATION

Sample Name:	Diluent	Acquired By:	VinitP
Sample Type:	Control	Sample Set Name:	071320_VALG_IMP_PR_SS
Vial:	2	Acq. Method Set:	VALG_IMP_ARD001_PR_MS
Injection #:	1	Processing Method:	VALG_IMP_PR_PM
Injection Volume:	20.00 ul	Channel Name:	254
Run Time:	57.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 254.0 nm (2998
		Result Id 4882	
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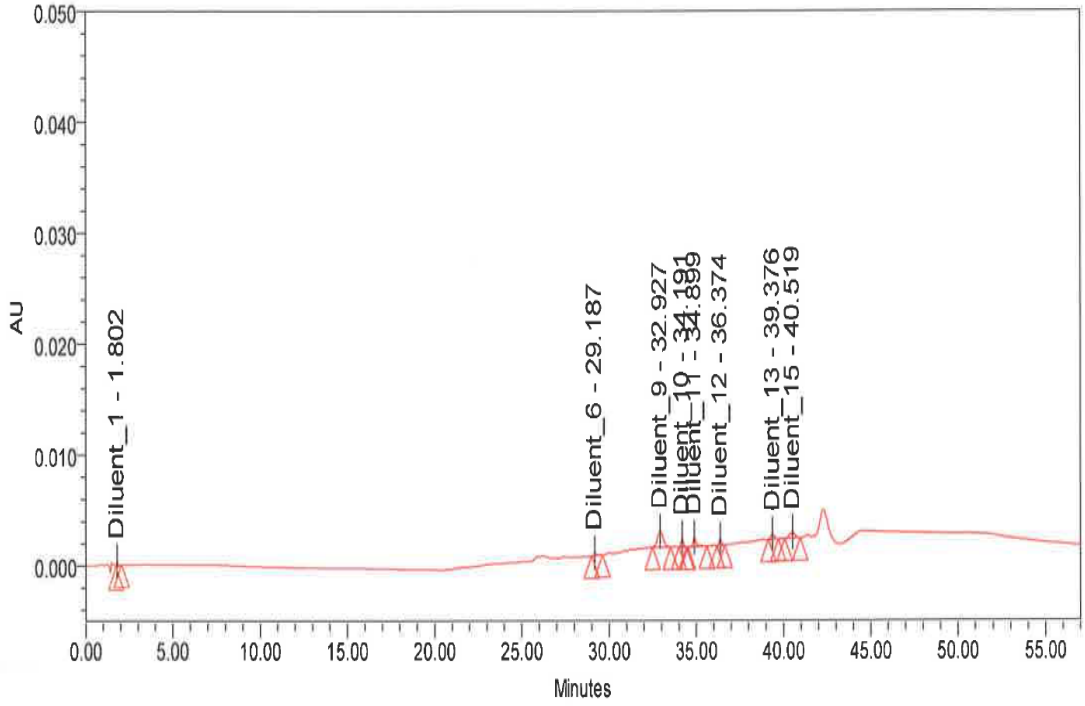
Date Processed:	7/16/2020 11:17:28 AM EDT -04:00		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Int Type	USP Plate Count	USP Tailing
1	Diluent_1	1.802	3063	BB	4791	1.2
2	Diluent_6	29.187	3082	BB	155086	1.0
3	Diluent_9	32.927	32711	BB	49226	1.2
4	Diluent_10	34.191	6714	BB	343001	1.3
5	Diluent_11	34.899	10726	BB	247810	1.9
6	Diluent_12	36.374	5361	BB	292992	1.1
7	Diluent_13	39.376	6180	BB	182619	1.0
8	Diluent_15	40.519	10602	BB	85377	0.9

Fig. 2: Representative Chromatogram of Diluent.

