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Spectrofluorimetric Technique Creation and Approval for Cabotegravir Estimate in Dose Formats for Medications

ASHOK GORJA[1], SANTHOSHI ABBINENI[1], DURGA PANIKUMAR ANUMOLU[1], and JAHNAVI BANDLA[2]*

[1]Faculty of Pharmacy, Gokaraju Rangaraju College of Pharmacy, Bachupally-500090, Hyderabad, Telangana, India.
[2]Faculty of Pharmacy, Vishnu Institute of Pharmaceutical Education and Research, Narsapur-502313, Medak, Telangana, India.
*Corresponding author E-mail: jahnavi.bandla@gmail.com

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ABSTRACT

The current investigation sought to create and approve specific spectrofluorimetric technique to cabotegravir estimate in dose formats for medications. The relative fluorescence intensity for the cabotegravir was measured at a wavelength of excitation of 395nm and wavelength of emission at 484nm. There was a linear response with a correlation coefficient of 0.999 between 5 and 25µg/mL. Developed technique was supported with the various validation parameters such as accuracy, sensitivity, specificity, and precision in keeping with the ICH recommendations. The devised approach was shown to be sensitive, the corresponding quantification and detection limits of 0.6 µg/mL and 0.21 µg/mL. The found results indicates the method developed for the estimation of cabotegravir were specific, accurate, precise and reproducible. Finally, it can be concluded that this spectrofluorimetric method could be used in routine analysis for cabotegravir estimate in dose formats for medications.

Keywords: Cabotegravir, Spectrofluorimetry, Accuracy, Precision, Method development, Validation.

INTRODUCTION

Chemically, cabotegravir (Fig. 1) was identified as (3S,11aR).2,4-difluorobenzyl) -N-3,2-a] hexahydrooxazolo (6-hydroxy-3-methyl-5,7-dioxo-2,3,5,7,11,11a) Pyrido[1,2-d]1-Pyrazine-8-carboxamide[1,2]. Its molecular weight is 405.36 g/mol and its chemical formula is $C_{19}H_{17}F_2N_3O_5$. It is a crystalline powder that ranges in color from white to off-white. It dissolves in DMSO[3-6] but is essentially insoluble in aqueous solutions like methanol and water. An antiviral drug called cabotegravir is used to treat and prevent HIV (Human Immunodeficiency Virus). It is a member of the integrase strand transfer inhibitor (INSTI) medication class[7,8]. In order to stop the virus from replicating, cabotegravir blocks the HIV integrase enzyme, which is necessary for the virus to incorporate its genetic material into the DNA of the host cell[9].

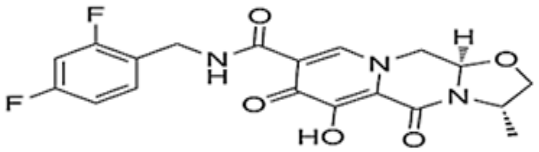

Fig. 1. Cabotegravir Structure

According to the literature review, there aren't many analytical approaches available such as UV spectrophotometry[10], HPLC[11] and LC-MS[12] methods were developed, but no spectrofluorimetric technique was created for cabotegravir estimate in marketed dose formats for medications. Spectrophotometry's outstanding sensitivity and specificity have led to its remarkable prominence in drug analysis. In contrast to spectrophotometry, spectrofluorimetry allows for examination at both emission and excitation wavelengths[13]. Hence the current investigation sought to create and approve new spectrofluorimetric technique to cabotegravir estimate in dose formats for medications.

MATERIAL AND METHODS

Chemicals and reagents

Hetero Drugs Pvt. Ltd., Hyd., Telangana, India, provided a complimentary sample of cabotegravir as part of their operating standard. Vocabria, the commercial version of cabotegravir, was bought from a nearby pharmacy. All of the solvents and chemicals that were employed were AR grade and were acquired from Merck in Mumbai, India.

Equipment

The current study made use of a number of instruments, including a spectrofluorometer (Shimadzu, RF 5301 PC, Japan), an ultrasonic cleaner (Sonica Ultrasonic Cleaner, Italy), a pH meter (Elico L120, Hyderabad, India), and a digital balance (Shimadzu, AUX 220D, Japan).

Preparation of cabotegravir solutions

Ten milliliters of dimethyl formamide (DMF) and ten milligrams of carefully weighed medication working standard were combined to make an initial solution with an amount of one milligram per milliliter. Later 10 µg/mL cabotegravir standard solution was prepared from the above stock solution using DMF as diluent.

Preparation of 2% ethyl acetoacetate reagent

0.2 mL of EAA was diluted with ethanol up to 10 mL.

Analytical method development

The development of the cabotegravir spectrofluorimetric method involved dissolving the analyte in a variety of solvents. It was discovered that DMF was chosen as the solvent because it yields clear spectra. Utilizing a standard solution including 10 µg/mL of cabotegravir in DMF, 2.5 mL of sulfuric acid, and 0.1 mL of 2% EAA reagent, excitation and emission wavelengths were determined through scanning within the 300-700nm wavelength range. Emission spectra were obtained by scanning solutions at a fixed excitation wavelength. After being excited at 395nm, cabotegravir exhibited fluorescence at an emission wavelength of 484nm.

