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Research article ***Pharmaceutical Analysis***

Development and validation of uv-visible spectrophotometric method for determination of elvitegravir in api form and pharmaceutical dosage form

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ABSTRACT

A new, simple, sensitive, precise, reproducible UV visible spectrophotometric method was developed for the determination of Elvitegravir in bulk form and Marketed Pharmaceutical dosage form with methanol solvent system. The method is based on the determination of Detection wavelength and plotting a calibration curve. The UV spectrum of Elvitegravir in Methanol Solvent System showed maximum wavelength at 275nm. Beer's law is valid in the concentration range of 10-60µg/ml. This method was validated for linearity, accuracy, precision, assay, ruggedness and robustness. The method has demonstrated excellent linearity over the range of 10-60µg/ml with the regression equation y=0.0228x+0.0107, and regression coefficient i.e. r2=0.9997 moreover, the method was found to be highly sensitive with LOD (0.2321µg/ml) and LOQ (0.7235µg/ml). Based on the results the proposed method can be successfully applied for the assay of Elvitegravir in various pharmaceutical dosage forms.

Keywords: Elvitegravir, UV Spectrum, Wavelength, Linearity, Calibration Curve.

INTRODUCTION

Elvitegravir is a human immunodeficiency virus type 1 (HIV-1) integrase strand transfer inhibitor (INSTI) used for the treatment of HIV-1 infection in antiretroviral treatment-experienced adults. Because integrase is necessary for viral replication, inhibition prevents the integration of HIV-1 DNA into the host genome and thereby blocks the formation of the HIV-1 provirus and resulting propagation of the viral infection. Although available as a single dose tablet, elvitegravir[1] must be used in combination with an HIV protease inhibitor coadministered with ritonavir and another antiretroviral drug. Elvitegravir in combination with an HIV protease inhibitor coadministered with ritonavir and with other antiretroviral drug(s) is indicated for the treatment of HIV-1 infection in antiretroviral treatment-experienced adults. Elvitegravir[2] is an HIV-1 integrase strand transfer inhibitor (INSTI). Integrase is an HIV-1 encoded enzyme that is required for viral replication. Inhibition of integrase prevents the integration of HIV-1 DNA into host genomic DNA, blocking the formation of the HIV-1 provirus and propagation of the viral infection. Elvitegravir undergoes primarily oxidative metabolism via CYP3A, and is secondarily glucuronidated via UGT1A1/3 enzymes. Metabolites are found in the plasma at very low concentrations, displayed considerably lower anti-HIV activity, and did not contribute to the overall antiviral activity of elvitegravir. Elvitegravir is a human immunodeficiency virus type 1 (HIV-1) integrase strand transfer inhibitor (INSTI) used for the treatment of HIV-1 infection in antiretroviral treatment-experienced adults. Because integrase is necessary for viral replication, inhibition prevents the integration of HIV-1 DNA into the host genome and thereby blocks the formation of the HIV-1 provirus and resulting propagation of the viral infection. Although available as a single dose tablet, elvitegravir[3] must be used in combination with an HIV protease inhibitor coadministered with ritonavir and another antiretroviral drug. Elvitegravir was first licensed from Japan Tobacco in 2008 and developed by Gilead Sciences. It was FDA approved on August 27,

2012. On September 24, 2014, the FDA approved the single pill form of elvitegravir. The IUPAC Name of Elvitegravir is 6-[(3-chloro-2-fluoro phenyl) methyl]-1-[(2S)-1-hydroxy-3-methyl butan-2-yl]-7-methoxy-4-oxo quinoline-3-carboxylic acid. The Chemical Structure of Elvitegravir is as follows

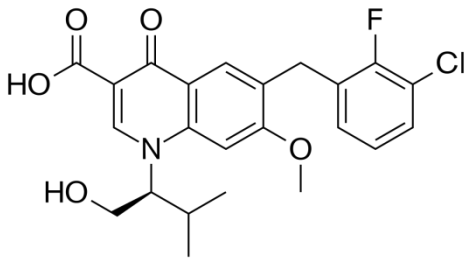

Fig 1: Chemical Structure of Elvitegravir

This novel proposed method (literature review[39-42]) contributes quick estimation, correct peak shape, precise, simple, and quick, use of smaller sample volumes and utilizing methanol as a mobile phase which is economical when compared with other existing methods. So, it is necessary to develop a simple, precise, and rapid RP-HPLC method for the quantitative determination of Elvitegravir. This work describes the validation parameters stated by the International Conference on Harmonization [ICH] guidelines Q2 (R1).

