

RECENT RESEARCH ON ANALYTICAL METHODS OF ANALYSIS OF RALTEGRAVIR AND ELVITEGRAVIR : A REVIEW.

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ABSTRACT

Highly Active antiretroviral therapy (HAART), a combination drug therapy is a topic of current interest in the treatment of HIV and AIDS. Techniques for the analysis and the quality control of antiretroviral drugs, particularly in the drug combinations are vital in achieving quality of these drugs and the treatments involved. Integrase inhibitor are a class of antiretroviral drug designed to block the action of integrase, a viral enzyme that inserts a viral genome into the DNA of the host cell. Since integration is a vital step in retroviral replication, blocking it can halt further spread of the virus. Integrase inhibitors were initially developed for the treatment of HIV infection. The HPLC, UV and HPTLC methods are available for the analysis of Raltegravir and Elvitegravir, the recently used drug for HIV and AIDS are reviewed in this article.

KEYWORDS :- Antiretroviral Drugs, Raltegravir, Elvitegravir, Integrase Inhibitor.

INTRODUCTION

ANTIRETROVIRAL DRUGS

Antiretroviral Drugs are the medication for the treatment of infection by retroviruses, primarily HIV. Different classes of Antiretroviral Drugs act at different stages of HIV life cycle. combination of several (typically three or four) antiretroviral drugs is known as Highly Active Anti Retroviral Therapy (HAART).^[1,2,3]

The Classes are ,

1. Nucleoside Reverse Transcriptase Inhibitor (NRTI) :-

Nucleoside Reverse Transcriptase Inhibitor (NRTI) binds and inhibits the action of reverse transcriptase to prevent the formation of viral RNA from pro viral DNA causing a decrease in the amount of virus in the body and subsequent spread to other healthy cells. The drugs under this category include Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Emtricitabine and Abacavir.

2. Nucleotide Reverse Transcriptase Inhibitor (NtRTI) :-

A Nucleotide Reverse Transcriptase Inhibitor (NtRTI) inhibits the activity of HIV-1 reverse transcriptase by competing with natural nucleic acid substances. The NtRTI is then incorporated in viral nucleic acid, causing termination of chain formation. Ex. Tenofovir

3. Non -Nucleoside Reverse Transcriptase Inhibitor (NNRTI) :-

A Non-Nucleoside Reverse Transcriptase Inhibitor (NRTI) inhibits the action of HIV reverse transcriptase but at a different site on the enzyme than the site targeted by NRTIs block RNA dependent DNA polymerase activities. The drugs under this category include Efavirenz, Nevirapine and Delavirdine.

4. Protease Inhibitor :-

A Protease Inhibitor (PI) inhibits the protease enzyme, which typically cleaves certain HIV protein precursors that are necessary for the replication of new infectious virions. This mechanism results in the production of immature, non infectious virions. These drugs are typically combined with other antiretroviral drugs and their use has led to marked clinical improvement and prolonged survival among HIV-infected patients. Because PIs are metabolized through cytochrome P-450, drug interactions are common and can be severe. The drugs under this category include Amprenavir, Fosamprenavir, Indinavir, Lopinavir, Nelfinavir, Ritonavir and Atazanavir.

5. Entry Inhibitor :-

A very early stage of the viral replication is viral entry, when the virus attaches to and enters the host cell. A number of “entry inhibiting” or “entry blocking” drugs are being developed to fight HIV. HIV

most heavily targets the immune system's white blood cells known as "helper T cells", and identifies these target cells through T-cell surface receptors designated "CD4" and "CCR5". Attempts to interfere with the binding of HIV with the CD4 receptor have failed to stop HIV from infecting helper T cells, but research continues on trying to interfere with the binding of HIV to the CCR5 receptor in hopes that it will be more effective.

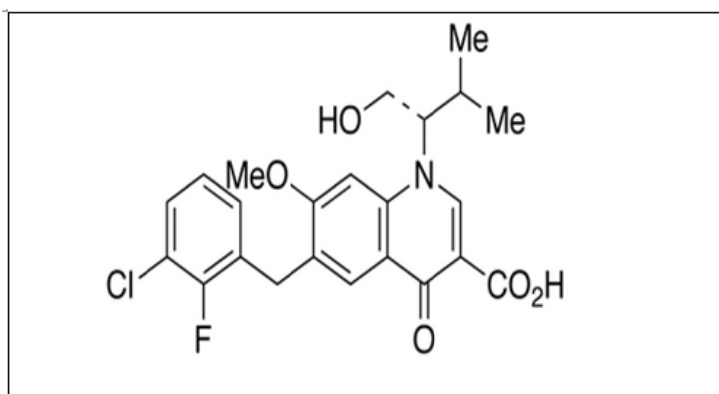
6. Integrase Inhibitors :-

Integrase Inhibitors (also known as integrase strand transfer inhibitor ,INSTI) are a class of antiretroviral drugs designed to block the action of integrase ,a viral enzymes that inserts the viral genome into the DNA of the host cell. Since integration is a vital steps in the retroviral replication,blocking it can halt the further spread of the virus. Integrase inhibitor were initially developed for the treatment of HIV infection. Raltegravir and Elvitegravir are the integrase inhibitors.^[1,2,3]

1. ELVITEGRAVIR (EVG) :-

Elvitegravir (EVG) is an HIV 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. The molecular formula of EVG is $C_{23}H_{23}ClFNO_5$ molecular weight of EVG is 447.9.

