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STABILITY INDICATING AND GREEN SOLVENT-ASSISTED CHROMATOGRAPHIC ANALYSIS OF AN ANTIVIRAL DRUG

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ABSTARCT:

This study presents a green chromatographic method for estimating the Ganciclovir, demonstrating validation parameters in alignment with ICH guidelines and green chemistry principles. The green analytical technique utilized the ethanol and acidic water at pH 3.0 as solvents, with an optimized mobile phase ratio of (80:20) and a flow rate of 1.0ml/min with a zorbax eclipse plus C18 (4.6 x 150mm, 5µm). Ethanol is listed as a green solvent in U.S Tri EPA with (CAS 64-17-5), as well as economical with manageable waste compared to traditional solvents. Green Evaluation tools such as AGREE, GAPI, and COMPLEX GAPI were employed to assess the greenness scores of the method. The AGREE tool provided an evaluation of the greenness of the method while the GAPI and COMPLEX GAPI offered details about the assessment of the method's adherence to the green chemistry principles. These tools were developed in adherence to 12 green analytical chemistry principles that provide a holistic approach toward qualitative and quantitative evaluations. The importance of greenness in analytical methods lies in reducing environmental impact, improving analyst safety, lowering cost and waste, and fostering environmental responsibility.

The linearity range was 10-50µg/ml with a regression coefficient of less than or equal to 0.999 indicating a strong linearship. RSD values are consistently below 2% and recovery rates for ganciclovir ranged from 98% to 102% within acceptable ranges. Under forced degradation conditions the desired amount of degradation was exhibited 8.02% degradation in acid, 9.25% in alkaline hydrolysis, 4.02% degradation in peroxide, 5.42 % in thermal, and 6.38 % in photolytic studies which are within limits as per 5-20% as per ICH Q1B guidelines. Incorporating ICHQ1(R2) the method validation parameters are within the range and thoroughly addressed.

Keywords: Ganciclovir, RP-HPLC, Ethanol, Green method, Validation.

1. INTRODUCTION:

The first anti-retroviral medication approved to treat CMV infection was ganciclovir 2-amino-9-[(1,3-dihydroxypropan-2-yl)oxy]methyl-6,9-dihydro-1H-purin-6-one [1]. It is often used to treat CMV infections in people with weakened cell-mediated immunity, such as those with poorly managed HIV/AIDS or transplant recipients who are at high risk of developing invasive CMV illness [2]. Valganciclovir, an oral prodrug that is converted to ganciclovir, is also used in immunocompromised people to prevent and treat CMV infections. [2].

High-performance liquid chromatography is one of the techniques in column chromatography that is used in analysis to separate, identify, and quantify the active compounds. It utilizes a stationary phase primarily a column and mobile phase to carry over the sample of analysis. The Quantitative analysis of Ganciclovir is carried out using liquid chromatography techniques such as RP-HPLC and acetonitrile as solvent as per the literature review [3][4][5][6]. All the methods utilized non-eco-friendly solvents such as acetonitrile ion pairing agents etc., for the estimation of the active compound. As a result, the application of the above methods is not environmentally safe and induces risk for the analyst.

However other anti-virals are estimated using different liquid chromatography techniques with green solvents such as gradient RP- HPLC-glibenclamide estimation using ethanol [7], Cefepime hydrochloride estimation using methanol [8], Rosuvastatin estimation using green solvent ethanol in RP-HPLC [9], Nevirapine and lamivudine using ethanol as solvent [10], Micellar chromatographic estimation of four antiviral drugs using Sodium Dodecyl Sulfate, Isopropanol, Triethylamine[11], Mixed micellar estimation of antiviral drugs using sodium lauryl sulfate[12] and other spectrophotometric-HPLC methods using green solvents[13][14], famciclovir by RP-HPLC [15]. Certain green methods were reported using ethanol as a solvent for estimating non-antiviral drugs using HPLC such as deferasirox in human plasma[16], azosemide-micellar chromatography [17], impurities in deferasirox[18]. No green methods were developed for the estimation of Ganciclovir.

Ethanol is considered as a green solvent as per the U.S Tri Environmental Protection Agency.The greenness of the method is evaluated based on the 12 GAC principles. Greeness evaluators are designed to assess the environmental impact of analytical methods helping analysts to develop more sustainable methods [19][20][21]. These tools evaluate various aspects of analytical procedures from consumables to waste generation. Three prominent evaluators are AGREE, GAPI, and COMPLEX GAPI. AGREE tool provides a comprehensive, user-friendly approach for evaluating the greenness of the method[22][23]. It incorporates various criteria such as sampling, number of steps involved in the sample preparation, automation, amount of waste generated, multi-analysis, energy saving, renewable reagents, and threats to operator safety. A score less than one at the center of the pictogram represents the method's greenness and sustainability.GAPI and COMPLEX GAPI are designed to assess the environmental impact of analytical procedures. It uses a color-coded hexagonal diagram to represent the different stages of the analytical process, with colors indicating the environmental friendliness of each stage [24][25][26].

MATERIALS

The materials used for the method development are listed in Table 1.

2. REAGENTS AND METHOD:

The reagents used for the method development are listed in Table 2.

Stock solution preparation:

A primary stock solution was produced by solubilizing 10 mg of Ganciclovir working standard in 7.0 mL of ethanol, sonicating to dissolve and remove any air, then bringing the quantity up to the mark with the identical amount of Ethanol.

As mentioned above, Pipette 0.3 mL of the ganciclovir stock solutions into a 10 mL volumetric flask, then dilute with ethanol to the appropriate level.

Optimizing the mobile phase:

A primary mobile phase investigated was Ethanol: Water in various ratios. At pH 3.0 the mobile phase was finally optimized for ethanol: acidic water; in the ratio of 80:20% v/v.

Mobile phase preparation:

Upon measuring precisely 800ml (80%) HPLC, Ethanol and 200 mL (20%) Water with pH 3.0 were combined and removed gases in an ultrasonicator bath for 10 minutes before being vacuum filtered via a 0.45 filter.

Column Optimization:

A procedure was done various C18 columns such as Symmetry, Zodiac, and Xterra. At 1ml/min flow, the Zorbax Eclipse Plus column C18 (4.6 x 150mm, 5m) provided good peak shape and resolution.

Sample Solution preparation :

Take a sample of Ganciclovir that weighs 10 mg equivalent and put it in a 10 mL flask that can measure volume. Add 7.0 mL of Diluent to the flask and use ultrasonication to dissolve the sample completely. Further, add the diluent to the flask to make up the volume.

From the Ganciclovir stock solution, draw 0.3 mL and transfer it to another 10 mL flask that can measure volume. Use Ethanol to fill up the flask to the mark to get the desired concentration.

System Suitability:

HPLC chromatographic conditions are optimised for analysis. 10 µL of standard drug solutions were injected into the HPLC in five replicates and validation criteria such as retention time, peak area, peak height, tailing factor, and theoretical plates were examined for system suitability.

Linearity :

Ganciclovir's primary stock solution was further diluted using diluents to generate a series of drug solutions consisting of 10, 20, 30, 40, and 50µg/ml for injection into the chromatographic apparatus. The linearity was assessed by plotting the calibration curve using Concentration vs Peak Area and obtaining the Regression Coefficient with intercepts by ICH recommendations.

Greenness Evaluation:

The proposed method was evaluated for the greenness metrics using AGREE software Analytical Greeness Calculator, GAPI, and Complex GAPI based on 12 Green Analytical Chemistry principles and was found to be safe and bio-friendly.

Forced Degradation Studies

Forced degradation studies were carried out under the conditions of hydrolysis(acid, base), peroxide, thermal and sunlight. 10 tablets were weighed, crushed and powdered, a quantity of equivalent to 10mg transferred into a 10mL volumetric flask and dissolved in diluent up to mark. This solution was used as a stock solution.

In acid degradation studies 0.2 mL of above stock solution was transferred to 10 mL volumetric flask , 3mL of 0.1N HCl was added and kept for 3 h, made up the volume up to the mark with the diluent and chromatogram were recorded.

In alkaline degradation studies 0.2 mL of above stock solution was transferred to 10 mL volumetric flask , 3mL of 0.1N NaOH was added and kept for 3 h, made up the volume up to the mark with the diluent and chromatogram were recorded.

In peroxide degradation studies 0.2 mL of above stock solution was transferred to 10 mL volumetric flask , 3mL of 0.1% H_2O_2 was added and kept for 3 h, made up the volume up to the mark with the diluent and chromatogram were recorded.

In thermal degradation studies 0.2 mL of above stock solution was transferred to 10 mL volumetric flask , made up the volume up to the mark with the diluent and exposed to 60-80°C for 3 h and chromatogram were recorded.

In Photolytic degradation studies 0.2 mL of above stock solution was transferred to 10 mL volumetric flask , made up the volume up to the mark with the diluent and exposed to sunlight for 3 h and chromatogram were recorded.

4. RESULTS:

The developed HPLC method estimates the Ganciclovir on the following optimized conditions after trial and error basis with a retention time of 3.155 minutes.

Optimized Conditions:

Instrument used : Waters HPLC with auto sampler and PDA 996 detector model.

Temperature : 35ºC

Column : Zorbax Eclipse plus C18 (4.6 x 150 mm, 5µm)

Mobile phase : Ethanol : acidic water at ; pH 3.0 adjusted by phosphoric acid; in the ratio of (80 : 20, v/v)

Flow rate : 1.0 mL/min

Wave-length : 245 nm

Injection- volume : 10 µL

Run time : 8 minutes

Validation :

The method was verified against ICH guidelines for the validation parameters.

System Suitability :

As per the optimized conditions the retention time of Ganciclovir was at 3.155 minutes for the standard and sample with a theoretical plate count of 2635 not less than 2000 and tailing factor of 0.9 not more than 2. The results are under the acceptance criteria of ICH Guidelines. The results are tabulated in table 3.

Specificity :

The ICH guidelines define specificity as the ability to measure the analyte accurately without reservation during the presence of other elements that might be expected to be there, such as elements from the matrix, products of degradation, and impurities. In order to precisely quantify Ganciclovir in a drug product, the method's specificity had been tested and obtained an optimized chromatogram as in Figure 1,2 and 3.

Assay of standard and Sample :

Standard and sample solutions were injected thrice, and their percentage were calculated. The purity was estimated to be 100% and is acceptable for estimating Ganciclovir in pharmaceutical dosage forms as reported in table 4 and 5.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity :

The method's linearity was tested using concentrations from 10µg/ml to 50µg/ml. Table 6 presents the calibration curve's concentration and peak areas. Figures 4,5,6,7,8,9 demonstrate the relationship between concentration and peak area, with a clear linear trend. The regression equation is y = 326.5x + 3710, and the regression coefficient (r^2) was found to be 0.998, which is well in the acceptance criteria of ICH Guidelines.