MATERIALS AND METHODS

Table 1: List of instruments used

S.No.	Instruments/Equipments/Apparatus
1	**LAB INDIA** UV – Vis spectrophotometer (T-60)
2	Electronic Balance **(SHIMADZU ATY224)**
3	Ultra Sonicator **(Wensar wuc-2L)**
4	P^H Analyzer **(ELICO)**
5	Triple Quartz Distillation Unit **(BOROSIL)**
6	Vaccum filtration Kit **(BOROSIL)**

Table 2: List of chemicals, reagents and standards used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	99.9%	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	A.R.	Loba Chem; Mumbai.
3.	Ethanol	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Chloroform	99.9%	HPLC	Loba Chem; Mumbai.
5.	Hydrochloric acid	99.9	L.R.	Sd fine-Chem ltd; Mumbai
6.	Sodium Hydroxide	99.9	L.R.	Sd fine-Chem ltd; Mumbai

METHOD DEVELOPMENT

Instrumentation

The Spectroscopic analysis was carried out using Double beam PG Instruments recording UV-Visible Spectrophotometer LAB INDIA (T-60) with 1mm path length matched quartz cells was used for analytical purpose.

Preparation of Standard Stock Solution of Elvitegravir

Accurately weighed 10mg of Elvitegravir was weighed accurately and transferred into 10ml volumetric flask. Add about 7ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 1000µg/ml of Elvitegravir.

Then pippetted out 1ml of Elvitegravir was weighed accurately and transferred into 10ml volumetric flask. Add about 7ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100µg/ml of Elvitegravir.

Then pippetted out 1ml of Elvitegravir was weighed accurately and transferred into 10ml volumetric flask. Add about 7ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 10µg/ml of Elvitegravir. Finally working standard solution of Elvitegravir containing 10µg/ml for method A and were prepared. It is scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the absorption maxima of Elvitegravir. The scanned UV spectrum is attached in the following page.

RESULTS AND DISCUSSION

METHOD OPTIMIZATION

Optimization of Selection of Solvent

It is well known that the solvents do exerts a profound effect on the quality and the shape of the peak. The choices of solvents for UV method development are: Chloroform, Acetone, Methanol, Dimethyl sulfoxide (DMSO), Dimethyl formamide etc. First optimize the different solvents. From that solvents methanol satisfied the all the optimized conditions.

Selection of Wavelength

The standard solutions are prepares by transferring the standard drug in a selected solvent or mobile phase and finally diluting with the same solvent/mobile phase. That prepared solution is scanned in the visible wavelength[4] range of 200-400nm.This has been performed to know the absorption maxima of Elvitegravir. While scanning the Elvitegravir solution we observed the maxima at 275nm. The visible spectrum has been recorded on LAB INDIA make UV – Vis spectrophotometer model T-60. The scanned UV spectrum[5] is attached in the following page. The λ_{max} of the Elvitegravir was found to be 275 nm in methanol as solvent system.