SAMPLE INFORMATION

Sample Name:	Repeatability_Control	Acquired By:	VinitP
Sample Type:	Standard	Sample Set Name:	071320_VALG_IMP_PR_SS
Vial:	10	Acq. Method Set:	VALG_IMP_ARD001_PR_MS
Injection #:	1	Processing Method:	VALG_IMP_PR_PM
Injection Volume:	20.00 ul	Channel Name:	254
Run Time:	57.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 254.0 nm (2998
		Result Id 4915	
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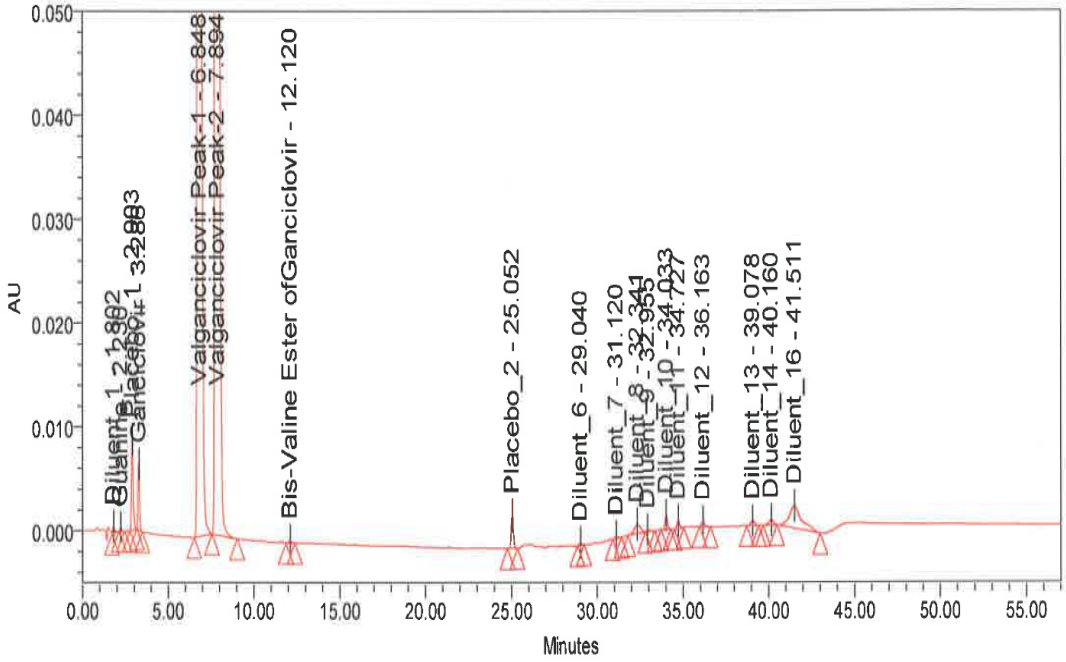
Date Processed:	7/16/2020 11:17:29 AM EDT -04:00		

Auto-Scaled Chromatogram



Peak Results

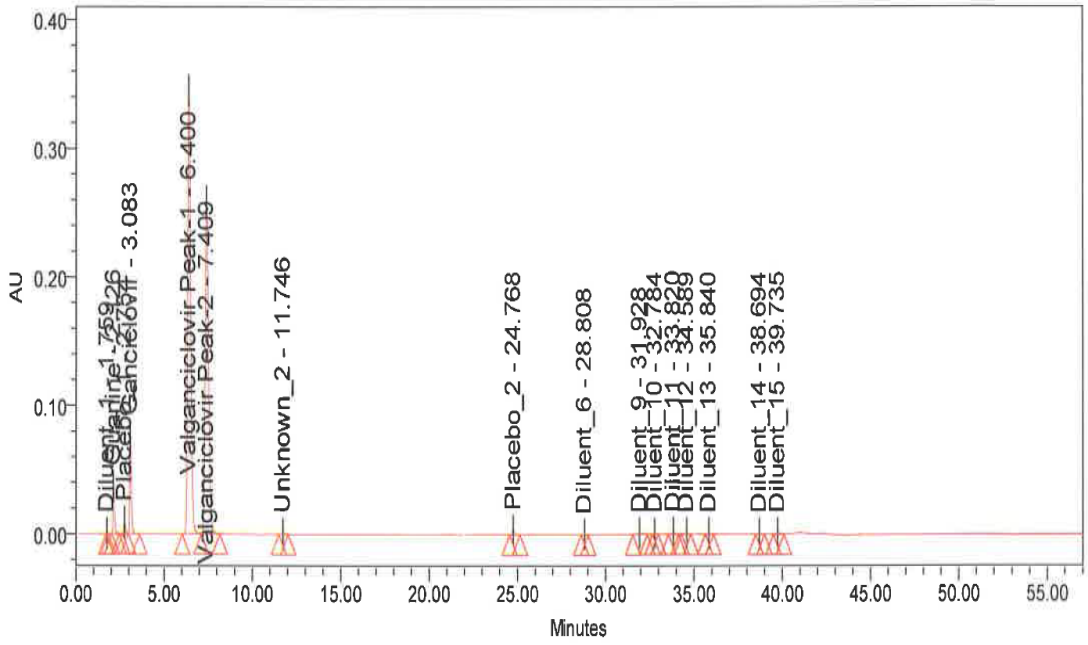
	Name	RT	Area	Int Type	USP Plate Count	USP Tailing
1	Diluent_1	1.802	4919	BB	4394	1.9
2	Guanine	2.230	1915	BB	4574	1.5
3	Placebo_1	2.903	61418	BB	4248	1.4
4	Ganciclovir	3.286	38859	BB	6613	1.2
5	Valganciclovir Peak-1	6.848	4664689	BB	7547	1.2
6	Valganciclovir Peak-2	7.894	3913854	BB	8955	1.2
7	Bis-Valine Ester ofGanciclovir	12.120	2411	BB	39365	1.4
8	Placebo_2	25.052	27084	BB	197278	1.2
9	Diluent_6	29.040	2164	BB	149288	1.1
10	Diluent_7	31.120	1807	BB	209294	0.7

Fig. 3: Representative Chromatogram of Sample Solution.

