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Systematic review on analytical techniques for the determination of anti-retroviral drugs

Monali P. Kunde[1*], Prof. Ashwini V. Shelke[1], Dr. Anil G. Jadhav[1]

Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra-422213

ABSTRACT

The concept involved in this article is to summarize different analytical methods used for the estimation of Dolutegravir, Raltegravir, Elvitegravir, Cobicistat and Elvitegravir from bulk and formulations. Drug investigation assumes an exceptionally conspicuous part in quality affirmation just as quality control of mass medications and drug plans. Quick expansion in drug businesses and creation of medication in different areas of the planet has gotten an ascent interest for new insightful methods in the drug enterprises. As a result, insightful technique advancement has turned into the fundamental movement of examination. From the bygone ages, individuals were attempting to track down completely secure ways of treating viral contaminations. In the current situation, because of the arising of new infections, the advancement of medications for their treatment is likewise acquiring equivalent significance. Prior to dispatching to the market, these medications ought to go through an approval interaction. Elite execution fluid chromatography (HPLC) combined with bright (UV), Photodiode cluster indicators (PDA), Mass spectrophotometer (MS) identifiers and so on is one of the quickest, protected and exact advancements utilized for assurance and partition of drug medications, pollutants and organic examples. HPLC is flexible and it requires some investment for measurement of medications when contrasted with old fluid chromatography strategies.

Key-words: Drug analysis, Analytical Methods, UV, HPLC, MS,

INTRODUCTION

The fundamental objective of the drug business is to furnish drug items with adequate quality, viability and wellbeing. The improvement of another medication item and its creation comprise of numerous drug processes, including scientific testing. The logical information created support further choices on how improvement ought to be sought after or give data on whether a medication item ought to be delivered. It is significant that each such turn of events or creation process furnish valid outcomes with steady quality and along these lines, it should be controlled and, if vital, constantly improved. By progress of the nature of a drug interaction, the nature of a medication item is likewise improved. Scientific techniques are among the most basic cycles in drug item improvement and creation. They assume a vital part in supporting other turn of events and creation processes all through all phases of a medication item's life cycle. It is fundamental that an insightful strategy be exact, precise and dependable, making it reasonable for its planned reason [1,2]. As a rule, the vitally working standard of a scientific strategy is detachment of the analytes present in the example. Fluid chromatography (LC) strategies are most regularly utilized, like HPLC or ultra execution fluid chromatography (UPLC), frequently in turned around stage mode with UV absorbance location. The reasons for investigation vary contingent upon the number, significance and connection of analytes that are needed not really set in stone. Insightful strategies for the measure of a functioning drug fixing (API) or assurance of its connected substances and corruption items are most generally applied [2]. Advancement of a particular and vigorous strength demonstrating LC technique for the assurance of related substances and debasement items is a complicated interaction. It requires a conscious constrained corruption of a medication substance and additionally a medication item under different pressure conditions, for example, hydrolytic, oxidative, photolytic, or warm conditions, to give focused on examples containing the analyte and its debasement items. The pressure conditions are more extreme than the sped up and long haul steadiness conditions endorsed in the ICH rules for solidness testing. A scientific technique for assurance of corruption items ought to be fit for distinguishing their expansion during the item's time span of usability and the strategy for the test ought to be equipped for identifying any abatement in the medication's substance during its time span of usability. Such strategies are solidness demonstrating [3-6].

Ongoing assessments demonstrate that 34 million individuals are presently living with HIV/AIDS around the world, with roughly 2.5 million new diseases happening every year [7]. The infection is communicated through the trading of infection containing liquids, including blood, bosom milk, semen and genital emissions [8-10]. Courses of viral disease incorporate sexual contact, infusion drug use, from mother to youngster during pregnancy, labor, or bosom taking care of, and openness of tainted body liquids to uncovered films or tissue [10, 11]. Antiretroviral treatment (ART) is the essential methodology for the treatment and the executives of the illness and can significantly lessen HIV-related dismalness and mortality [12-14]. Workmanship is firmly suggested for all HIV-contaminated people, paying little mind to