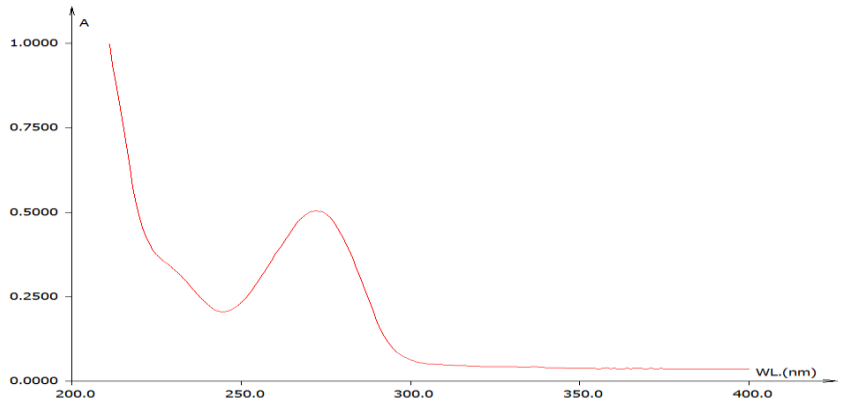

Fig 2: Visible Spectrum of Elvitegravir at 275nm

Preparation of calibration curve for Elvitegravir

Standard solutions of Elvitegravir in the concentration range of 10 µg/ml to 60 µg/ml were obtained by transferring (1, 2, 3, 4 and 5, 6ml) of Elvitegravir stock solution (100ppm) to the series of clean and dry 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed.

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The absorbencies of the solutions were measured at 275nm against the solvent system as blank and calibration curve is plotted. The Lambert-Beer's Law[6] is linear in concentration range of 10 to 60 µg/ml at 275nm for Elvitegravir respectively. The Calibration Curves results were shown in Fig-3

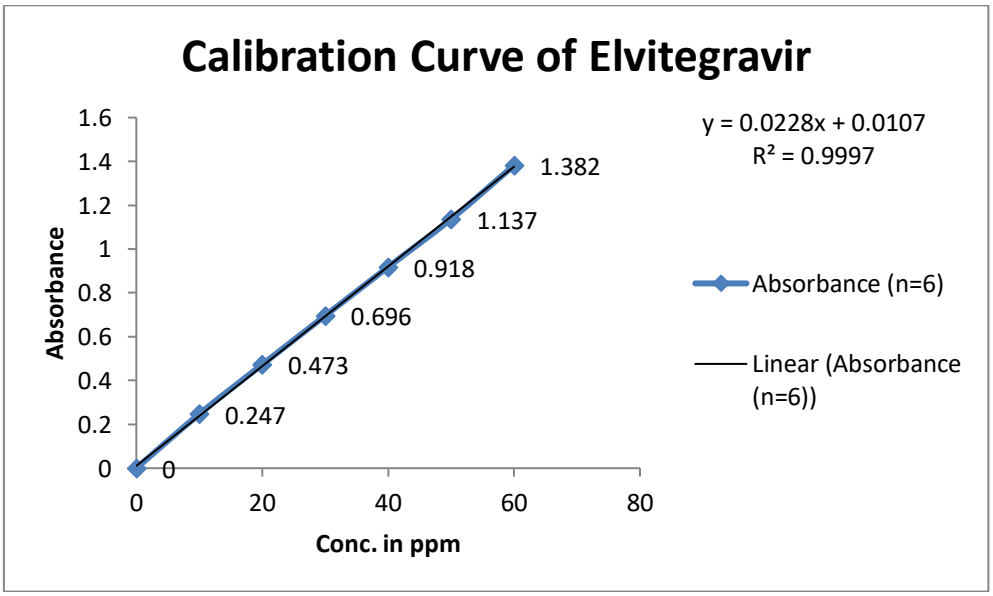

Fig 3: Calibration Curve for Elvitegravir at 275 nm.

For Elvitegravir the Beer- Lambert's law is obeyed in concentration range of 10 to 60 µg/ml at 275nm. Moreover, in the linearity study[7,8] at consecutive wavelength, the linear regression equation[9] for Elvitegravir, calibration curve at 275nm was calculated by y = 0.0228x + 0.0107 (R^2 = 0.999).

METHOD VALIDATION

Validation of the developed method according to I.C.H guidelines

Following parameters were taken into consideration for validation[10-16] of proposed method:

Linearity

As per assessed under section in above.

Preparation of dilutions of Elvitegravir for Linearity Study

Standard solutions of Elvitegravir in the concentration range of 10 µg/ml to 60 µg/ml were obtained by transferring (1, 2, 3 and 4, 5, 6ml) of Elvitegravir stock solution (100ppm) to the series of clean & dry 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed.