Analytical method validation

The devised procedure was validated for a number of criteria in compliance with the ICH guidelines[13,14].

Linearity

Aliquots of cabotegravir in the 5–25 µg/mL range were produced in several volumetric flasks with a capacity of 10 mL containing 2.5 mL sulfuric acid, 0.1 mL 2% EAA reagent, and DMF. The fluorescence intensities of the previously stated solutions have been evaluated at an emission wavelength of 484nm using an appropriate blank. The calibration curve's characteristics, including its slope, intercept, and correlation coefficient, were calculated[16].

Accuracy

By calculating the percentage recoveries of cabotegravir using the conventional addition method, the correctness of the results was ascertained. From the tablet powder stored in volumetric flasks, A predetermined concentration of cabotegravir was achieved by adding cabotegravir to standard solutions at 80, 100, and 120% levels. At emission wavelength of 484nm, the produced solutions' fluorescence intensities were determined. Analyte preparations were examined in triplicate at each concentration level in order to confirm the recovery.

Precision

The current method's repeatability (intra-day precision) was determined by measuring the corresponding response for three different cabotegravir concentrations (5, 15 and 25 µg/mL)

three times in a single day. Throughout a week, three separate days were used to estimate the response in triplicate for three distinct concentrations (5, 15 and 25 µg/mL) in order to discuss the intermediate (inter-day) precision. The percentage relative standard deviation (% RSD) was used to express the findings of both investigations.

Sensitivity

According to ICH criteria, samples with extremely low concentrations of cabotegravir were used in order to identify the lowest quantitative quantity (LOQ) and the lowest detected amount (LOD), which demonstrated the analytical method's sensitivity. The formulas were used to compute LOD and LOQ, respectively. To demonstrate the analytical method's robustness, by slightly altering the emission wavelength, the fluorescence intensity of the analyte solutions was also evaluated[17].

Assay of cabotegravir

Average weight of 20 tablets of Vocabria of 30mg of cabotegravir were taken and crushed into powder. To get a concentration of 1 mg/mL, 10mg of precisely weighed cabotegravir was dissolved in 10 mL of DMF. Whatmann filter paper was used to filter the aforementioned solution, and an aliquot of the clear filtrate was removed. This was diluted to make 10 mL with diluent, then 2.5 mL of sulfuric acid and 0.1 mL of 2% EAA were added. Additionally, the assay percentage was determined by evaluating fluorescence intensity of the final solution at an emission wavelength of 484 nm.

RESULTS AND DISCUSSION

The current investigation was developed for estimating cabotegravir using spectrofluorimetric method in pharmaceutical dosage form.

Analytical method development

The standard cabotegravir solution was prepared by dissolving the working standard in DMF solvent. For the selection of solvent, a variety of solvents such as methanol, water, DMSO and DMF were tested for obtaining spectrofluorimetric spectrum. Out of which DMF was chosen as the solvent because it yielded a clean spectrum. The standard solution for analysis was prepared by 2.5 mL sulfuric acid, 0.1 mL of 2% EAA reagent addition. When the standard solution was scanned in the range of 300-700nm, it has shown excitation wavelength at 395nm and emission wavelength at 484nm. The spectrum was shown in Figure 2.

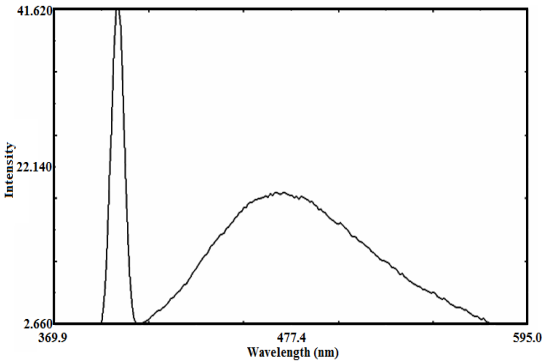

Fig. 2. Spectrofluorimetric spectrum of cabotegravir standard solution

Analytical method validation

The created technique was approved to meet the ICH criteria.

Linearity

The linearity of the calibration curve (emission intensity vs. concentration) was investigated between the concentration 5 and 25 µg/mL. The cabotegravir overlay spectrum for the linearity range, as displayed in Fig. 3, is listed below. The correlation coefficient value (R) for captopril in the linear regression analysis was 0.9994, and the regression equation was y = 0.4141x + 0.014. Here are the cabotegravir linearity data from Table 1 and the calibration graph from Fig. 4, which demonstrated an increase in intensity at 484nm emission wavelength with increasing medication concentration.

