LC-MS approach for the contaminant's quantification in anti-retroviral API

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Abstract

The current work focuses on procedure development for analysis and then further authentication for quantifying contaminant G (4-Dimethylaminopyridine) and contaminant S (N-[(Phenylmethoxy)carbonyl]-L-valine 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl) methoxy]ethyl ester) in Valaciclovir Hydrochloride Hydrate anti-retroviral active pharmaceutical ingredient (API) powder using Liquid Chromatography-Mass Spectrometry (LC-MS) tactic. This newly settled LC-MS procedure of analysis is proposed to complement the existing method (Thin layer chromatography) for quantifying contaminant G and contaminant S in the Valaciclovir Hydrochloride Hydrate API monograph.

The LC-MS method with Ascentis Express C18 (2.7µm, 4.6 mm X 15cm) analytical HPLC column was hired to fix the levels of Contaminant G and Contaminant S in Q1 Multiple ion mode. A gradient system consisting of cyanomethane (Reservoir B) and formic acid ammonium ion salt (0.01M), pH 3.0 (Reservoir A) was used for the elution of analytes, with separate compositions. The method developed was authenticated in agreement with the strategies mentioned in International Conference on Harmonization. Contaminant G quantitation limit was found to be 204.16ppm whereas contaminant S limit was found to be 215.60 ppm respectively.

Keywords: Q1 Multiple Ion, ICH guidelines, Contaminant G, Contaminant S.

Introduction

l-valyl ester of acyclovir salt is valaciclovir hydrochloride and chemical title is 2-((2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy)ethyl ester monohydrochloride, L-Valine (Table 1-1C). Docter prescribed valaciclovir hydrochloride hydrate medicine to eradicate the zoster herpes viruses and simplex. Herpes virus DNA replication is inhibited by the phosphorylation of valaciclovir to acyclovir triphosphate by viral thymidine kinase. Two contaminants relevant to the process are present in the active ingredient, valaciclovir hydrochloride hydrate contaminant G and contaminant S. 4-Dimethylaminopyridine, whose molecular formulation is $C_7H_{10}N_2$, is contaminant G (Table 1-1A).

N-[(Phenylmethoxy)carbonyl]-L-valine 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy]ethyl ester is the chemical title of contaminant S. Contaminant S's molecular formulation is $C_{18}H_{28}N_6O_6$ (Table 1- 1B). With a somewhat high level of harm, contaminant G can damage the eyes and lungs and is absorbed via the skin[2]. According to European Pharmacopoeia, contaminants G and S are recognized as official Contaminants of Valaciclovir Hydrochloride Hydrate API.

For the medication valacyclovir and related linked constituents' acyclovir, guanine and unidentified contaminant utilizing high-performance liquid chromatography, a chiral method validation is available. Acyclovir and valacyclovir, two antiviral medications, have been evaluated in the literature utilizing micellar electrokinetic chromatography (MEKC) in conjunction with their contaminant guanine[2]. There is a dearth of material on valaciclovir hydrochloride hydrate, whereas there is a wealth of publicly available literature on acyclovir[1,3,5,6,8,10,12-14]. Strong contaminant p-toluene sulfonic acid used API-4000 LC-MS/MS which was designed to measure the contaminant at residual or trace levels in pharmaceutical medicinal compounds[4].

Material and Methods

Pharmacopeial contaminates S and G were purchased from a legalized dealer. Cyanomethane and formic acid ammonium ion salt were acquired from Honeywell. Organise Valaciclovir Hydrochloride Hydrate API sample for the research work from the active pharmaceutical ingredient manufacture.

Table 1
Structural Details

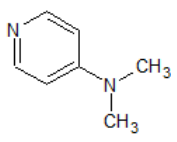
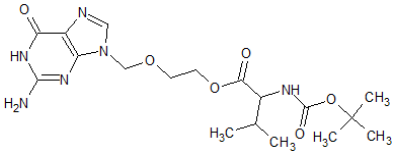
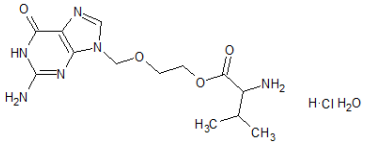
Contaminant G-1A	Contaminant S- 1B	Valaciclovir Hydrochloride Hydrate-1C
		

Table 2 lists the analytical tool utilized designed for the creation of the contaminant S and contaminant G method for quantification in the valaciclovir Hydrochloride Hydrate API.

Solutions preparation procedure: Sample and standard solutions were arranged in accordance with the preparation procedures provided in table 3 to conduct the study. Prior to the analysis, every prepared solution shown in table 3 was thoroughly sonicated.

Method Development Details: Evaluate various type of analytical columns during method development study including Waters Xbridge C18 (5.0µm, 4.6mm X 15cm), Ascentis (2.7µm, 4.6mm X 10cm) Express Octadecylsilane, Ascentis (2.7µm, 4.6mm X 5.0cm) Express Octadecylsilane and Inertsil ODS (5.0µm, 4.6 mm X 25cm). On the Ascentis (2.7µm, 4.6mm X 15cm) Express Octadecylsilane analytical performance column, the recovery of contaminants G and S was found to be within the acceptable limits when used in conjunction with a gradient system and reservoir A (Formic acid ammonium salt 0.01M, pH 3.0) and B (Cyanomethane). Select multi-reaction monitoring mode of Mass Spectrometer. Contaminant G, contaminant S and sample solution were injected on the Ascentis (2.7µm, 4.6mm X 15cm) Express Octadecylsilane column while maintaining the transitions 123.20 -> 107.20 for contaminant G and 425.20 -> 369.30 for contaminant S. contaminant G and contaminant S's responses were deemed adequate. Different mobile phase flow rates were used with various compositions.