SAMPLE INFORMATION

Sample Name:	Sample Acid 0.5N HCL 80°C@1Hr	Acquired By:	NehareddyC
Sample Type:	Unknown	Sample Set Name:	071320_VALG_IMP_SIS_SS
Vial:	11	Acq. Method Set:	VALG_IMP_ARD003_MS
Injection #:	1	Processing Method:	VALG_IMP_PM
Injection Volume:	20.00 ul	Channel Name:	254 nm
Run Time:	57.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 254.0 nm (2998
		Result Id 5828	
Date Acquired:	7/14/2020 2:50:05 AM EDT -04:00	Processing Method Id 5593	
Date Processed:	7/17/2020 10:57:54 AM EDT -04:00		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Int Type
1	Diluent_1	1.759	1950	BB
2	Guanine	2.126	202555	BB
3	Placebo_1	2.754	58705	BB
4	Ganciclovir	3.083	491659	BB
5	Valganciclovir Peak-1	6.400	4111507	BB
6	Valganciclovir Peak-2	7.409	3492186	BB
7	Unknow n_2	11.746	4525	BB
8	Placebo_2	24.768	25501	BB
9	Diluent_6	28.808	2057	BB
10	Diluent_9	31.928	14317	BB
11	Diluent_10	32.784	1628	BB

	Name	RT	Area	Int Type
12	Diluent_11	33.820	12100	BB
13	Diluent_12	34.589	5974	BB
14	Diluent_13	35.840	5251	BB
15	Diluent_14	38.694	4061	BB
16	Diluent_15	39.735	9324	BB

Fig. 4: Representative Chromatogram of Acid Hydrolysis Sample Solution.

SAMPLE INFORMATION

Sample Name:	Sample Base_0.01N NaOH	Acquired By:	NehareddyC
Sample Type:	Unknown	Sample Set Name:	071320_VALG_IMP_SIS_SS
Vial:	12	Acq. Method Set:	VALG_IMP_ARD003_MS
Injection #:	1	Processing Method:	VALG_IMP_PM
Injection Volume:	20.00 ul	Channel Name:	254 nm
Run Time:	57.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 254.0 nm (2998
		Result Id 5829	
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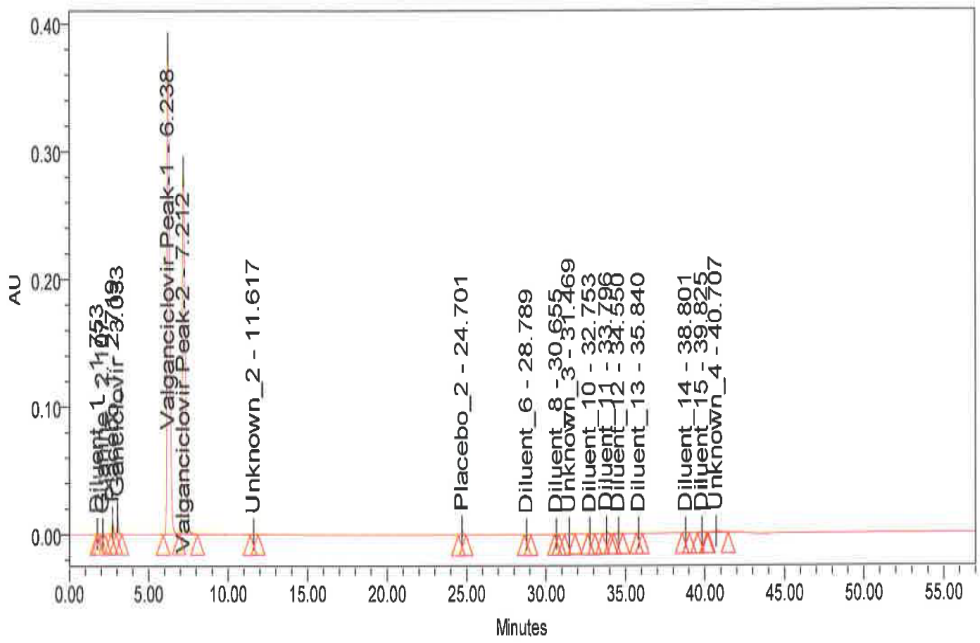
Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Int Type
1	Diluent_1	1.753	1878	BB
2	Guanine	2.107	1956	BB
3	Placebo_1	2.719	58965	BB
4	Ganciclovir	3.033	84705	BB
5	Valganciclovir Peak-1	6.238	4474560	BB
6	Valganciclovir Peak-2	7.212	3773304	BB
7	Unknown_2	11.617	2837	BB
8	Placebo_2	24.701	24843	BB
9	Diluent_6	28.789	2124	BB
10	Diluent_8	30.655	1538	BB
11	Unknown_3	31.469	7016	BB

