International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.13, No.01, pp 06-19, 2020

A Comprehensive Review on The Estimation of Emtricitabine Individually and in Combination With other Drugs

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Abstract : Emtricitabine is a nucleoside reverse transcriptase inhibitor for the prevention of HIV infection. This drug's, clinical and pharmaceutical analysis requires effective analytical procedures and stability studies for quality control and pharmacodynamics and pharmacokinetic studies. A comprehensive literature survey published in various journals related to analytical and pharmaceutical chemistry was conducted and instrumental analytical methods were developed and used in bulk drugs and pharmaceutical dosage form as single and combined with other drugs. This review will critically examine UV spectroscopy analytical methods (simultaneous equation method, derivative spectrophotometric method, absorption ratio and Q-based method), High-performance liquid chromatography (HPLC), High-performance thin-layer chromatography (HPTLC), Liquid chromatography coupled with tandem mass spectrometry (LC-MS).
Keywords : Emtricitabine, HIV infection, Analytical method, HPLC.

Introduction[1-3]

Emtricitabine is an NRTI (Nucleoside reverse transcriptase inhibitor) for the prevention of HIV infection in children and adult. It is a synthetic fluro derivative of thiocytidine with potent antiviral activity. It is freely soluble in various aqueous solvents, acetonitrile, methanol and slightly soluble in isopropyl acetate. Appearance solid, white to off white powder, chemical name is 2', 3'-dideoxy-5-fluoro-3'-thiacytidine. Anti-HIV drugs such as emtricitabine slow down or prevent damage to the immune system, and reduce the risk of developing AIDS-related illnesses. Emtricitabine is additionally active against Hepatitis B virus.

Mechanism of Action:

When HIV infects a cell, the reverse transcriptase enzyme copies the single-stranded viral RNA genome to two-stranded viral DNA. This viral DNA is then integrated into the deoxyribonucleic acid (DNA) chromosomal CD4 and can be reproduced in the body. The synthesis of DNA is completed by four natural nucleosides: adenosine, cytidine, guanosine and thymidine. A nucleoside reverse transcriptase inhibitor (NRTI) replaces a defective version of one of the nucleosides that cause the proviral DNA chain to be terminated prematurely.

DOI= http://dx.doi.org/10.20902/IJPTR.2019.130102

Most common adverse reactions in Paediatric patients are diarrhoea, nausea, fatigue, dizziness, depression, sleeplessness, abnormal dreams, rash, abdominal pain, asthenia, increased cough and rhinitis. Hyperpigmentation of the skin in paediatric patients has been common.

Marketed formulation of Emtricitabine

Emtricitabine formulation is approved by the USFDA and is commercially available under the following brands either individually and in different combinations.

1.Emtriva, Coviracil

Emtricitabine and it's Combination

1. Tenof EM (200+300) tenofovir+emtricitabine
2. Descovy (emtricitabine&tenofoviralafenamide)
3. Genvoya (tenofoviralafenamide+emtricitabine+elvitegravir+cobicistat)
4. Stribild (tenofovirdisoproxil+emtricitabine+elvitegravir+cobicistat)
5. Odefsey (tenofoviralafenamide+emtricitabine+rilpivirine)
6. Eviplera (tenofovirdisoproxil+emtricitabine+rilpivirine)
7. Trustiva (tenofovir+efavirenz+emtricitabine)

Reported Analytical Methods:

Spectrophotometric Methods

Many analytical methods involving spectroscopic analysis of the drug individually and as multicomponent samples have been reported. These methods include a simultaneous equation method, derivative spectrophotometric method, absorption ratio and a method based on Q analysis.

Chromatographic Method

Liquid chromatographic analysis for the determination of Emtricitabine individually and in combination has been reported covering different phases of analytical research viz; Profiling of impurities, Stability indicating analytical methods, Bioanalytical method development in different biological fluids to determine the concentration of Emtricitabine in human serum and to determine simultaneously in synthetic mixture or combination dosage form such as Elvitegravir, Cobicistat, Rilpivirine.