Another method employed methanol as eluent B, 0.1 volume percent of formic acid and 0.02 volume percent of trifluoracetic acid in water as reservoir A. The gradient approach was later proven, but the isocratic method was first attempted to set. Multi-reaction monitoring (MRM) mode was used to take method development trials, although the accuracy and contaminants responses were not determined to be within a satisfactory range. Ultimately, an eluent A (Formic acid ammonium salt 0.01M, pH 3.0) and B (Cyanomethane) with gradient run were chosen after examining the data obtained from the analytical development study. Table 4 offered a gradient program.

Table 2
Instrument Details

LC Pump -20AD - Shimadzu	
SPD Detector -20A - Shimadzu	
SIL-20AC/HT - Shimadzu	
CTO-10ASvp- Shimadzu	
Method of Analysis Information	
A Reservoir	Formic acid ammonium ion salt 0.01M, pH 3.0
B Reservoir	Cyanomethane
Analysis Stainless Steel Column	Ascentis (2.7µm, 4.6mm X 15cm) Express Octadecylsilane
Eluent-Flow Velocity	1.0mL/min, 0.5mL/min via Splitter
Temperature of the Column	15^0 C
Temperature of the Sampler cooler	5^0 C
Volume of Injection	5.0µl
Method Duration time	20.0 Minutes
Parameter Mass Spectrometer	
Equipment	AB Sciex 4000 API
Ionization Technique	ESI
Ionization Polarity	+ve
Type of Scan	Multiple Ions Quadrupole 1(Q1)
Molecular Mass of G Contaminant	123.2 $(m+H)^+$
Molecular Mass of S Contaminant	425.2 $(m+H)^+$
Declustering potential (DP)	50 V
EP	10 V
CUR	35
IS	5500 V
Gas Source 1	30
Gas Source 2	50
Details -Valco Valve	The initial interval for emitting was between 4.1 - 7.4 minutes, followed by 9.9 - 18 minutes.
Details -Software Version and Name	1.6.3 Analyst

Table 3
Solutions Preparation Procedure

Solvent Blend	Ethanol: Water (80:20 *v/v*)
Stock solution preparation procedure	
Contaminant G stock solution having 0.51mg/mL concentration and Contaminant S stock solution having 0.54mg/mL prepared in solvent Blend	
Dilutant	Cyanomethane: Formic acid ammonium ion salt 0.01M, pH 3.0 (10:90)
Stock Solution preparation procedure 0.05 mg per mL	
Transported 1.0ml volume stock (0.05 mg per mL) solution of impurities S and G into 10mL capacity graduated flask. Further diluted up to the line with dilutant to make 0.05mg/mL concentration of solution.	
Stock solution preparation 0.001 mg/mL	
Transported 1.0ml volume of solution having 0.05mg per mL concentration into graduated flask having 50mL capacity. Further diluted to 50mL volume with dilutant up line to make 0.001mg per mL concentration of solution.	
Standard Solution Preparation	
Transported 0.50ml volume of solution having 0.001 mg per mL concentration into graduated flask having 10ml capacity. Further diluted the solution to 10ml volume with dilutant. Concentration of solution of contaminant G was 510.40 ppm and contaminant S was 539.00 ppm respectively against sample concentration.	
Preparation- Sample	
Valaciclovir Hydrochloride Hydrate 0.1 mg/mL solution prepared in the dilutant.	
Preparation for Recovery Study	
Prepared recovery solution LOQ, 50%, 100% and 150% levels for contaminants G and S against Valaciclovir Hydrochloride Hydrate API sample concentration by weakening stock solution of contaminants with the required volume of dilutant.	
preparation for Linearity Study	
Linearity solutions prepared for contaminant G (204.16 ppm, 255.20 ppm, 408.32 ppm, 510.40 ppm, 612.48 ppm, 767.60 ppm) and for contaminant S (215.60 ppm, 269.50 ppm, 431.20 ppm, 539.00 ppm, 646.80 ppm, 808.50 ppm) by weakening stock preparation (0.05 mg per mL) of contaminants with the required volume of diluent.	

Table 4
System program

Time	(%) Reservoir A	(%) Reservoir B
0.01	95	5
4.0	95	5
7.5	20	80
10	20	80
12.5	95	5
20	95	5

Results and Discussion

LC-MS technique advantages compared to thin layer chromatography (TLC) procedure: Thin layer chromatography (TLC) procedure is available in the Valaciclovir Hydrochloride Hydrate monograph of European Pharmacopoeia for determining the contaminants S and G. Contaminants G and S limits are 0.05% against the concentration of the Valaciclovir Hydrochloride Hydrate API sample. Figure 1 is an image of a thin-layer chromatography plate using the monograph approach. Quantification using the TLC technique displays problems with reproducibility. Because the TLC plate's length is limited, a limited separation quality can be attained. One major issue with the TLC approach is its time consumption.

The recent LC-MS methodology was established by trying various types of stationary phases with different column chemistry to attain important resolution of the contaminants S and G with Valaciclovir Hydrochloride Hydrate API and accuracy inside the receipt standards, taking into consideration the drawbacks associated with the current method as described in the monograph.

In comparison to the current European Pharmacopoeia TLC approach, the method developed using LC-MS was more delicate and selective. It may be used to accurately evaluate contaminants G and S in the antiviral medication valaciclovir hydrochloride hydrate. The mobile phase flow rate in the LC-MS technique was 1.0 ml and the run time was 20 minutes. The separation of contaminant G and contaminant S in the LCMS analysis method demonstrates the high resolving power and the LCMS-developed method also proves the orthogonality with the available TLC method.