Precision :

Method Precision (Repeatability)

Five replicates of the 100% accuracy solution are injected as per experimental conditions. Measured the peak areas and computed the % relative standard deviation. The % RSD had been with in the acceptance criteria >2 as shown in the Table 7 and Figures 10,11,12,13,14.

Ruggedness

In order to assess the intermediate precision of the method, testing had been conducted on different days while preserving the similar conditions. Six sample solutions were injected into HPLC by two different analysts, and the %RSD was calculated. As shown in table 8 and 9 and figures 15,16,17,18,19,20 (analyst-1) 21,22,23,24,25,26 (analyst-2), the %RSD was found to be greater than 2.

Accuracy :

HPLC was injected with three replicates of individual concentrations of 50%, 100% and 150%, and chromatograms were recorded in figures 27,28,29, the peak responses and the amounts of Ganciclovir found and added were measured. The individual and mean recovery values were then evaluated and presented in table 10. According to the ICH criteria, the percentage recovered fell within a permitted range.

LOD for Ganciclovir

The LOD for ganciclovir is the minimum amount of the analyte that can be found in a sample but not always quantified.

$LOD = 3.3 \times \sigma / s$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve(3)

Result:

= 3.3 × 1314.685 /3265

= 1.3µg/ml

LOQ for Ganciclovir

An analytical procedure can quantitatively measure the amount of analyte in a sample, but only if it is above the "quantitation limit," which is the lowest amount possible.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

=10 × 1314.685 /3265

= 4.0µg/ml

Method Robustness

The method for Ganciclovir was tested for its robustness by varying the flow rate from 0.9ml/min to 1.1ml/min and the ratio of the mobile phase from more organic to less organic. The method was found to be robust only at less flow rates and was able to withstand changes in the mobile phase by up to ±5%. Both standard and sample injections were performed under different chromatographic conditions. As per table 11 and figures 30,31,32,33 however, there were no significant changes observed in the resolution, tailing factor, asymmetric factor, and plate count.

Analytical Method Greenness and comparison :

1. **AGREE**

The method's greenness was assessed utilizing an e-platform of "Agree Analytical Greenness Calculator V.0.5. Beta", GAPI, Complex GAPI. Based on the 12 GAC principles(4), the analytical greenness score aids in the evaluation and development of more sustainable and efficient techniques. For 12 parameters, previous analytical approaches were compared to the suggested method. The Analytical greenness calculator's scoring Figure. 34 indicates that certain characteristics, such as 7, 10, 11, and 12, are colored in red, indicating that they are not ecologically

safe or a renewable resource. Using a green solvent and the proposed approach, the Greenness measures were green and close to one, indicating an environmentally safe and sustainable method.

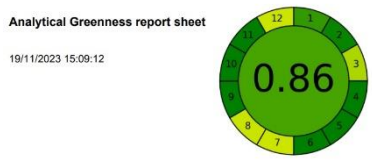
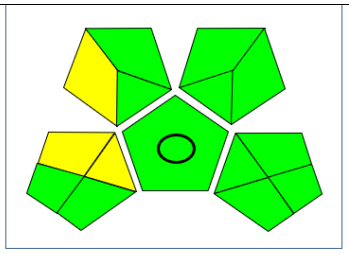
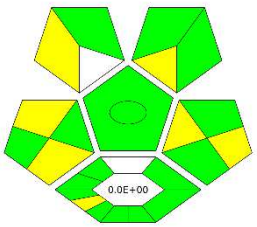
2. GAPI

The Green Analytical Procedure Index is a new tool to assess the greenness of the analytical method. The greenness in the chart is indicated with three colours Green, Yellow, Red. Green indicates the parameter is greenness and safety and yellow indicates slight greenness of the method and Red indicates that is not ecologically safe.The parameters are indexed as per the 12 GAC principles for the determination and assessment of sample handling, waste assessment, and energy consumption. The method was assessed with the tool and found that this approach is environmentally safe Figure 35.

3. COMPLEX GAPI

The complex Green analytical procedure index is used to assess the greenness of the method Figure 36. as well as the pre-analysis process greenness of the method. It helps to analyze the environmental friendliness of the method. The method was assessed using the software and found that it is environmentally safe and less hazardous to the analyst.

Comparision :

S.No	Analyte	Agree	GAPI	Complex GAPI
1	Ganciclovir			

Forced Degradation Studies :

The degradation studies show that the drug degrades by comparing the area of sample to standard peak areas. The percentage degradation was calculated for in each degradation study and tabulated in table 12. The percentage of degradation was found to be with in 5-20% or recovery percentage

was between 90-110% as per ICH Q1B guidelines for each study, In comparison with peroxide the degradation was less in thermal and photolytic conditions where as the degradation was less in acid compared to alkaline conditions and chromatograms were recorded in Figures 37,38,39,40,41.

5. Discussion:

The present study aimed at developing and validating green analytical method for the determination of Ganciclovir, an anti-viral agent commonly used in the treatment of herpes virus, particularly cytomegalo virus. No green solvent-assisted method development and validation was carried out to estimate the Ganciclovir in bulk and Pharmaceutical dosage forms. The development of such methods is important to ensure the safety of analysts as well as the environment.

The green analytical method proposed in this study utilized the eco-friendly solvents Ethanol , acidic water and reagents as per the U.S Tri EPA list with (CAS 64-17-5) and the remaining aspects like sample handling, low toxicity, waste and energy management in compliance with the 12 green analytical chemistry principles.

The chromatographic conditions optimized in this method (80:20) ethanol and acidic water, 1.0ml/min with Zorbax Eclipse plus C18 (4.6 x 150mm, 5µm) exhibited the satisfactory separation of Ganciclovir with a 3.15min retention time. The validation results demonstrate the method's reliability and sustainability for its intended purpose. The parameters such as linearity (10-50µg/ml) show the linear relationship, precision, robustness, and ruggedness are within the acceptance criteria as per the ICHQ1(R2) guidelines confirming the ability of the method for quantifying Ganciclovir.

Forced degradation studies were conducted to evaluate the method's ability to detect and quantify the Ganciclovir and its potential degradation products under stress conditions. The method demonstrated adequate stability-indicating capability with clear separation and quantification of Ganciclovir from its degradation products in acid (8%), alkaline (9%), peroxide (4%), thermal (5%), photolytic (6%) which are within the limits of 5-20% acceptance criteria under stress conditions as per ICH guidelines Q1A, Q1B, Q2B.

The method's greenness was evaluated by the three different Greenness evaluator tools AGREE, GAPI, and COMPLEX GAPI. The results of these tools suggest the method is greener with less than 1 score in AGREE and green color indication by GAPI and COMPLEX GAPI in color-coded hexagonal suggesting the method is greener and sustainable in all 12 conditions of green analytical chemistry. A comparison is carried out between acetonitrile as solvent [27][28] and ethanol as

solvent with the evaluation tools, the results indicate that ethanol as a solvent is more greener than acetonitrile in terms of waste generation, renewability, toxicity, and threat to the operator.

The developed method offers a reliable, sensitive, eco-friendly approach for the determination of Ganciclovir in Pharmaceutical Formulations. By adhering to the principles of green analytical chemistry, this method presents a significant advancement towards environmentally responsible Pharmaceutical Analysis.

6. Conclusion :

A novel green solvent-assisted, stability-indicating HPLC method with a reliable and environmentally friendly approach to analyse ganciclovir in bulk and pharmaceutical dosage forms has been developed. The ICH Guidelines verified the approach to have a linear regression coefficient of no more than 0.999 and a linearity range of 10-50μg/ ml. The accuracy and precision of the method were demonstrated by % RSD values of <2% and the percentage recovery varied from 98-102% for ganciclovir. LOD and LOQ were within the acceptable limits. The percentage degradation of the active compound was found to be within 5-20% in forced degradation studies and acceptance criteria. This method exhibited good selectivity, linearity, accuracy, precision, robustness, and ruggedness along with system suitability and stability. This method can be used for regular analysis and measurement of pharmaceutical products that contain ganciclovir, ensuring that they are safe and effective.

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Disclosure Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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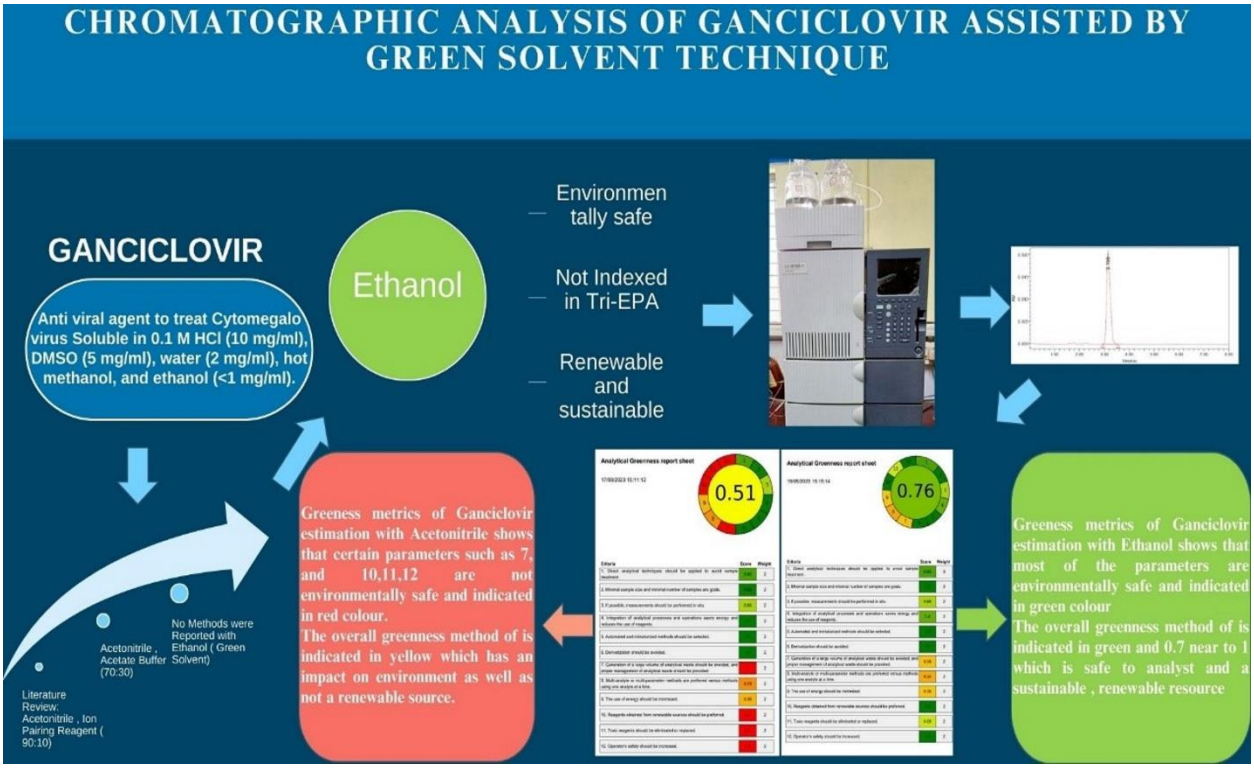
FIGURES :

