A REVIEW ON EFAVIRENZ IN VARIOUS ANALYTICAL METHODS

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Abstract

A type of medication known as non-nucleoside reverse transcriptase inhibitors includes efavirenz. EFV is used to treat AIDS along with other anti-retroviral medications. It functions by reducing the level of blood-borne HIV infection. Acquired immunodeficiency syndrome is not cured, however, it does prevent catastrophic infections like TB and HIV-related cancer. In antiretroviral therapy regimens, it is administered in conjunction with other inhibitors. The most important kind of treatment for persons with human immunodeficiency virus infection is adequate antiretroviral therapy since it increases survival and guards against future infections. The task of the analysts in creating and validating such pharmaceutical substances is difficult as a result.The review that has been presented consequently completely covers the efavirenz analytical results that have been gathered from a number of sources, including Scopus, Web of Science, Google Scholar, Pub-Med, and other literature databases. Several analytical techniques were employed during the development of the medicine to meet the qualitative and quantitative evaluation of efavirenz from diverse pharmaceutical and biological matrices. Analyzing the use of spectrophotometric and sophisticated chromatographic methods in the past and present to analyze efavirenz in biological samples and pharmaceutical formulations, either alone or in combination with other medications, is the main emphasis of this study's examination. Furthermore, it has been noted that the majority of EFV examination methods use high-performance liquid chromatography, both individually and together.

Keywords: Chromatography, ICH, EFV, HPLC, Validation.

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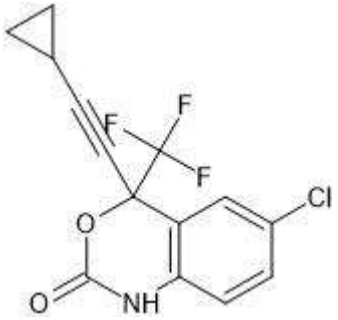
1. Introduction

Efavirenz is a non-nucleoside reverse transcriptase inhibitor of the first generation. identical to other NNRT antagonists. It is used in antiretroviral therapy regimens in conjunction with other inhibitors.Adequate antiretroviral therapy is the most important form of treatment for people diagnosed with the human immunodeficiency virus in order to improve survival and prevent dangerous infections. [1] The most important parts of antiretroviral therapy used to treat human immunodeficiency virus are called non-nucleoside reverse transcriptase inhibitors. It changes its ribonucleic acid into reverse transcription with the help of an enzyme that catalyses reverse transcriptase. These agents stop reverse transcriptase and reverse transcription from making more human immunodeficiency virus.[1]
The seven kinds of HIV medications are post-attachment inhibitors, nucleoside reverse transcriptase inhibitors, nucleoside reverse transcriptase antagonists, protease inhibitors (PI), CCR5 antagonists, and integrase strand transfer inhibitors (INSTIs). [1] Since 1998, the U.S. Food and Drug Administration (USFDA) has approved Efavirenz as an NNRTI for the treatment of HIV. It grew rapidly prevalent in industrialized nations for the same function. EVZ in combination with two NNRTIs, either abacavir/lamivudine or emtricitabine/tenofovir, is the ideal first-line regimen for the treatment of HIV-infected individuals, according to current guidelines. [1]

CHEMICAL PROPERTIES:
EVZ is a byproduct of benzoxazinone, which is a white or slightly yellow crystalline substance having a melting temperature between 136 and 141 degrees Celsius. (S) -6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl) is the chemical formula for EVZ. -2H-3,1-benzoxazin-2-one. It has the formula C14H9ClF3NO2 and the mass 315.68 g/moL.At position 4, a chiral carbon atom was discovered on EVZ. The 4S stereoisomer is primarily used in published papers for commercially available pharmaceutical matrices. At 20 oC, it exhibited a specific optical rotation of 89°-100° in a 3 mg/mL methanol concentration solution. [1]

PHYSICAL PROPERTIES:
The density was determined to be 1.50.1 g/cm3. The boiling point of efavirenz at 760 mmHg is 422.755.0 °C, and the flash point is 209.431 °C. The psa of efv is 38.33000, and the log p value is 3.72. At 25 °C, the vapour pressure and index of refraction are 0.01.1 mmHg and 1.582, respectively.CAS number of efavirenz was found to be 154598-52-4.[2]

