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A NEW SIMPLE ANALYTICAL METHOD FOR SIMULTANIOUS ESTIMATION OF COBICISTAT AND ELVETIGRAVIR BY RP-HPLC-PDA IN THEIR TABLET DOSAGE FORMS

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ABSTRACT

A simple, rapid, accurate and precise RP-HPLC analytical method has been developed and validated for the quantitative analysis of Cobicistat (COB) and Elvitegravir (ELV) in bulk drugs and combined dosage forms. The proposed method was carried out using on an Agilent Xterra C_{18} (5µm, 15cm X 4.6mm) column. The composition of Mobile phase containing 0.05M Phosphate buffer and methanol (30:70v/v) pH adjusted to 7 with orthophosphoric acid, is reported for the simultaneous estimation of COB and ELV in pharmaceutical dosage forms has been developed. The injection volume of samples was 10µl at 1ml/min flow rate. The column temperature was ambient. UV detection was carried out using a UV-PDA detector at 260 nm. The validation of this method was done as per ICH guidelines. The retention times were observed as 2.39 and 3.90 min for Cobicistat, and Elvitegravir respectively. Linearity ranges were observed at 20 -100 µg/ml for Cobicistat and 10 – 50 µg/ml for Elvitegravir. Relative Standard Deviation did not exceed 2. The present study demonstrates the applicability of chromatographic method to develop a new, sensitive, single RP-HPLC method for the simultaneous quantitative determination of two antiviral agents in fixed pharmaceutical dosage form.

INTRODUCTION:

Cobicistat and Elvitegravir combined dosage form is used for the treatment of HIV-1infection in adult patients. Cobicistat (Fig-1) acts as an HIV integrase inhibitor[1, 2]. It has a molecular formula of C40H53N7O5S2 and a molecular weight of 776.0. It is a new pharmacokinetic enhancer, metabolized by CYP3A and especially used to increase elvitegravir levels when administered[3-4]. Chemically it is thiazol - 5 - ylmethyl N - [1-benzyl-4-[[2-isopropylthiazol-4-yl) methyl – methyl-carbamoyl] amino] - 4 - morpholino - butanoyl] - amino -5- phenyl-pentyl] carbamate.

Elvitegravir (Fig-1) is 6- (3- Chloro-2-fluorobenzyl) -1- [(2S) -1- hydroxy -3- methyl butan -2- yl] -7- methoxy -4- oxo-1, 4 dihydro quinoline -3- carboxylic acid. Elvitegravir inhibits the strand transfer activity of HIV-1 integrase (integrase strand transfer inhibitor; INSTI), 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 does not inhibit human topoisomerases I or II[5].

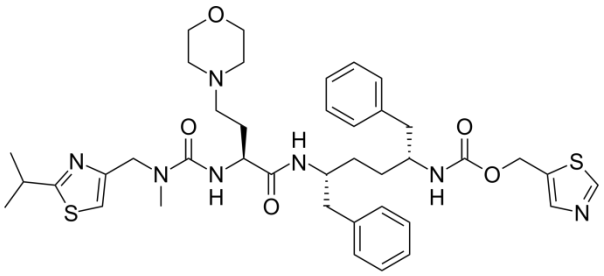

Figure 1: Chemical Structure of Cobicistat

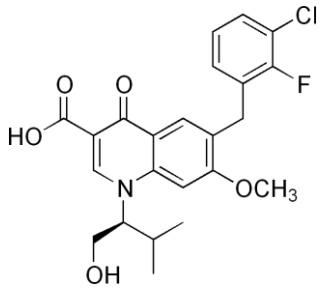

Figure 2: Chemical Structure of Elvitegravir

Various UV, HPLC and LC/MS/MS assay methods were reported in the literature for the estimation of Cobicistat and elvitegravir individually and in combination with other drugs. These methods include; UV spectroscopy method[6-8], Ion pair HPLC method[9], HPLC method[10-13], HPTLC method[14-15] and LC/MS/MS[16-18].

On the contrary to the best of our knowledge, there is no official method for the simultaneous estimation of Cobicistat and elvitegravir by RPHPLC in tablet dosage form. In view of the lack of information, present study was conducted with an objective of development and validation as per ICH guidelines[19-20] of a simple RP-HPLC method for the simultaneous determination of COB and ELV in tablets dosage form.

MATERIALS & METHOD:

The Pharmaceutical grade working standards of Cobicistat and Elvetigravir were obtained as a gift from Richer Pharmaceuticals (Prasanthinagar, Hyderabad, India). Tablets Cobicistat and Elvetigravir was purchased from local market Hyderabad, India. All the chemicals were HPLC grade purchased from SD Fine Chem., Mumbai. MilliQ water was used, prepared inhouse.