Validation-Analytical Method: By subjecting the dilutant, specified contaminants and valaciclovir hydrochloride hydrated API sample, the method's specificity was confirmed. Figures 2-5 show the chromatograms of the associated solutions. Figure 2's dilutant chromatogram demonstrated that neither contaminants nor an interfering peak could be found during the retention periods of valaciclovir hydrochloride hydrate API. Figures 2-5's extracted chromatograms showed that 2.70 minutes and 8.18 minutes are the retention times of contaminants G and S. Chromatograms using the recognized method show that there are no conflicting peaks at the retention times for contaminants G, S and valaciclovir hydrochloride hydrate API powder. The API powder, valaciclovir hydrochloride hydrate and contaminants G and S could be distinguished from one another using a newly developed analytical approach.

For contaminants G and S, the ratio of signal to noise was used to establish the lower limit of quantification (LLOQ) and limit of detection (LOD). Prepare the standard solution concentrations at lower levels to achieve the procedure's lower limit of quantitation. The LLOQ solutions of contaminants S and G yield a signal/noise ratio of 219.0 (Figure 7) and 245.5 (Figure 6) correspondingly. 215.60 ppm and 204.16 ppm are the lower limits of quantification for contaminants S and G.

The linearity of the devised analytical technique was established in the Q1 multiple ions scan type through the injection of impurities G and S at various concentration levels ranging from LLOQ to 150% of the target concentration.