The absorbances of the solutions were measured at 275 nm against the solvent system as blank and calibration curve is plotted. The Lambert-Beer's Law is linear in concentration range[17] of 10 to 60 µg/ml at 275 nm for Elvitegravir. The results are shown in Fig 4.

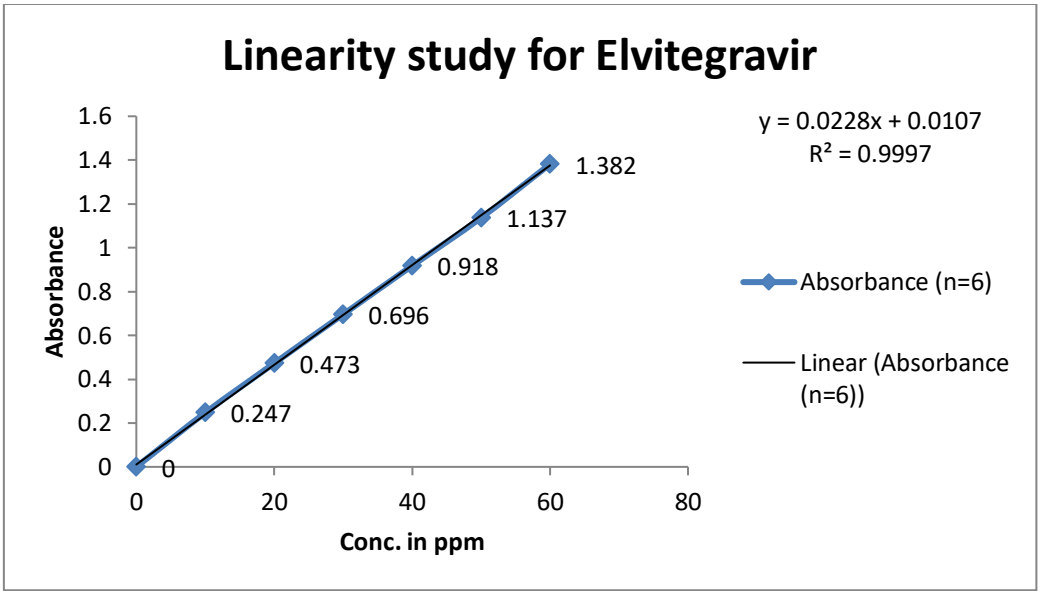

Fig 4: Calibration Curve for Elvitegravir at 275nm.

Linearity range was found to be 10-60 µg/ml for Elvitegravir at 275 nm. The correlation coefficient was found to be 0.999, which shows good linearity between above range. The slope[18] was found to be 0.022 and intercept was found to be 0.010 which was close to zero intercept.

Range

Range[19] of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. It includes working range, linearity range and target range and 100% concentration or test concentration. The range my developed method concentrations are 10-60 µg/ml.

Accuracy

The accuracy[20] is nothing but the comparison of obtained value with the standard value. After completion of analysis of Elvitegravir containing 3 group 3 replicates with the bulk and pharmaceutical dosage form.

Method

The accuracy of the developed method can be studied by preparing the solutions of various concentrations i.e. 80%, 100% and 120%. In these concentrations the amount of marketed pharmaceutical dosage form was kept as constant and the quantity of pure drug (API) is varied. The prepared solutions in triplicates and here determined is percentage recovery[21] of pure drug. The results obtained from the accuracy studies are shown in Table-16.

In nine different 10 ml volumetric flasks, 1 ml of the pre-analysed tablet solution (100 µg/ml) was taken and added 1, 2, 3 ml of standard solution of bulk (API) mixture (100µg/ml) and the volume was made up to 10 ml with methanol. The results are shown in Table 3.

