



DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UPLC METHOD FOR THE DETERMINATION OF RELATED SUBSTANCES IN VALACYCLOVIR HCL DRUG SUBSTANCE

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Article Received on 15/11/2018

Article Revised on 05/12/2018

Article Accepted on 26/12/2018

ABSTRACT

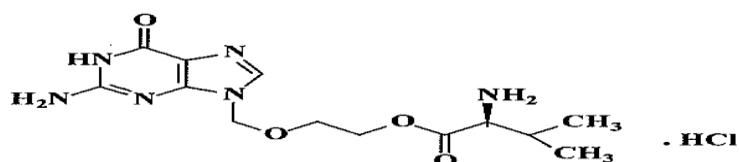
Method development, validation and stability indicating RP-UPLC method for the determination of Valacyclovir HCl and its related substances in pharmaceutical products. This method has high degree of performance to separation and quantification of Valacyclovir HCl in the presence of its related substances. This efficient Separation can be achieved by using new Waters Acquity CSH Phenyl hexyl with dimensions (100mm x 2.1 mm, 1.7µm). The buffer used in this method Trifluoro Acetic Acid with a flow rate of 0.3 ml/min and the absorbance was monitored at 254 nm. The total run time was 10.0 min. The correlation coefficient of the method shows good linear relationship with 0.998 to 0.999 for all related substances. The limit of detection and quantification are determined for Valacyclovir HCl related substances are 0.01µg/ml and 0.03µg/ml. The signal to noise ratio has been observed about 3 and 10 for LOD & LOQ for all related substances. The precision of the method %RSD is less than 6.4% and the % recovery of Valacyclovir HCl and its related substances is between 80.1 – 116 %. When the drug is subjected to different stress conditions and the resulting degradation products obtained were not interfere during the determination of Valacyclovir HCl and its related substances.

KEYWORDS: Valacyclovir HCl, RP-UPLC, Method development, Validation, Stability-indicating.

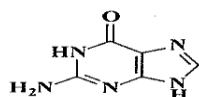
1.0. INTRODUCTION

Valacyclovir^[1-3] is l-valyl ester pro-drug of the antiviral drug acyclovir. The chemical name of valacyclovir is "l-valine-2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]ethyl ester" and the chemical structure of Valacyclovir HCl and its related substances are shown in Figure 1. Acyclovir is selective inhibitor for herpes viral replication and moderately soluble with limited oral bioavailability (~20%) this inversely proportional with dosage. Valacyclovir has high bioavailability about four times higher than acyclovir, and quickly hydrolysed to acyclovir with oral administration observed. Its transport mechanism reveals, high bioavailability is because of intestinal peptide transporter. Valacyclovir is quickly converted to acyclovir and its antiviral activity to herpes simplex virus type 1 and type 2 and varicella zoster virus both in vitro and in vivo. Drug absorption and pharmacokinetics improve by the action of pro-drugs. Valacyclovir better absorbed than acyclovir in the gastrointestinal tract than the parent drug, and is cleaved by enzymatic reaction in to valine an amino acid and acyclovir. There have been reports dealing with the determination of valacyclovir based on

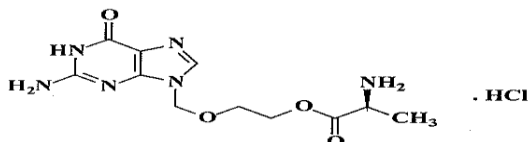
spectrophotometric analysis^[4-10], high performance liquid chromatography^[11-24], high performance liquid chromatography with mass spectrometry.^[25-27] The increasing interest from regulatory authorities for possible potential impurities are more concern a number of sophisticated analytical techniques such as HPLC and LC-MS methods are available for estimation of related substances in Valacyclovir HCl drug substances and drug products. However there is no UPLC method describing the determination of related substances in Valacyclovir HCl drug substances. UPLC methods are more sensitive than HPLC because of the holistic design of the system, at low volume injections of samples with corresponds to HPLC. Hence the aim of the present work is development and validation^[28] of simple, accurate and precise reversed phase UPLC method for determination of related substances in Valacyclovir HCl.



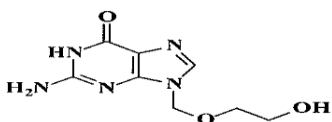
2-[(2-Amino-1, 6-Dihydro-6-Oxo-9h-Purin-9-Yl) Methoxy] Ethyl-L-Valinate Hydrochloride [Valacyclovir Hcl]



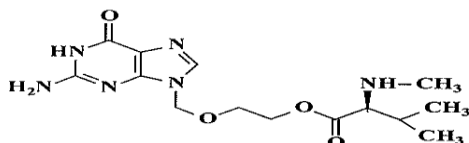
2-AMINO-1,9-DIHYDRO-6H-PURIN-6-ONE [GUANINE]



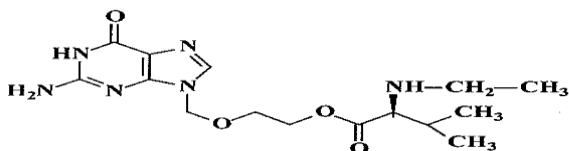
2-[(2-AMINO-1,6-DIHYDRO-6-OXO-9H-PURIN-9-YL)METHOXY]ETHYL-L-ALANINATE HYDROCHLORIDE [ALANINE ANALOG OF VALACYCLOVIR]



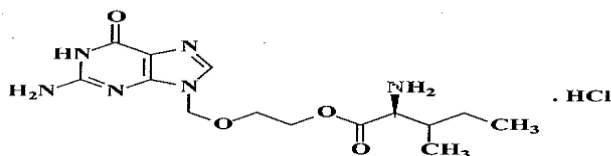
2-AMINO-1,9-DIHYDRO-9-[(2-HYDROXYETHOXY)METHYL]-6H-PURIN-6-ONE [ACYCLOVIR]



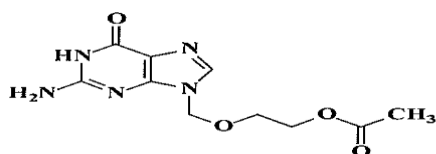
2-[(2-AMINO-6-OXO-1,6-DIHYDRO-9H-PURIN-9-YL)METHOXY]ETHOXY-N-METHYL-L-VALINATE [N-METHYL VALACYCLOVIR]



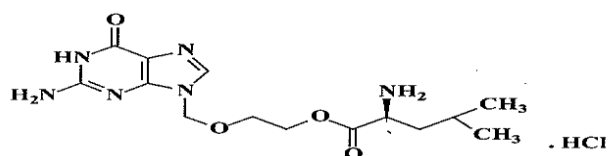
2-[(2-AMINO-6-OXO-1,6-DIHYDRO-9H-PURIN-9-YL)METHOXY]ETHOXY-N-ETHYL-L-VALINATE [N-ETHYL VALACYCLOVIR]



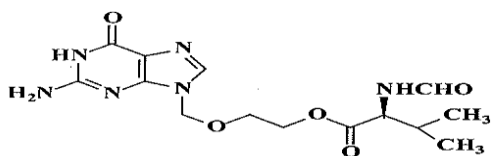
2-[(2-AMINO-1,6-DIHYDRO-6-OXO-9H-PURIN-9-YL)METHOXY]ETHYL-L-ISOLEUCINATE HYDROCHLORIDE [ISOLEUCINE ANALOG OF VALACYCLOVIR]



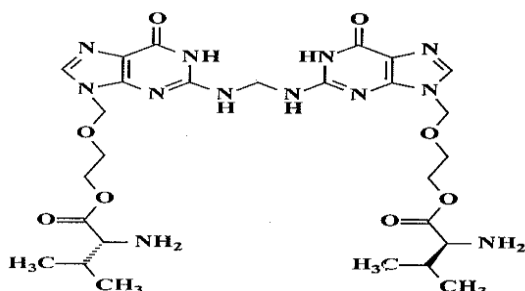
2-[[2-AMINO-1,6-DIHYDRO-6-OXO-9H-PURIN-9-YL)METHOXY]ETHYL ACETATE [O-ACETYLACYCLOVIR]



2-[(2-AMINO-1,6-DIHYDRO-6-OXO-9H-PURIN-9-YL)METHOXY]ETHYL-L-LEUCINATE HYDROCHLORIDE [LEUCINE ANALOG OF VALACYCLOVIR]



2-[(2-AMINO-6-OXO-1,6-DIHYDRO-9H-PURIN-9-YL)METHOXY] ETHOXY-N-FORMYL-L-VALINATE [N-FORMYL VALACYCLOVIR]



2,2'-(METHANEDIYLDIIMINO) BIS[[(6-OXO-1,6-DIHYDRO-9H-PURIN-9-YL)METHOXY]ETHYL L-VALINATE] [VALACYCLOVIR METHYLENE DIMER]

Fig. 1.

Table: 1.

Product Name	Valacyclovir Hydrochloride
Chemical Name	2-[(2-amino-1, 6-dihydro-6-oxo-9H-purin-9-yl) methoxy] ethyl-L-valinate hydrochloride
CAS Reg.No:	[124832-27-5]
Molecular Formula	C ₁₃ H ₂₀ N ₆ O ₄ .HCl
Molecular Weight	360.8

2.0. EXPERIMENTAL

2.1. Chemicals and reagents

Samples of Valacyclovir HCl were contributed from Aurobindo pharma Ltd, Pydibhimavaram, India. HPLC grade Acetonitrile, Methanol, Ethanol, AR grade Trifluoro Acetic Acid, Ammonium dihydrogen orthophosphate and Orthophosphoric Acid were purchased from Merck India Ltd, Mumbai. High pure water was prepared by using Millipore Milli-Q water purification system. New Waters Acquity CSH Phenyl hexyl (100 x 2.1 mm, 1.7 µm) column (Part no. # 186005407), waters Acquity BEH (50mm×2.1mm, 1.7µm) and waters Acquity HSS C18 (100mm×2.1mm, 1.8µm) was procured from Waters India Ltd, Bangalore.