STRUCTURE:



SOLUBLITY:
Efavirenz (EFV) is an anti-IgG immunodeficiency virus type 1 oral antiviral drug with very poor water solubility.As a result, the drug's dissolution rate restricts its gastrointestinal absorption. [2] The Food and Drug Administration's system for classifying biopharmaceuticals says that this is a class II drug (low solubility, high permeability). Drugs that are highly permeable but not very soluble in GI fluids are often poorly absorbed because they don't dissolve well enough in GI fluids. With an intrinsic solubility of 8.3 g/mL in pH ranges 1-8, EVZ is almost impossible to dissolve in water. The tests of solubility were done at different pH levels by adding acid (HCL) and base (NaoH) solutions to distilled water. Solubility was measured in bio-relevant media, and it was found to be strong in an aqueous buffer.[1]

MECHANISM OF ACTION:
EVZ is attached to the active site of the reverse transcriptase enzyme in a way that is not a chemical bond. Reverse tran-scriptase tells viral DNA to make more copies of itself. By moving essential amino acids around, these NNRTIs stop the polymerization of the same..[1]

PHARMACOKINETICS:

Peak plasma concentrations of efavirenz (Cmax) in healthy volunteers ranged from 0.51 mg/L to 2.9 mg/L 5 hours after a single oral dose of 100 to 1600 mg of efavirenz. Even though the amount of the drug increased in Cmax, the increase was not proportional. This suggests that higher doses of the drug are not absorbed as well. Efavirenz gets into the brain from the blood. Efavirenz strongly binds to proteins in human plasma (99.5 to 99.75%), especially albumin. Efavirenz is mostly broken down by the cytochrome P450 (CYP) 3A4 and 2B6 isoenzymes in the liver. The half-life of efavirenz in plasma at the end was between 52 and 76 hours after a single dose and between 40 and 55 hours after multiple doses (dose not specified). [3]

PHARMACODYNAMICS:

In primary lymphoid and monocytic cell cultures, efavirenz shown good inhibitory effect against wild-type cell cycle progression, with 95% inhibitory efficacy. When coupled with zidovudine, didanosine, or indinavir, efavirenz displayed synergistic antagonistic effect against HIV-1 in vitro. Efavirenz was cytotoxic to HIV-infected cell lines and a T lymphocyte line at an 80 mol/L concentration.[3]

DOSAGE AND ADMINSTRATION:

Usual dosage in clinical trials : 200 to 600 mg/day in combination therapyRoute of administration : Oral
Frequency of administration : Once daily[3]

ADVERSE EFFECTS:

Headache, lightheadedness, lethargy, sleeplessness, and nervous system symptoms, as well as a maculopapular rash.

UV –VISIBLE SPECTROSCOPY:

A simple, delicate, and specific spectroscopic technique for identifying efavirenz in biological sample and pharmaceutical formulations [4] UV-spectrophotometry is a widely used analytical technique for obtaining both qualitative and quantitative statistics from unresolved band spectra.[5]This methodology is widely used in a variety of practical and theoretical applications due to its low cost and ease of use. Only chromophores that absorb in the UV-visible spectrum can be utilized as samples. Absorption spectroscopy enhances fluorescence spectroscopy. [5]

NMR:

It is a type of spectroscopy that is based on the ability of atom nuclei to acquire electromagnetic energy between 4 and 900 MHz. According to NMR theory, the majority of nuclei have spin, and all nuclei have electrical charges. When a magnetic field is present, energy is transmitted from a lesser energy state to a higher one. Whenever the spin comes back to its base state, energy is released at the same frequency as the wavelength of the energy transfer.To generate an NMR spectrum for the target nucleus, the signal value that corresponds to this transfer is subjected to various measurements and processing stages. CPMAS NMR is used to investigate the formations of micronized EFZ and heptane-recrystallized polymorphs. [6].