Chromatographic Conditions:

Waters 2695 series HPLC consisting pump, Auto sampler, Auto injector, VWD & photo diode array detector, thermostatic column compartment connected with Empower 2 software connected with a Xterra C_{18} (5µm, 15cm X 4.6mm) column.

Mobile Phase:

Weighed 0.50 g of KH_2PO_4 and 0.30 g of potassium dihydrogen phosphate was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 7 with ortho phosphoric acid. A mixture of pH 7 Phosphate buffer 300 mL (30%), 700 mL of Methanol (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration. Mobile phase is used as Diluent**.**

Preparation of Standard solutions

Cobicistat

10mg of Cobicistat working standard was accurately weighed and transferred into a 10mL clean dry volumetric flask and about 2ml of Dimethyl formamide is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent.

Elvetigravir

10mg of Elvetigravir working standard was accurately weighed and transferred into a 10mL clean dry volumetric flask and about 2ml of Dimethyl formamide is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent.

Preparation of Sample Solution: (Assay of tablet dosage form)

Accurately 10 tablets were weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Cobicistat and Elvetigravir (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluent.

Table 1: Optimized chromatographic conditions

S. No	Chromatographic Parameter	Condition
1.	Column	Xterra C_{18} (5µm, 15cm X 4.6mm)
2.	Mobile Phase	0.05M Phosphate buffer and methanol (30:70v/v) pH adjusted to 7 with orthophosphoric Acid.
3.	Flow Rate	1 ml/min
4.	Column Temperature	Ambient
5.	Injection Volume	10µl.
6.	Detection Wavelength	260 nm
7.	Rts	2.39 and 3.90 min for Cobicistat, and Elvitegravir respectively
8.	Diluent	Mobile Phase

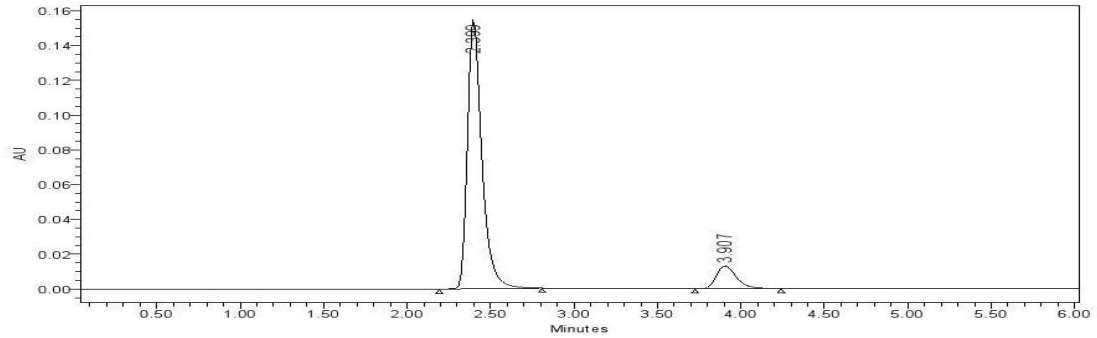

Figure 3: Chromatogram of Optimized method showing the elution of COB at 2.399 and ELV at 3.907

Table 2: System suitability data

S. No	Peak name	Rt	Area	Height	USP Plate	USP Tailing	USP Resolution
1	Cobicistat	2.399	946124	155429	5105	1.3	8.1
2	Elvitegravir	3.907	111541	13239	3788	1.4	6.3

Table 3 Accuracy results of Cobicistat

%Concentration level	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	353867	5.0	5.0	101.3%	100.0%
100%	4735088	10	9.94	99.4%	
150%	5911798	15	14.8	99.2%	

Table 4: Accuracy results of Elvitegravir

%Concentration Level	Area	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
50%	2332744	5	5.10	101.8%	100.5%
100%	3132697	10	9.99	99.9%	
150%	3918997	15	14.9	99.1%	

Table 5: Accuracy results of Elvitegravir and Cobicistat

Injection No.	Elvitegravir		Cobicistat	
	Area	% Assay	Area	% Assay
1	1501417	100.68	2235319	99.59
2	1486940	99.70	2240678	99.83
3	1490656	99.95	2249490	100.22
4	1487329	99.73	2245822	100.05
5	1490384	99.94	2251694	100.32
6	1491345	100.00	2244601	100.00
Mean	1491345.17	100.00	2244600.67	100.00
Std. Dev	5260.72	0.35	5953.98	0.27
%RSD	0.35	0.35	0.27	0.27