It has the following structural formula,



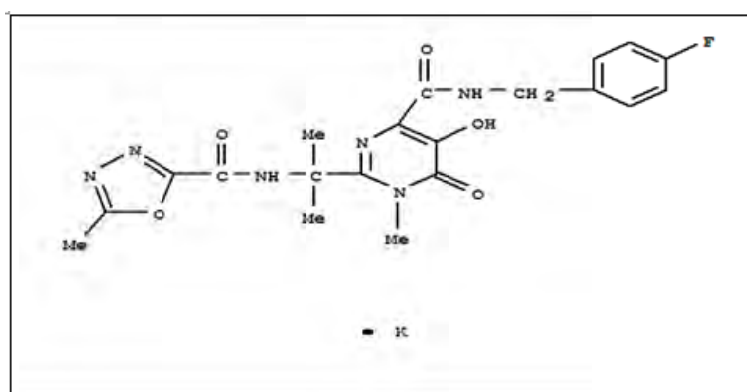
Elvitegravir is white to pale yellow solid with a solubility in DMSO,Acetonitrile and methanol.The partition coefficient(log p) for EVG is 5.5 and PKA is 6.6.^[1,2]

2. RALTEGRAVIR POTASSIUM (RALP) :-

Raltegravir Potassium (RALP) is a an enzyme integrase inhibitor used for the treatment of Human Immunodeficiency Virus 1(HIV-1)infection in the treatment experienced adult patient who have evidence of viral replication and HIV -1 strain resistant to multiple antiretroviral agent. RALP targets integrase, an HIV enzyme that integrates the viral genetic material into the human chromosomes,a critical step in pathogenesis of HIV .The drug is metabolized away via glucuronidation.^[1,2,3]

Raltegravir Potassium chemically known as is N-[(4-Fluorophenyl)methyl]-1,6-dihydro-5-hydroxy-1-methyl-2-[1-[[[(5-methyl-1,3,4-oxadiazol-2-yl)carbonyl]amino]ethyl]-6-oxo-4-pyrimidinocarboxamide mono potassium salt. The empirical formula is $C_{20}H_{20}FKN_6O_5$ and the molecular weight is 482.51.

The structural formula is



It is a white powder, soluble in water. The pKa is 6.6 in water. The octanol/water partition at PH 7.4 is 2.80.

HPLC Method of Analysis of Antiretroviral Drugs :-

High Performance Liquid Chromatography (HPLC) is a separation process, it separates mixture containing two or more components under high pressure. In HPLC Stationary phase is packed in one end of column which is attached to a source of pressurized liquid mobile phase. HPLC is a fastest growing analytical technique for the analysis of the drug. Its simplicity, high specificity and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage form and biological fluids. Several hplc methods were reported for the analysis of antiretroviral drug in the bulk, dosage form and biological fluids.

A summary of research work on several analytical methods (HPLC,UV,HPTLC,UPLC & MS) reported for the Raltegravir and Elvitegravir alone and in combination is give in Table 1.

CONCLUSION :-

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

Table1- A summary of research work on the analytical methods for the Estimation of Raltegravir and Elvitegravir alone and in the combination.