Table 3: Data of Recovery Studies

Level of Recovery	Sample Conc. (µg/ml)	Standard Conc. Added (µg/ml)	Total Conc. (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Mean % Recovery ± SD	% RSD
80%	8	10	18	7.984	99.80	100.025 ± 0.253722	0.253659
80%	8	10	18	8.024	100.30		
80%	8	10	18	7.998	99.975		
100%	10	10	20	10.032	100.32	100.1733 ± 0.133167	0.132936
100%	10	10	20	10.014	100.14		
100%	10	10	20	10.006	100.06		
120%	12	10	22	11.979	99.825	99.944 ± 0.282005	0.282163
120%	12	10	22	12.032	100.266		
120%	12	10	22	11.969	99.741		

The results obtained for the accuracy study (recovery method) from three sample studies (n = 3) for each level indicated that the mean of the % recovery was 100.025% and 100.1733% and 99.944% and R.S.D was 0.253659%, 0.132936 % and 0.282163% for Elvitegravir in mixture (ELV 10 µg/ml). Here the mean % recovery[22] is in between 98-102 % thus showing that the analytical technique has a good recovery study.

Precision

The precision[23] of developed analytical method said to be the closeness of agreement between a series of measurement obtained from the multiple sampling of the homogenous sample solution under the prescribed experimental conditions. The precision of the developed method can be analysed by the 5 or 6 different homogenous solutions and the respective are noted down. The results are shown in the precision is % RSD. The results obtained from the precision studies are shown in the Table-6.

The precision can be divided into following types. 1. Repeatability and 2. Intermediate precision. In this first one is Repeatability[24] or Intra-day precision[25] was determined on five/six replicates of same sample solutions on the same day. Inter-day precision was estimated by analyzing newly prepared sample solutions in triplicate over the 3 consecutive days. Both inter day and intraday precision was expressed as % RSD. The % RSD values for intraday precision for Method A was 0.19-1.03. The % RSD for inter day precision for Method A are 0.17- 0.98. The results were summarized in Table-2. The low value of % RSD for the method indicates the high precision of the method.

Repeatability

Repeatability was assessed using:

Six time repetition of target concentration 100 % that is (10µg/ml).

Intermediate precision can be assessed by intra-day and inter day analysis.

Method: In the study of the repeatability precision which was conducted on the solution which has the concentration value 100 % of the target concentration (n = 6). The results are shown in Table-4.

Table 4: Data of Repeatability of Absorbances

S.No.	Conc. (µg/ml)	Wavelength (nm)	Absorbance
1	10	275	0.245
2	10	275	0.243
3	10	275	0.242
4	10	275	0.241
5	10	275	0.246
6	10	275	0.242
Mean ± S.D.	**0**	**0**	**0.243167**
Standard Deviation	**0**	**0**	**0.001941**
% RSD	**0**	**0**	**0.798132**

Repeatability study showed a R.S.D of 0.798132 % for Elvitegravir. Thus it is concluded that the analytical technique has a good repeatability precision as %R.S.D for the drug were less than 2 %.

Intermediate Precision

Intra-assay & Inter-assay

The intra & inter day variation of the method[26] was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Elvitegravir revealed that the proposed method is precise.

Table 5: Intra-Day and Inter-Day Precision for Method A

	Observed Conc. of Elvitegravir (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Con. found (µg/mL)	% RSD	Con. found (µg/mL)	% RSD
8	8.03	1.03	7.81	0.98
10	10.09	0.51	9.94	0.29
12	12.14	0.19	12.19	0.17

Robustness

Robustness[27-29] of the method was determined by carrying out the analysis under different temperature condition i.e. at 23°C, 25°C and at 28°C. The respective absorbances of 10µg/ml. Were noted and the result was indicated as % RSD.

Table 6: Temperature-23°C

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.243	Mean = 0.245 SD = 0.002608 % RSD = 1.06436
10	0.246	
10	0.247	
10	0.245	
10	0.241	
10	0.248	

Table 7 Temperature-25°C

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.245	Mean = 0.242667 SD = 0.003141 % RSD = 1.29442
10	0.238	
10	0.243	
10	0.242	
10	0.241	
10	0.247	

Table 8: Temperature-28°C

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.242	Mean = 0.244167 SD = 0.003601 % RSD = 1.474782
10	0.243	
10	0.249	
10	0.247	
10	0.245	
10	0.239	

Ruggedness

In the ruggedness[30-33] study, the influence of small, deliberate variations of the analytical parameters on the absorbance of the drug was examined. The factor selected was a change in the analyst. The Ruggedness of the method was determined by carrying out the analysis by different analyst and the respective absorbance of 10µg/ml was noted. The result was indicated as %RSD (Table No-9).