Stability Indicating Method:

Stability indicating method is used to check drug stability under different conditions. Here, Emtricitabine is studied by RP- HPLC and UPLC for stability studies.

Table no: 1 Spectrophotometric method used to detect Emtricitabine

Title	Method	Solvent	Concentration Range	Ref
Simultaneous estimation of emtricitabine (EMT), tenofovirdisoproxilfumarate (TDF), and rilpivirineHCl (RPV) in tablet dosage form by Vierordt's method	UV- Spectroscopic	Methanol	Concentration ranges 4–12 μg/ml for EMT, 6–18 μg/ml for TDF, and 0.5–1.5 μg/ml for RPV.	4

Two methods for simultaneous determination of Emtricitabine and TenofovirDisoproxilFumarate by spectroscopy have been developed. First method is Simultaneous equation method and second method is Absorbance ratio Method.	UV- Spectroscopic	Methanol	Concentration range of 6-48 µg/mL and 4-32 µg/mL was	5
Development and validation of uvspectrophotometeric method for simultaneous estimation of emitricitabine and tenofovirdisproxilfumarate in bulk and tablet formulation by simultaneous equation method.	UV- Spectroscopic	Distilled water	5-30µg/ml	6
simple spectrophotometric method for the determination of tenofovir and emtricitabine in tablet dosage form	UV- Spectroscopic	Methanol and acetonitrile	5 µg/ml to 30 µg/ml for tenofovir ,for Emtricitabine in the range of 2 µg/ml to 20 µg/ml	7
Spectrophotometric simultaneous determination of Tenofovirdisoproxilfumarate and Emtricitabine in combined tablet dosage form by ratio derivative, first order derivative and absorbance corrected methods and its application to dissolution study.	UV- Spectroscopic	Methanol and 0.1NHCl	Concentration range of 3-21 µg/ml for TE and 2-14 µg/ml for EM for first two methods, concentration range for third method was 6-30 µg/ml of TE and 4-20 µg/ml of EM.	8
New simple spectrophotometric method for determination Of the antiviral mixture of emtricitabine and tenofovirDisoproxilfumarate	_	Distilled water	2-40µg/ml	9
Spectrophotometric methods for the determination of emtricitabine in bulk and in its pharmaceutical formulations using aromatic aldehydes as	UV- Spectroscopic	Distilled water and methanol	1-40µg/mlfor method A and 2.5-35.0µg/ml for method B	10

chromogenic reagents				
Development and validation of emtricitabine and tenofovirdisoproxilfumerate in pure and in fixed dose combination.	UV- Spectroscopic	Distilled water	Concentration range of 4 – 24 µg/ ml.	11

Table no. 2 Chromatographic methods used to detect Emtricitabine

Title	**Method**	**Mobile Phase&Stationary Phase**	**Results/Parameters**	**Ref**
Development and Validation for the Simultaneous Estimation of TenofovirAlafenamide and Emtricitabine in Bulk and Tablet Dosage Form	RP-HPLC	M.P: Methanol:distill water(60:40v/v) S.P: C18(4.6X250 mm, 5µ) column	Retention time-3.10 min and 7.38 min,Linearity range- 5-30µg/ml, 40-240µg/ml, recovery studies-<2	12
Development and validation of analytical methodfor quantitation of Emtricitabine, Tenofovir,Efavirenz	HPLC	M.P: Methanol (A) and buffer at pH 4.5(B) S.P: Zorbax SB CN, (250 · 4.6 mm, 5 lm) column	Linearity range-20-140µg/ml, % assay-99-100.5	13
Simulataneous Estimation of Emtricitabine and TenofovirDisoproxilFumarate in a Tablet Dosage Form	RP-HPLC	M.P: Acetonitrile: Potassium dihydrogen phosphate buffer (pH 3.0 ± 0.05 adjusted with orthophosphoric acid): triethylamine in the ratio of 70:30:0.5(v/) S.P: Luna C18 (25cm x 4.60 mm, particle size 5µm)	Retention time-1.78 and 2.27 min, Linearity range- 5-50 µg/mL for EMT ,5-50 µg /ml for TDF, LOD and LOQ values-0.015 and 0.045 µg/ml for EMT and 0.039 and 0.117 µg/ml for TDF	14
simultaneous estimation of Emtricitabine, TenofovirDisoproxilFumarate and Rilpivirine in bulk and pharmaceutical tablet dosage forms	RP-HPLC	M.P: mixture of 0.01M Potassium dihydrogen phosphate (pH adjusted to 4 with orthophosphoric acid) and Acetonitrile (30:70, v/v) S.P: Inertsil ODS 3V C18 column (250mm×4.6 mm, 5mm particle size)	linearity range-50-300µg/ml for Emt, 75-450µg/ml for Tdf and 6.25-37.5µg/ml for Rilpivirine, %recovery-99.68% to 100.05%	15
Simultaneous estimation of emtricitabine, tenofovir disoproxilfumarate, and	RP-HPLC	M.P: Acetonitrile and Phosphate buffer PH 3(60:40) S.P: Thermo Hypersil ODS C-18	Linearity range-10-50µg/ml for tenofovir,	16