	Name	RT	Area	Int Type
12	Diluent_10	32.753	2803	BB
13	Diluent_11	33.796	12271	BB
14	Diluent_12	34.550	6616	BB
15	Diluent_13	35.840	4768	BB
16	Diluent_14	38.801	4074	BB
17	Diluent_15	39.825	8512	BB
18	Unknown_4	40.707	32075	BB

Fig. 5: Representative Chromatogram of Base Hydrolysis Sample Solution.

SAMPLE INFORMATION

Sample Name:	Sample Water 60°C @ 5Hr	Acquired By:	NehareddyC
Sample Type:	Unknown	Sample Set Name:	071320_VALG_IMP_SIS_SS
Vial:	13	Acq. Method Set:	VALG_IMP_ARD003_MS
Injection #:	1	Processing Method:	VALG_IMP_PM
Injection Volume:	20.00 ul	Channel Name:	254 nm
Run Time:	57.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 254.0 nm (2998
		Result Id 5830	
Date Acquired:	7/14/2020 4:45:51 AM EDT -04:00	Processing Method Id 5593	
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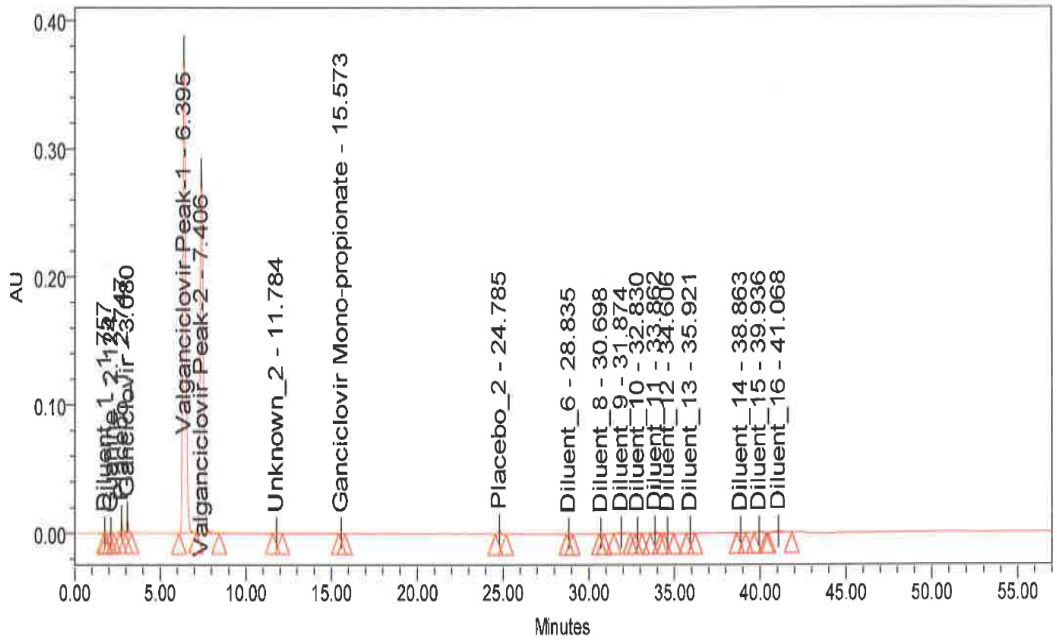
Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Int Type
1	Diluent_1	1.757	1905	BB
2	Guanine	2.124	2000	BB
3	Placebo_1	2.747	59893	BB
4	Ganciclovir	3.080	77482	BB
5	Valganciclovir Peak-1	6.395	4506303	BB
6	Valganciclovir Peak-2	7.406	3793528	BB
7	Unknown_2	11.784	2903	BB
8	Ganciclovir Mono-propionate	15.573	1305	BB
9	Placebo_2	24.785	25467	BB
10	Diluent_6	28.835	2505	BB
11	Diluent_8	30.698	1341	BB