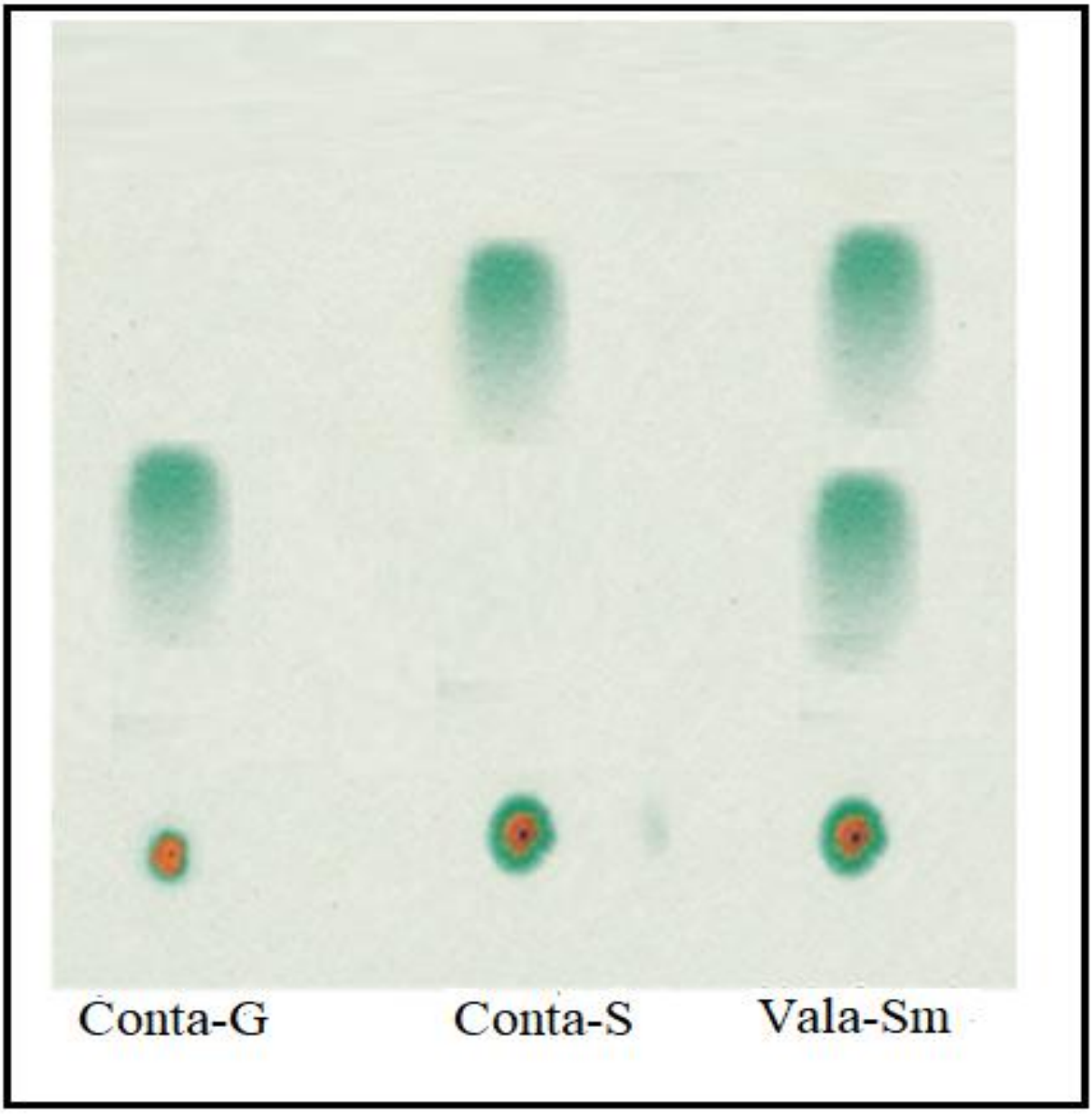


Figure 1: TLC Plate Contaminants G and S

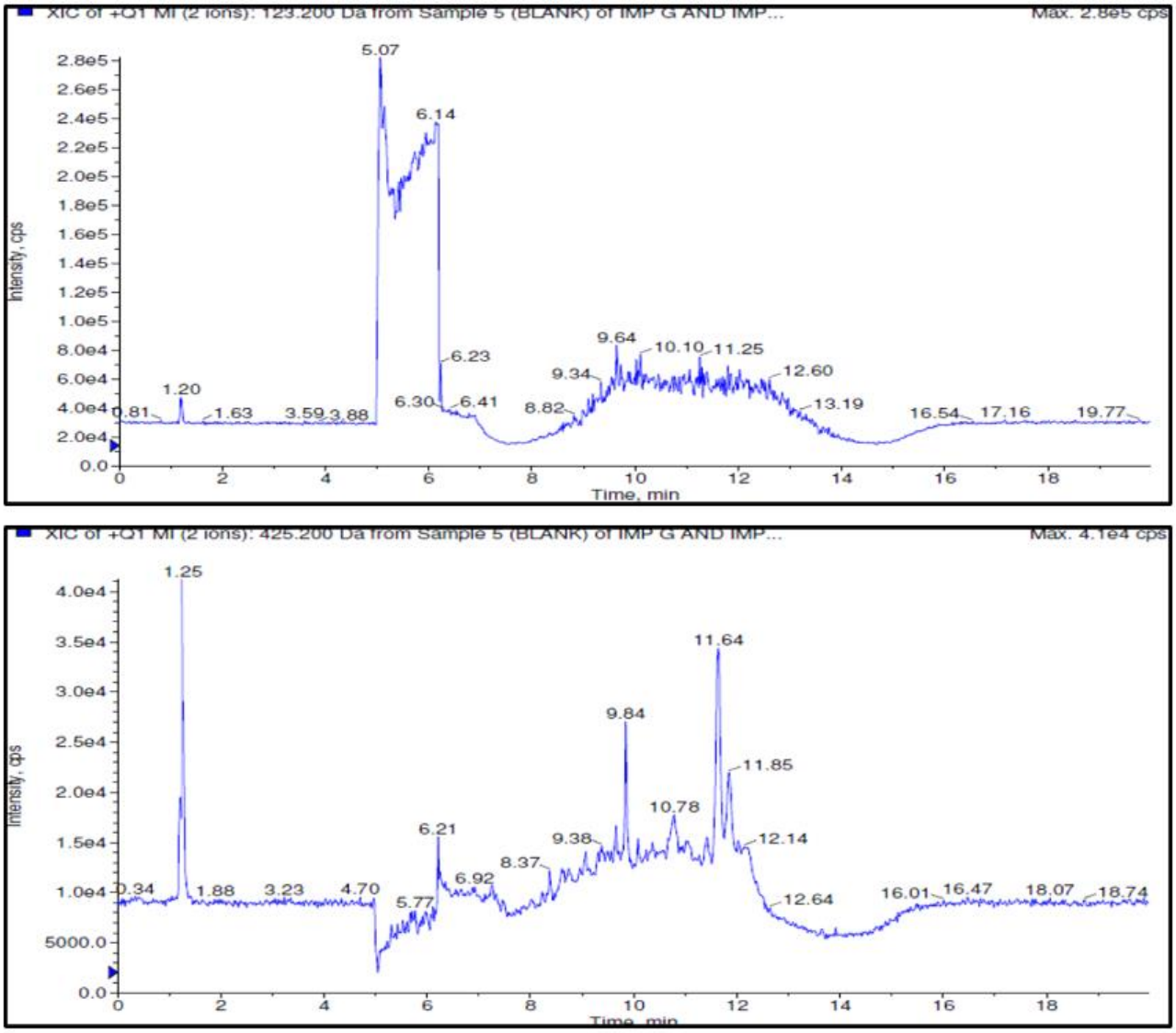


Figure 2: Blank XIC- Contaminant G and S

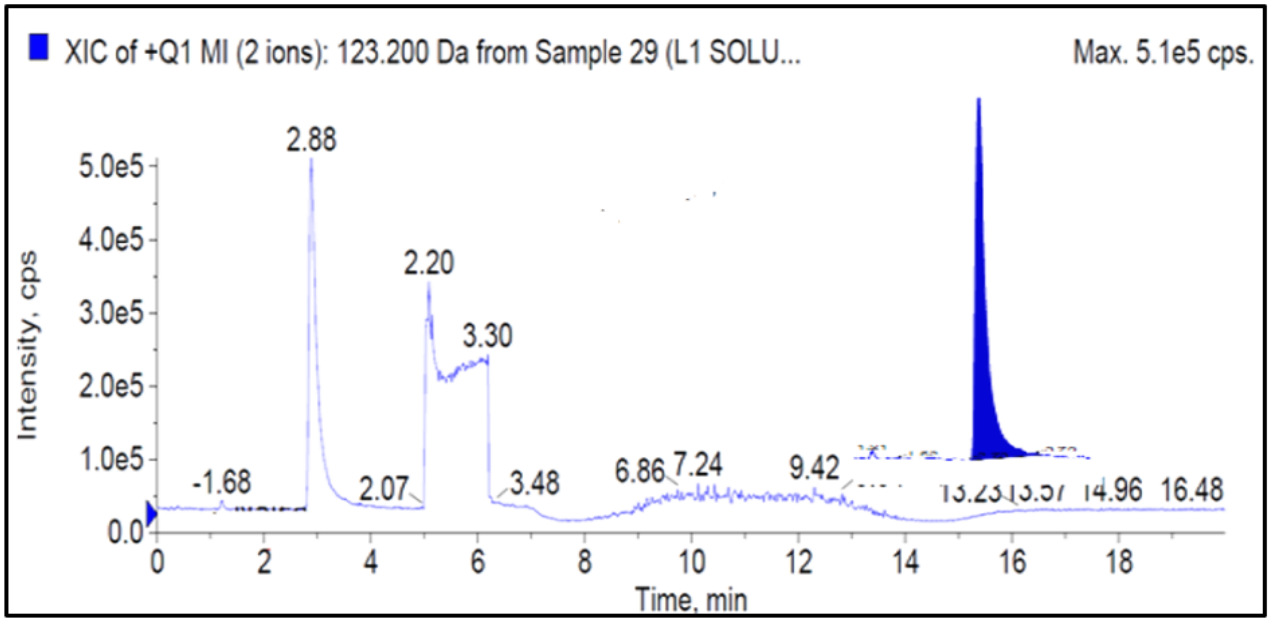


Figure 3: Contaminant G XIC

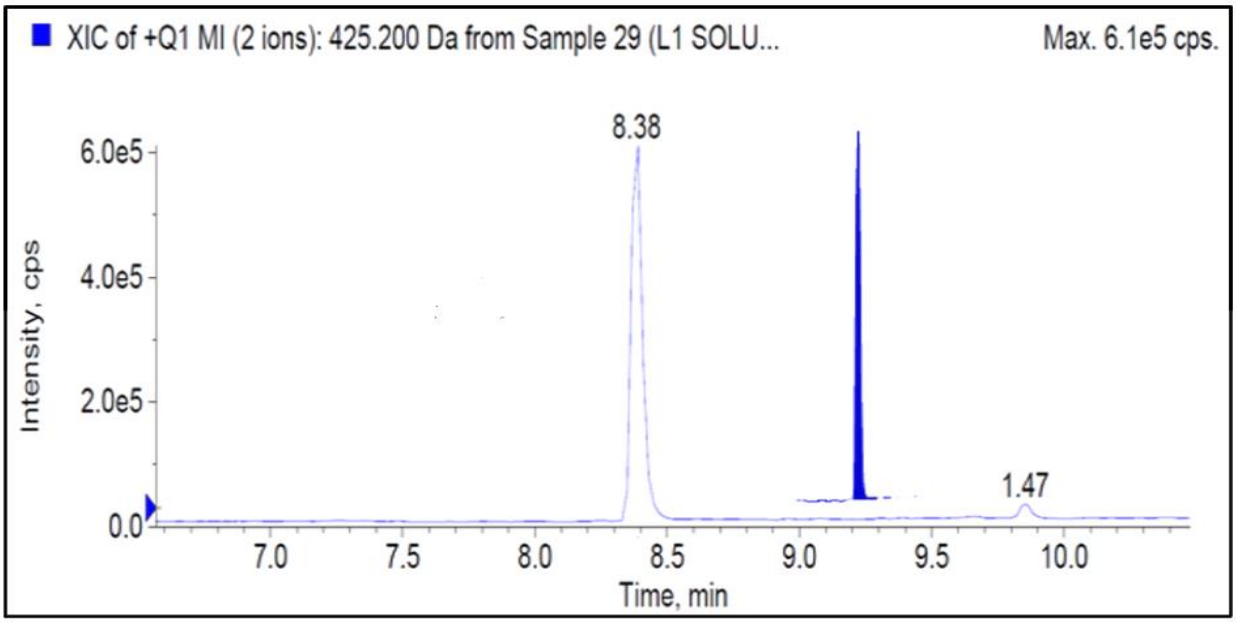


Figure 4: Contaminant S XIC

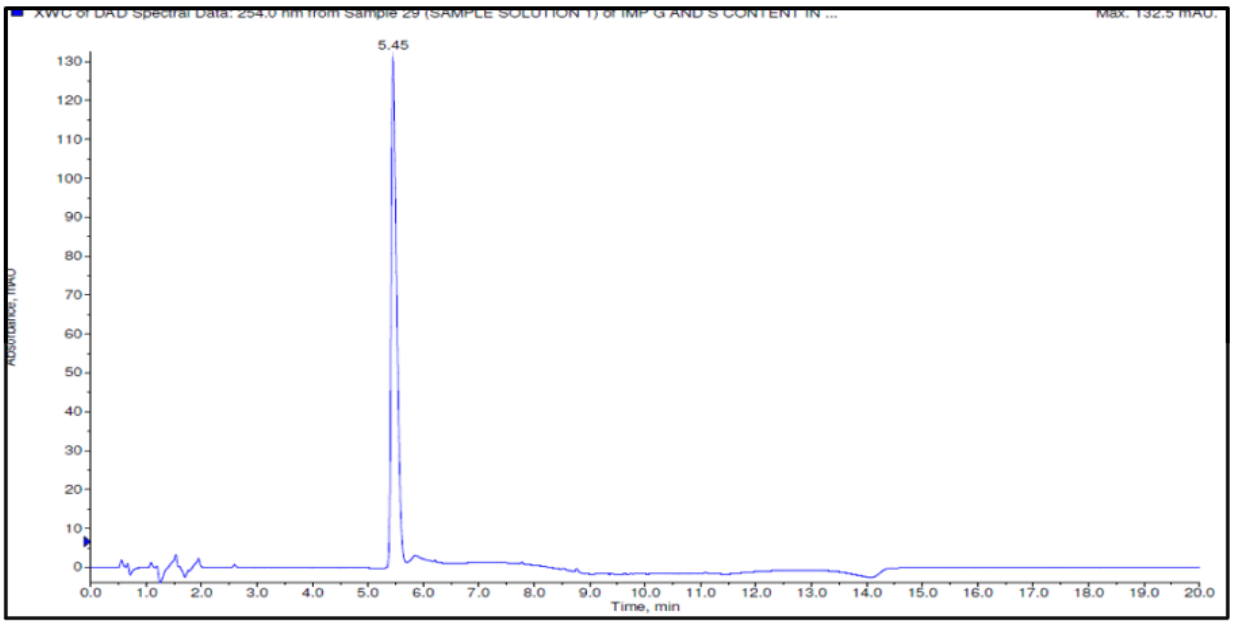


Figure 5: Chromatogram UV-Sample

Table 5
Contaminants S and G Linearity

Level	Contaminant G Strength in ppm	Area-Contaminant G	Contaminant S Strength in ppm	Area-Contaminant S
Linearity L 1	204.16	2263699	215.60	231387
Linearity L 2	255.20	2750275	269.50	326611
Linearity L 3	408.32	4525298	431.20	558097
Linearity L 4	510.40	5577965	539.00	705821
Linearity L 5	612.48	6567244	646.80	829698
Linearity L 6	767.60	8691043	808.50	1137061
(r^2) Correlation Coefficient		0.9984	**(r^2) Correlation Coefficient**	0.9976
Slope		11095.8	**Slope**	1473.2
Intercept		-110972.1	**Intercept**	-84065.9