rilpivirine in bulk form		column (150×4.6mm, 5µ)	emtricitabine and 4-12µg/ml for rilpivirine LOD-0.0085µg/ml, 0.23µg/ml and 0.26 µg / ml , LOQ-0.025 µg / ml, 0.7041µg/ml and 0.8137 µg / ml for tenofovir, emtricitabine and rilpivirine %Recovery-99.25 % - 99.84%.	
Development and validation Of analyticalMethod for simultaneousEstimation of tenofovir andEmtricitabine inPharmaceutical dosage forms	HPLC	M.P: Buffer, Methanol and Acetonitrile (40: 50: 10) S.P: Columnof Hi Q C18 W (150 mm: 4.6 mm, 5 µ)	_	17
Development and validation of method for simultaneous estimation of emtricitabine, rilpivirine, tenofovirdisoproxilfumarate and its pharmaceutical dosage forms	RP-HPLC	M.P: 0.02M sodium dihydrogenorthophosphaste as mobile phase A and mixture of Methanol and water in ratio of 85:15as mobile phase B at a flow rate of 1.5 ml/min S.P: Inertsil ODS 3V column	Linearity range-3-21, 1-10 and 0.5-3 µg/ml, LOD- 2.830, 0.079, 0.070 , LOQ- 8.576, 0.239, 0.21, Precision (%RSD) 0.294 0.479 0.878	18
Simultaneous estimation of emtricitabine andtenofovirdisproxilfumerate	HPLC	M.P: Methanol: Phosphate Buffer (65:35 v/v) S.P: C18 column [250mm, 4.6m, 5µm]	Retention time-2.461 and 6.231 min,Linearity range-10 to 50µg/ml,recovery(%)-100.23,99.52, LOD & LOQ-0.00752, 0.00218ug/ml, 0.00851,0.0315ug/ml	19
Simultaneous Method for Determination ofEmtricitabine, TenofovirDisoproxilFumarate, Elvitegravir and Cobicistat in Tablets	HPLC	M.P: gradient mixture of 0.1% Trifluoroacetic acid and Acetonitrile S.P: Atlantis C18 column (100×4.6 mm, 5 µm)	_	20
Development and Application of Liquid	LC	M.P: A -KH2PO4 (0.02M) in 1000 ml of water and by	Linearity range-emt 60-180	21