2.2. Instrumentation (Apparatus)

Acquity UPLC system equipped with an LC pump (model ACQ-BSM) used for method development and validation its contain, an online degasser, auto sampler (model ACQ-SM) with thermostat, and detector (TUV) (model ACQ-TUV). The data was acquired, monitored and processed by using Empower3 software. Design

expert version 9 (Stat-Ease Inc., Minneapolis, USA) was used for the optimizing chromatographic conditions. The buffers p^H was monitored by using Metrohm 780 p^H meter and weights taken by using the Sartorius CPA225D balance. In this research work we have attempted various chromatographic columns for method development. After number of trails we have been selected Waters Acquity CSH Phenyl hexyl (100 x 2.1 mm, 1.7 µm) column based on its performance.

2.3. Chromatographic conditions

The chromatographic separations were performed by using New Waters Acquity CSH Phenyl hexyl (100 x 2.1 mm, 1.7 µm) (Part no. # 186005407). The preparation mobile phase-A by using the 2ml of Trifluoro acetic acid in 1000ml water, mobile phase-B 800ml methanol and 200ml of Mobile phase-A in 1000 ml beaker. The flow rate of the mobile phase is 0.3 mL/min. The column oven temperature was maintained at 15°C and the absorption was measured at 254nm. The total Run time of the method was found to be 10.0 min and the injection volume was 1 µL. Water and Ethanol in the ratio of

(80:20 v/v) used as a diluent. Mobile phase and diluent Filter through 0.22µ finer porosity membrane filter. The

chromatographic conditions were given in table-2.

Table: 2.

Instrument	:	RP-UPLC make by Waters
Mode of analysis	:	Gradient
Flow rate	:	0.3 mL/min
Detector wave length	:	254 nm
Column temperature	:	15°C
Injection volume	:	1.0 µL
Column	:	WatersAcquityCSHPhenylhexyl(100x2.1mm, 1.7µ)
Run time	:	10.0 min
Delay time	:	2.0 min
Sample Manager Temp	:	10°C

Inference: Results from the table-2 indicated that the eluting run time was completed within 10 minutes compared with other HPLC methods for related substances determination in the literature. Moreover the

injection volume of the sample required for RP-UPLC method is 1 µL is sufficient but in the case of HPLC methods it needs about 20 µL.

Table: 3.

S. No.	NAME	RRT	LOD	LOQ	Spec
1	Acyclovir+Guanine	~0.39	0.010	0.030	NMT 1.5
2	Alanine Analog of Valacyclovir	~0.45	0.010	0.030	NMT 0.1
3	Methoxymethylguanine	~0.52	0.015	0.030	NMT0.15
4	Valacyclovir HCl	1.00	---	---	---
5	N-Methyl Valacyclovir	~1.11	0.010	0.030	NMT0.3
6	O-Acetyl Acyclovir	~1.18	0.010	0.030	NMT0.1
7	N-Ethyl Valacyclovir	~1.33	0.010	0.030	NMT0.1
8	Isoleucine Analog of Valacyclovir	~1.54	0.010	0.030	NMT0.1
9	Leucine Analog of Valacyclovir	~1.59	0.010	0.030	NMT0.1
10	N-Formyl Valacyclovir	~1.78	0.015	0.030	NMT0.15
11	Valacyclovir methylene dimer	~1.89	0.010	0.030	NMT0.1

2.4. Preparation of sample solution

Preparation of sample solution

Weighed and transferred accurately 40 mg of Valacyclovir HCl sample into a 100 mL clean, dry volumetric flask add 50 mL of diluent and sonicate to dissolve. Make up to volume with diluent. Filter through 0.22µ finer porosity membrane filter.

2.5. Calculation of Val acyclovir HCl related substances

By percentage area normalization method.

3.0. RESULTS AND DISCUSSION

3.1.0. Method development and Optimization of RP-UPLC conditions

The RP-UPLC conditions were optimized by using trials with different columns, several mobile phase compositions; flow rate and p^H were studied. In this method less quantity of solvents are used and total consumption of solvents is not more than 1ml per run time. One of the principles of green chemistry is prevention of waste. Further advantages of RP-UPLC method is able to increase the speed, sensitivity and

resolution compared to HPLC methods. By considering above aspects, RP-UPLC instrument is a most suitable technique for the Related substances determination of Val acyclovir HCl.

3.1.1. Selection of stationary phase

Based on the structure, molecular weight of API and related substances present in the products C18 columns like Waters BEH C18 column were initially screened for the separation. But these columns failed to provide acceptable separation and peak shape. For also several other HSS C18 columns with other stationary phases are screened for separation but a remarkable selectivity was achieved with New Waters Acquity CSH Phenyl hexyl (100mm x 2.1 mm, 1.7 µm) was finalised. This column was not yet used in any other methods.

Waters AcquityBEH C18 column; The trifunctionally bonded BEH Column particle gives a widest usable p^H range i.e's 1-12range, superior low p^H stability and ultra low column bleed for high sensitivity applications.

Waters Acquity CSH Phenyl hexyl column; this column are used to provide an alternative selectivity and

are a valuable tool for method development. The trifunctionally bonded C₆phenyl ligand is a robust and

low bleed sorbents that selectively retains poly aromatic compounds through Π - Π interactions.

Table: 4 Stationary phases Information.

Brand	Acquity UPLC	Particle size(dp)	1.7 μ m
%Carbon Load	14	Particle Substrate	Hybrid
Bonding Technology	Phenyl-Hexyl	Pore size	130Å
Chemistry	Phenyl	Silanol Activity	Low
Endcapped	Yes	Surface Area	185
ID	2.1 mm	Technology	CSH
Length	100mm	USP Classification	L11
Particle Shape	Spherical	Units in package	1/pkg
Mode	Reversed-phase	p ^H range	1-11

3.1.2. Selection of Mobile phase

As one of the objectives of the method is to develop a different buffer, i.e ammonium dihydrogen orthophosphate with p^H 2.5 adjusted with Orthophosphoric acid and Trifluoro acetic acid+water buffers were evaluated. It is observed that in Trifluoro acetic acid buffer is a capable contestant for efficient separation of related substances. The organic modifiers, methanol and acetonitrile were used at different composition conditions. Based on the results, methanol was finalised as a organic modifier.

3.1.3. Selection of Diluent

Freely soluble in water, practically insoluble in anhydrous ethanol and 1-octanol. But It is stable for at least 24 hrs at 10°C at a composition of water + ethanol (80:20) ratio. Hence, water + ethanol (80:20) were selected as a diluent.

3.2.0. METHOD VALIDATION

The developed analytical method was subjected to validation with respect to various parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, precision, recovery studies, specificity and reproducibility as per the ICH guidelines.

3.2.1. SYSTEM SUITABILITY

In the optimized RP-UPLC conditions, system suitability parameters were evaluated for Valacyclovir HCl. Tailing factor for Valacyclovir HCl was not more than 1.5. The USP plate count for Valacyclovir HCl is not less than 8000. The resolution between Valacyclovir HCl and N-Methyl Valacyclovir is not less than 1.5. The results are summarised in Table: 5.

System suitability chromatogram

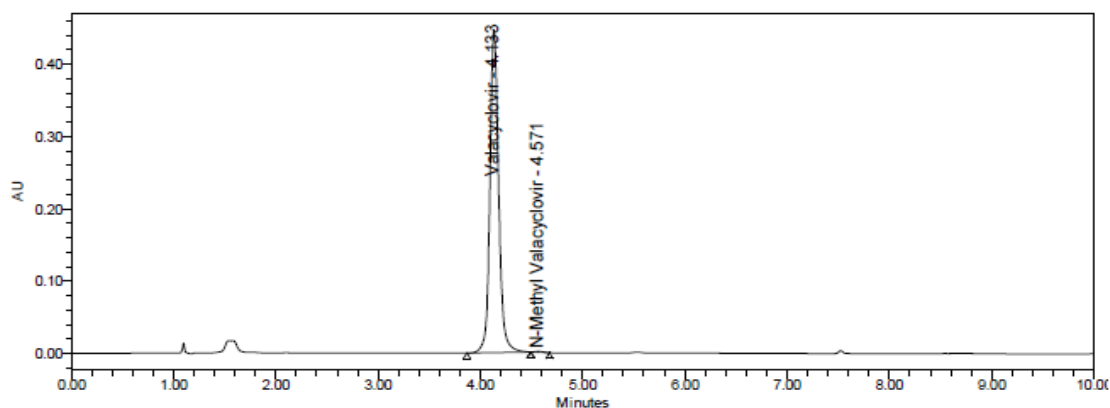


Fig: 2.

Table: 5.

Parameters	Valacyclovir HCl		Limit
	Initial SST	Bracketing SST	
Tailing factor	1.13	1.12	NMT 1.5
USP Plate count	12038	12054	NLT 8000
Retention time (min)	4.13	4.15	~4.3
Resolution between Valacyclovir HCl and N-Methyl Valacyclovir	3.0	3.1	NLT 1.5

3.2.2. SPECIFICITY

The specificity of the proposed method was demonstrated by interference study. It was found that presence of some common excipients did not interferences at the retention time of Valacyclovir HCl and its related substances. Thus the developed method can be successfully applied for determination of Valacyclovir HCl its related substances in bulk drug form.