pretreatment CD41 T cell count. Besides, ART has shown adequacy in infection the board as well as in viral anticipation as pre-openness prophylaxis in high-hazard populaces [15-18]. There are right now more than 25 antiretroviral (ARV) specialists endorsed for HIV treatment by the U. S. Food and Drug Administration (FDA) in both single-and multi-drug details [19]. Combinatorial ART regimens are ordinarily needed for the supported concealment of viral replication and clinical advantage [20]. At present, in excess of 100 regimens exist for the treatment of HIV [21]. ARVs evoke their remedial impacts through the designated hindrance of different phases of the viral contamination cycle. Hence, drug classes are delineated as CCR5 bad guys, viral combination inhibitors, nucleoside/nucleotide switch transcriptase inhibitors (NRTIs/NtRTIs), non-nucleoside invert transcriptase inhibitors (NNRTIs), integrase strand move inhibitors (INSTIs), and protease inhibitors (PIs). Numerous combinatorial ART regimens consolidate drugs from more than one ARV class, and the U. S. Branch of Health and Human Services (DHHS) has shown suggested and elective regimens for sickness the executives [22]. Furthermore, new treatments are constantly being looked for that exploit new popular targets, have movement against safe viral strains, have a lower frequency of unfavorable impacts, and proposition helpful dosing. New specialists of existing classes are as of now in cutting edge phases of clinical advancement [23]. The developing interest for these specialists animates a quest for new considerably more viable medications, yet in addition calls for more significant level of value control of these helpful substances and arrangements, so they are in the most noteworthy conceivable degree liberated from any debasements that might come from the creation interaction, just as from disintegrations results of dynamic or assistant substances. Accordingly, it appears to be proper to foster new insightful strategies with respect to their subjective and quantitative examination. For this point, distinctive logical techniques are utilized for deciding enemy of HIV drugs. Against HIV drugs are the new advancements of medications and there is an extraordinary need to audit the insightful work announced so far in the writings. Endeavors have been made to gather the writing from 2000 up to the present. Logical techniques permitting the assurance of TDF, FTC and EFV drugs in different media, like drug definitions, organic networks and natural examples, is examined. As of now, there are five significant classes of ARV drugs viz. nucleoside turn around transcriptase inhibitor [NRTI], non-nucleoside switch transcriptase inhibitor [NNRTI], Protease inhibitors [PI], combination inhibitor and integrase inhibitor [IIs].

The main single-tablet fixed-portion mix (FDC) antiretroviral (ARV) has been economically accessible starting around 2006 and is promoted as Atripla® [24]. A conventional item has been monetarily accessible in South Africa since April 2013 [25, 26] and comprises of efavirenz (EFV), emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) in a proportion of 600 mg/200 mg/300 mg. TDF in this amount is comparable to 245 mg tenofovir disoproxil (TNF) and 136 mg of tenofovir [27]. The tablet is taken once every day for the treatment of HIV-1 disease [28, 29]. Once-every day FDC tablets are the least difficult antiretroviral treatment accessible [30]. FDC ARV treatment is advantageous for patients as it diminishes the "pill trouble" which thus further develops adherence to treatment [28-30]. FDC were at first shown for

treating HIV-positive antiretroviral gullible patients and HIV-positive pregnant ladies and the people who are breastfeeding. It is presently accessible to all patients on the proposal of a doctor [26]. Treatment with EFV, FTC and TNF is the favored first-line treatment for antiretroviral gullible HIV-1-contaminated people [30]. Bioequivalence between the measurements structure containing a solitary particle and the FDC notwithstanding great pharmacokinetics works with once day by day dosing of EFV, FTC and TNF [24, 30].

