DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF ELVITEGRAVIR (EVG) IN BULK AND PHARMACEUTICAL FORMULATIONS.

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ABSTRACT

A simple, rapid, precise and economical spectrophotometric method has been developed for quantitative analysis of Elvitegravir (EVG) in manufactured tablet formulation. The stock solution and subsequent dilution of Elvitegravir was done in methanol. The standard solution of Elvitegravir in methanol showed absorption maxima at 313.00 nm. The drug obeyed Beers- Lamberts Law in the concentration range of 1-100 µg/mL with coefficient of correlation (R^2) was 0.999 . The method can be adopted in routine analysis of Elvitegravir in bulk and tablet dosage form and it involves relatively low cost solvents and no complex extraction technique.

KEYWORDS :- Method Validation, Elvitegravir(EVG), Spectrophotometric.

INTRODUCTION :-

Elvitegravir (EVG) 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 prevent the integration of HIV-1 DNA into host genomic DNA, blocking the formation of the HIV-1 provirus and propogation of the viral infection.The chemical name of elvitegravir is 3-quinolinecarboxylic acid ,6-[(3-chloro-2-fluorophenyl)-methyl]-1,4-dihydro-1-[(1S)-1-(hydroxymethyl)-2-methylpropyl]-7-methoxy-4-oxo.It has molecular formula of $C_{23}H_{23}CLFNO_5$. And the molecular weight of the 447.9.[1,2]

It has the following structural formula,

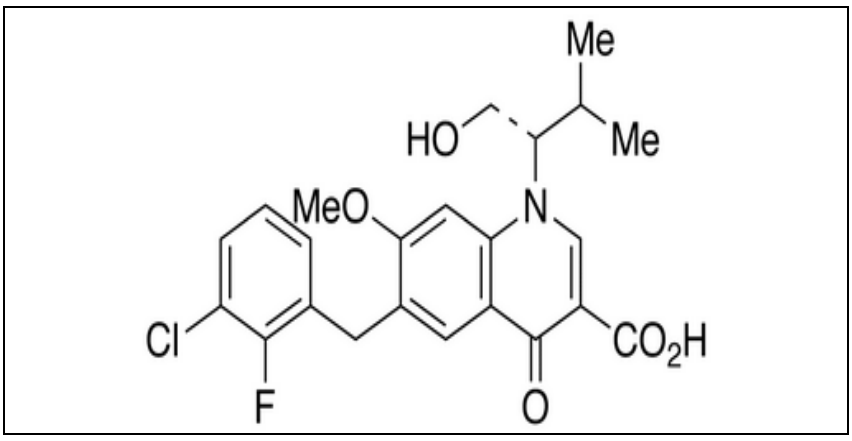

Figure 2 :- UV spectrum of pure drug EVG in Methanol.

Elvitegravir is a white to pale yellow solid with a solubility in DMSO,ACN and Methanol.The partition coefficient (log p) for elvitegravir is 5.5 and the PKA is 6.6.[1,2]

MATERIALS AND METHODS

Materials :-

Elvitegravir bulk drug was obtained from Mylan Labs Ltd.(Hyderabad ,India).The commercially tablets of elvitegravir are not available in the indian market; hence we have manufactured Elvitegravir immediate release tablets containing 50 mg Elvitegravir.The other ingredients used was Lactose Monohydrate,Microcrystalline Cellulose ,Starch and Magnesium Stearate. Other chemical used was analytical and glasswares used was Class A grade.

Instruments :-

SHIMADZU UV-1800 UV/VISIBLE Spectrophotometer with UV probe 2.10 softwares and 1 cm matched quartz cells were used for the absorbance measurement. Analytical balance used for weighing standard and sample was SHIMADZU AUX 220 Uni Bloc PAT 1987.

Preparation of Standard Stock Solution :-

Accurately weighed 50 mg of elvitegravir working standard was transferred into a 100ml volumetric flask, it was dissolved with Methanol which further sonicated for 10 min.The volume was made up to 50 ml with Methanol to give solution containing 100 µg/mL of EVG.