3.2.3. Blank and Impurity interference

The blank were prepared and injected in RP-UPLC. No interference was observed at any of the peaks of interested in sample and related substances chromatogram. Reference chromatograms are depicted bellow figure 3-5.

Blank chromatogram

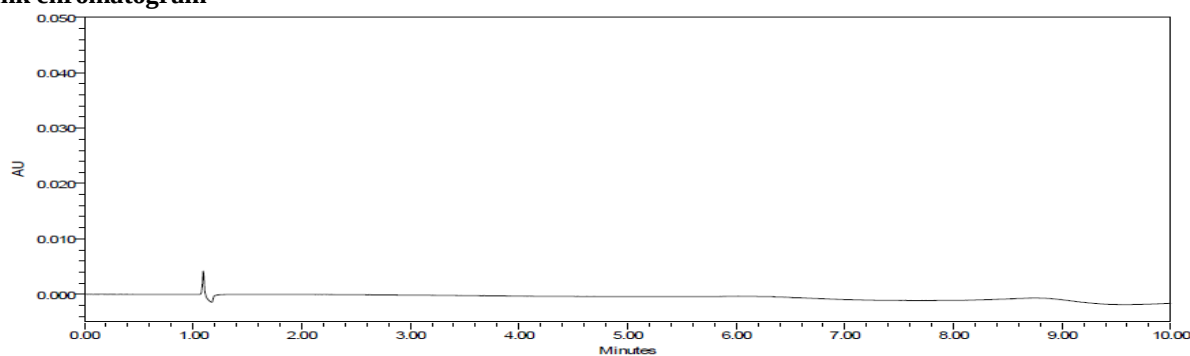


Fig: 3.

Spec level related substances spiked with sample chromatogram

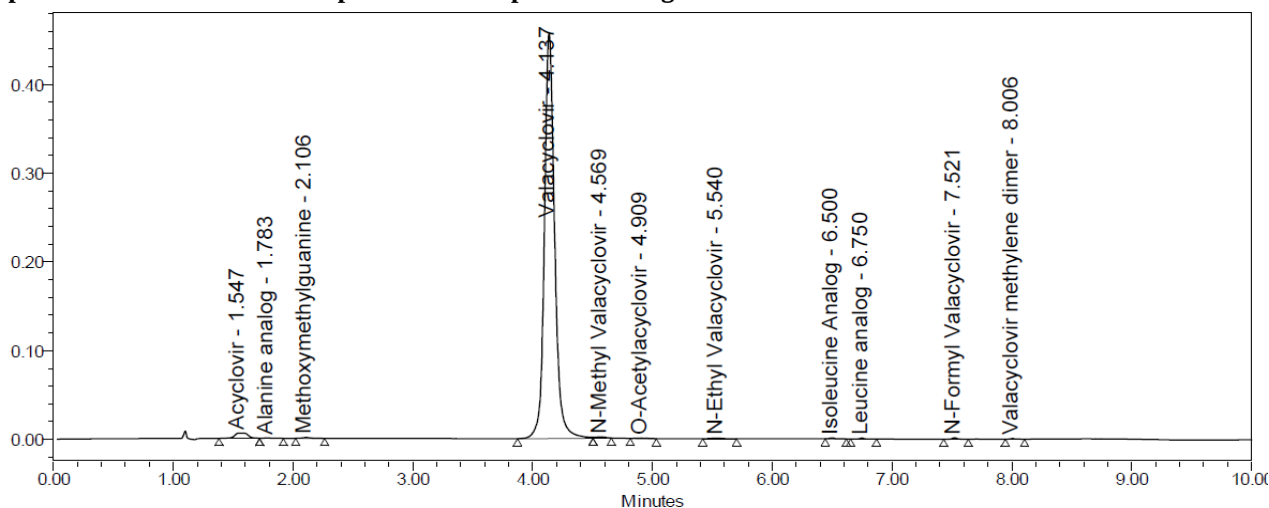


Fig: 4 Spec level spiked chromatogram.

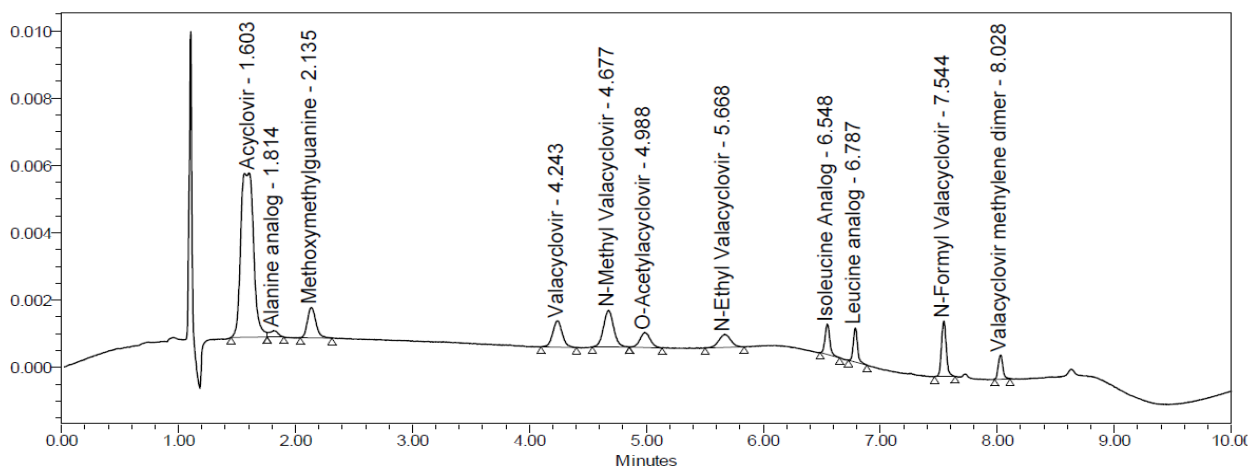


Fig: 5.

3.2.4. Forced degradation/ Stress study

In order to determine whether the analytical method was stability-indicating, Valacyclovir HCl bulk drug form was stressed under various conditions to conduct forced degradation studies. Valacyclovir HCl was soluble in ethanol+water in the composition of (20:80). All solutions for forced degradation studies were prepared to yield concentration 400µg/g of Valacyclovir HCl.

Acid degradation (1M HCl/85°C/24hours)

Solution of Valacyclovir HCl (400µg/g) for acid degradation study was prepared using 1N hydrochloric acid solution and the resultant solution was 24 hours to facilitate acid degradation of Valacyclovir HCl.

Alkali degradation (1M NaOH/85°C/24hours)

Solution of Valacyclovir HCl (400µg/g) for base degradation study was prepared by using 1N NaOH resultant solution was 24 hours to facilitate alkali degradation of Valacyclovir HCl.

Oxidation (30% H₂O₂/85°C/120min)

Solution of Valacyclovir HCl (400µg/g) for peroxide degradation study was prepared using 30% H₂O₂

resultant solution was 120min to facilitate peroxide degradation of Valacyclovir HCl.

Thermal degradation (105°C/120Hours)

Valacyclovir HCl bulk drug was kept in hot oven for 120 hours at 105°C to determine the effects of thermal degradation on the stability of Valacyclovir HCl by using the analytical method.

Hydrolysis

Solution of Valacyclovir HCl (400µg/g) for Hydrolysis degradation study was 24 hour to facilitate Hydrolysis degradation of Valacyclovir HCl.

Photo stability (1.2 million Lux Hours)

Photo irradiated Valacyclovir HCl was accurately weighed and transferred to volumetric flask to make the final concentration 400µg/g of Valacyclovir HCl. The proposed method evaluates the ability to separate the related substances of Valacyclovir HCl from its degradation products shown in Figure: 6 to 12. The results summarised in Table: 6.

Undegraded Sample chromatogram.

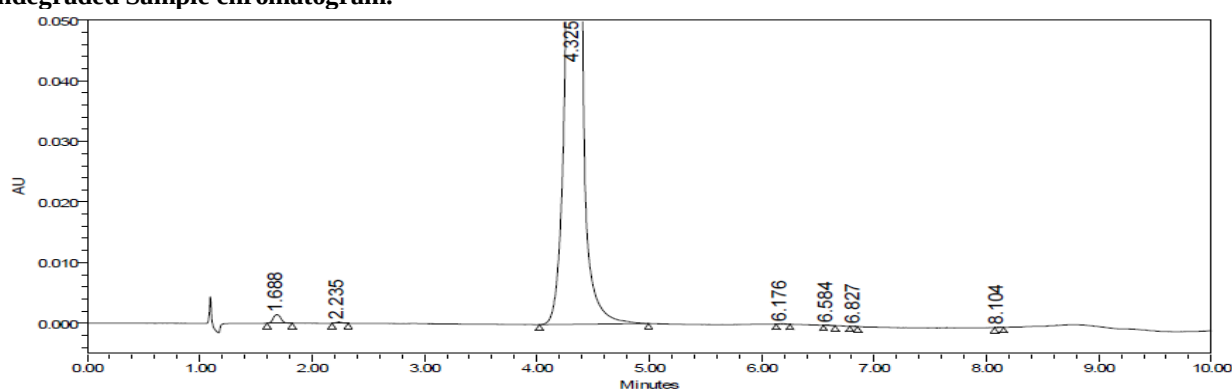


Fig:6.