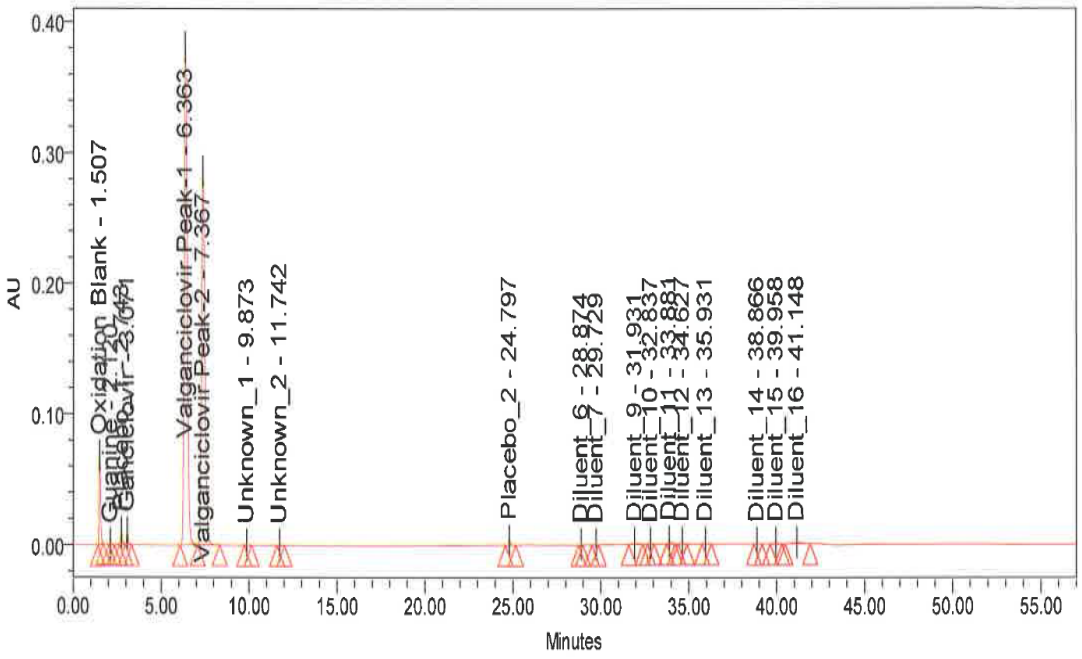
	Name	RT	Area	Int Type
12	Diluent_9	31.874	15000	BB
13	Diluent_10	32.830	2108	BB
14	Diluent_11	33.862	13067	BB
15	Diluent_12	34.606	6040	BB
16	Diluent_13	35.921	5852	BB
17	Diluent_14	38.863	4268	BB
18	Diluent_15	39.936	9333	BB
19	Diluent_16	41.068	34691	BB

Fig. 6: Representative Chromatogram of Water Hydrolysis Sample Solution.

SAMPLE INFORMATION

Sample Name:	Sample Oxidation_5%H2O2	Acquired By:	NehareddyC
Sample Type:	Unknown	Sample Set Name:	071320_VALG_IMP_SIS_SS
Vial:	10	Acq. Method Set:	VALG_IMP_ARD003_MS
Injection #:	1	Processing Method:	VALG_IMP_PM
Injection Volume:	20.00 ul	Channel Name:	254 nm
Run Time:	57.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 254.0 nm (2998
		Result Id 5827	
Date Acquired:	7/14/2020 1:52:14 AM EDT -04:00	Processing Method Id 5593	
Date Processed:	7/17/2020 10:57:54 AM EDT -04:00		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Int Type
1	Oxidation Blank	1.507	364452	BB
2	Guanine	2.120	2334	BB
3	Placebo_1	2.743	60261	BB
4	Ganciclovir	3.071	56008	BB
5	Valganciclovir Peak-1	6.363	4547315	BB
6	Valganciclovir Peak-2	7.367	3812664	BB
7	Unknown_1	9.873	2940	BB
8	Unknown_2	11.742	2482	BB
9	Placebo_2	24.797	26010	BB
10	Diluent_6	28.874	1424	BB
11	Diluent_7	29.729	1645	BB