Selection of Wavelength :-

The standard stock solution was further diluted with Methanol to get a 10 µg/mL of concentration. The solution was scanned between 200 and 400 nm using water as blank. The UV spectrum of EVG in water had shown λ max at 313.00 nm. Hence, it was selected for the analysis of EVG (Figure 2).

Preparation of calibration curve :-

Aliquots of standard stock solution were further diluted with methanol to get the solutions of concentration 10-50 µg/mL. The absorbances were measured at 313.00 nm against water as blank. All measurements were repeated three times for each concentration. The calibration curve was constructed by plotting mean of absorbance against corresponding concentration.

Preparation of the sample solution :-

The tablets of EVG are not available in Indian market; hence tablets manufactured in laboratory were assayed. These were labeled to contain 50 mg of EVG as an active substance per tablet. Twenty tablets containing 50 mg of EVG were accurately weighed and powdered. The powder equivalent to 50 mg of EVG was weighed and transferred to a 50 mL volumetric flask; 10 Ml methanol was added and sonicated for 20 min. The volume was adjusted to 50 mL with methanol. The solution was filtered through Whatman filter paper. From this filtrate, 1 mL was transferred to a 100 mL volumetric flask and diluted with methanol to 100 mL in order to obtain the final concentration of 10 µg/mL. The absorbance was measured at 313.00nm using methanol as blank. This procedure was repeated for six times. The amount of EVG present in formulation was calculated by comparing it with standard absorbance.

Method Validation :-

The developed method was validated as per ICH Guidelines for the following parameters.

Linearity :-

The standard solution was prepared by dilution of stock solution containing 1000 µg/ml. Linearity test solution for the method were prepared at five different concentration level ranging from 10-50 µg/ml of analyte concentration. Linear calibration graph was obtained between absorbance versus concentration of EVG drug. Beers law was valid over the concentration range from 10-50 µg mL^{-1} of EVG drug.

Specificity and Selectivity :-

The spectra obtained from tablet solution were identical with that obtained from standard solution containing an equivalent concentration of EVG. This showed that there was no any interference from Excipients. Therefore, it could be said that developed method was highly selective.

Recovery studies :-

To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%,100% and 120% level to preanalysed sample and subsequent solution were reanalyzed. At each level, three determinations were performed.

Precision :-

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and interday precisions.

Repeatability :-

Repeatability of the method was determined by analyzing six samples of same concentrations of drug. Graphs were recorded, and the area of each graph was measured.

Intraday and Interday Precision :-

Intraday precision was determined by analyzing the drugs at three different concentrations and each concentration for three times, on the same day. Interday precision was determined similarly, but the analysis being carried out daily, for three consecutive days.

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ±2 nm. For changes of conditions, the sample was assayed in triplicate.

Ruggedness

To determine ruggedness, two different analyst performed assay on manufactured tablets of the drug in similar operational and environmental conditions using developed method.

Solution stability

The stability of the standard solution was tested at intervals of 1, 6, 12 and 24 h. The stability of solutions was determined by comparing absorbance of EVG.[3]

RESULTS AND DISSCUSION :-

The overlay UV spectra of standard and tablet solutions of EVG in methanol was found to be same. The UV spectrum of EVG in methanol has maximum absorption (λmax), at 313.00 nm. The absorbance of excipients in tablet solution did not interfere with EVG at 313.00 nm. As a result, the wavelength was selected for quantitative analysis. The developed method was applied for estimation of EVG in tablet formulation. The results obtained are shown in (Table 1). The drug showed linearity in the concentration range of 10-50 µg/mL. Linear regression data is shown in (Table 2) and (Figure 3).