Sample chromatogram of peroxide degradation

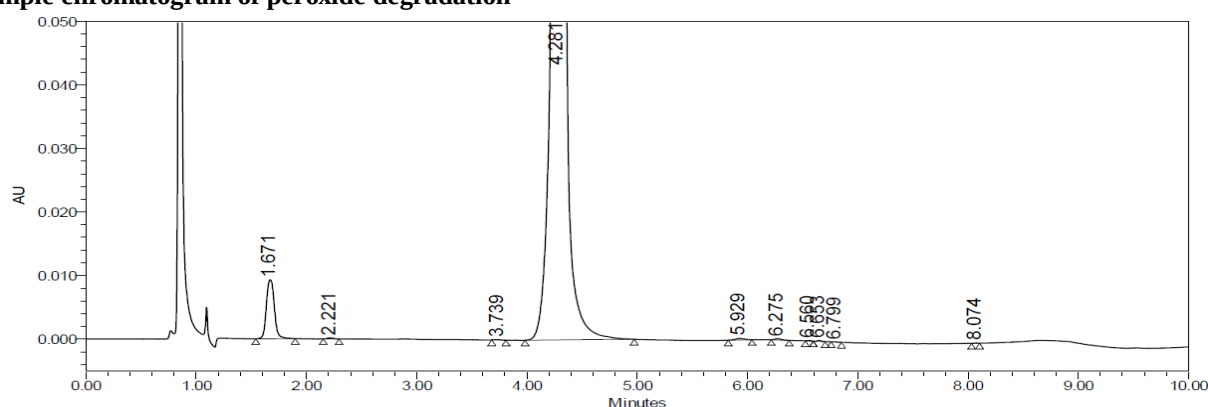


Fig: 7.

Sample chromatogram of thermal degradation

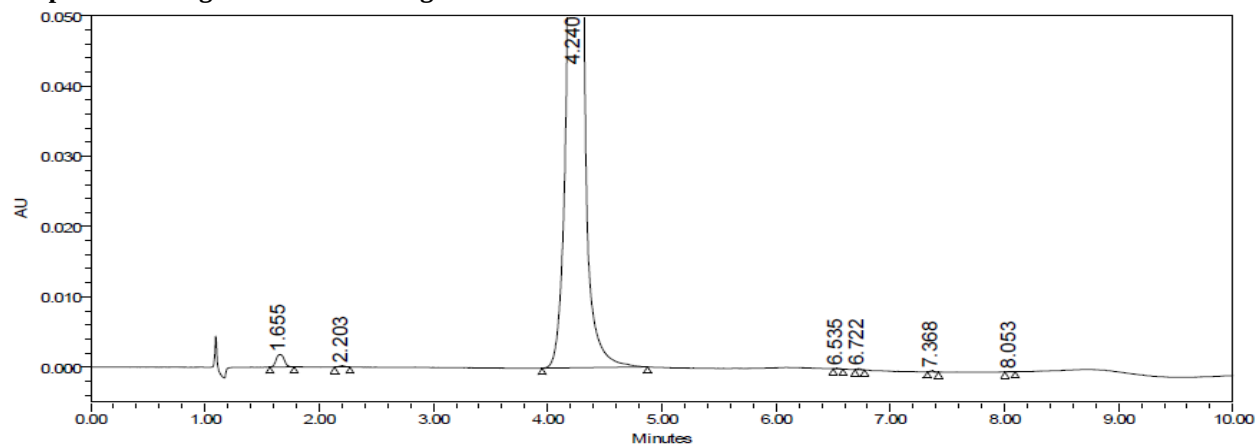


Fig: 8.

Sample chromatogram of hydrolysis degradation

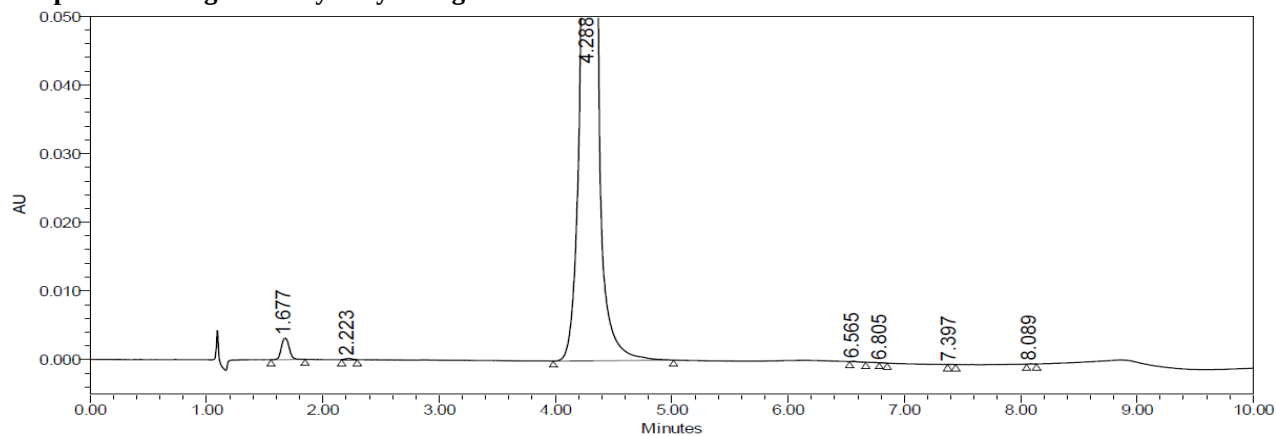


Fig: 9.

Sample chromatogram of photo degradation

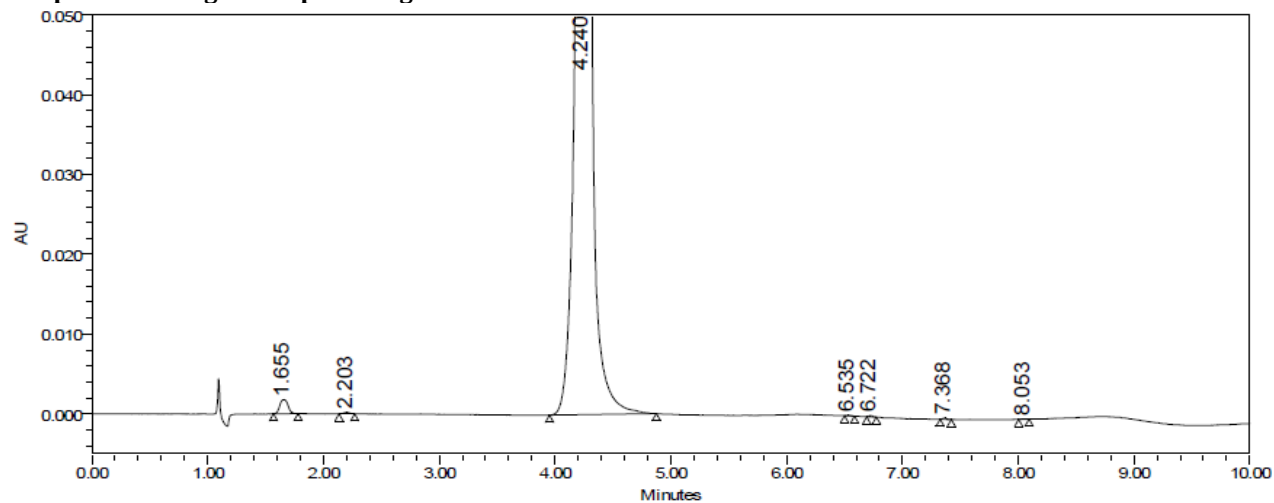


Fig: 10.

Sample chromatogram of acid degradation

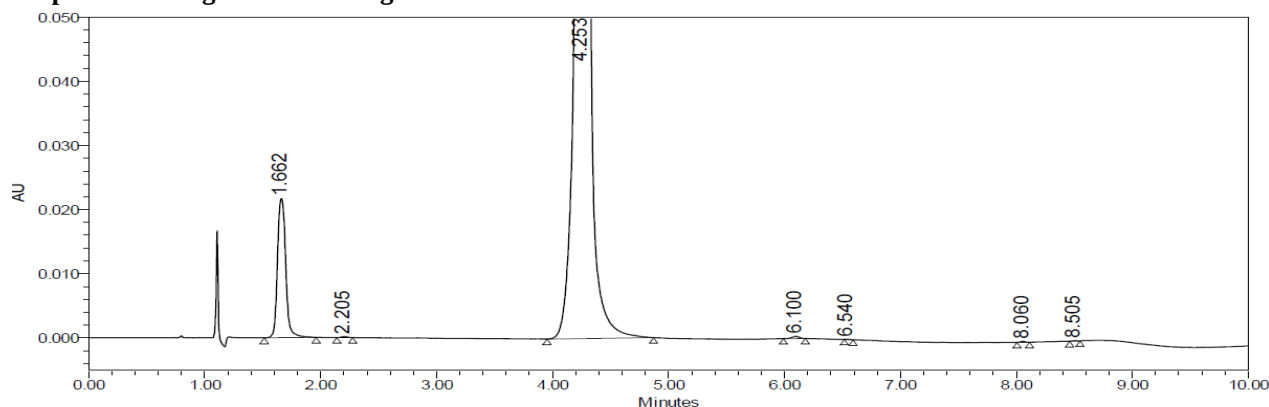


Fig: 11.