The contaminant G concentrations at 204.16 ppm, 255.20 ppm, 408.32 ppm, 510.40 ppm, 612.48 ppm and 767.60 ppm and the contaminant S concentrations at 215.60 ppm, 269.50 ppm, 431.20 ppm, 539.00 ppm, 646.80 ppm and 808.50 ppm were charted to create the calibration curve. A study was conducted using regression line to determine the intercept, slope and values of the correlation coefficient. Table 5 offered access to linearity data.

By doping the specified quantities of contaminants S and G at different levels (LLOQ, 150%, 100%), against the sample concentration, a spike study was conducted to determine the correctness of the recently proposed analytical method. Figure 8 and 9 are the extracted chromatograms of contaminant G and S of LLOQ doping study. LLOQ, 150% and limit levels of relevant accuracy data are shown in table 6. The coefficient of variation should be below 10.0. The regaining of contaminants G and S at three stages (LLOQ, 150% and 100%) should fall between 85.0% and 115.0%. Recovery percentages 98.63 % to 103.41 % with percentage RSDs of 2.94 were found for contaminant S and 96.03 % to 98.31 %, with percentage RSDs 1.51 respectively for contaminant G.

The ruggedness and repeatability study of the analysis method was evaluated by doping requirement level contaminants S and G in six newly organized prepared sample solutions and the coefficients of variation of the contaminants G and S content were checked. A coefficient of variation of not more than 10.0 is appropriate and table 7 provides the relevant statistics. Contaminant G's and contaminant S's relative standard deviations were 1.91 and 2.12 respectively. Examine the precision of the LLOQ level and the coefficients of variation of the six repeat injections for contaminants G and contaminants S were 1.70 and 3.91 respectively. Table 8 displays connected LLOQ precision data.