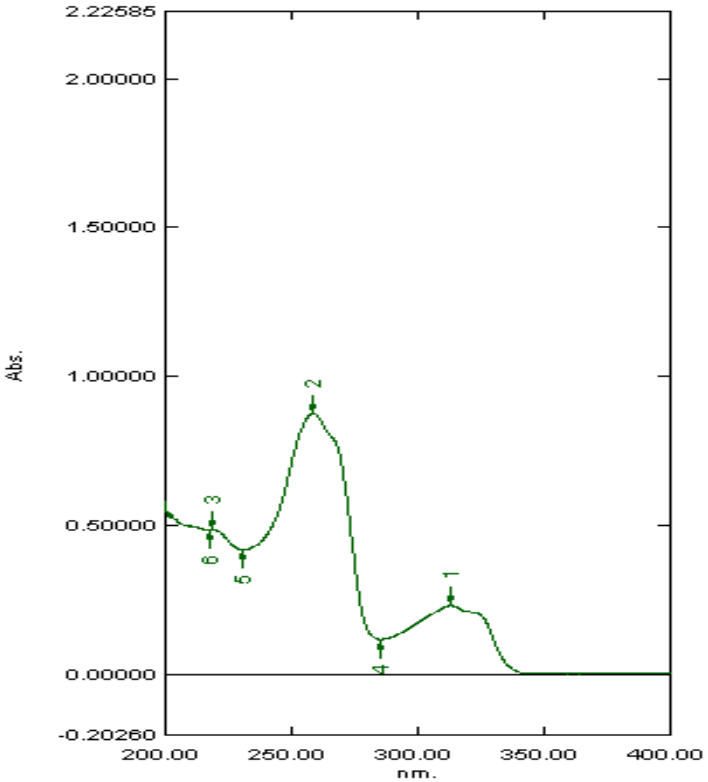

Figure 3 :- Linearity plot of EVG

The developed method was found to be accurate, indicated by means of % recoveries ranging from 99.04 to 99.666 % in table 3.The method was also found to be preciseas the %RSD values for intraday and interday precision were found to be less than 2%. The results are summarized in Table 4. Assay of EVG for all deliberate changes of conditions was within 98.0–102.0 % as shown in Table 5, which indicates robustness of the method. These results of stability studies indicate that the solution was stable for 24 h at ambient temperature. The % RSD of assay was 0.43 % after 24 h. The results are shown in (Table 6).

Table 1: Assay of tablet formulations

Labeled claim (mg)	**% Assay***	**%RSD***
50	101.56 %	0.81

*Mean of six determinations.

Table 2: Linear regression data

Sr.No	**Parameters**	**Results**
1	λmax (nm)	313.00
2	Beer's law limit (µg/ml)	1-100
3	Correlation coefficient(r)	0.999
4	Regression equation	Y=0.027x+0.0009
5	Slope (m)	0.027
6	Intercept (c)	0.0009

Table 3: Results of recovery studies

Level of addition (%)	**Amount of std drug added (µg/mL)**	**Amount recovered (µg/mL)***	**% Recovery ***
80	12	11.96	99.666
100	15	14.85	99.04
120	18	17.88	99.35

*Mean of three determinations

Table 4: Result of Repeatability, Intraday, and Interday precision studies

Repeatability

Sr.No.	**Conc (µg/ml)**	**Absorbance**	**Mean**	**S.D.**	**% RSD**
1		0.423			
2		0.411			
3	15 µg/ml	0.421	0.4204	0.0081	1.91 %
4		0.432			
5		0. 415			
6		0.420			

Intraday Precision :-

Sample concentration(µg/ml)	%RSD*	Average %RSD
10	1.43	0.90
20	0.97	
30	0.3	

*Mean of three determinations.

Interday Precision :-

Sample concentration(µg/ml)	%RSD*				Average %RSD
	Day 1	Day2	Day3	Mean	
10	0.256	0.250	0.248	0.2513	0.85
20	0.541	0.540	0.537	0.5393	
30	0.829	0.828	0.821	0.826	

*Mean of three determinations

Table 5: Result of robustness studies

Method wavelength (nm)	Condition	% Assay*	% RSD
313.00	Analyst - 1	99.25%	1.22%
	Analyst - 2	99.45%	1.43%

*Mean of three determinations

Table 6: Stability data

Sr. No	Ingredient	Time	% Assay*	% RSD
1	EVG	1	99.85 %	0.22 %
		6	100.22 %	0.22 %
		12	100.56 %	0.33 %
		24	101.02 %	0.43 %

*Average of three determinations.