GRAPHICAL ABSTRACT



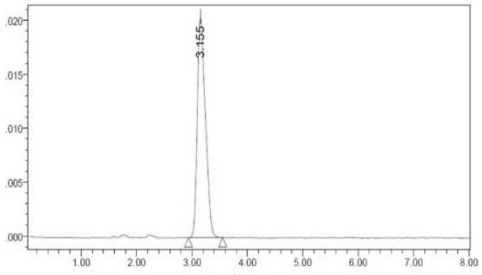


Fig. (1). Optimized chromatogram of Ganciclovir sample

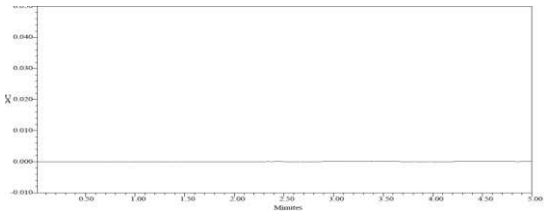


Fig. (2). Chromatogram of Blank

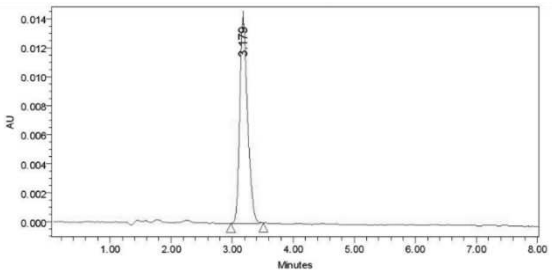


Fig. (3). Chromatogram of standard

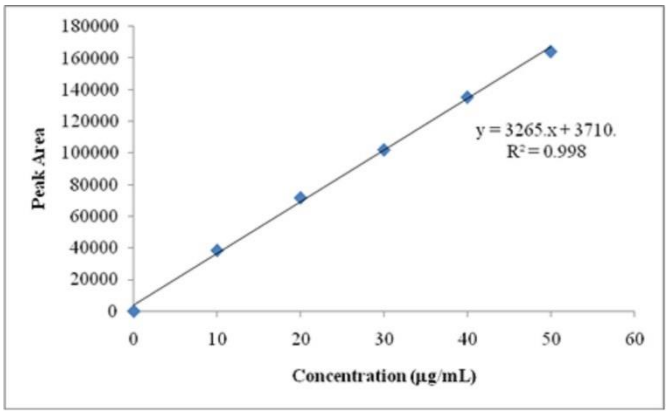
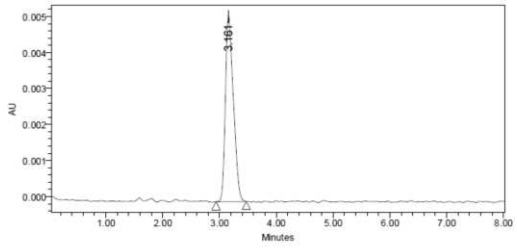


Fig. (4). : Linearity Graph of Ganciclovir



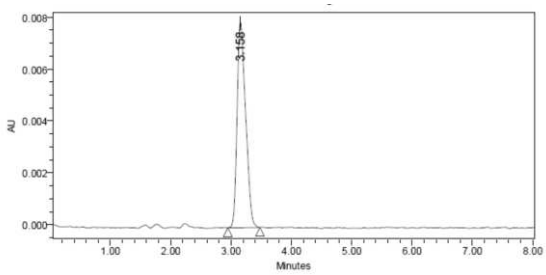
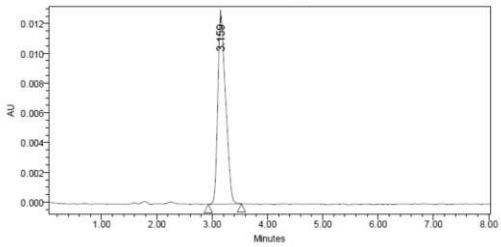


Fig. (5). Chromatogram showing linearity level-1 Fig. (6). Chromatogram showing linearity level-2



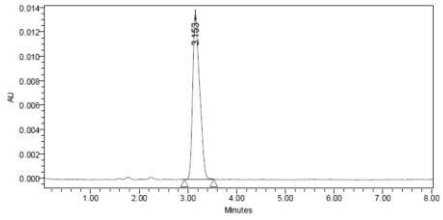
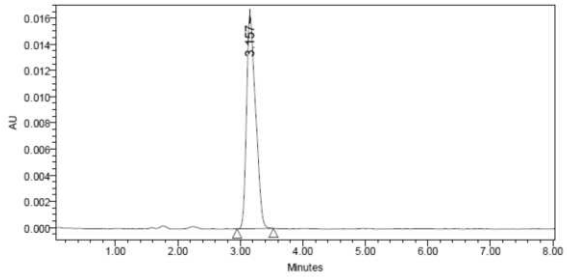


Fig. (7). Chromatogram showing linearity level-3 Fig. (8). Chromatogram showing linearity level-4



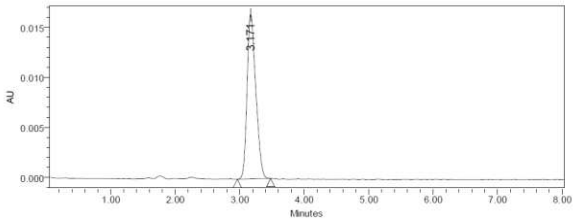


Fig.(9). Chromatogram showing linearity level-5 Fig. (10). Chromatogram showing precision injection -1