Sr . No.	Drug	Method	Instrument, Mobile Phase, RT, Flow Rate & Results of Validation	Reference
1.	Raltegravir Potassium	Simple UV Spectro photometric Method	M.Phase :- Water. λ_{max} :- 331.6 nm Results :- $R^2=0.999$, $Y=0.0201x-0.0008$ Slope (m) :-0.0201 , Intercept (c) :- 0.0008 Detection Limit($\mu\text{g/mL}$)- 0.2 Quatification limit ($\mu\text{g/mL}$)-0.5	Girija B. Bhavar et.al. ^[4]
2.	Maraviroc and Raltegravir	Simultaneous Estimation of Maraviroc & Raltegravir in Human Plasma by UV-HPLC method.	C-18(150mm x 4.6 mm I.D.) M.Phase :- KH_2PO_4 λ_{max} :- 197 & 300 nm Flow Rate :-1ml/min R.T. – 10 min	Notari S et.al. ^[5]
3.	Raltegravir Potassium	RP-High Performance Liquid Chromatography And HPTLC .	HPLC:- C-18 symmetry (150mm x 4.6 mm.5 μ) M.Phase :- phosphate buffer :Methanol (45:55%)(PH 3.0) HPTLC:- Toluene:Ethyl Acetate:Methanol: Glacial Acetic Acid (4:5:0.6:0.4% v/v) λ_{max} :- 218 nm , Result-Flow Rate :- 0.6ml/min , R.F.Value :-0.12 Recovery value at 98.36 % to 100.18 % , %RSD of 0.9213 at $r^2=0.9998$. R.T. 4.3 Min	T. Sudha et.al. ^[6]
4.	Raltegravir Potassium	Validated Reverse Phase HPLC Method	C-8 M.Phase :- Phosphate Buffer and Acetonitrile 40:60 v/v λ_{max} :- 247nm , Flow Rate :- 0.6 ml/min Result :- RSD :-less than 2% , % Recovery :- 99.36% & 101.85 % R^2 :- 0.999	A. Lakshmana Rao et.al. ^[7]
5.	Raltegravir Potassium	RP- High Performance Liquid Chromatography & UV Spectro photometric Method	Column :- Symmetry C-18 (4.6 X 150 mm,5 μm XTerra) M.Phase :- phosphate buffer : Methanol (45:55%)(PH 3.0) Flow Rate :-0.6ml/min at 219 nm HPLC :- Slope-70827 , Intercept :- 8620 LOD :- 0.027g/ml , LOQ:- 0.09g/ml	T. Raghu pathi et.al. ^[6]

			UV :- $R^2 -0.999$, $Y:-0.0433 x -0.0024$ Slope :-0.0433 , Intercept :-0.0024 LOD :- 0.0959 g/ml , LOQ:-0.290g/ml	
6.	Raltegravir Potassium	Validated stability – indicating UPLC assay method and degradation behavior of raltegravir potassium	BEH Shield 100 x 2.1mm,1.7 μ m column./mixture of sodium perchlorate(0.2g I 1000 ml of water,PH 2.5 \pm 0.05 with perchloric acid)and Acetonitrile 65:35(v/v) Flow Rate :- 0.3 ml/min , λ_{max} :- 240 nm Result :- $R^2 - 0.9998$ Intercept :- 37805 , Y intercept :- 0.62 Slope(m):-30248945 , R.T. 28 min	B.V. Rami Reddy Et.al. ^[8]
7.	Raltegravir	HPLC-PDA method for simultaneous quantification of HIV integrase inhibitors	Gradient (Acetonitrile and Phosphate Buffer) C – 18 Reverse Phase	D'Avolio et.al. ^[9]
8.	Raltegravir	Determination of HIV integrase inhibitor ,MK-0518(Raltegravir), in human plasma using 96-well liquid-liquid extraction and HPLC-MS/MS	Hexane: Methylene chloride in 96-well format with 200 μ L plasma sample size.C-18(50 x 3.0 mm,3 μ m,titanium frits) M.Phase - 42.5/57.5(v/v%)0.1Mm EDTA in 0.1% formic acid /Methanol Flow Rate :-0.5mL/min Result :- Intraday precision :-3.2% . Accuracy :-94.8 to 106.8 %	Merschman S. A. et.al. ^[11]
9.	Elvitegravir And Rilpivirine	Liquid Chromatography Tandem Mass Spectroscopy	M.Phase :- 20-m Mammonium acetate /MeOH (50:50) Result :- Range :- RPV- 5-2500 ng/ml , EVG-50-5000 ng/ml RPV:-Interday :- 3-6.3% and accurate (3.8-7.2%)ml for EVG	Aouri M. et.al. ^[12]
10	Elvitegravir, Tenofovir DF,Emtricitabine and cobicistat	Liquid Chromatographic Method.	Inertsil ODS 3V C ₁₈ column(250m x 4.6 mm, 5 μ m particle size,100 A ⁰ pore size) Flow Rate - 1 ml/min. M.Phase :- KH ₂ PO ₄ (0.02 M) in 1000 ml of Water:Acetonitrile. λ_{max} :-240 nm Result :- Conc.Range -15-180 μ g/ml LOD-0.75 μ g/ml , LOQ-2.25 μ g/ml	Raghu Ram Jampala et.al. ^[13]
11	Elvitegravir, Raltegravir,Maraviroc,Etravirine,Tenofovir, Boceprevir.	New UPLC-MS-MS	Evaluation of Accuracy Shows A Deviation <15% from target concentration At Each Quality control level. Interday and intraday assay variation. R.T.- 4.2 min.	Zoubir Djerada Et.al.
12	Elvitegravir	RP-HPLC	Inertsil ODS 3V,250 x 4.6 mm, Column 5 μ m particle size M.Phase :- 0.03 M KH ₂ PO ₄ :Methanol(80:20)v/v & Acetonitrile & Buffer 60:40 v/v . Flow Rate :- 1.0 ml/min , λ_{max} :- 257 nm. Result :- R.T.- 6.250 min. Range :-80 – 960 μ g/mL. $Y - 9474.289 x + 147734.8116$ LOD & LOQ - 0.4 & 1.2 % Assay – 98.60 %	Padigela Swetha et.al. ^[15]