CONCLUSION

The developed UV Spectrophotometric method for the determination of EVG has the advantage of being fast,simple,inexpensive and applicable over the wide concentration range with high precision and accuracy.The method was validated as per the guidelines laid by ICH. The results of the validation tests were found to be satisfactory and therefore this method can be applied successfully to analysed drug formulations.

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REFERENCES

[1] B.A.Dessimmie, R.Schrijvers, Z.Debyzer. Elvitegravir : once a daily alterative to Raltegravir. Lancet Infect Dis.2012,12,3-5.

[2] Gilead Sciences,Stribild® (Elvitegravir/Cobicistat/Emtricitabine/Tenofovir)Prescribing Information.Foster City,CA.August 2012.

[3] Validation of Analytical Procedure,Text and Methodology,European Medicines Agency,(June 1995)229-244.

[4] Ramanathan S, Wright M, West S, Kearney B.P. Pharmacokinetics, Metabolism and excretion of ritonavir boosted GS-9137(Elvitegravir)[abstract 30].8th International workshop on Clinical Pharmacology of HIV Therapy. Budapest, Hungary, April16-18, 2007.

[5] Ramanathan S ,West.S, Hui J, Chuck SL, Kearney B.P. Clinical Pharmacokinetics of once daily elvitegravir boosted by atazanzvir versus ritonavir [abstract18] 9th International workshop on Clinical Pharmacology of HIV Therapy. New Orleas, USA, April 7-9,2008.

[6] German P et.al. Effect of Food on Pharmacokinetics of elvitegravir,emtricitabine,tenofovir and the pharmacoenhacer GS-9350 as a fixed dose combination tablet[abstract A1-1300].49th Interscience Conference on Antimicrobial agent and chemotherapy.San Francisco,CA,September 12-15,2009.

[7] Ramanathan S.,Mathias A.A, German P., Kearney B.P., Cliical Pharmacokinetics and Pharmacodynamic profile of the HIV integrase inhibitor elvitegravir,Cliniacal Pharmacokinetics 50(2011)22244

[8] Aouri M. , Calmy A., Hirschel B., Telenti A., Bucli T., Cavassini M., et.al. A Validated assay by liquid chromatography-tandem mass spectroscopy for the simultaneous quantification of elvitegravir and rilpivirine in HIV positive patients, journal of mass spectrometry 48 (2013) 616-625.

[9] Padigela S*1, V.V.S.Rajendra Prasad2,M.Bhagavan Raju3,N.Suresh Kumar 4.Estimation Of Elvitegravir In Tablet Dosage Forms By RP-HPLC.Indo American Journal of Pharmaceutical Reasearch,2013ISSN O:2231-6876.

[10] Post et.al.Elvitegravir /Cobicistat/Tenofovir DF/Emtricitabine in HIV infected patients with mild to moderate renal impairment[abstract].IAS 2013June 30-July 3 Kuala Lumpur.

[11] Djerada Z., Feliu C, Tournois C,Vautier D,Binet L.Robinet A,Marty H,Gozalo C,Lamiable D,Millart H.Validation of a fast method for quantitativeanalysis of elvitegravir, raltegravir, maraviroc, etravirine, tenofovir, boceprevir and 10 other antiretroviral agents in human plasma samples with a new UPLC-MS/MS SA technology.J Pharm Biomed Anal.2013 Dec;86:100-11.doi:10.1016/j.jpba.2013.08.002.Epub 2013 Aug 12.

[12] Custodio JM, RheeM, Shen G.et.al. Pharmacokinetics and safetycof boosted-elvitegravir in subjects with hepatic impairment .Antimicrob Agents Chemother.2014 feb 18.

[13] Custodio JM, Liu Y,Graham H, et.al.Bioequivalence of two pediatric formulations vs adult tablet formulation of elvitegravir [abstract].Conference on Retroviruses and Opportuistic Infections,Boston MA,March 3-6.2014.

[14] Jampala R.R ,V.Kiran Kumar. Developement and application of Liquid Chromatographic Method for Simultaneous Determination of Elvitegravir,Tenofovir DF,Emtricitabine and Cobicistat in fixed Dosage Form.23rd (1):7-13.