Sample chromatogram of Base degradation

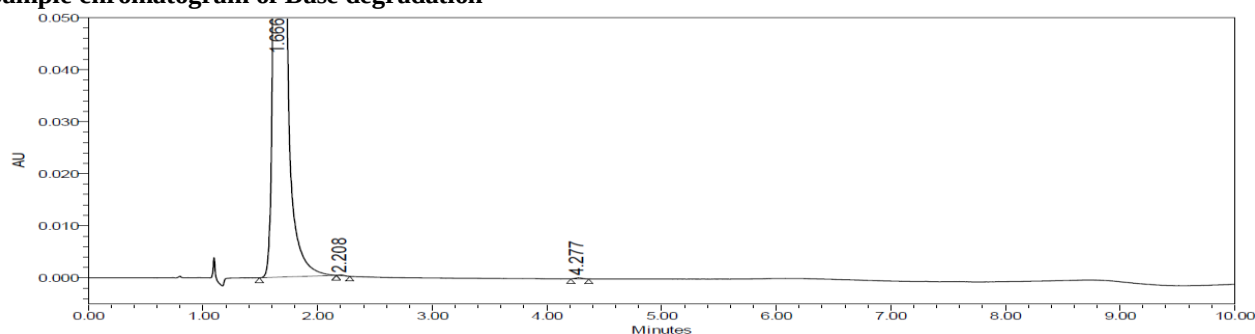


Fig: 12.

Table:6.

Stress study									
S.NO	Related substances	Spec	Undegraded	1.0N Acid	1.0N Base	Peroxide	Thermal	Tyrosolysis	Photo
1	Acyclovir+Guani	NMT 1.5	0.241	4.01	99.9	1.73	0.32	0.54	0.23
2	Alanine Analog	NMT 0.1	ND	ND	ND	ND	ND	ND	ND
3	MMG	NMT 0.15	0.027	0.024	0.019	0.026	0.026	0.025	0.025
4	Valacyclovir	NA	99.7	95.9	0.034	98.12	99.6	99.4	99.7
5	N-Methyl	NMT 0.3	ND	ND	ND	ND	ND	ND	ND
6	O-Acetyl	NMT 0.1	ND	ND	ND	ND	ND	ND	ND
7	N-Ethyl	NMT 0.1	ND	ND	ND	ND	ND	ND	ND
8	Isoleucine	NMT 0.1	0.008	0.006	ND	0.007	0.008	0.009	0.008
9	Leucine	NMT 0.1	0.002	ND	ND	0.002	ND	ND	0.002
10	N-Formyl	NMT 0.15	ND	ND	ND	ND	ND	ND	ND
11	Dimer	NMT 0.1	0.004	ND	ND	0.003	0.005	0.004	0.003
12	Unknown-RRT	NMT 0.1	ND	ND	ND	0.01	ND	ND	ND
13	Unknown-RRT	NMT 0.1	ND	0.056	ND	0.032	ND	ND	ND
14	Unknown-RRT	NMT 0.1	ND	ND	ND	0.014	0.013	ND	ND
15	Unknown-RRT	NMT 0.1	ND	ND	ND	ND	0.014	0.002	ND
16	Unknown-RRT	NMT 0.1	ND	0.005	ND	ND	ND	ND	ND

Inference: Based on the above data clearly indicated that base was more impacted the main product and

converted into a acyclovir product. 1N HCl and Hydrogen peroxide also impact the molecule moreover

any of the degradation peaks not interfere with main product and also related substances.

3.2.5 LINEARITY

3.2.6. Linearity for related substances of Valacyclovir HCl

To establish linearity of the related substances method, solutions were prepared by diluting the impurity stock

solution to obtain the required concentrations at different levels ranging from 0.5% to 150%. The correlation coefficient, slope and y-intercept of the calibration curve were calculated. Further the relative response factors for all related substances were evaluated based on the S/N ratio method. All peaks response nearer to 1. So considered as 1 for all related substances. Results are summarized in table: 7.

Overlay chromatogram of Linearity solutions

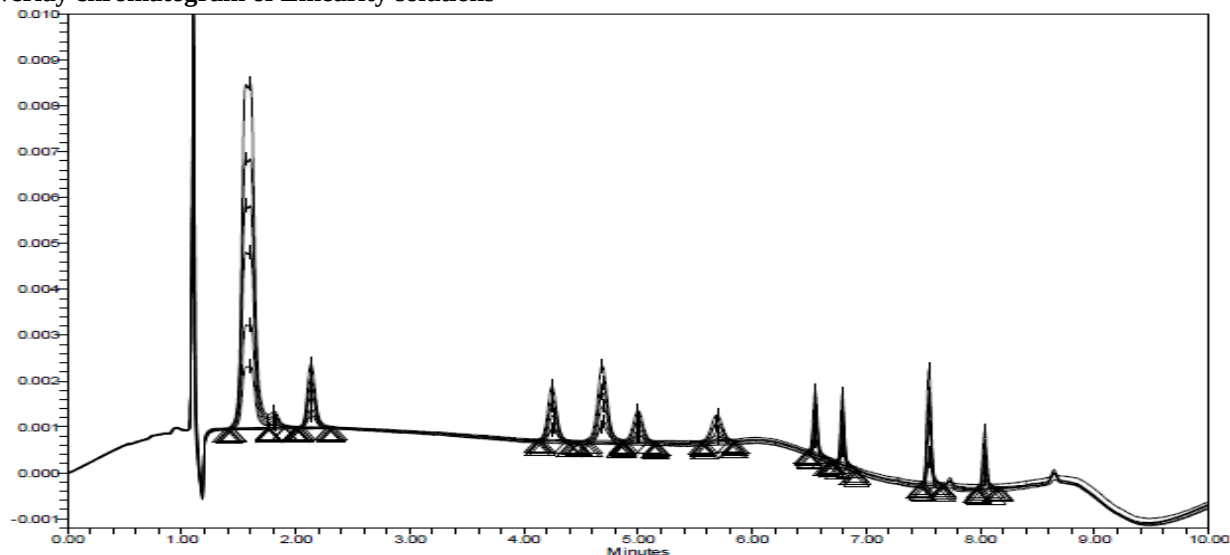


Fig: 13.

Linearity of graph Acyclovir + Guanine.

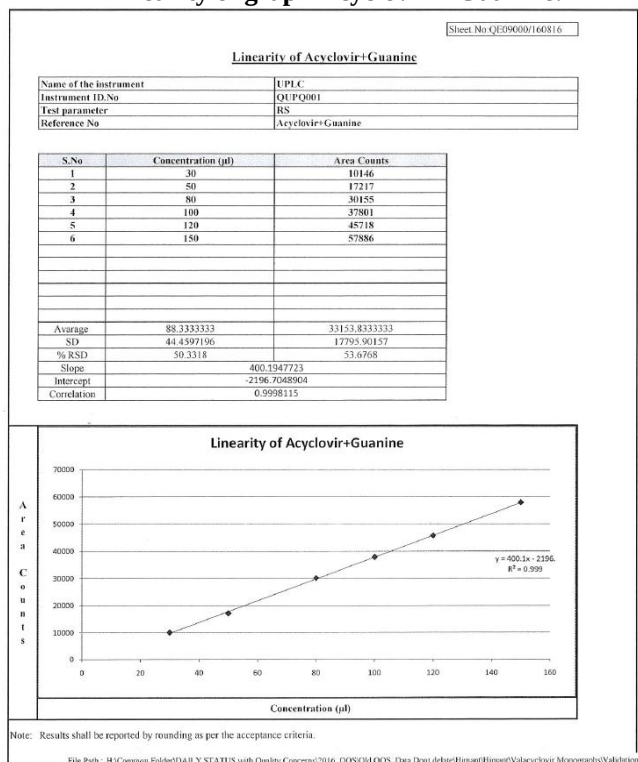


Fig:14

Linearity of Alanine analog Of Valacyclovir.

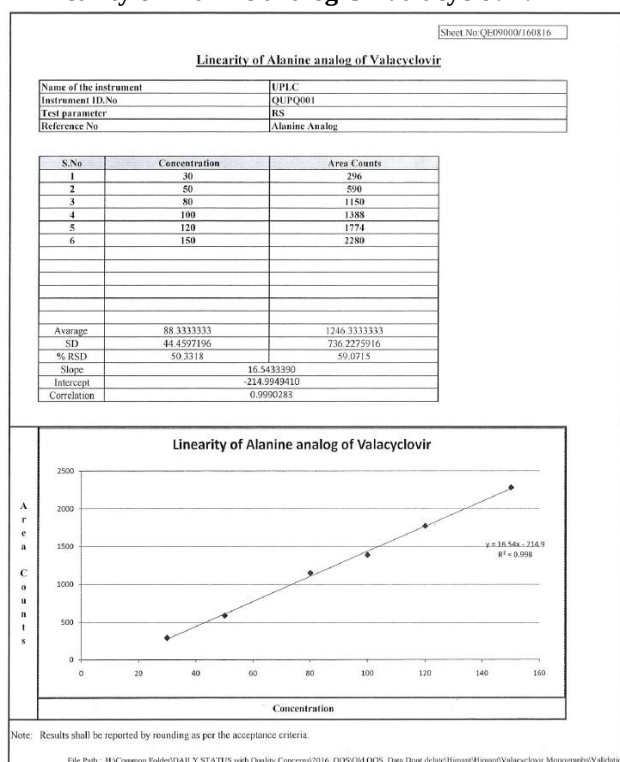


Fig: 15

Linearity of Valacyclovir HCl:

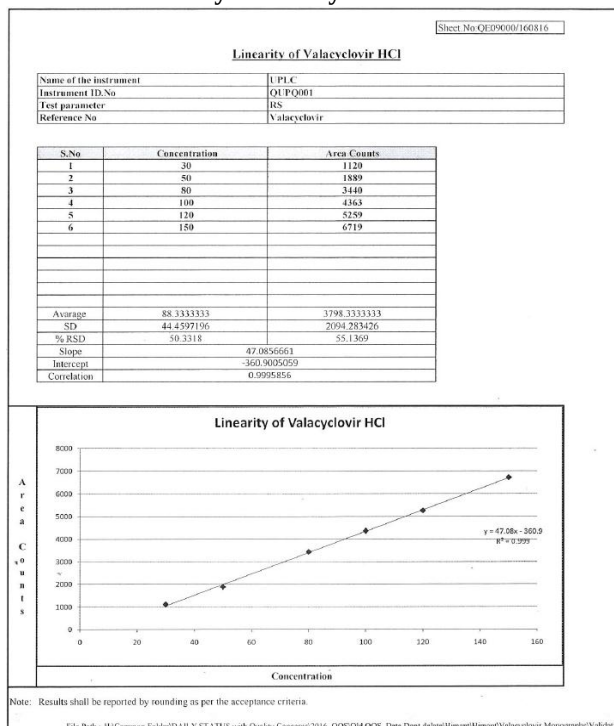


Fig: 17

Linearity graph of O-Acetyl acyclovir:

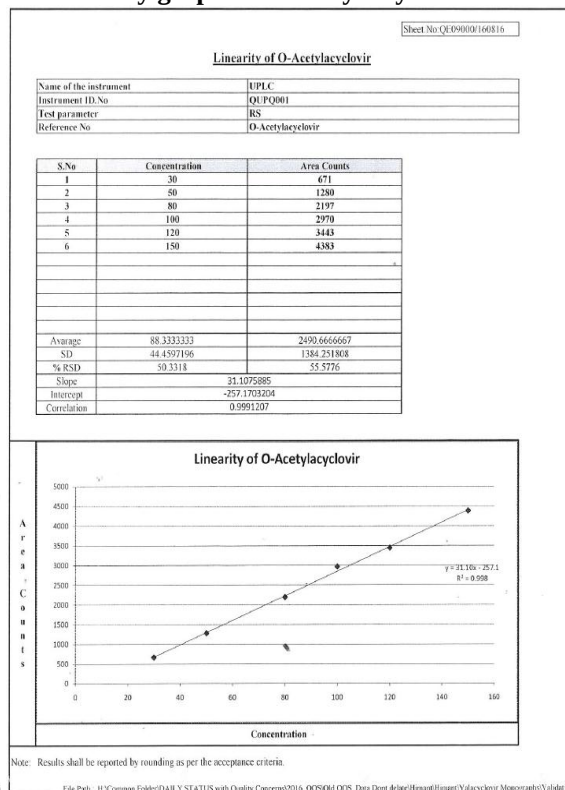


Fig: 19

Linearity graph of N-Ethyl Valacyclovir:

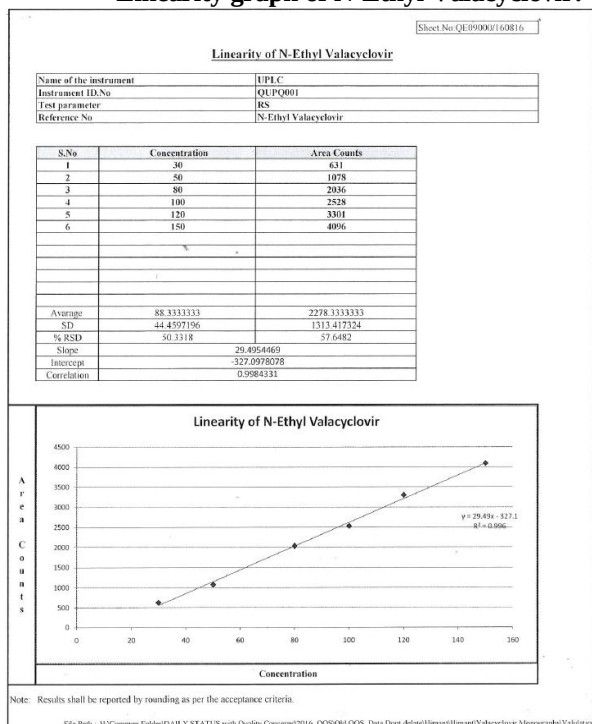


Fig: 20

Linearity graph of Isoleucine Analog:

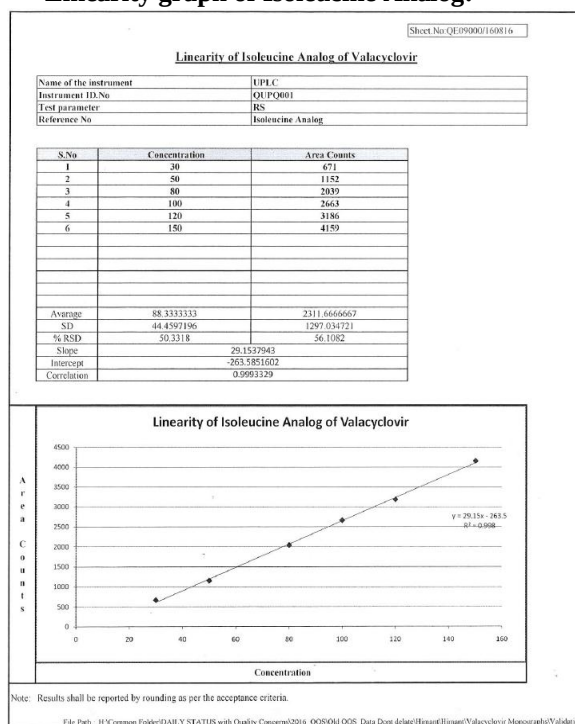


Fig: 21

Linearity graph of N-Formyl Valacyclovir:

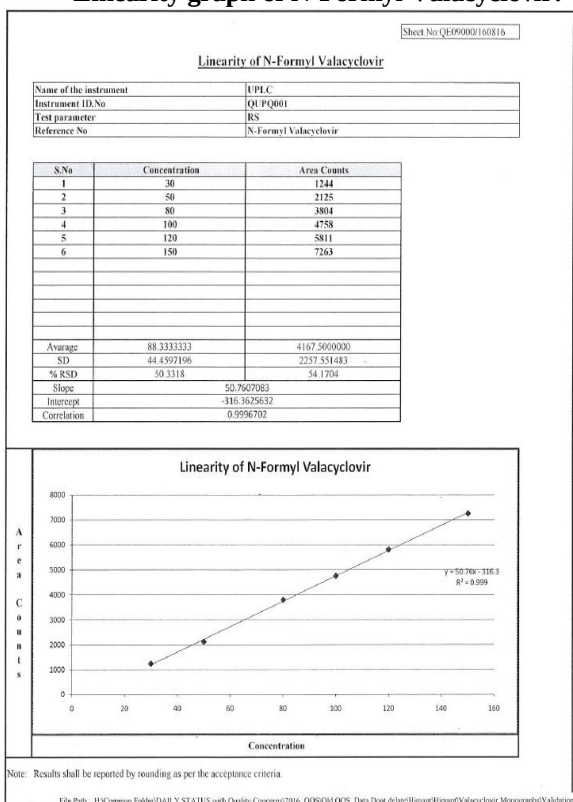


Fig: 22

Linearity of Leucine Analog Valacyclovir:

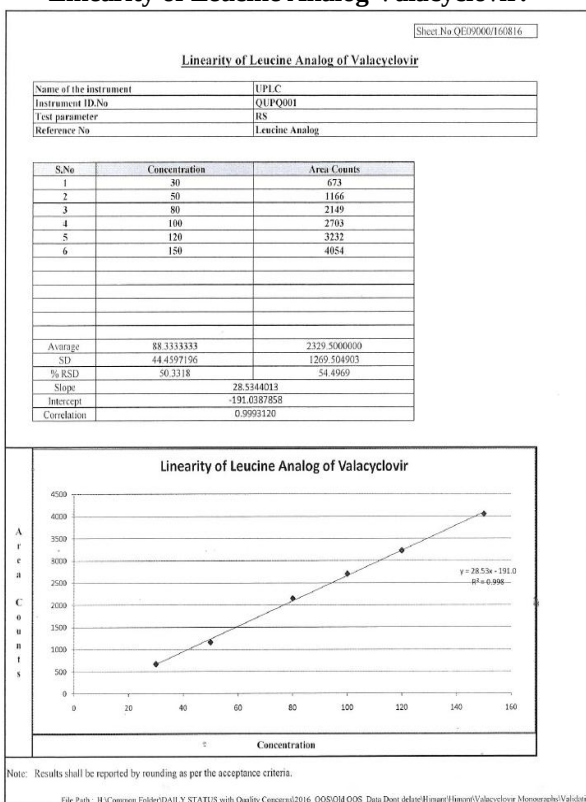


Fig: 23

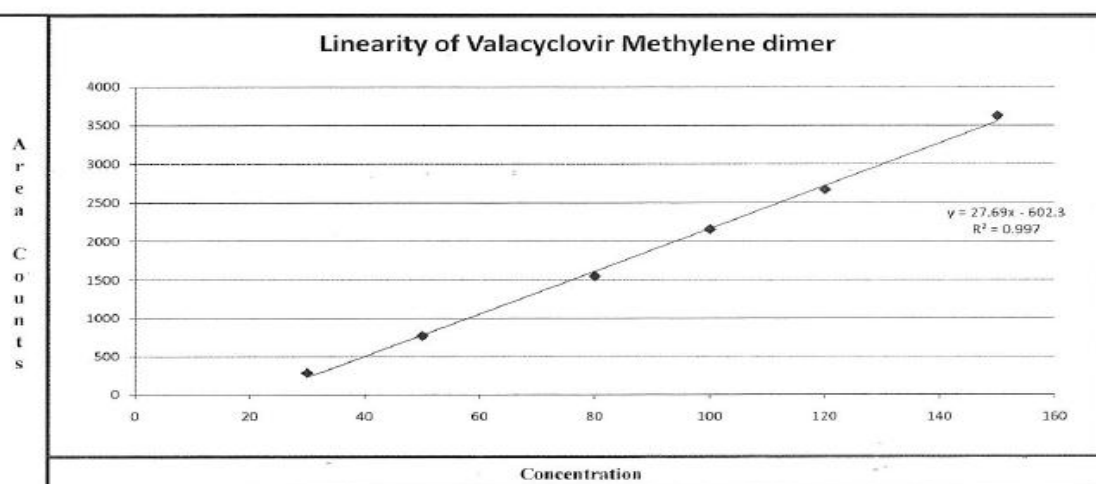
Linearity graph of Valacyclovir Methylene dimer