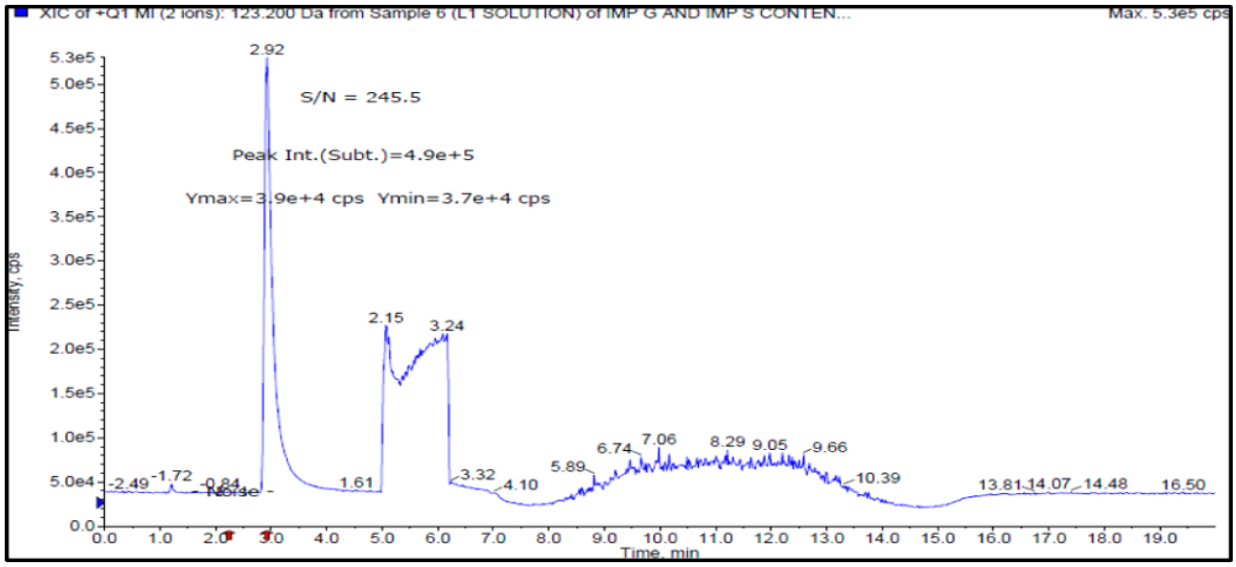


Figure 6: Contaminant G- signal/noise ratio

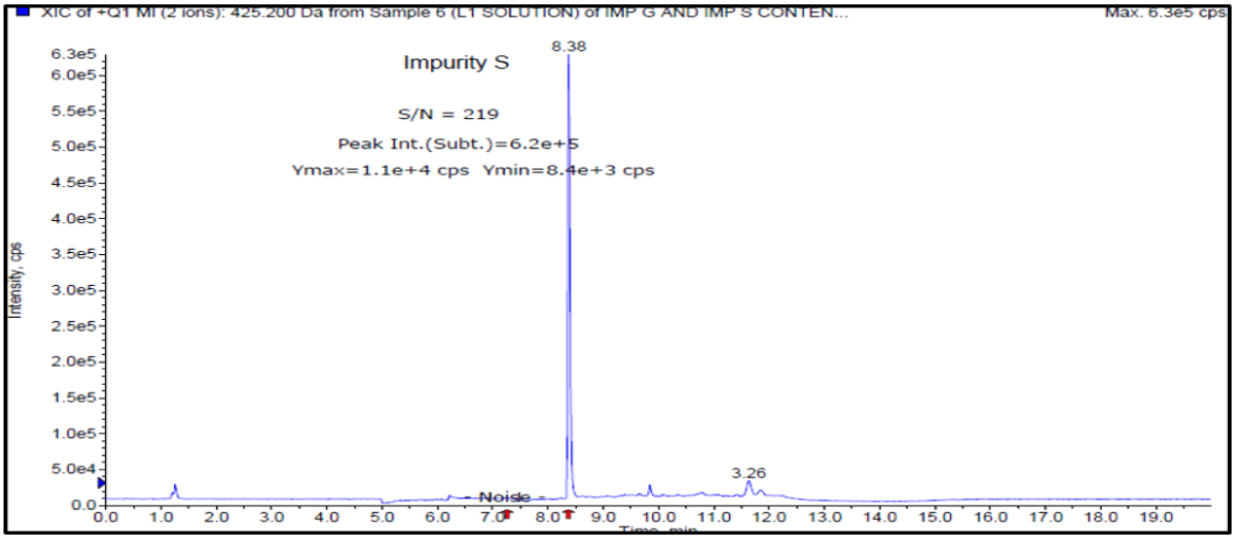


Figure 7: Contaminant S- signal/noise ratio

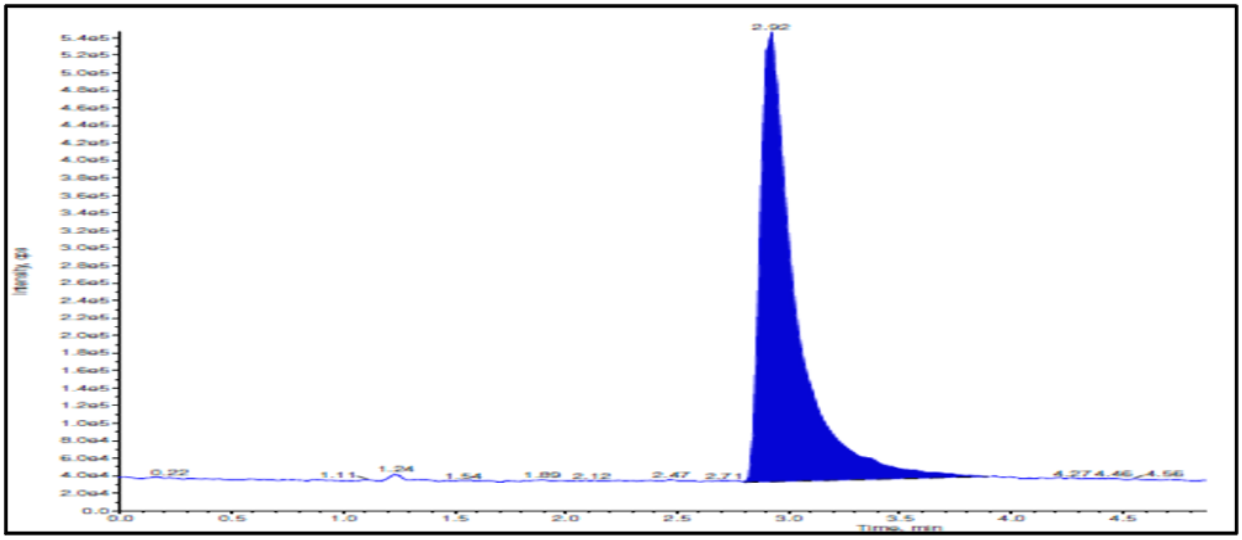


Figure 8: Contaminant G- LLOQ Spiked Sample XIC

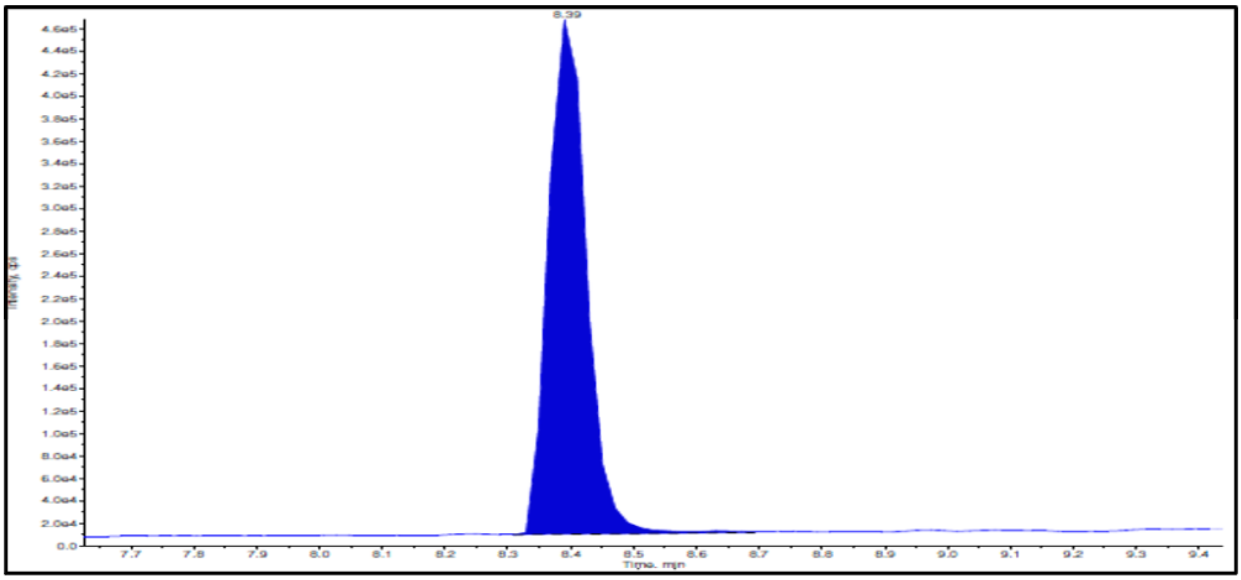


Figure 9: Contaminant S - LLOQ spiked sample XIC

Table 6
Accuracy result for Contaminants G and S

	Contaminant G			Contaminant S		
Accuracy Stages	**Theoretical Strength (ppm) against Sample**	**Measured Strength (ppm) against Sample**	**Regaining Percentage**	**Theoretical Strength (ppm) against Sample**	**Measured Strength (ppm) against Sample**	**Regaining Percentage**
(40%) LLOQ	204.16	200.7	98.31	215.60	212.65	98.63
100 %	510.40	504.31	98.80	539.00	528.41	98.04
150 %	767.60	737.11	96.03	808.50	836.10	103.41
	Coefficient of variation		1.51	Coefficient of variation		2.94

The target level standard concentrations of contaminants G and S were spiked in six newly prepared sample solutions at different times to test for ruggedness. The commutative percent relative standard deviation of each contaminant's content among the intermediate precision and the spike precision should not exceed 10.0. Contaminant G's and contaminant S's relative standard deviations were 1.92 and 1.90 respectively. Table 9 displays the connected data. Method of analysis robustness was verified through deliberate adjustments to the flow speed of the mobile phase and mobile phase pH. 10% adjustment (0.90 to 1.00 mL/min) was done in the eluent flow speed which was 1.0 mL in the analytical technique.

Table 7
Precision (Spike) Result for Contaminants G and S

Sequence	Strength of Contaminants G gained in sample	Strength of Contaminants S gained in sample
1	510.41	540.52
2	515.35	552.22
3	497.96	523.77
4	491.45	547.77
5	509.44	537.23
6	514.77	555.16
Average	506.56333	542.7783
Standard Deviation	9.695949	11.52882
Coefficient of variation	1.91	2.12

Table 8
LLOQ Level Precision data

Injection Sequence	Contaminant G Area in LLOQ Solutions	Contaminant S area in LLOQ Solutions
1	2253188	217424
2	2267776	236222
3	2197895	243271
4	2274617	235323
5	2312592	225580
6	2276123	230145
Average	2263698.52	231327.49
Standard Deviation	37740.52	9059.79
Coefficient of variation	1.70	3.91

Table 9
Ruggedness result for Contaminants S and G

Injection	Contaminant G Strength obtained in sample	Contaminant S Strength obtained in sample
Precision-1	510.41	540.52
Precision-2	515.35	552.22
Precision-3	497.96	523.77
Precision-4	491.45	547.77
Precision-5	509.44	537.23
Precision-6	514.77	555.16
Ruggedness -1	515.22	530.13
Ruggedness -2	521.17	554.23
Ruggedness -3	525.22	553.77
Ruggedness -4	520.17	548.10
Ruggedness -5	517.14	537.77
Ruguddness-6	520.87	551.21
Average	513.26417	544.3233
Standard Deviation	9.8594435	10.32366