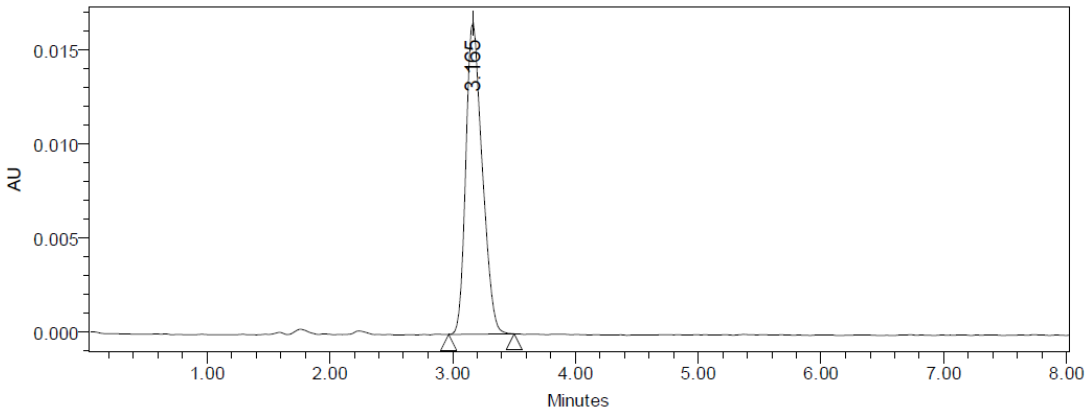


Fig. (11). Chromatogram showing precision injection -2

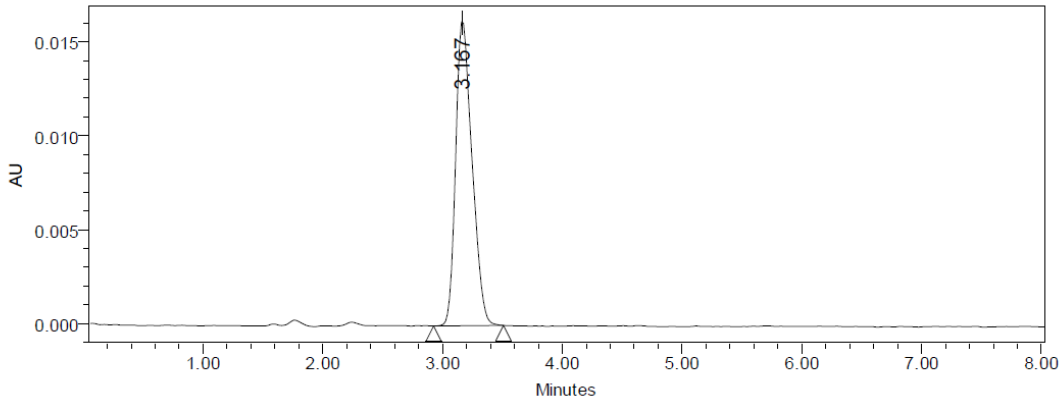


Fig. (12). Chromatogram showing precision injection -3

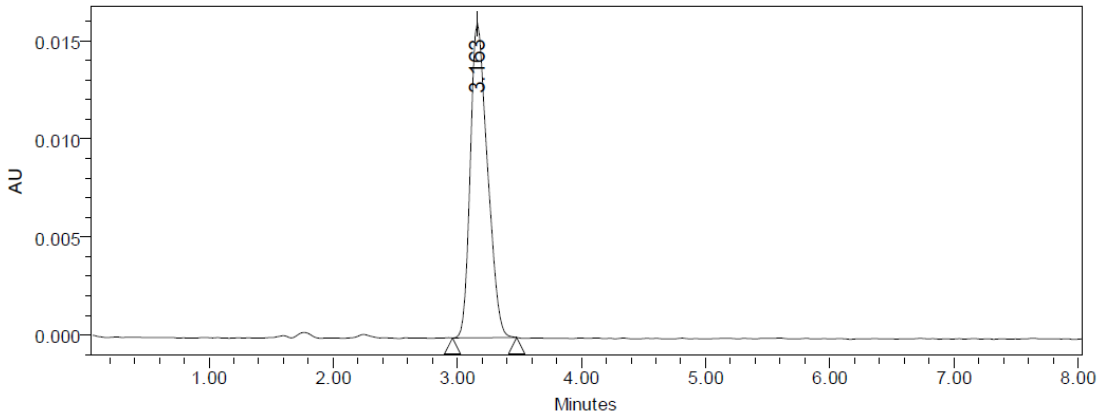


Fig. (13). Chromatogram showing precision injection -4

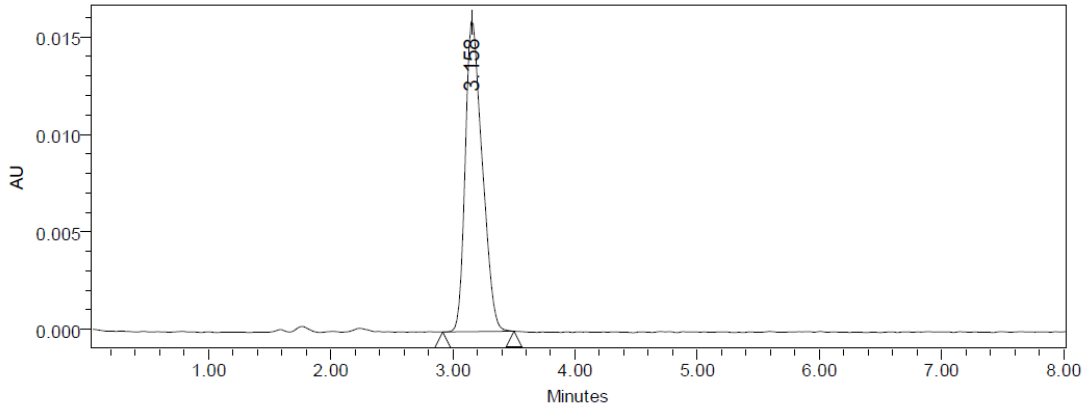


Fig. (14). Chromatogram showing precision injection -5

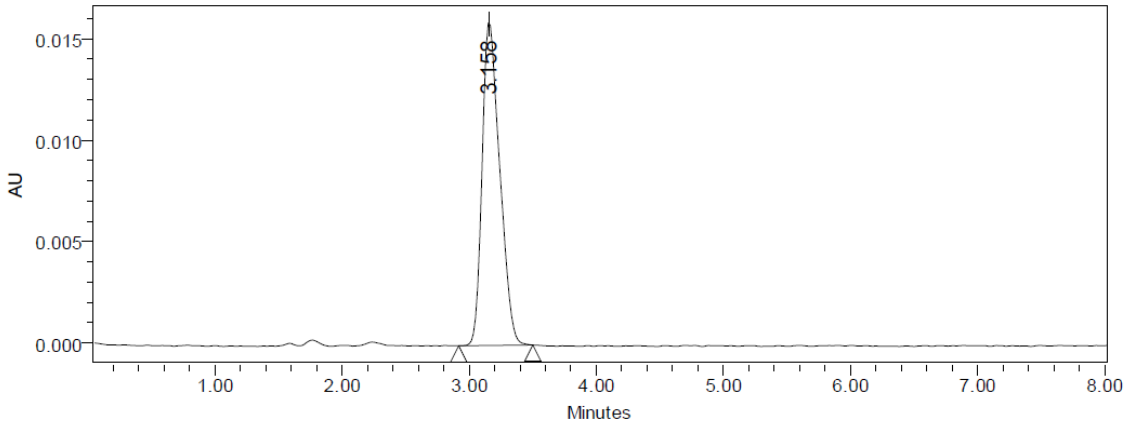


Fig. (15). Chromatogram showing Analyst 1 injection -1

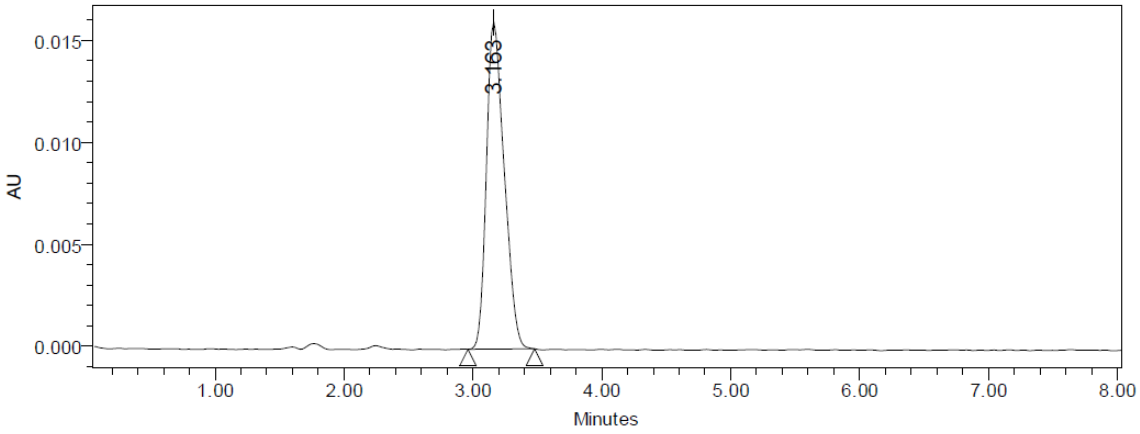


Fig. (16). Chromatogram showing Analyst 1 injection -2

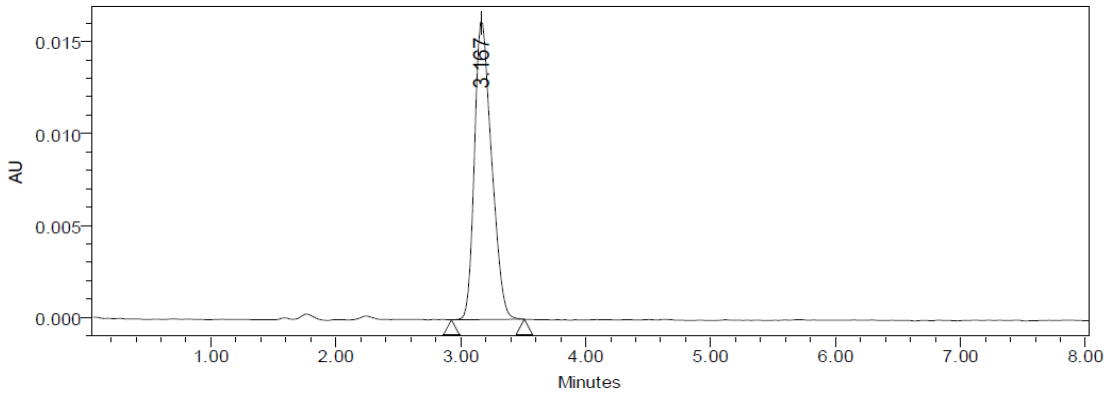


Fig. (17). Chromatogram showing Analyst 1 injection -3

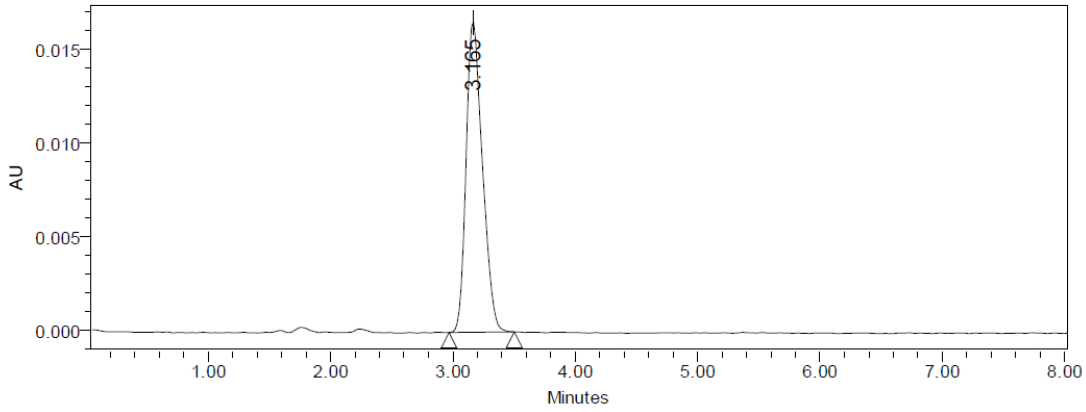


Fig. (18). Chromatogram showing Analyst 1 injection -4

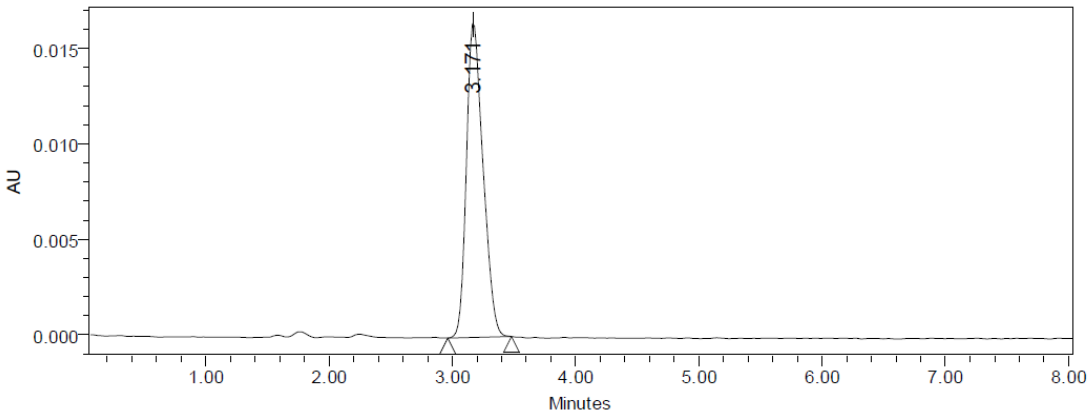


Fig. (19). Chromatogram showing Analyst 1 injection -5

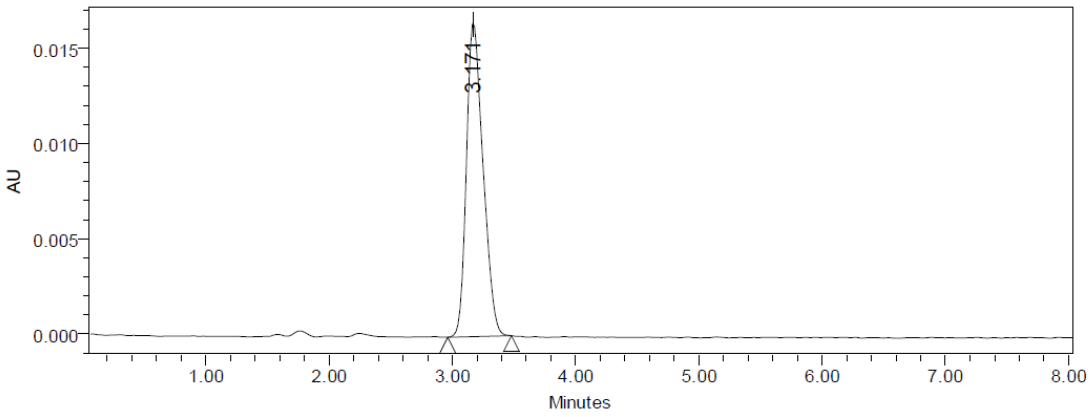


Fig. (20). Chromatogram showing Analyst 1 injection -6