REFERENCE :-

- [1] Esposito D, Craigie R.HIV integrase structure and function. *adv Virus Res.*1999;52:319-33.
- [2] Hazuda DJ, Felock P, Witmer W, Wolfe A, Stillmock K, Grobler JA, et.al. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science*2000;287(5453): 646-50.
- [3] Pommier Y.,Johnson A. A. ,Marchand C.Integrase inhibitors to treat HIV/AIDS. *Nat Rev Drug Discov.*2005;4(3):236-48
- [4] Girija B.Bhavar*,Sanjay S.Pekamwar. Kiran B.Aher, Sanjay R.Chaudhari .Simple Spectrophotometric Method for Estimation of Raltegravir Potassium in Bulk and Pharmaceutical formulations. *Journal of Applied Pharmaceutical Sciences* Vol.3(10).pp.147-150,October,2013 at DOI:10.7324/JAPS.2013.31026
- [5] Notari S.Tommasi C.,Nicastri E.Bellagamba R,Tempestilli M,Percillo LP et.al.Simultaneous Determination of Maraviroc and Raltegravir in human plasma by HPLC-UV method .*Inter Union Biochem.Mol Biology life* 2009;61(Suppl 4):470-475
- [6] T.Sudha,T.Raghupathi.Reverse Phase –High Performance Liquid Chromatography and Ultra Violet Spetrophotometric Method for the Estimation of Raltegravir Potassium in Bulk and in Tablet Dosage Form.*Global Journal of Medical Research.*Volume 11 Issue 2 Version 1.0 July 2011
- [7] A.Lakshamana Rao and MS. Raghu Ram. Validated Reverse Phase HPLC Method for Determination of Raltegravir in Pharmaceutical Preparation.*Ijrpc*2012,2(1) Issn:2231:2781
- [8] Rami Reddy B.V, Reddy B.S., Raman N .V.V.S.S. , Jyothi G.,Chader Reddy KS,Rambabu C. Validated stability –indicating UPLC assay method and degradation behavior of raltegravir potassium.*Int JPharm Technol* .2012;4(1):4045-59.
- [9] D'Avolio A ,Baietto L, Siccardi M, Simiele M, Oddone V, Bonora S,Di Perri G.An HPLC-PDA method for the simultaneous quantification of the HIV integrase inhibitor Raltegravir,the new nucleoside reverse transcriptase inhibitor etravirine ,and 11 other antiretroviral agents in the plasma of HIV infected patients.*Ther Drug Monit* .2008;30(6):662-69
- [10] .Rezk NL,White N,Kashuba AD. An Accurate and Precise High Performance Liquid Chromatography method for the rapid quantification of the novel HIV integrase inhibitor raltegravir in huma blood plasma after solid phase extraction.*Anal Chim Acta.*2008;628(2):204-13
- [11] Merschman S A, Vallano PT, Wenning LA, Matuszewki BK, Woolf EJ. Determination of the HIV integrase inhibitor ,MK-0518(Raltegravir),in the human plasma using 96-well liquid-liquid extraction and HPLC-MS/MS .*Chromatogra B Analyt Technol Biomed Life Sci.*2007;857(1):15-24.
- [12] Long MC, Bennetto-Hood C, Acosta EP. A sensitive HPLC-MS-MS Method for the determination of raltegravir in the human plasma.*J Chromatogr B Analyt Technol Biomed Life Sci* 2008;867(2):165-71
- [13] Raghu Ram Jampala , V.Kiran Kumar . Appala Raju Nemala.Development and Application of Liquid Chromatographic Method for Simultaneous Determination of Elvitegravir ,Tenofovir DF,Emtricitabine and Cobicistat in fixed Dosage form.23rd June 2014.5(1):7-13
- [14] Herve Millart. Validation of a fast method for quantitative analysis of Elvitegravir ,Raltegravir, maraviroc, etravirine, tenofovir, boceprevir and 10 other antiretroviral agents in human plasma samples with a new UPLC-MS/MS technology.
- [15] Padigela Swetha, V.V.S.Rajendra Prasad, M.Bhagava Raju, N.Suresh Kumar.Estimation of Elvitegravir in the Tablet Dosage Forms by RP-HPLC.*IAJPR.*2013;3(6):4697-4705.
- [16] http://en.wikipedia.org/wiki/Discovery_and_Development_of_Integrase_Inhibitor.