HPLC:

HPLC is one of the most common methods used in research and industry to solve problems in the analysis of pharmaceuticals. For the analysis of EVZ, a fast, precise, stability-indicating HPLC method with an isocratic mode of evaluation was made and tested. [1] Depending on the chemical make-up of the analyte, particles move slowly or steadily through the solid phase. HPLC can be used in different ways in the lab and clinical science fields. It is a common step in the process of making pharmaceuticals because it is a reliable way to get pure products. [7] Even though HPLC can sell good products. A solid adsorbent material section is moved by pumps through a pressurised fluid medium that holds a testing mixture. Depending on how the molecules of the analyte are made, they move through the sorbent slowly or gradually. HPLC can be used in many different ways in the lab and in clinical science. It is often done when making medicines because it is an effective way to get pure products and make sure they stay that way. Even though HPLC can make the highest-quality products.[8]

HPTLC :

The HPTLC method, an automated, high-tech thin-layer chromatography technology, can effectively replace both high-performance liquid chromatography (HPLC) and gas chromatography. It improved detection thresholds and separation efficiency (GC). technique is also known as "flat-bed chromatography." HPTLC employs similar core concepts to TLC, such as the sorbent theory of isolation. A concentration gradient enables the mobile phase or diluents to flow. Sample solution affinities govern how particles move toward the adsorbate. Greater interactions slow the module's movement in the direction of the adsorbate. Low-affinity constituents disperse rapidly from the adsorbate. A chromatographic column is used to distinguish the components.[9]

MASS SPECTROMETRY:

Mass spectrometry is an analytical method for determining a compound's molecular mass that has

aided in the indirect identification of isotopes. Mass spectrometry is a practical method for figuring out the chemical composition of a sample or molecule. More recently, it has been used to classify biological products from many species, especially proteins and protein complexes. Measurements of molecular weight are frequently used to classify unidentified chemicals, weigh existing compounds, and determine the structure and chemical properties of molecules.(Theron et al., 2010)

SFC :

A supercritical fluid, such as carbon dioxide, serves as the mobile phase. It can be used to evaluate and purify lesser to intermediate molecular masses, low boiling point substances, as well as separate chiral molecules. Since carbon dioxide is widely used as the solvent system, the whole separation flow channel should be pressurised. The concepts are comparable to those employed in high-pressure liquid chromatography (HPLC). Convergence chromatography, also known as SFC, is where liquid and gas characteristics converge with one another. [10]

2. Discussion

Efavirenz a well known anti-retroviral drug used in treatment of AIDS. RP-HPLC and UV-Visible spectrophotometric techniques are most widely used methods for qualtification efavirenz. Reports have shown that accountable number of LC-MS and NMR is used in quantification of efavirenz in different drug formulation. Due to its high cost efficient and tedious time consuming process it is less suitable for analysis. HPLC and uv holds high preference for the analysis of drug both in pharmaceutical formulation and biological fluids. Acetronitrile and phosphate buffer are widely used M.P in HPLC. C18 Column is used to produce more reproducible results in hplc analysis. Methanol and water is used as solvent in UV spectrophotometric method. High resolution peaks of efavirenz is obtained in 247nm.Bulk formulation of efavirenz tablets analysis can be performed using RP-HPLC method.The sensitivity and selectivity are greatly increased by employing LC-MS/MS Techniques.A simple and accurate method of analysis efavirenz capsules can be performed using HPTLC method.Aluminium precoated plates with silica gel is more commonly used stationary phase.Organic solvents such as methanol,chloroform and toluene are most commonly used M.P in HPTLC estimation. As from the resuts obtained RP-HPLC technique is used in qualtifiaction of different types of anti –retroviral drug combinations simentaneously.

3. Conclusion

The review of present work highlights the past and present scenario of spectrophotometric and advance chromatography techniques of efavirenz in pharmaceutical formulation and biological specimens as a single moiety or with combinations of other drugs . It also seen that high performance of liquid chromatography methods are predominant for the analysis of EFV as single entity and in comination.

Although there are several other well defined methods are used to validate and quantifiy efavirenz in quality control process HPLC produce more accurate results.