Chromatographic Method for Simultaneous Determination of Elvitegravir, TenofovirDisoproxilFumarate, Emtricitabine, and Cobicistat in Fixed Dosage Form		adjusting the pH to 2.5 with diluteorthophosphoric acid, B was Acetonitrile. S.P: Inertsil ODS 3V C18 column (250 m×4.6 mm, 5 µm particle size, 100Å pore size)	mcg/ml, Tdf-40-120 mcg/ml, Efv-120-360 mcg/ml,LOD-0.3µg/ml, 0.4µg/ml and 0.12µg/ml, LOQ- 0.9µg/ml, 0.12µg/ml and 0.36µg/ml	
A novel stability indicating method development and validation for the determination of tenofovirdisoproxilfumarate and emtricitabine in bulk and pharmaceutical formulations	RP-HPLC	M.P: Methanol and Phosphate buffer (30:70 v/v, pH 4) S.P: C18 column (Agilent TC-C18 (2) column. 5µm, 4.6*250 mm)		22
Selective Determination of Antiretroviral Agents Tenofovir, Emtricitabine, and Lamivudine in Human Plasma by a LC–MS–MS Method for a Bioequivalence Study in Healthy Indian Subjects	LC-MS/MS	M.P: 0.5% Formic acid in Water and Acetonitrile (55:45, v/v) S.P: ACE 5 CN column (150 mm × 4.6 mm, 5 µm) under isocratic conditions		23
Analytical method development and validation for the simultaneous estimation of emtricitabine and tenofovirin bulk and tablet dosage forms	HPLC	M.P: Methanol:Water (70:30 v/v) pH 3 S.P: Symmetry Premsil C_{18} (250 mm×4.6 mm, 5 µm)	Linearity range-10-10,000 ng/mL,% recovery-98 to 105% for emt and 97 to 103% for tdf, Precision- 1.7 to 3.7% and 3.7 to 5.2%	24
Method Development and Validation for Simultaneous Estimation of Emtricitabine and TenofovirDisoproxilFumarate in Pure and Tablet Dosage Form	RP-HPLC	M.P: Methanol: Phosphate buffer pH-3 (70:30 v/v) S.P: Phenomenax Luna C18 (250mm x 4.6mm i.d; particle size 5µm) column		25
Determination of TenofovirFumarate and Emtricitabine in Bulk Powder and in Tablets	RP-HPLC DAD	M.P: Disodium hydrogen phosphate–Acetonitrile (50:50, *v*/*v*). contains 0.1% triethylamine (TEA) and was adjusted to pH 6.0. S.P: Zorbax SB-C8 column, 5 µm, 4.6 × 250 mm	2.5–650 ng mL^{-1} for tenofovir and 10–4000 ng mL^{-1} for emt	26

Method development and validation by rp-hplc for simultaneous estimation of emtricitabine and tenofovirdisoproxilfumarate	RP-HPLC	M.P: Acetonitrile: Phosphate buffer (60:40 v/v) S.P: Isocratically on C8 Phenomenex Luna (4.6X250 mm) column	Linearity rangr-40-240 µg/ml, 60-360 µg/ml ,%recovery-99.84%, 99.75%	27
Development and validation of an LC method for the determination of emtricitabine and related compounds in the drug substance	LC	M.P: ACN, Phosphate buffer (pH 4.4), and Water S.P: RP C18 column (25 cm64.6 mm i.d.), 5 µm		28
The simultaneous assay of tenofovir and emtricitabine in plasma using LC/MS/MS and isotopicallylabeled internal standards	LC/MS/MS	M.P: 3% acetonitrile/1% acetic acid, aq.) stream flowing at 200_L/min. S.P: Synergi Polar-RP, 2.0mm×150mm, reversed-phase analytical column	Linearity range-10 ng/mL to 1500 ng/mL.Accuracy and precision within ± 20% at the LLOQ and ± 15%	29
Determination of Emtricitabine in Human Plasma using HPLC with FluorometricDetection	HPLC	M.P: Phosphate buffer and Methanol S.P: Atlantis dC18 analytical column is used along with a 15 min linear gradient elution	Linearity range-0.01 to 5.0 mg/L,%recovery-100% to 107%	30
Liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for simultaneous determination of tenofovir and emtricitabine in human plasma and its application to a bioequivalence study	LC/MS/MS	M.P: Methanol ,Acetonitrile and ammonium acetate (pH 3.0, 40mM) (20:80, v/v) S.P: Chromolith Speed Rod RP18 column (50mm×4.6mm)	10–600 ng/ml for TEN and 25-2500 ng/ml for EMT, Precision within 12.0% for TEN and 15.6% for EMT	31
Development and validation of a LC–MS/MS method for the quantification of tenofovir and emtricitabine in seminal plasma	LC-MS/MS	M.P: Deionized water with 0.05% formic acid(A) and methanol with 0.05% formic acid (B). At time zero the flow consisted of 95% of mobile phase A and 5% mobile phase B S.P: Reversed-phase Atlantis T3 C18column(2.1 × 100 mm i.d., 3 µm particle size)	Linearity range-3.13–1000 ng/mL for tenofovir and 6.25–2000 ng/mL for emtricitabine., Accuracy-0.48% and 8.43% for tenofovir, and between 0.64% and 13.87% for emtricitabine.	32