Coefficient of variation	1.92	1.90

Table 10
Solution constancy

Situations	Area of Contaminant G	Area of Contaminant S
Initial At 0 hrs.	8715434.0	664232.0
39.30 hrs. At Room Temperature	7505262.0	583583.0
Coefficient of variation	15.7	12.8

Hydrogen ion concentration of mobile phase was modified by +0.2 units (2.8 pH and 3.2 pH) to check the impact of pH adjustment on the sample analysis. The chromatographic performance and the ability to separate contaminant G and contaminant S from the hydrated valaciclovir hydrochloride were not significantly affected by any of the aforementioned parameter modifications. Prepared sample solution and specification level solutions of contaminants were spiked

into it and reserved at 25 °C (Room temperature) to demonstrate the constancy of the contaminant G and contaminant S solutions. By figuring out the contaminants G and S solution's area coefficient of variation among 0 and 40 hours, the stability of the solution was assessed. Coefficient of variation of area under the curve of contaminants G and S solution should be below 20.0%. Table 10 showed that at room temperature, the contaminant G and S solution remained stable for 39.30 hours.

Conclusion

Using a liquid chromatograph mass spectrometer, an accurate, sensitive, selective and specific analytical method was created to quantify contaminants S and G in the Valaciclovir Hydrochloride Hydrate API at the lower level (0.05 percent) against the concentration of Valaciclovir Hydrochloride Hydrate API sample. In the positive mode of ionization, an electrospray ionization source/probe was employed. It has also been confirmed that the LC-MS method has lower detection capability and is efficient for quantifying contaminants G and S than the TLC method.

To validate the analytical method, validation experiments were conducted on specificity, precision, linearity, accuracy and stability of the solution. Acceptable contaminant resolution using the Valaciclovir Hydrochloride Hydrate API demonstrated the method's specificity. The linearity of this method was found to span from 204.16 ppm to 767.60 ppm for contaminant G and from 215.60 ppm to 808.50 ppm for contaminant S against the Valaciclovir Hydrochloride Hydrate API sample concentration. The coefficient correlation for contaminant G and contaminant S was 0.9984 and 0.9976 respectively.

The developed method demonstrated high accuracy with recovery values ranging from 96.03% to 98.31% for contaminant G and 98.04% to 103.41% for contaminant S with relative standard deviations of 1.51% and 2.94% respectively. Additionally, the method's sensitivity was confirmed, with 204.16 ppm being a quantification limit at the lower side for contaminant G whereas 215.60 ppm is a quantification limit at the lower side for contaminant S.

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