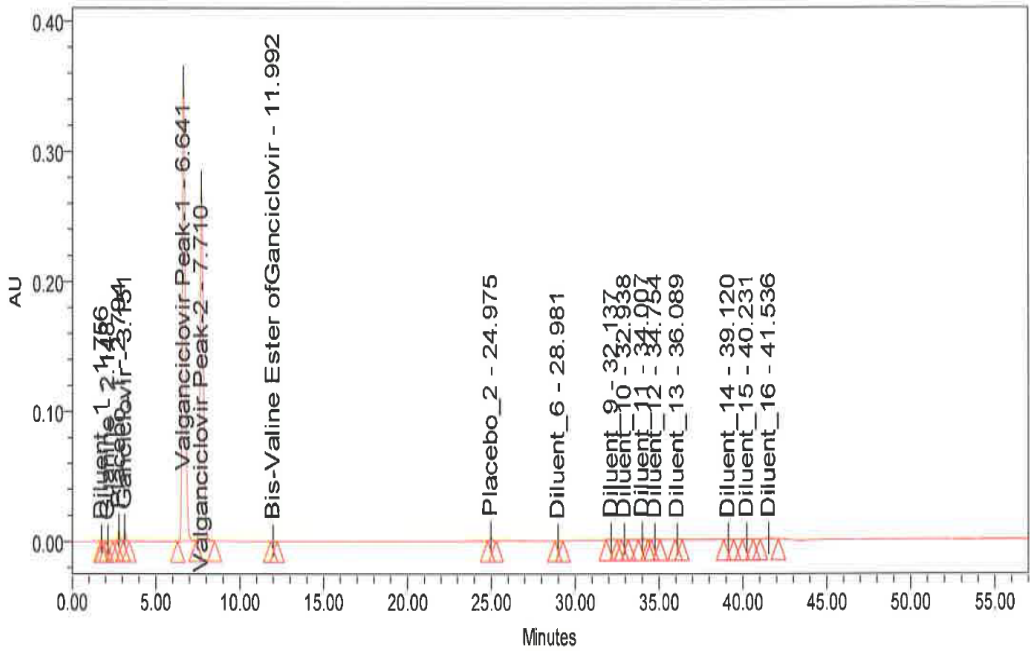
	Name	RT	Area	Int Type
12	Diluent_9	31.931	13817	BB
13	Diluent_10	32.837	1670	BB
14	Diluent_11	33.881	13351	BB
15	Diluent_12	34.627	5709	BB
16	Diluent_13	35.931	4916	BB
17	Diluent_14	38.866	4200	BB
18	Diluent_15	39.958	9821	BB
19	Diluent_16	41.148	35009	BB

Fig. 7: Representative Chromatogram of Oxidation Sample Solution.

SAMPLE INFORMATION

Sample Name:	Sample Temp 105°C for 3 days	Acquired By:	NehareddyC
Sample Type:	Unknown	Sample Set Name:	071320_VALG_IMP_SIS_SS
Vial:	14	Acq. Method Set:	VALG_IMP_ARD003_MS
Injection #:	1	Processing Method:	VALG_IMP_PM
Injection Volume:	20.00 ul	Channel Name:	254 nm
Run Time:	57.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 254.0 nm (2998
		Result Id 5831	
Date Acquired:	7/14/2020 5:43:41 AM EDT -04:00	Processing Method Id 5593	
Date Processed:	7/17/2020 10:57:55 AM EDT -04:00		