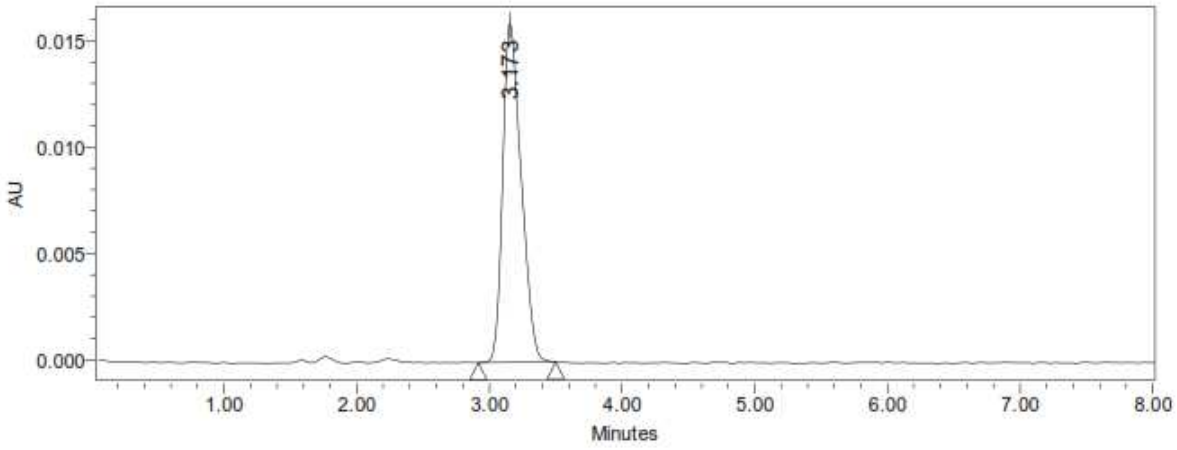


Fig. (21). Chromatogram showing Analyst 2 injection -1

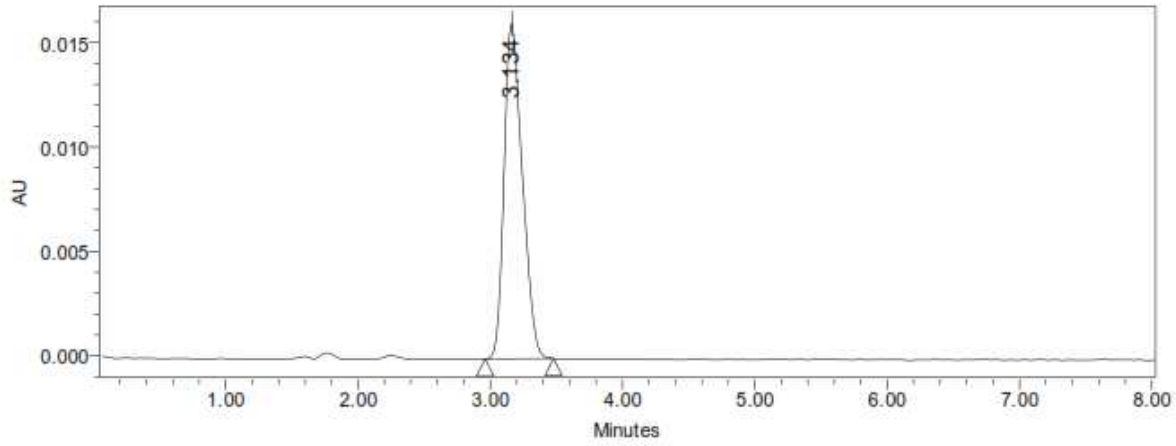


Fig. (22). Chromatogram showing Analyst 2 injection -2

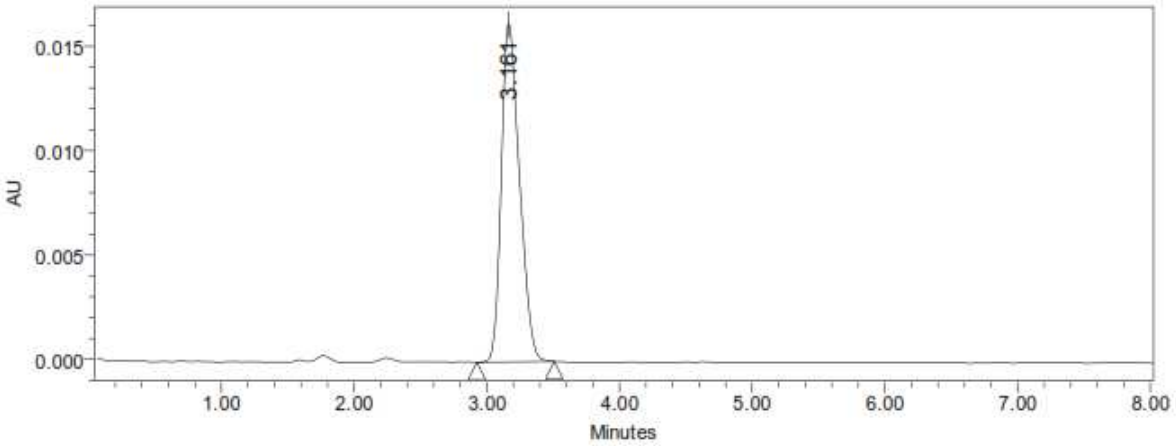


Fig. (23). Chromatogram showing Analyst 2 injection -3

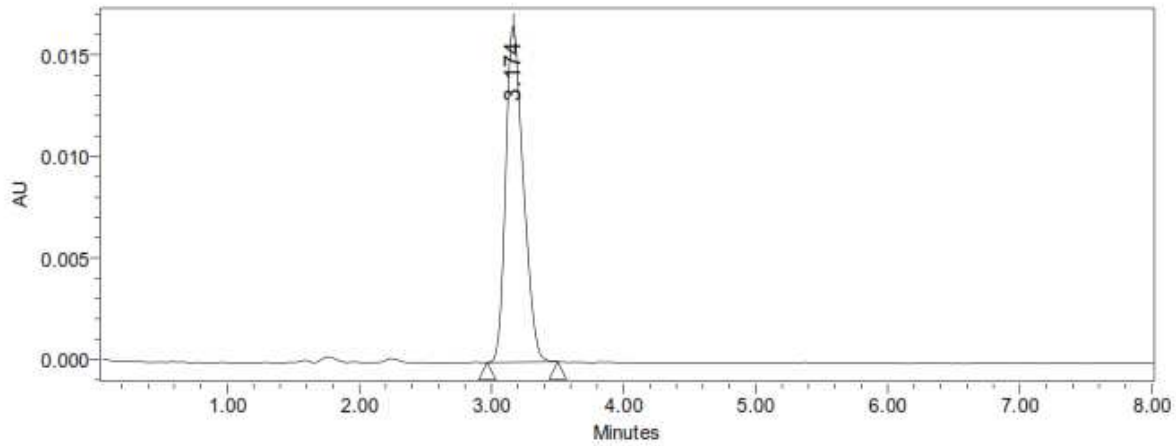


Fig. (24). Chromatogram showing Analyst 2 injection -4

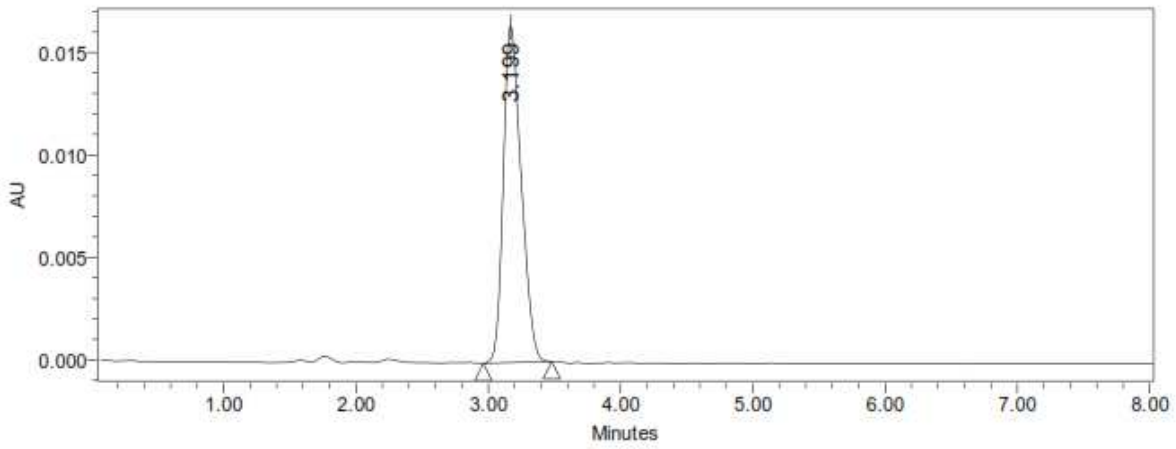


Fig. (25). Chromatogram showing Analyst 2 injection -5

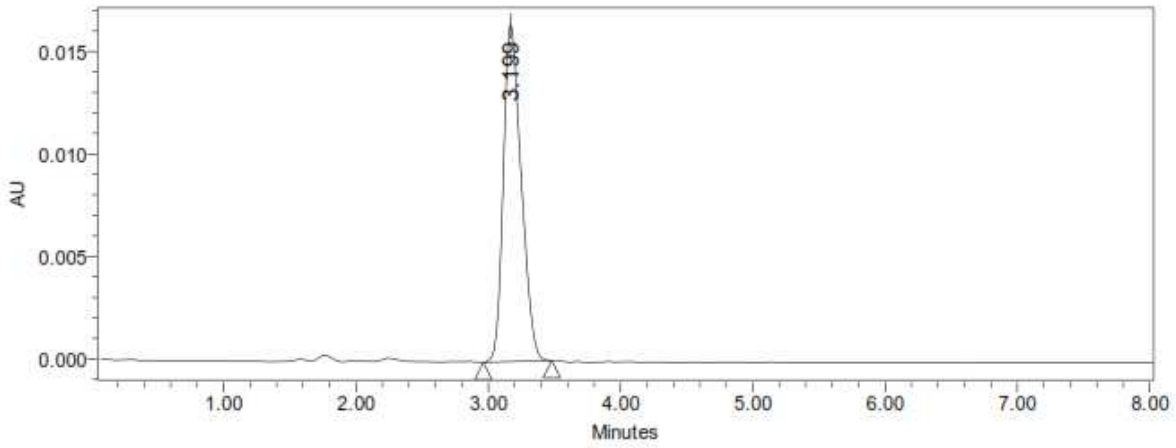


Fig. (26). Chromatogram showing Analyst 2 injection -6

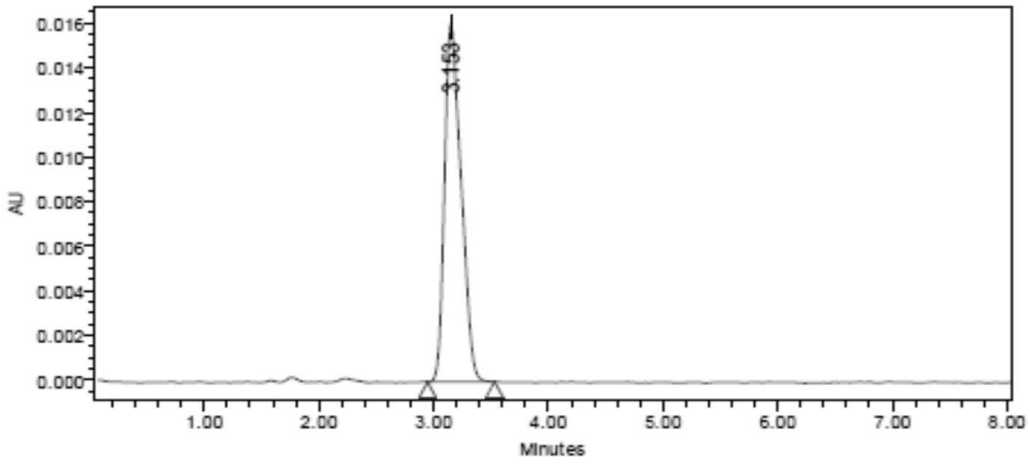


Fig. (27). Chromatogram showing accuracy-50%

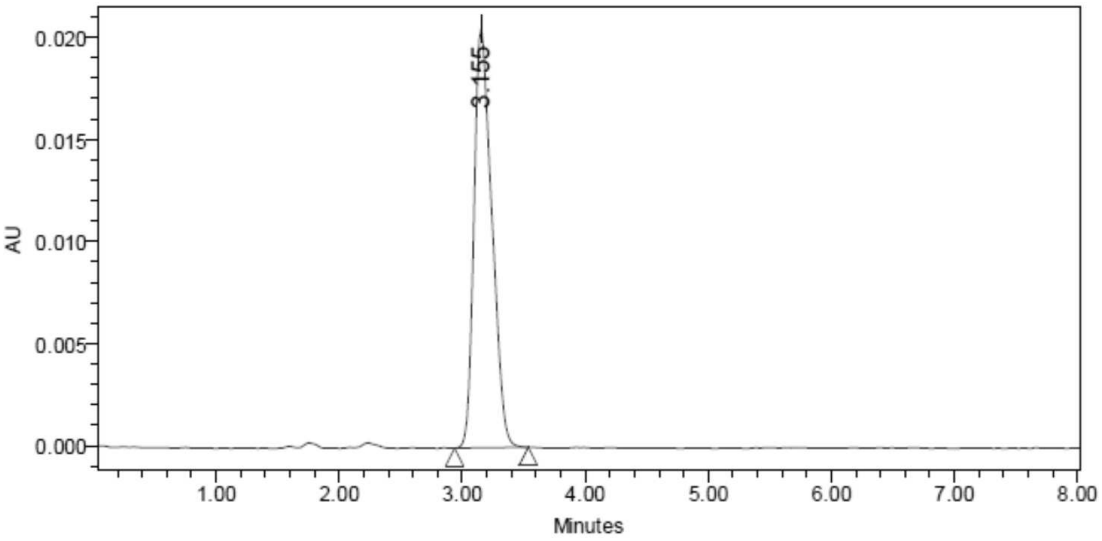


Fig. (28). Chromatogram showing accuracy-100%

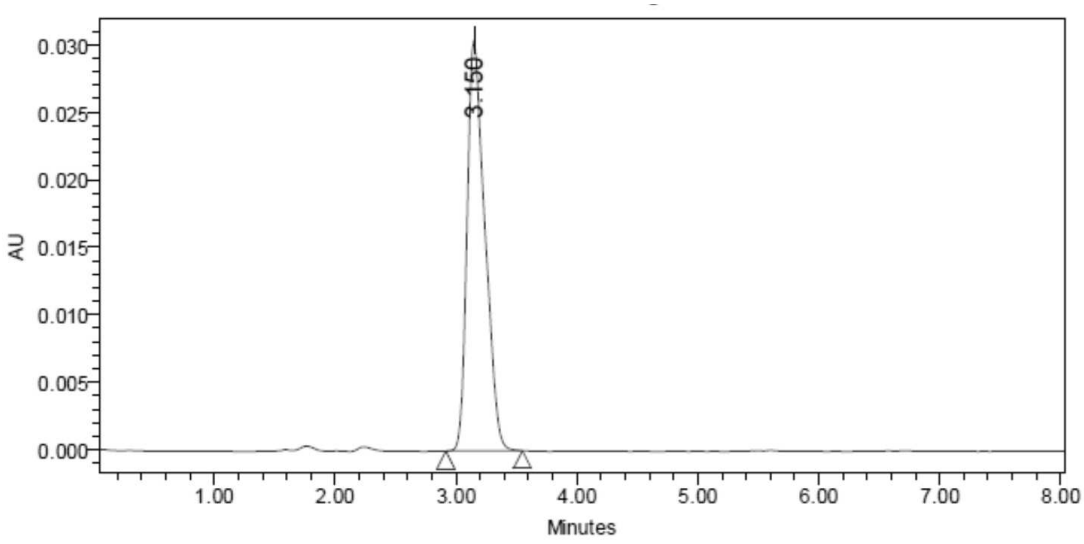


Fig. (29). Chromatogram showing accuracy-150%

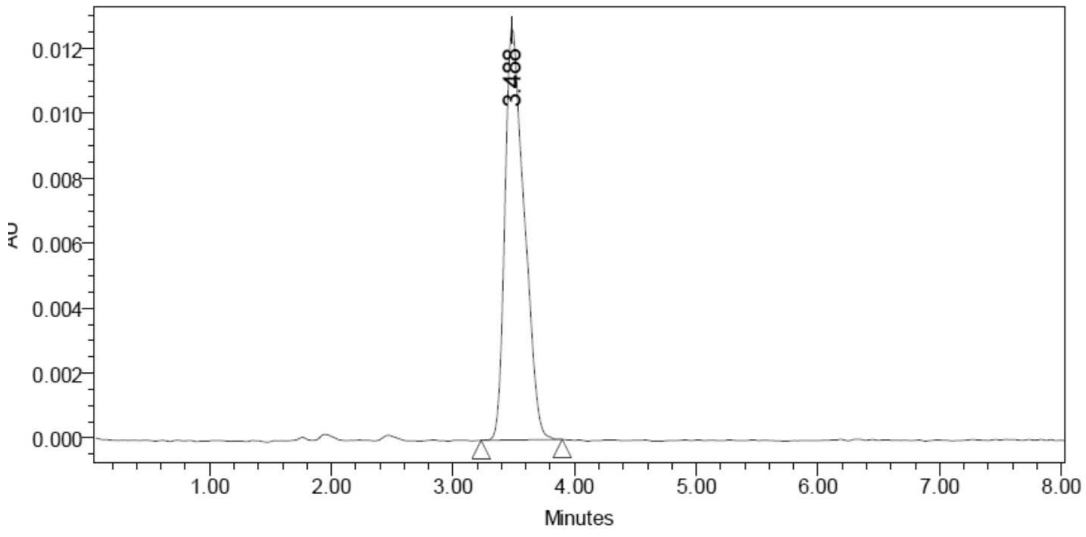


Fig. (30). Chromatogram showing less flow of 0.9ml/min

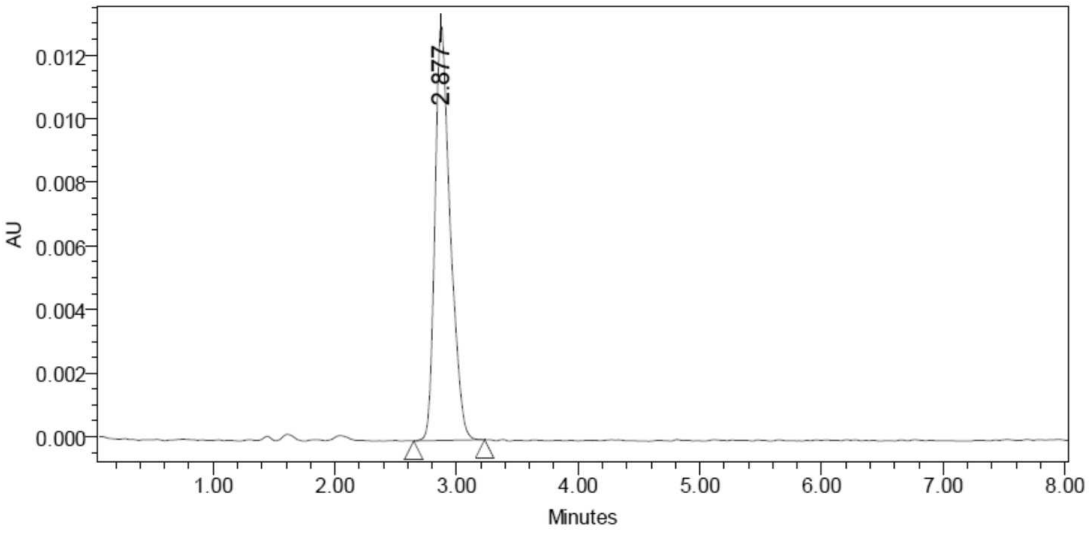