Tables 9: Results showing Ruggedness for Elvitegravir
Analyst-1

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.239	Mean = 0.243167 SD = 0.004355 % RSD = 1.790983
10	0.238	
10	0.241	
10	0.246	
10	0.248	
10	0.247	

Analyst-2

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.247	Mean = 0.244 SD = 0.004427 % RSD = 1.814422
10	0.246	
10	0.250	
10	0.241	
10	0.238	
10	0.242	

Analyst-3

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.252	Mean = 0.245 SD = 0.004243 % RSD = 1.73169
10	0.241	
10	0.243	
10	0.246	
10	0.247	
10	0.241	

Specificity

The presence of excipients in formulation does not interfere with the drug absorbances. Therefore, the proposed method was found specific[34] and selective for the drug Elvitegravir.

Limit of detection and limit of quantification

The limit of detection[35] (LOD) and the limit of quantification limit[36] (LOQ) are measured by using the following equations:

L.O.D. = 3.3 (SD/S).
L.O.Q. = 10 (SD/S)

Where,
SD = Standard deviation of the response
S = Slope of the calibration curve[37]
The slope S and the SD may be estimated from the calibration curve of the analyte/sample.

The LOD was found to be 0.2321 µg/ml and LOQ was found to be 0.7235 µg/ml for Elvitegravir respectively which represents that sensitivity of the method is high.

Analysis of Marketed Formulations

Assay of Marketed Tablet Formulation Brand - Vitekta Tablet

Elvitegravir was procured from the local market as tablets of strength having 85mg marketed with brand names of Vitekta Tablet. This marketed formulation was manufactured by the Gilead Sciences, Inc. Weighed accurately about twenty tablets and calculate the weights of individual tablets and finally calculate the average weight. They were triturated to fine powder by using a mortar and pestle. The powdered tablet equivalent to 25mg of Elvitegravir was dissolved in 15ml of methanol with the help of sonication process and the final volume was made up to the mark with the methanol in 25 ml volumetric flask. The resulted solution was filtered using Whatman filter paper (0.45µm). This final solution was further diluted to obtain 10µg/ml concentration of the solution by using methanol used as a solvent and observed by UV analysis. This procedure was repeated in triplicate. The observed assay[38] for commercially available tablets Vitekta Tablet (100mg) and validation parameters were summarized in following Table-10.

Table 10: Assay Results of Marketed Formulations

Marketed Formulations	Actual concentration of Elvitegravir [Label Claim] (µg/ml)	Amount obtained of Elvitegravir (µg/ml)	% Elvitegravir
Vitekta Tablet	85	84.865	99.76%

The amount of drug in Vitekta Tablet was found to be 84.865 (± 0.865) mg/tab for Elvitegravir & % assay was 99.76 %.

SUMMARY AND CONCLUSION

The standard solutions of Elvitegravir in Methanol (10µg/ml) subjected to a scan individually at the series of wavelengths of 200 nm to 400 nm. Absorption maximum of Elvitegravir was found to be at 275 nm. Therefore, 275nm was selected as λ_{max} of Elvitegravir for the present study. The calibration curve of Elvitegravir was found to be linear in the range of 10-60 µg/ml at 275 nm. Therefore, it was clear that Elvitegravir can be determined without interference of any irrelevant substance in single component pharmaceutical products. The used technique was initially attempted on bulk drugs in their synthetic sample and concentrations were estimated. The % recovery was carried out at 3 levels, 80%, 100% and 120% of Elvitegravir standard concentration. Three samples were prepared for each recovery level. The solutions were then analysed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet dosage form. The newly developed method was validated according to the ICH guidelines and the method validation parameters. The developed method was subjected to do the various method validation parameters such as specificity, accuracy, precision, linearity and range, limit of detection and limit of quantification, robustness and ruggedness etc.

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