1. **Cobicistat and Elvitegravir**

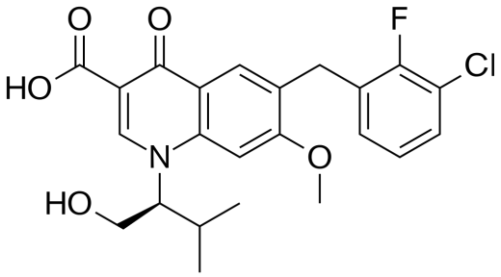

Fig. Structure of Elvitegravir

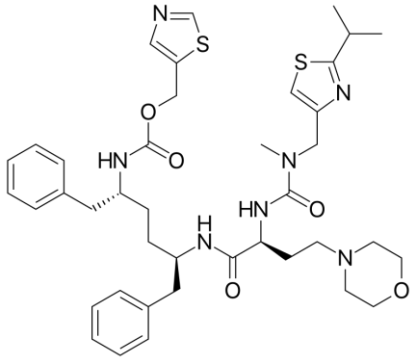

Fig. Structure of Cobicistat

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the simultaneous estimation of cobicistat and elvitegravir in the pharmaceutical dosage form. A column of ODS (250mm 4.6mm; i.d and 5µ particle size) was used along with the mobile phase comprising of 0.02M dipotassium hydrogen orthophosphate buffer (pH adjusted to 3.3) and methanol in the ratio of 80:20 (v/v). The flow rate was maintained at 1.0 ml/min and the effluents monitored at 254 nm. The retention time for cobicistat was found to be 2.58 ± 0.3 min and elvitegravir was 3.71 ± 0.3 min. The detection concentration was linear over 125-750 µg/ml for cobicistat and 12.5-75 µg/ml for elvitegravir. Regression equations of cobicistat and elvitegravir were found to be y = 25883x + 19711 and y = 27696x + 6046 respectively with regression co-efficient 0.999. The % RSD for Intra and Inter day precision was < 2%. The accuracy of method was validated by recovery studies and found to be significant within acceptable range 98-102%. [31]

2. Raltegravir

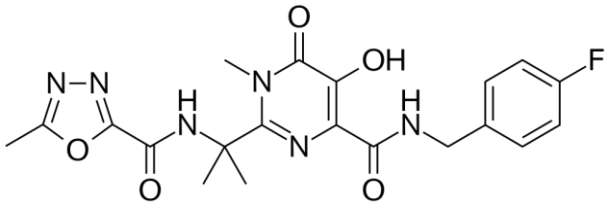

Fig. Structure of Raltegravir

Raltegravir has obeyed Beer-Lambert's law 1–150 in zero order (D0) and 10–150 µg/mL in both first-order derivative (D!) and difference spectroscopic methods. Conclusion**:** Raltegravir has shown a wide range of linearity in all the methods, and all the methods were validated as per the ICH guidelines. These simple methods can be successfully applied for the assay of Raltegravir in tablets.[32]

3. Dolutegravir

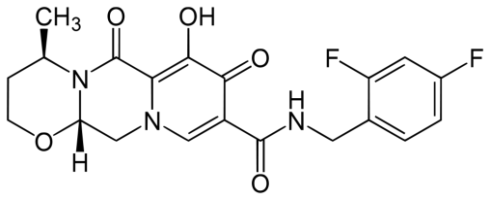

Fig. Structure of Dolutegravir

A simple, rapid and robust reverse phase HPLC method was developed and validated for the determination of impurities in Dolutegravir drug substance. The main aim of this study is to reduce the run time of the HPLC method by developing and validating a new, less expensive HPLC method. The chromatographic separation of Dolutegravir and its related impurities is carried out by using C8 column (150 × 4.6 mm), 5 µm with 0.1% trifluoroacetic acid in water as mobile phase A, methanol as mobile phase B. The flow rate is 1.0 mL/min with gradient elution mode. The wave length for detection is 240 nm (UV detector). The developed method was validated and proved that the method was specific, accurate and precise as per ICH. The system suitability criteria found to be within the limits. The limit of detection and limit of quantification demonstrate that the method is sensitive. The linearity curve was found to be linear and the correlation coefficient obtained is not less than 0.998. The average percentage recoveries of impurities were in the range of 97 to 101%.[33]

Table no. 01: Analytical methods used for the estimation of Cobicistat and Elvitegravir