Fig. (31). Chromatogram showing less flow of 1.1 ml/min

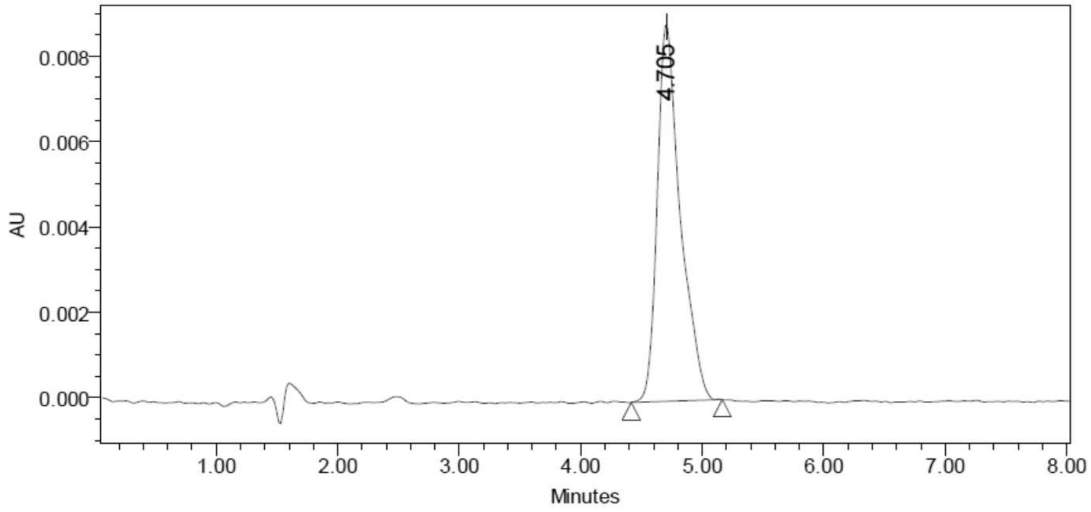


Fig. (32). Chromatogram showing less organic composition

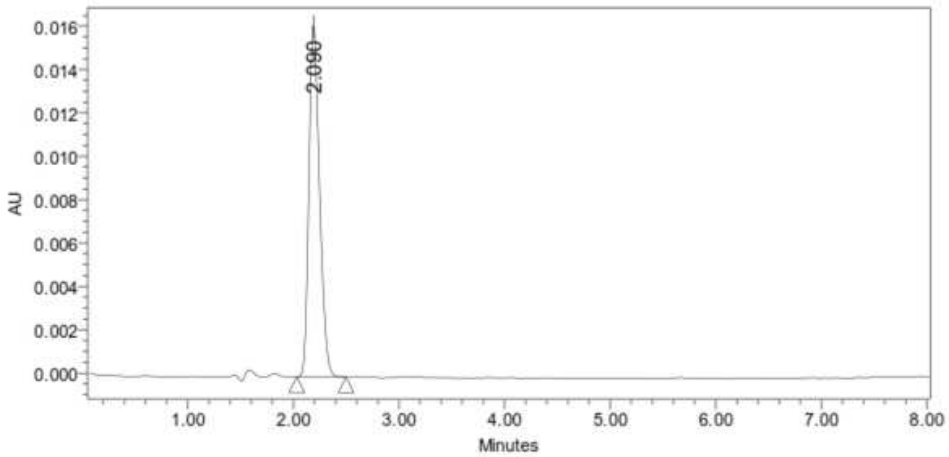


Fig. (33). Chromatogram showing more organic composition

Analytical Greenness report sheet

19/11/2023 15:09:12

0.86

Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.9	2
2. Minimal sample size and minimal number of samples are goals.	0.98	2
3. If possible, measurements should be performed in situ.	0.66	2
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	1.0	2
5. Automated and miniaturized methods should be selected.	1.0	2
6. Derivatization should be avoided.	1.0	2
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	0.6	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.61	2
9. The use of energy should be minimized.	1.0	2
10. Reagents obtained from renewable sources should be preferred.	1.0	2
11. Toxic reagents should be eliminated or replaced.	1.0	2
12. Operator's safety should be increased.	0.6	2