TABLE :1 : Hyphenated Techniques

SNO	DRUG	INSTRUMENT	MOBILE PHASE	STATIONARY PHASE	LINEARITY	RETENTION TIME (min)	ref
1	EFV,ZDV,SVD,LAD,NEV	HPLC-MS/MS	0.30% formic acid in methanol (20:80)	Eclipse XDB-C18 (150 mm × 4.6 mm, 5 µm)	25-3,200 µg/mL-1 50-6400 µg/mL-1 50-3200 µg/mL-1	N/A	[1]
2	EFV	LC-MS-MS	ACN: ammonim acetate (90:10)	reversed-phase C18 column	N/A	1.93, 1.70, 1.52, and 1.82	[2]
3	EFV	LC-TOF-MS	ACN ethanol	N/A	N/A	N/A	[3]
4	EFV	LC MS	methanol	SPE) on C18 cartridges.	(r 2, 0.989-0.992) over the concn	N/A	[4]

Table:2: HPLC method

S. NO	DRUG	IN-STRUMENT	MOBILE PHASE	SATIONARY PHASE	DE-TECTION	REN-TION TIME	LINE-ARITY	FLOW RATE	REF
1	TSF, EMT, EFV	RP HPLC	acetonitrile Tri-etheyl amine (60:40)	C18 column with(250 × 4.6 mm, 5 µm)	265nm	11.849	60-180 mcg/mL, 40-120 mcg/mL, and 120-360 mcg/mL	1.0 mL/ min	[5]
2	EFV	RP HPLC	Methanol acetonitrile	sunfire c18 column	249nm	N/A	10-400 µg/mL	N/A	[6]
3	EFV	HPLC	acetonitrile,wa-ter,orthophos-phoric acid (70:30:0.1).	(C18, 250 mm × 3.9 mm, 10 µm)	252 nm	N/A	N/A	N/A	[7]
4	EFV	RP HPLC	Acetonitrile,0.03 M KH2PO4, water.	BDS C18, 250 × 4.6 mm,	260 nm.	10.549 min	N/A	0.8 mL/ min	[8]
5	EFV	HPLC	Acetonitrile,am-monium dihydro-gen phosphate	deactivated octade-cylsilyl silica gel	N/A	N/A	40 µg/mL to 160 µg/Ml	N/A	[9]
6	EFV	RP HPLC	Acetonitrile:Phos-phate Buffer	Phenomenex-Luna RP-18(250 × 4.6 mm, 5 µm)	247 nm.	4.611 min.	N/A	1.0 mL min-1	[6]
7	EFV	RP HPLC	acetonitrile: water	Luna 5 µ C18 col-umn,	270 nm.	N/A	N/A	1 mL/ min,	[10]
8	EFV	RP HPLC	buffer and acetoni-trile	C18 COLUMN	252nm	N/A	N/A	N/A	[11]
9	EFV, TSF, LAD, TEN	RP HPLC	N/A	N/A	N/A	2.3, 3.6 13.6 min	N/A	N/A	[12]

10	EFV, LAD	RP HPLC	triethylamine acetonitrile	(Luna 5µ C18(2) 100A 250 × 4.60 mm i.d.) column	245nm	2.271 ± 0.177, 7.267 ± 0.513 min	N/A	1.0 mL/ min.	[13]
11	EFV	HPLC-UV	formic acid ,acetonitrile	C18 column.	247 nm.	5.57 min	r (r=0.9997).	0.3 mL/ min	[14]
12	EFV	HPLC	2-propanol, ethanol, trifluoro acetic acid	cellulose derivatized chiral column.	N/A	7.5 and 9.2 min,	(R2) of 0.9999	N/A	[15]
13	.EFV, NEV	HPLC-MS	N/A	N/A	N/A	N/A	(r2 = 0.867 and 0.972, resp.),	N/A	[16]
14	EFV	RP HPLC	methanol water	N/A	N/A	2.58 min.	0.9999.	1 mL/ min	[17]
15	EFV, LAD, TEN	HPLC	ammonium acetate , acetonitrile ,methanol	C18	265 nm.	N/A	N/A	1.5 mL/ min	[18]
16	EFV	HPLC	Acetonitrile , acetate buffer	N/A	292nm	4.007 min	N/A	1.5ml/min	[19]
17	LAD, TEN, EFV	RP HPLC	methanol:phosphate buffer	c18	N/A	0.432, 0.657, 2.281 min,	N/A	0.3 mL/ min.	[20]