Sr.No.	Method	Mobile Phase composition	Detection (nm)	Ref.
1	RP-HPLC	0.05 M Sodium dihydrogen phosphate (pH 5): Acetonitrile(70:30 v/v)	235nm	34
2	RP-HPLC	Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v)	255nm	35
3	RP-HPLC	aceto- nitrile : phosphate buffer (10 : 90 at pH 6.5)	240 nm	36
4	RP-HPLC	water (adjusted to pH 3 with 0.1% Orthophosphoric acid): Acetonitrile (55:45 % v/v)	245 nm	37
5	RP-HPLC	ortho phosphoric acid buffer, Acetonitrile, (50:50v/v)	210 nm	38
6	RP-HPLC	Buffer 0.1% Formic acid: Acetonitrile	293 nm	39
7	RP-HPLC	0.05M Phosphate buffer and methanol (30:70v/v)	260 nm	40
8	RP-HPLC	0.1 M NaH2 PO4 and methanol	260 nm	41

		(70:30 v/v)		
9	RP-HPLC	0.05M Phosphate buffer pH 3.0 (adjusted with dilute phosphoric acid) and Acetonitrile in the ratio 95:5	240 nm	42
10	RP-UPLC	0.1% Orthophosphoric acid buffer and acetonitrile (35:65 v/v)	250 nm	43

Table no. 02: Analytical methods used for the estimation of Elvitegravir

Sr.No.	**Method**	**Mobile Phase composition**	**Detection (nm)**	**Ref.**
1	RP-HPLC	gradient mixture of 0.1% trifluoroacetic acid and acetonitrile	240nm	44
2	Liquid Chromatography Tandem Mass Spectroscopy	20-m M ammonium acetate /MeOH (50:50)	250 nm	45
3	Liquid Chromato Graphic Method	KH2PO4 (0.02 M) in 1000 ml of Water:Acetonitrile	240 nm	46
4	UPLC-MSMS	Evaluation of Accuracy Shows A Deviation <15% from target concentration At Each Quality control level. Interday and intraday		47

		assay variation. R.T.- 4.2 min.		
5	RP-HPLC	0.03 M KH2PO4 :Methanol(80:20)v/v & Acetonitrile & Buffer 60:40 v/v	257 nm	48

Table no. 03: Analytical methods used for the estimation of Raltegravir

Sr.No.	**Method**	**Mobile Phase composition**	**Detection (nm)**	**Ref.**
1	Simple UV Spectro photometric Method	Water	331.6 nm	49
2	UV-HPLC	KH2PO4	297 nm	50
3	HPTLC	Toluene:Ethyl Acetate:Methanol: Glacial Acetic Acid (4:5:0.6:0.4%v/v)	218nm	51
4	RP-HPLC	Phosphate Buffer and Acetonitrile 40:60 v/v	247nm	52
5	RP-HPLC	phosphate buffer : Methanol (45:55%)(PH 3.0)	219nm	53
6	UPLC	/mixture of sodium perchlorate(0.2g I 1000 ml of water,PH 2.5±0.05 with perchloric acid)and Acetonitrile 65:35(v/v)	240 nm	54
7	HPLC-PDA	Acetonitrile and Phosphate Buffer) C	240 nm	55

		– 18 Reverse Phase		
8	HPLC-MS/MS	42.5/57.5(v/v%)0.1Mm EDTA in 0.1% formic acid /Methanol	240 nm	56

Table no. 04: Analytical methods used for the estimation of Dolutegravir

Sr.No.	**Method**	**Mobile Phase composition**	**Detection (nm)**	**Ref.**
1	UV Spectrophotometric Method	Water	259.80 nm	57
2	UV Spectrophotometric Method	8M Urea as Hydrotropic Solubilizing Agent	256 nm	58
3	UV-Spectrophotometry And RP-HPLC	Methanol	259.80 nm	59
4	HPLC	acetonitrile: water (pH 7.5) in the ratio 80:20 v/v	260 nm	60
5	High-Performance Thin-Layer Chromatographic Method	methanol:chloroform:formic acid in the proportion of 8:2:0.5 v/v/v	265 nm	61
6	HPLC MS/MS	acetonitrile and water in the ratio 60:40 containing 0.1% formic acid	260 nm	62

CONCLUSION

As there are several analytical methods (HPLC, UV, HPTLC, UPLC & MS) were reported there is a continued need for developing more efficient, sensitive, accurate and precise methods for the analysis of the Dolutegravir, Raltegravir, Elvitegravir, Cobicistat and Elvitegravir alone and in combination in the dosage forms and in the biological fluids.

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