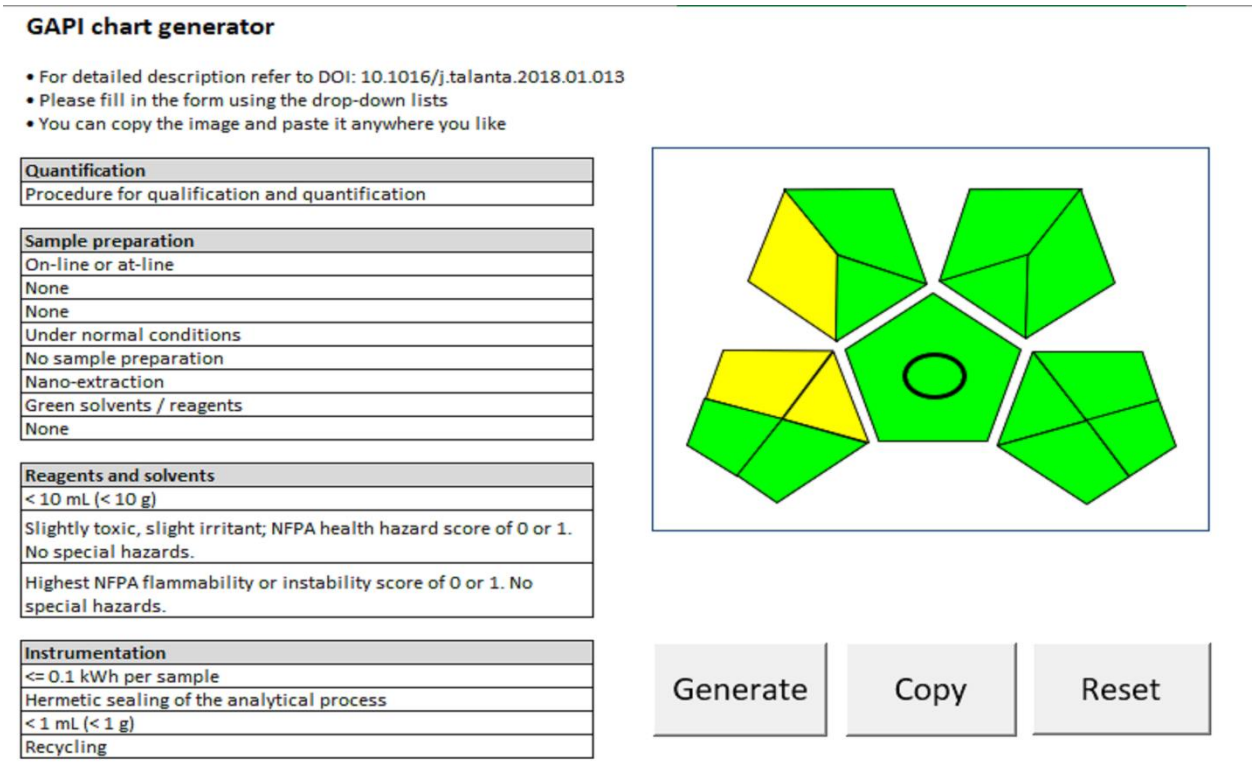
Analytical Greenness report sheet

19/11/2023 16:14:55

0.53

Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.9	2
2. Minimal sample size and minimal number of samples are goals.	0.65	2
3. If possible, measurements should be performed in situ.	0.66	2
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	0.6	2
5. Automated and miniaturized methods should be selected.	1.0	2
6. Derivatization should be avoided.	1.0	2
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	0.08	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.51	2
9. The use of energy should be minimized.	1.0	2
10. Reagents obtained from renewable sources should be preferred.	0.0	2
11. Toxic reagents should be eliminated or replaced.	0.0	2
12. Operator's safety should be increased.	0.0	2

Fig. (34). Green metrics comparision of Ganciclovir analysis with Acetonitrile and Ethanol by Agree software



GAPI chart generator

- For detailed description refer to DOI: 10.1016/j.talanta.2018.01.013
- Please fill in the form using the drop-down lists
- You can copy the image and paste it anywhere you like

Quantification
Procedure for qualification and quantification

Sample preparation
On-line or at-line
None
None
Under normal conditions
No sample preparation
Nano-extraction
Green solvents / reagents
None

Reagents and solvents
< 10 mL (< 10 g)
Slightly toxic, slight irritant; NFPA health hazard score of 0 or 1. No special hazards.
Highest NFPA flammability or instability score of 0 or 1. No special hazards.

Instrumentation
<= 0.1 kWh per sample
Hermetic sealing of the analytical process
< 1 mL (< 1 g)
Recycling