Auto-Scaled Chromatogram

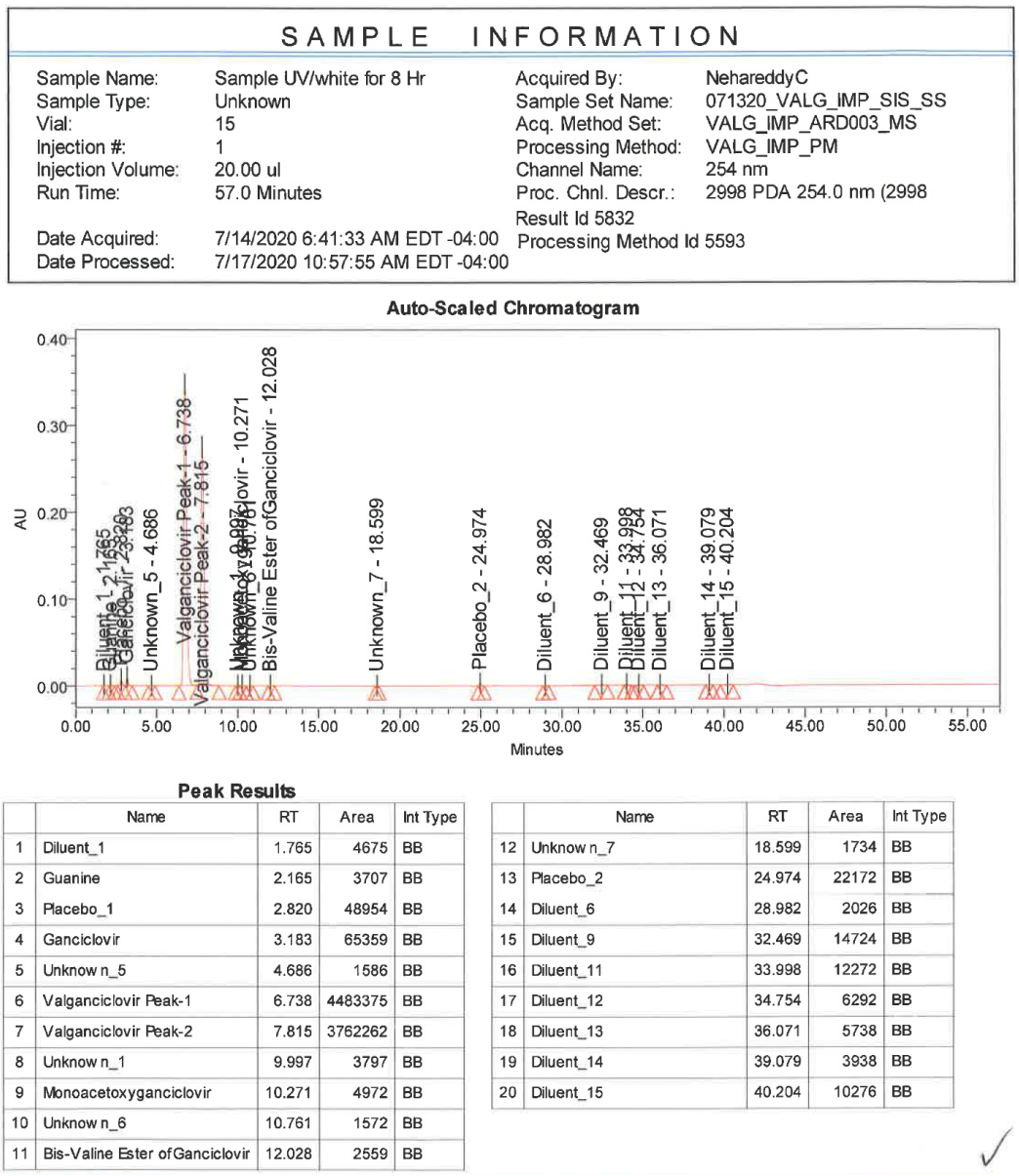


Peak Results

	Name	RT	Area	Int Type
1	Diluent_1	1.756	1801	BB
2	Guanine	2.148	2308	BB
3	Placebo_1	2.794	59006	BB
4	Ganciclovir	3.151	54647	BB
5	Valganciclovir Peak-1	6.641	4465761	BB
6	Valganciclovir Peak-2	7.710	3743137	BB
7	Bis-Valine Ester ofGanciclovir	11.992	2200	BB
8	Placebo_2	24.975	24438	BB
9	Diluent_6	28.981	2637	BB
10	Diluent_9	32.137	9918	BB
11	Diluent_10	32.938	1966	BB

	Name	RT	Area	Int Type
12	Diluent_11	34.007	12758	BB
13	Diluent_12	34.754	5858	BB
14	Diluent_13	36.089	4299	BB
15	Diluent_14	39.120	4629	BB
16	Diluent_15	40.231	8983	BB
17	Diluent_16	41.536	21200	BB

Fig. 8: Representative Chromatogram of 105°C Sample Solution



SAMPLE INFORMATION

Sample Name:	Sample UV/white for 8 Hr	Acquired By:	NehareddyC
Sample Type:	Unknown	Sample Set Name:	071320_VALG_IMP_SIS_SS
Vial:	15	Acq. Method Set:	VALG_IMP_ARD003_MS
Injection #:	1	Processing Method:	VALG_IMP_PM
Injection Volume:	20.00 ul	Channel Name:	254 nm
Run Time:	57.0 Minutes	Proc. Chnl. Descr.:	2998 PDA 254.0 nm (2998
		Result Id 5832	
Date Acquired:	7/14/2020 6:41:33 AM EDT -04:00	Processing Method Id 5593	
Date Processed:	7/17/2020 10:57:55 AM EDT -04:00		

Peak Results

	Name	RT	Area	Int Type
1	Diluent_1	1.765	4675	BB
2	Guanine	2.165	3707	BB
3	Placebo_1	2.820	48954	BB
4	Ganciclovir	3.183	65359	BB
5	Unknown_5	4.686	1586	BB
6	Valganciclovir Peak-1	6.738	4483375	BB
7	Valganciclovir Peak-2	7.815	3762262	BB
8	Unknown_1	9.997	3797	BB
9	Monoacetoxyganciclovir	10.271	4972	BB
10	Unknown_6	10.761	1572	BB
11	Bis-Valine Ester ofGanciclovir	12.028	2559	BB

	Name	RT	Area	Int Type
12	Unknown_7	18.599	1734	BB
13	Placebo_2	24.974	22172	BB
14	Diluent_6	28.982	2026	BB
15	Diluent_9	32.469	14724	BB
16	Diluent_11	33.998	12272	BB
17	Diluent_12	34.754	6292	BB
18	Diluent_13	36.071	5738	BB
19	Diluent_14	39.079	3938	BB
20	Diluent_15	40.204	10276	BB

Fig. 9: Representative Chromatogram of UV/White Light Sample Solution.

Robustness

The solution stability & mobile phase stability

The standard and sample solution kept for bench top, under refrigerator were injected initially, after 24 hours and 48 hours. The difference between initial, 24hrs and 48hrs of individual impurity less than 0.03% and total impurities less than 0.1% and the similarity factor after 24 hours and after 48 hours is between 0.95 to 1.05 indicates the solution is stable up to 48hrs and the results were tabulated in Table 8. for mobile phase stability the standard and sample solutions injected initially, after 24 hours and after 48 hours, a slight variation of parameters like theoretical plates, asymmetry and % RSD indicates the mobile phase is stable up to 48 hours.