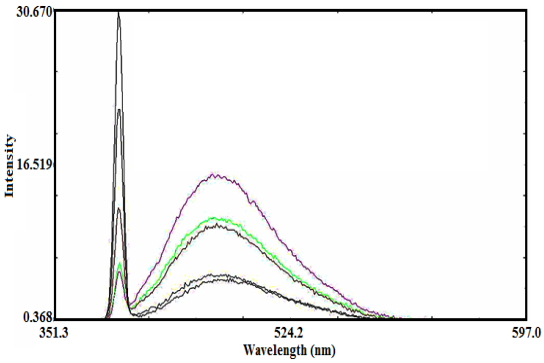

Fig. 3. Spectrofluorimetric overlay spectrum of cabotegravir for linearity study

Table 1: Linearity data of cabotegravir

Sr. No	Concentration (µg/mL)	Intensity (n=5)
1	0	0
2	5	2.22
3	10	4.084
4	15	6.166
5	20	8.157
6	25	10.515

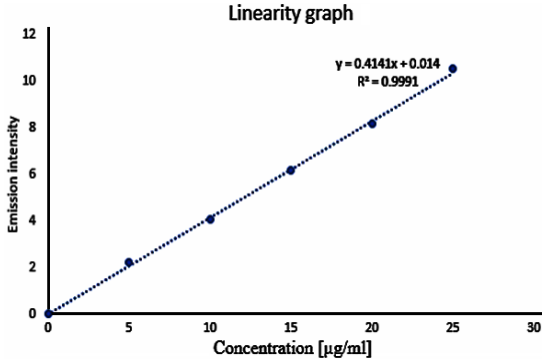

Fig. 4. Cabotegravir plot for linearity study

Accuracy

The accuracy of the standard addition process was assessed using the computation of cabotegravir recoveries. A standard cabotegravir solution at concentrations of 80%, 100%, and 120% was added to pre-qualified sample solutions containing 30 mg of cabotegravir. The concentration was determined by measuring the intensities of the spiking solutions at 484nm [λ_{ems}]. At each of the designated concentration levels, the recovery was found to be between 98.44 and 102%, which was excellent. The % recovery data for cabotegravir were summarized in the Table 2.

Table 2: Accuracy results for cabotegravir

Drug	Level of accuracy,%	Theoretical Concentration, (µg/mL)	Concentration found (µg/mL) (AM±SD)(n=3)	% Recovery	% RSD
Cabotegravir	80%	18	16.54±0.017	98.02%	0.25
	100%	20	20.63±0.042	100.04%	0.55
	120%	22	22.64±0.040	101.90%	0.41

Arithmetic Mean (AM); Standard Deviation (SD), Relative Standard Deviation (RSD), and number of samples (n)

Precision

The precision within and between days of the fluorometric technique was achieved by computing the responses to the three different concentrations of cabotegravir (5, 15, and 25 µg/mL), and the responses are recorded three times on various days, including the same day. The results showed the %RSD for both within-day-precision, and between-days-precision. The created technique was deemed accurate as the percentage RSD values were found to be less than 2.0. Table 3 presented a summary of the precision data.

Table 3: Precision data for cabotegravir

Drug	Concentration (µg/mL)	Inter-Day Precision (AM±SD)(n=3)	% RSD	Intra-Day Precision (AM±SD)(n=3)	%RSD
Cabotegravir	5	2.663±0.05	0.46	2.623±0.0196	0.13
	15	6.583±0.038	0.32	6.621±0.0317	0.17
	25	10.624±0.0594	0.43	10.57±0.0316	0.21

Arithmetic Mean (AM); Standard Deviation (SD), Relative Standard Deviation (RSD), and number of samples (n)

Sensitivity

Spectrofluorimetric method's LOD and LOQ values were presented in Table 4.

Table 4: Cabotegravir LOD and LOQ data

Parameters for Validation	Values (µg/mL)
LOD	0.21
LOQ	0.60

Assay of marketed formulation

The evaluation of the proposed approach was done using the assay of 30 mg cabotegravir tablets. The cabotegravir test results were shared and contrasted with the recommended dosages on the package. 29.4 mg of cabotegravir (30 mg) was obtained. The assay result percentage for 30 mg was 98%. The result obtained fell between reasonable boundaries and two percent RSD was discovered. The assay data for commercial tablets of cabotegravir were presented in Table 5.

Table 5: Assay of cabotegravir tablets [30mg] by spectrofluorimetric method

Drug name	Label claim [mg]	AM±SD[mg] [n=3]	%Assay	%RSD
Cabotegravir	30 mg	29.4 ± 2	98 %	1.018

Arithmetic Mean (AM); Standard Deviation (SD), Relative Standard Deviation (RSD), and number of samples (n).

CONCLUSION

A brand-new, very accurate spectrofluorimetric

technique was created to estimate the amount of cabotegravir present in pharmaceutical dosage forms. The drug solutions were prepared using the developed method, which employed dimethyl formamide as the solvent. The devised procedure was validated for a number of criteria in compliance with ICH guidelines. It was discovered that the procedure was sensitive, linear, accurate, and precise. Ultimately, it can be said that cabotegravir in medication dosage forms can be routinely analyzed using the established spectrofluorimetric approach.

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Conflict of interest

No conflicts of interest are disclosed by the writers.

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