Generate Copy Reset

Fig. (35). Greeness metrics of Ganciclovir analysis by Simple GAPI

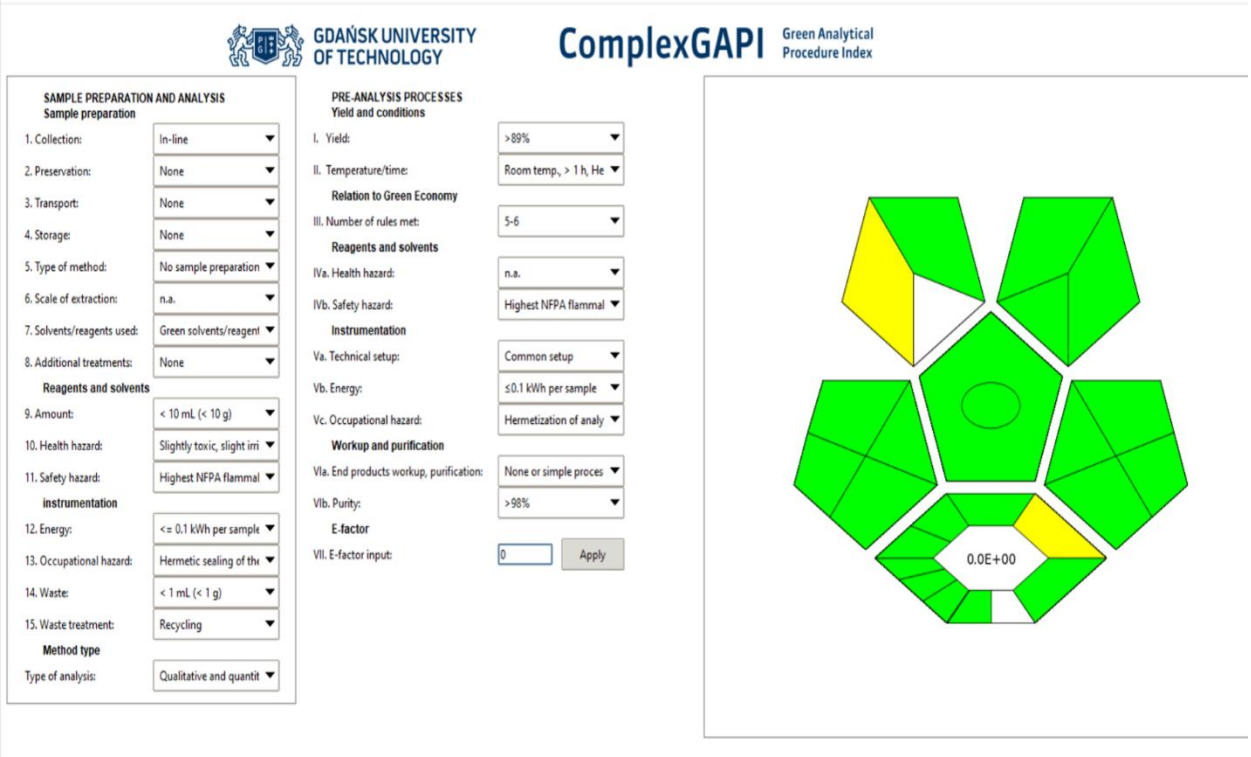


Fig. (36). Greeness metrics of Ganciclovir analysis by Complex GAPI

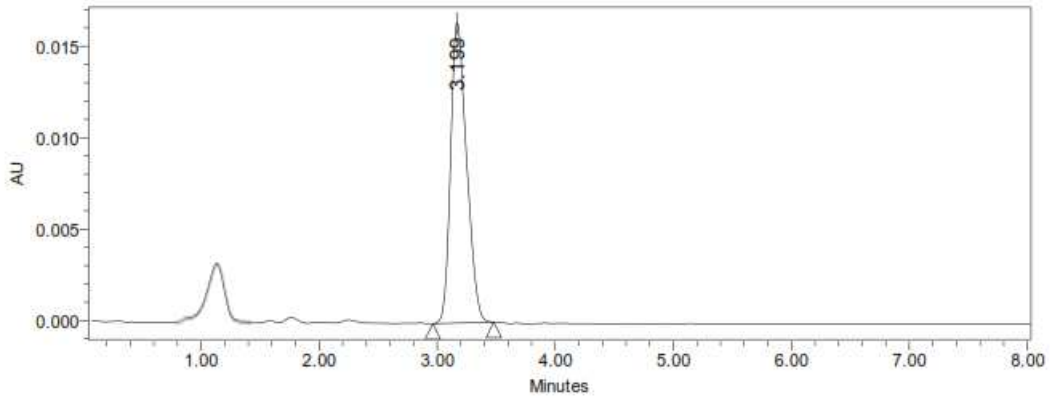


Fig. (37). showing acid degradation

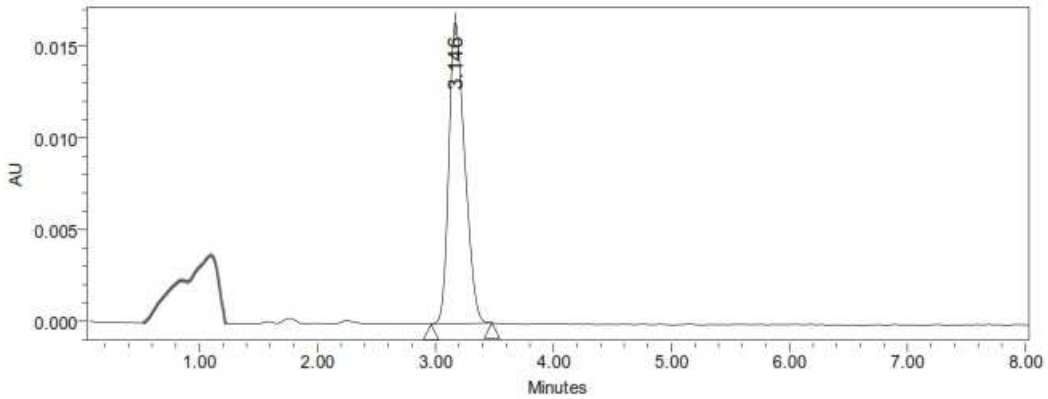


Fig. (38). showing Alkaline degradation

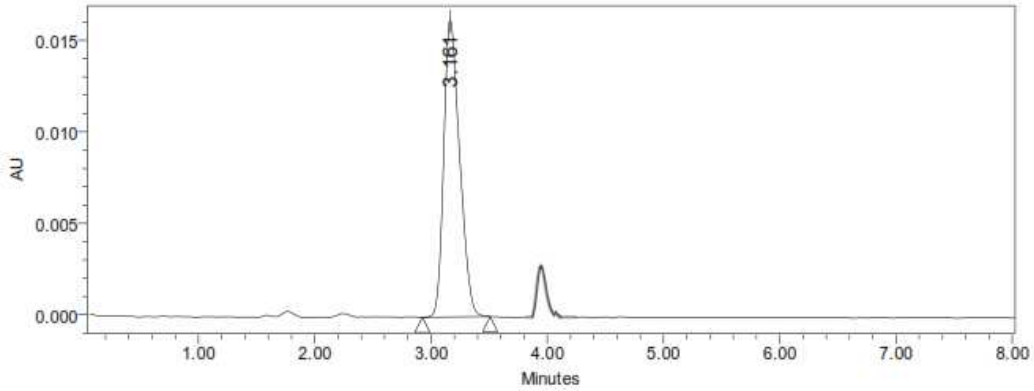


Fig. (39). showing Thermal degradation

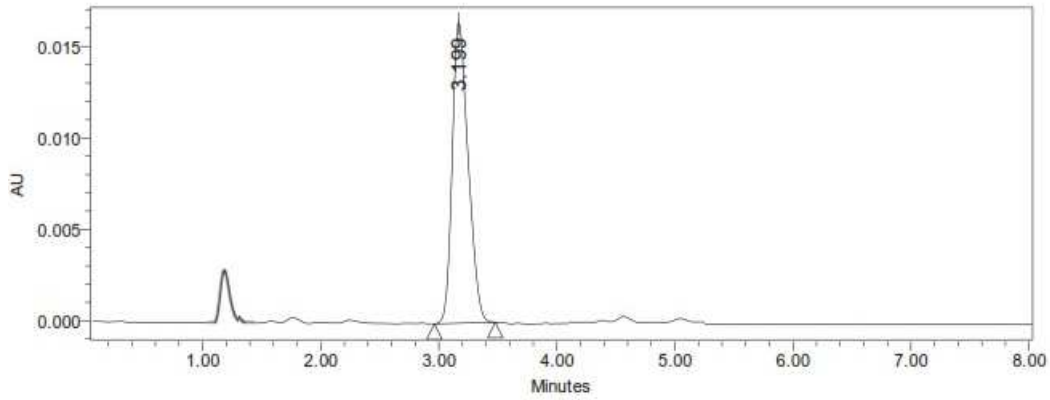


Fig. (40). showing peroxide degradation

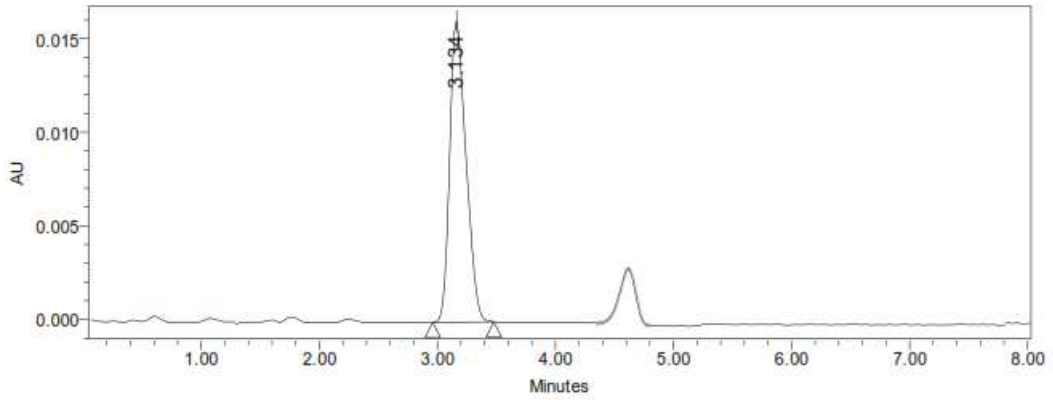


Fig. (41). showing Photolytic degradation

TABLES :

Table 1 : Materials used for method develpment

S.No	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector.
2	pH meter	Labindia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table 2: Reagents used for estimation.

S.No	Chemical	Brand names
1.	Ganciclovir	API procured from Lotus Pharmaceuticals Ltd., Bangalore, India

2.	Water : Ethanol for HPLC	LICHROSOLV (MERCK) Solvents
3.	Phosphoric acid for HPLC	Merck

Table 3 : Results of Idealized Chromatogram for Ganciclovir standard and sample

S.No	Standard	RT in Mins	Peak Area (µVsec)	Height (µV)	USP Plate Count	USP Tailing
1.	Ganciclovir	3.179	125867	14160	2876	1.32
2.	Ganciclovir	3.176	125815	14030	2754	1.31
3.	Ganciclovir	3.175	125534	13906	2682	1.31

Table 4 : Assay results of Ganciclovir standard

S.No	Sample	RT in Mins	Peak Area (µVs)	Peak Height (µV)	USP Tailing	USP Plate Count	Injection
1	Ganciclovir	3.170	154627	16995	1.29	2635	1
2	Ganciclovir	3.174	154620	16965	1.29	2605	2
3	Ganciclovir	3.170	153996	16754	1.26	2617	3

Table 5 : Assay results of Ganciclovir sample

S.No	Name	RT	Area	Height	USP Tailing	USP Plate	Injection
1	Ganciclovir	3.170	154627	16995	1.29	2635	1
2	Ganciclovir	3.174	154620	16965	1.29	2605	2
3	Ganciclovir	3.170	153996	16754	1.26	2617	3

Table 6 : Ganciclovir Linearity

S. No	Concentration, µg/ml	Average, Peak Area of Ganciclovir
1	1.4	38456
2	1.8	71757
3	2.2	102084
4	2.6	135425
5	3.0	164355

Table 7 : Method Precision data of Ganciclovir

S.No	Sample	RT in Mins	Area ,(µV*sec)	Height ,(µV)	USP Plate count	USP Tailing
1.	Ganciclovir	3.163	153478	16569	2510.1	1.34
2.	Ganciclovir	3.164	153540	16248	2245.0	1.35
3.	Ganciclovir	3.158	153952	16043	2174.1	1.31
4.	Ganciclovir	3.162	154073	16321	2352.5	1.32
5.	Ganciclovir	3.172	154322	16564	2438.4	1.37
6.		3.170	154382	16754	2438.2	1.39
Mean			153892.802			
Std. Dev.			338.9			
% RSD			0.21			

Table 8: Result of ruggedness for Ganciclovir- Analyst 1

S. No	Sample	Retention time in mins	Peak Area,(µV*sec)	Height, (µV)	USP Plate Count	USP Tailing
1	Ganciclovir	3.165	153478	16589	2510.0	1.37
2	Ganciclovir	3.153	153850	16148	2255.1	1.35
3	Ganciclovir	3.148	153752	16123	2174.1	1.39
4	Ganciclovir	3.157	154063	16144	2352.4	1.32
5	Ganciclovir	3.161	154362	16544	2438.2	1.31
Mean			153842.8			
SD			329.9			
% RSD			0.2			

Table 9 : Result of ruggedness for Ganciclovir- Analyst 2

S.No	Sample	RT in Mins	Area-(µVs)	Height, (µV)	USP Plate count	USP Tailing
1.	Ganciclovir	3.163	153624	16594	2376	1.32
2.	Ganciclovir	3.144	153621	16568	2373	1.30
3.	Ganciclovir	3.163	153173	16520	2827	1.31
4.	Ganciclovir	3.165	153457	16492	2236	1.31
5.	Ganciclovir	3.198	153877	16593	2173	1.32
6.	Ganciclovir	3.194	154526	16495	2927	1.30
Mean			153493			
Std. Dev.			450.3451			
% RSD			0.293348			

Table 10 : Results of Accuracy for Ganciclovir

S.No	Percentage Concentration, (at specification Level)	Area of the peak	Amount Added in (ppm)	Amount Found in (ppm)	%Recovery	Average Recovery
1	50 %	53261.67	15.0	15.9	99.3	99.4%
2	100 %	103318	30.0	29.87	99.5	
3	150%	151061.7	45.0	44.79	99.5	

Table 11 : Results of Robustness for Ganciclovir

S.No	sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
1	1.0 mL/min	126066	3.145	2145	1.33
2	0.9 mL/min	132330	3.487	2919	1.36
3	1.1 mL/min	115279	2.856	2514	1.42
4	Low organic phase	118484	4.709	3260	1.45
5	High organic phase	113280	2.191	2248	1.26

Table 12 : Results of Degradation studies for Ganciclovir

S. No	Type of degradation	Weight of sample (µg/ml)	Area of sample	Assay content (% w/w)
			Ganciclovir	**Ganciclovir**
1	Acid (0.1N HCl)	50µg/ml of Ganciclovir	153109	92%±0.02 (8.02%)
2	Alkaline (0.1N NaOH)	50µg/ml of Ganciclovir	153009	91.5%±0.75 (9.25%)
3	Peroxide (0.1% H_20_2)	50µg/ml of Ganciclovir	153425	96%±0.02 (4.02%)
4	Thermal (at 60^0 c)	50µg/ml of Ganciclovir	153397	95%±0.42 (5.42%)
5	Photolytic (Sunlight)	50µg/ml of Ganciclovir	153359	94%±0.38 (6.38%)