Sheet No:QE09000/160816

Linearity of Valacyclovir Methylene dimer

Name of the instrument	UPLC
Instrument ID.No	QUPQ001
Test parameter	RS
Reference No	Valacyclovir Methylene dimer

S.No	Concentration	Area Counts
1		
2		
3	30	290
4	50	775
5	80	1550
6	100	2155
7	120	2670
8	150	3626
Average	88.3333333	1844.3333333
SD	44.4597196	1232.771944
% RSD	50.3318	66.8411
Slope	27.6984823	
Intercept	-602.3659359	
Correlation	0.9989413	



Note: Results shall be reported by rounding as per the acceptance criteria.

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Fig: 24

Table 7.

S.NO	Related substances	Linearity	Specification
1	Acyclovir+Guanine	0.999	NLT 0.99
2	Alanine Analog	0.999	
3	Methoxymethylguanine	0.999	
4	Valacyclovir HCl	0.999	
5	N-Methyl Valacyclovir	0.999	
6	O-Acetylacyclovir	0.999	
7	N-Ethyl Valacyclovir	0.998	
8	Isoleucine Analog	0.999	
9	Leucine Analog	0.999	
10	N-Formyl Valacyclovir	0.999	
11	Valacyclovir Methylene Dimer	0.998	

Inference: Based on correlation coefficient values from the table-7 were within the Acceptance criteria.

3.2.7. LIMIT OF DETECTION AND LIMIT QUANTIFICATION

The limit of detection (LOD) and limit of quantitation (LOQ) were established for Valacyclovir HCl by diluting the standard stock solution. The concentration at

0.01µg/ml, 0.030µg/ml LOD and LOQ for Valacyclovir HCl and its related substances. The signal to noise ratios was found to more than 3 and 10 for the all related substances respectively. Hence these concentrations were finalised as LOD and LOQ concentrations. The results are summarised in Table: 8 & 9. Overlay chromatograms of LOD & LOQ are representing below.

Table 8: LOD and LOQ Precision.

LOD Precision				NMT 33.0%
S.No	Related substances	Mean	SD	RSD
1	Acyclovir+Guanine	4195	46	1.11
2	Alanine Analog	125	26	21.07
3	Methoxymethylguanine	478	32	6.69
4	Valacyclovir HCl	441	44	10.06
5	N-Methyl Valacyclovir	645	54	8.36
6	O-Acetylacyclovir	207	69	30.12
7	N-Ethyl Valacyclovir	196	51	26.2
8	Isoleucine Analog	259	9	3.29
9	Leucine Analog	276	15	5.31
10	N-Formyl Valacyclovir	509	15	2.97
11	Valacyclovir Methylene Dimer	138	34	24.42

Table 9.

LOQ Precision				NMT 10.0%
S.No	Related substances	Mean	SD	RSD
1	Acyclovir+Guanine	10062	29	0.28
2	Alanine Analog	262	23	8.76
3	Methoxymethylguanine	1258	20	1.56
4	Valacyclovir HCl	1121	41	3.69
5	N-Methyl Valacyclovir	1751	81	4.64
6	O-Acetylacyclovir	756	102	13.5
7	N-Ethyl Valacyclovir	628	44	7.05
8	Isoleucine Analog	669	18	2.69
9	Leucine Analog	666	32	4.85
10	N-Formyl Valacyclovir	1242	18	1.46
11	Valacyclovir Methylene Dimer	433	20	4.56

Inference: Based on above values from the table-8 & 9 were within the Acceptance criteria. LOD & LOQ overlay chromatograms are depicted bellow.

Overlay Chromatogram of LOD

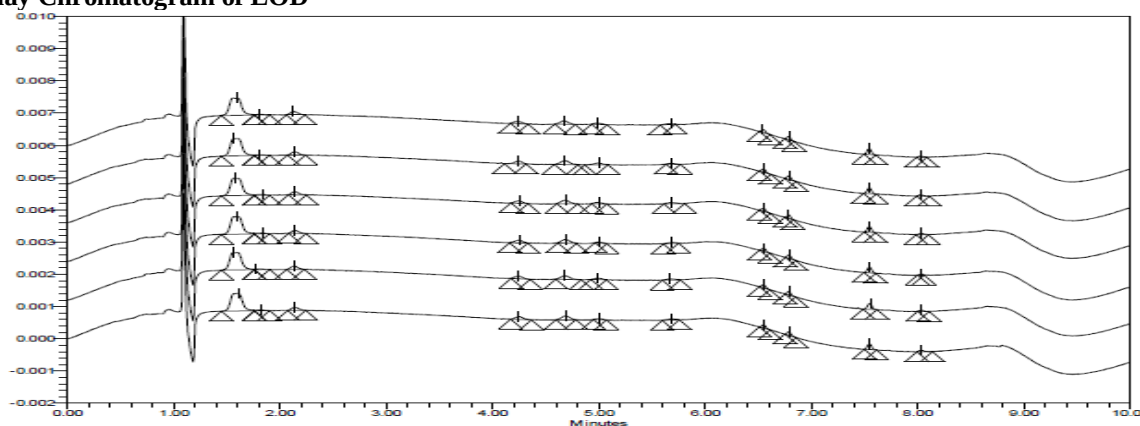


Fig-25.

Overlay Chromatogram of LOQ

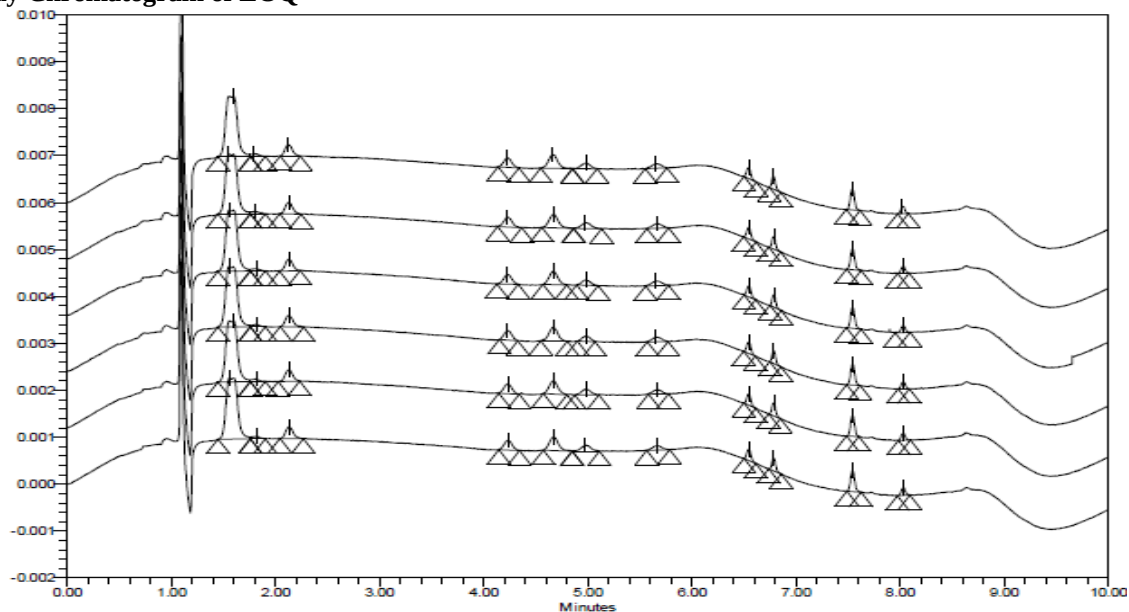


Fig-26.

3.2.8. ACCURACY

3.2.9. Accuracy for related substances of Valacyclovir HCl

The accuracy of the method was determined by calculating recoveries of Valacyclovir HCl by the standard addition method. Known amounts of standard

solutions of Valacyclovir HCl (80, 100, and 120 % level) were added to previously analyzed sample of bulk drug. The percentage of recoveries was calculated. The percentages of recoveries were between 80% to 120%. The results are summarised in the following Table: 10.