18	EFV, LAD, TSF	RP HPLC	water acetonitrile	Thermo Hypersil ODS C-18 column	261 nm.	1.55 , 2.60 ,12.77 min	N/A	1.0 mL/ min.	[21]
19	LFT	RP HPLC	0.01% TFA in 0.1 M ammonium acetate (solvent A) and 0.1% TFA in acetonitril	C16, column	275 nm (NVP), 255 nm (EFV), and 300 nm (LUM)	N/A	N/A	N/A	[22]
20	EFV	RP-LC	ammonium acetate buffer :acetonitrile	C18 column.	N/A	N/A	N/A	N/A	[23]
21	LAD, DAN, EFV	RP-HPLC	water: tetrahydrofuran: acetonitrile	Oyster BDS premium C18	245 nm.	2.01, 3.01, and 8.61 min	N/A	1.15 Ml/ min	[24]
22	LAD, TSF, EFV	RP-HPLC	acetonitrile: MeOH: water a	Phenomenex Luna C18 column	258 nm;	3.27, 4.58, 10.90 min,	N/A	0.5 mL/ min.	[25]
23	EMT, TSF, EFV.	RP-HPLC	N/A	N/A	262 nm.	2.6, 5.4, 7.9 min,	N/A	N/A	[26]

24	EFV	RP-HPLC	methanol , phosphate buffer	C 18	245 nm.	2.48 ± 0.02 min	N/A	1.0ml/min,	[27]
25	TSF, EFV.	RP-HPLC	Acetonitrile and phosphate buffer	Agilent Zorbax Eclipse XDB C18	255nm.	2.44min for TDF and 5.52 min for Efavirenz	3-18 µg/Ml, 3-18 µg/mL	0.6 mL/min	[13]
26	EFV, LAD, ZDV	RP-HPLC	Acetonitrile, potassium dihydrogen orthophosphate buffer	C18 column,	275nm	N/A	N/A	1.0 mL/min	[28]
27	EMT, TEN, EFV.	HPLC	methanol and buffer	Zorbax SB CN,	260 nm.	N/A	r (r2 > 0.999),	1.5 mL/min	[29]
28	EFV	HPLC-ECD	phosphate buffer acetonitrile	Nova-Pak C18 cartridge column	N/A	3.70 and 8.89 minutes.	(R2 = 0.9979)	N/A	[30]
29	EFV	RP-HPLC	Acetonitrile, 10 mM phosphate buffer	Waters Spherisorb® 5 µm ODS	246 nm	N/A	N/A	N/A	[31]

30	EFV	HPLC-UV	methanol, 10 mM ammonium acetate buffer	Nucleosil C18	247 nm.	N/A	N/A	1.0 mL/min	[32]
31.	EFV	UHPLC	methanol, acetonitrile and 0.1 M formic acid	CSH C18 column	245nm	N/A	0.078 to 10 µg/mL	0.3 mL/min	[51]

Table:3. UV Spectroscopy Methods

S.NO	DRUG	INSTRUMENT	RANGE	WAVELENGTH	LINEARITY	REF
1.	EFV	UV VISIBLE	N/A	247nm	5-15 µg/mL	[33]
2.	EFV	UV VISIBLE	5-50µg/mL	245nm	N/A	[34]
3.	EFV	UV -VISIBLE	range of 5-40 µg/mL	N/A	N/A	[36]
4.	EFV	UV-VISIBLE	1-4 µg/mL	320 nm	linear (r2 = 0.09657)	[37]
5.	EFV	UV-VISIBLE	0-60 µg/mL and 4-24 µg/m	494nm.	N/A	[38]
6.	EFV	UV-VISIBLE	N/A	523nm.	10-60 µg/mL and 4-24 µg/mL	[39]

7.	EFV,LAD	UV -VISI-BLE	10-100 µg/mL and 10-70 µg/mL resp.	271 and 247 nm	N/A	[40]
8.	EFV,LAD ,ZVD	UV-VISI-BLE	4-12 µg/mL, 1-3 µg/mL and 2-6 µg/mL	305 nm, 250 nm and 254 nm	N/A	[41]
9.	EFV	UV-VISI-BLE	N/A	267nm.	2 to 12 µg/mL	[42]

ABBREVATION:

ZVD- ZIDOVUDINE

EFV-EFAVIRENZ

LAD-LAMIVUDINE

NEV- NEVIRAPINE

SVD- STAVUDINE

TSF-TENOFOVIR DISPROXIL FUMARATE

EMT- EMTRICITABINE

TEN-TENOFOVIR

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