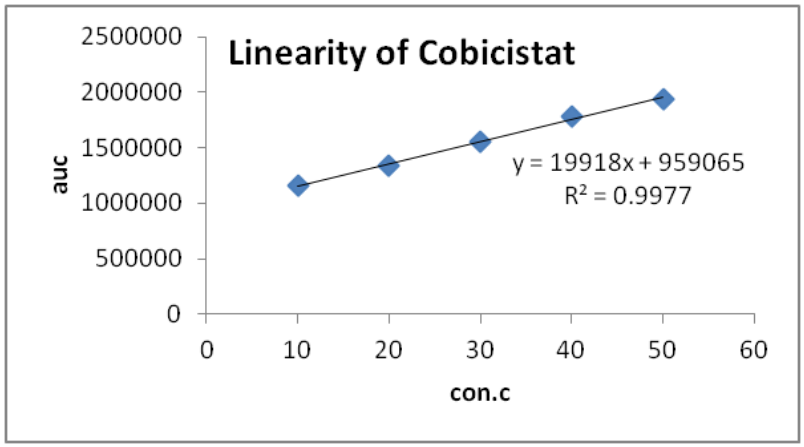

Figure 4: Linearity of COB

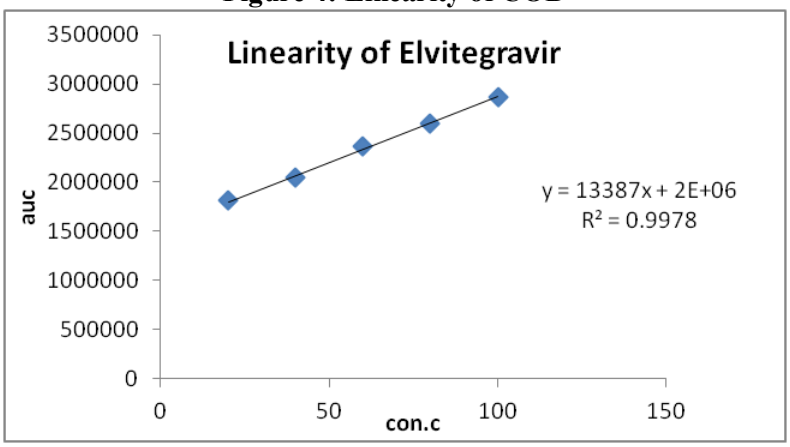

Figure 4: Linearity of ELV

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions:

In order to achieve good separation between the two components different buffer pH-conditions and different proportions of solvents like methanol, Acetronitrile and water tested binary and tertiary eluents. However, in 0.05M Phosphate buffer and methanol (30:70v/v) pH adjusted to 7 with ortho phosphoric acid, achieved good satisfactory results at a flow rate of 1.0 ml/minute measured at a detection of 260 nm. The chromatogram of optimized standard mixture was shown in Figure 3. The system suitability parameters such as retention time, assymetry, resolution and theoritical plates for optimized standard mixture tabulated in Table 1.

Method Validation:

System suitability:

System suitability is an integral part of the method validation to evaluate the parameters like tailing factor, theoretical plates, resolution and %RSD for replicate injections. The results were within the limits and were presented in Table 2. Figure 2 shows the Standard chromatogram.

Accuracy:

To determine the Accuracy of the proposed method, recovery studies were conducted; known amount of pure drug concentrations was spiked in sample at three different levels, ie, 50%, 100% and 150% and was calculated. Accuracy was calculated as the percentage of recovery. The results were tabulated in Table 3 & 4.

Precision

The precision was evaluated at three levels, repeatability, reproducibility and intermediate precision each level of precision was investigated by six replicate injections of concentrations. The result of precision was expressed as % of RSD and was tabulated in Table 5.

Linearity:

The linearity was evaluated by measuring different concentrations (25% to 150%) of the standard solutions to Cobicistat (20-100 µg/ml) and Elvitegravir (10-50 µg/ml). The calibration curve was constructed by plotting concentration of standard solutions against mean peak areas and the regression equation was computed. Lineaity curves of COB and ELV were shown in figures 4 and 5.

Detection limit (DL) and quantitation limit (QL):

Estimation of DL and QL considered the acceptable signal-to-noise ratios 3:1 and 10:1 respectively. The limit of detection and quantitation to be determined as 2.95 and 9.87µg/ml for Cobicistat and 3.04 and 10.30 µg/ml for Elvitegravir respectively.

CONCLUSION:

A simple, specific and reliable isocratic HPLC-PDA method was developed for the estimation of Cobicistat and Elvitegravir in their pharmaceutical formulation. The Proposed method is specific, sensitive accurate & precise. Hence the developed method can be adapted to regular quality control analysis of COB and ELV tablets.

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