Extraction time of analyte

The difference between as such condition and different extraction samples for % of individual impurity less than 0.03% and % of total impurities 0.1% found within the limits.

Filter variation

The filter variation was injected the test solution of centrifuged and filtered through 0.22µ nylon filter 0.45µ nylon filter and 0.22µ PVDF, 0.45µ PVDF filter and the difference between filtered portions of individual impurity less than 0.03% and total impurities were less than 0.1% with respect to centrifuged sample shows no effect of filter variation.

Effect of Column Temperature and Flow Variation

The standard preparation was injected under normal condition (i.e. as such condition) and of the altered conditions column temperature 35±5°C and flow rate 1±0.1ml the difference between as such for all changed conditions parameters like theoretical plates, asymmetry and % RSD within the limits proves the method is robust.

Forced Degradation Studies

Acid Hydrolysis Stress study

Pipetted 2.0 mL of Constituted Oral Solution Sample or pipetted 2.0 mL of Constituted Placebo Oral Solution Sample into a separate 100-mL volumetric flask. Pipetted 5.0 mL of 0.5N Hydrochloric Acid Solution into the flask, mixed well and tightly closed the flask. Kept the solution under 80°C for 1 hour.

After 1 hour of acid hydrolysis, pipetted 5.0 mL of 0.5N Sodium Hydroxide solution into the flask to neutralize the solution make up with diuent, Filtrate the solution through 0.45 micron nylon filter and injected into the chromatography and the chromatogram shown in (fig. 4a).

Base Hydrolysis Stress Study

Pipetted 2.0 mL of Constituted Oral Solution Sample or pipetted 2.0 mL of Constituted Placebo Oral Solution Sample into a separate 100-mL volumetric flask.

Pipetted 5.0 mL of 0.01N Sodium Hydroxide Solution into the flask, mixed well and tightly closed the flask. Kept the solution under 60°C for 1 hour.

After 1 hour of base hydrolysis, pipetted 5.0 mL of 0.01N Hydrochloric Acid solution into the flask to neutralize the solution and make up with diluent. Filtrate the solution through 0.45 micron nylon filter and injected into the chromatography.

Peroxide Oxidation Stress Study

Pipetted 2.0 mL of Constituted Oral Solution Sample or pipetted 2.0 mL of Constituted Placebo Oral Solution Sample into a separate 100-mL volumetric flask. Pipetted 5.0 mL of 5% Hydrogen Peroxide into the flask, mixed well and tightly closed the flask. Kept the solution under room temperature condition for 6 hours. After 6 hours of oxidation make up with diluent. Filtrate the solution through 0.45 micron nylon filter and injected into the chromatography. The homogeneity of the peak is pure, and the chromatogram shown in (fig. 4c).

Water degradation Stress study

Pipetted 2.0 mL of Constituted Oral Solution Sample or pipetted 2.0 mL of Constituted Placebo Oral Solution Sample into a separate 100-mL volumetric flask.

Pipetted 5.0 mL of water into the flask, mixed well and tightly closed the flask. Kept the solution under 60°C for 5 hours. After 5 hours of water hydrolysis and make up with diluent. Filtrate the solution through 0.45 micron nylon filter and injected into the chromatography.

Heat Stress Study

Pipetted 2.0 mL of Constituted Oral Solution Sample or pipetted 2.0 mL of Constituted Placebo Oral Solution Sample into a separate 100-mL volumetric flask. Tightly closed the flasks. Kept the flasks under 105°C for 3 days. After exposure under 105°C for 3 days and make up with diluent. Filtrate the solution through 0.45 micron nylon filter and injected into the chromatography. The homogeneity of the peak is pure.

Photolytic Stress study

UV Light

Pipetted 2.0 mL of Constituted Oral Solution Sample or pipetted 2.0 mL of Constituted Placebo Oral Solution Sample into a separate quartz culture dish with cover. Kept the dishes under UV/White light (SUNTEST CPS+) for 8 hours. After exposure under UV/White light, with aid of the diluent, transferred Constituted Oral Solution Sample or Constituted Placebo Oral Solution Sampleinto a separate 100-mL volumetric flask make up with diluent. Filtrate

the solution through 0.45 micron nylon filter and injected into the chromatography. The homogeneity of the peak is pure.

CONCLUSIONS

The proposed RP-HPLC method satisfies the parameters like system suitability, specificity, precision, accuracy, linearity, and robustness, ruggedness. The obtained results from the validation as per the ICH guidelines and drug stability were indicates this method is accurate, sensitive and best suitable Method for determination of known and unknown impurities in Valganciclovir HCl regular laboratory analysis.

ACKNOWLEDGEMENTS

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