Table: 10 Accuracy of the all related substances

S.No	Related substances	Spec	80%			
			Sample-01	Sample-02	Sample-03	Recovery
1	Acyclovir+Guanine	NMT 1.5	1.3	1.29	1.26	105-108
2	Alanine Analog	NMT 0.1	0.07	0.07	0.07	87.5-87.5
3	Methoxymethylguanine	NMT 0.15	0.13	0.14	0.13	108-116
4	N-Methyl Valacyclovir	NMT 0.3	0.26	0.25	0.25	104-108
5	O-Acetylacyclovir	NMT 0.1	0.07	0.07	0.07	87.5-87.5
6	N-Ethyl Valacyclovir	NMT 0.1	0.08	0.08	0.07	87.5-100
7	Isoleucine Analog	NMT 0.1	0.08	0.08	0.08	100-100
8	Leucine Analog	NMT 0.1	0.08	0.08	0.08	100-100
9	N-Formyl Valacyclovir	NMT 0.15	0.14	0.14	0.14	116-116
10	Methylene Dimer	NMT 0.1	0.07	0.07	0.07	87.5-87.5
S.No	Related substances	Spec	100%			
			Sample-01	Sample-02	Sample-03	Recovery
1	Acyclovir+Guanine	NMT 1.5	1.5	1.5	1.52	100-101
2	Alanine Analog	NMT 0.1	0.09	0.08	0.09	80-90
3	Methoxymethylguanine	NMT 0.15	0.16	0.16	0.16	106-106
4	N-Methyl Valacyclovir	NMT 0.3	0.31	0.3	0.3	100-103
5	O-Acetylacyclovir	NMT 0.1	0.09	0.083	0.084	83-90
6	N-Ethyl Valacyclovir	NMT 0.1	0.09	0.09	0.1	90-100
7	Isoleucine Analog	NMT 0.1	0.09	0.09	0.09	90-90
8	Leucine Analog	NMT 0.1	0.1	0.1	0.1	100-100
9	N-Formyl Valacyclovir	NMT 0.15	0.17	0.17	0.17	113-113
10	Methylene Dimer	NMT 0.1	0.09	0.09	0.08	80-90

Table: 12.

Intermediate Precision										
S.No	Acyclovir+ Guanine	Alanine	MMG	N- Methyl	O- Acetyl	N-Ethyl	Isoleucine	Leucine	N- Formyl	Dimer
Sample-1	1.65	0.11	0.16	0.3	0.08	0.09	0.1	0.1	0.18	0.08
Sample-2	1.8	0.11	0.16	0.31	0.09	0.09	0.09	0.09	0.17	0.08
Sample-3	1.57	0.11	0.16	0.29	0.09	0.09	0.09	0.1	0.17	0.09
Sample-4	1.58	0.11	0.16	0.32	0.08	0.09	0.1	0.09	0.17	0.08
Sample-5	1.59	0.12	0.16	0.3	0.08	0.091	0.1	0.09	0.17	0.08
Sample-6	1.56	0.12	0.17	0.32	0.09	0.09	0.09	0.1	0.17	0.08
Average	1.625	0.113	0.162	0.307	0.085	0.090	0.095	0.095	0.172	0.082
SD	0.091	0.005	0.004	0.012	0.005	0.0004	0.005	0.005	0.004	0.004
RSD	5.6	4.6	2.5	3.9	6.4	0.5	5.8	5.8	2.4	5.0

Inference: Results from the table-12, it is illustrated %RSD of all related substances was found to be within the acceptance criteria i.e. NMT 10.0%.

Table: 13 Cumulative RSD of the Method precision and Intermediate Precision.

Overall % RSD										
S.No	Acyclovir+ Guanine	Alanine	MMG	N- Methyl	O- Acetyl	N-Ethyl	Isoleucine	Leucine	N- Formyl	Dimer
Sample-1	1.49	0.09	0.16	0.29	0.09	0.1	0.09	0.1	0.17	0.08
Sample-2	1.48	0.09	0.17	0.31	0.09	0.1	0.09	0.1	0.17	0.09
Sample-3	1.49	0.09	0.16	0.26	0.08	0.09	0.09	0.1	0.17	0.08
Sample-4	1.51	0.09	0.16	0.3	0.09	0.09	0.09	0.1	0.17	0.09
Sample-5	1.49	0.09	0.16	0.29	0.09	0.1	0.091	0.11	0.16	0.09
Sample-6	1.51	0.08	0.16	0.29	0.08	0.09	0.09	0.1	0.17	0.08
Sample-7	1.65	0.11	0.16	0.3	0.08	0.09	0.1	0.1	0.18	0.08
Sample-8	1.8	0.11	0.16	0.31	0.09	0.09	0.09	0.09	0.17	0.08
Sample-9	1.57	0.11	0.16	0.29	0.09	0.09	0.09	0.1	0.17	0.09
Sample-10	1.58	0.11	0.16	0.32	0.08	0.09	0.1	0.09	0.17	0.08
Sample-11	1.59	0.12	0.16	0.3	0.08	0.091	0.1	0.09	0.17	0.08
Sample-12	1.56	0.12	0.17	0.32	0.09	0.09	0.09	0.1	0.17	0.08
Ave	1.56	0.101	0.162	0.30	0.09	0.09	0.09	0.098	0.17	0.083
SD	0.092	0.014	0.004	0.016	0.005	0.004	0.004	0.006	0.004	0.005
%RSD	5.90	13.7	2.41	5.5	6.00	4.8	4.8	5.9	2.5	5.9

Inference: Sample Results from the table-13 it was observed that cumulative RSD's were found to be within the limit i.e. NMT15.0%.

3.3. FILTER COMPATIBILITY

Filter compatibility to the sample is concluded from the recovery study indicated that there is no absorption of these component to filter.

3.4. SOLUTION STABILITY

The standard and samples solutions were kept on temperature at 10°C and injected the aged samples (Every one hour) into the RP-UPLC. The peak areas corresponding to Valacyclovir HCl and its related substances were measured. Calculated the similarity factor and found that the values are below 10.0% RSD. Thus indicates the sample solutions are stable for at least 24 hrs when stored on room temperature condition.

3.5. METHOD ROBUSTNESS

Method robustness was performed by applying small changes in the ratio of mobile phase, column temperature, flow rate and wavelength. The results of change in ratio of mobile phase, column temperature, wavelength, and flow rate are shown in Table 15. The flow rate 0.27–0.33 mL/min, wavelength 252 to 256nm, Column temperature 13.5°C to 16.5°C and Study of the mobile phase composition B $\pm$ 20%. Based on this, the method is proved to be robust and can easily be implemented in quality control laboratories for the regular analysis of Valacyclovir HCl samples with great confidence.

Table-14.

S.No	Related substances	Spec	Regular Method	Flow Rate		Column Oven		Wavelength		Organic Solvent variation	
				0.33	0.27	16.5	13.5	256	252	20% Less	20% More
1	Acyclovir+Guanine	NMT 1.5	0.241	1.97	1.94	1.95	1.96	1.96	1.95	1.86	1.98
2	Alanine	NMT 0.1	ND	0.12	0.12	0.105	0.12	0.107	0.106	0.08	0.114
3	MMG	NMT 0.15	0.027	0.2	0.19	0.185	0.183	0.188	0.187	0.185	0.187
4	Valacyclovir	NA	99.7	96.9	96.9	97	96.9	96.6	96.9	97.2	96.9
5	N-ethyl	NMT 0.3	ND	0.3	0.31	0.29	0.3	0.32	0.31	0.31	0.29
6	O-acetyl	NMT 0.1	ND	0.089	0.09	0.086	0.091	0.091	0.089	0.083	0.092
7	N-Ethyl	NMT 0.1	ND	0.103	0.106	0.087	0.091	0.087	0.085	0.11	0.091
8	Isoleucine	NMT 0.1	0.008	0.104	0.099	0.101	0.101	0.099	0.104	0.101	0.101
9	Leucine	NMT 0.1	0.002	0.108	0.088	0.084	0.084	0.086	0.086	0.09	0.085
10	Nformyl	NMT 0.15	ND	0.19	0.17	0.17	0.172	0.173	0.168	0.174	0.171
11	Dimer	NMT 0.1	0.004	0.082	0.072	0.072	0.076	0.076	0.074	0.076	0.075

Inference: Based on the above data there are no impacts observed. This method is highly robust can easily implemented in Quality control laboratories.

4.0. DISCUSSION

The purpose of the present work was to develop a short, robust, precise RP-UPLC method for the accurate quantification of Valacyclovir HCl related substances. This method was developed for drug substance. As mentioned in the introduction section, limited reports are available for quantification of Valacyclovir HCl by HPLC but there is no UPLC analytical method for quantification of 11 related substances. Present method was highly sensitive. The developed method was successfully validated for drug substance as per the ICH guideline. The proposed method is much superior to reported methods in terms of solvent consumption, run time, instrumental technique (RP-UPLC), selectivity, and applicability to Assay analysis.

5.0. APPLICATION TO PHARMACEUTICAL INDUSTRY:

This work will help industry to develop, manufacture and launch the product in a fast and economical way which in turn reduces the cost of the medicine and help the patient to avail quality, innovative and affordable medicine.

6.0. CONCLUSION

A stability indicating RP-UPLC related substances method has been developed for determination of Valacyclovir HCl. Developed method was proved to be robust using the experimental design, this method can be successfully implemented in the quality control lab for the routine analysis of this product. Further this RP-UPLC method was successfully validated as per ICHQ2 (R1) guideline and proved to be precise, linear, sensitive, accurate, and robust. This method is short and simple, hence implementation of this method in quality control and analytical development labs can yield high throughput. As low amounts of solvents are required, implementation of this method will be eco friendly. This was the first RP-UPLC related substances method that

can accurately quantitative the Valacyclovir HCl and its related substances in drug substance.

ACKNOWLEDGEMENTS

The authors are heartily thankful to acknowledge Aurobindo Pharma Ltd and Andhra University for providing all the facilities to carry out the research work.

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