Table.no:3 Stability indicating profile for Emtricitabine

Title	Method	Mobile Phase, Stationary Phase	Results	Ref
Stability-Indicating Method for the Simultaneous Determination of Tenofovir, Emtricitabine, and Efavirenz	RP-HPLC	M.P: Phosphate buffer (pH 3.5): Acetonitrile S.P: Reverse-phase C18 column	Linearity range-20–300 µg mL^{-1}, 24.5–367.5 µg mL^{-1} and 60–900 µg mL^{-1} for FTC, TDF, and EFV	33
Development and Validation of Stability Indicating RP-HPLC Method for the Simultaneous Estimation of EmtricitabineTenofovirAlafenamide Bulk and their Combined Dosage Form	RP-HPLC	M.P:Phosphate buffer: Acetonitrile (80:20) as mobile phase at a flow rate of 1 mL/min Column: Inertsil ODS (4.6 × 250 mm, 5 µm)	2–12 µg/mL for EMT, 3 –18 µg/mL for TNDF, 1.5– 9 µg/mL for ELV and COB, %Recovery-99.93–100.08 ± 0.5%	34
Stability indicating method for simultaneous estimation of emtricitabine, tenofovirdisoproxylfumarate , cobicistat and elvitegravir in pharmaceutical dosage form	HPLC	M.P: combination of 0.1%TFA and Acetonitrile in gradient mode employing at a flow rate of 1.2 ml/min, S.P: Inertsil ODS 3V(4.0x250mm, 5µm,)	Retention time-3.43 min., 4.75 min., 5.27, and 7.56 min, Concentration Range (µg/ml)-100-300, 150-450, 75-225, 75-225 µg/ml,%assay-99.8%	35
Stability Indicating method for the Simultaneous estimation of Rilpivirin, Emtricitabine and Tenofovir in Bulk and Combined Pharmaceutical Dosage Form	HPTLC	M.P: Methanol:Toluene:Ethylacetate:Ammonia (1.5:5.5:1.5:0.1 v/v/v/v), S.P: Silica gel 60 F254		36
Simultaneous Determination of Emtricitabine, Elvetegravir, Cobicistat and Tenofovir in their Tablet Dosage Forms	HPLC-DAD	M.P; 0.05M Phosphate buffer pH 3.0 (adjusted with dilute phosphoric acid) and Acetonitrile in the ratio 95:5 from 0 min to 4 minutes, further increased the Acetonitrile ratio from 5 to 50 from 4 min to 10 minutes S.P: reverse phase C_{18} column (250x4.6mm, 5 µ)	Retention time-1.5, 5.4, 6.6 and 7.5 min,%Assay-98-100%,Linearity range(mcg/ml)-10 to 60 7.5 to 45 7.5 to 45 15 to 90	37
Stability Indicating Ultra Performance Liquid Chromatographic Method for the Quantitation of Emtricitabine	UPLC Photodiode array detector	M.P: 0.015 M potassium dihydrogen phosphate buffer pH 2.2 and acetonitrile in ratio 75:25 v/v. S.P: Waters ACQUITY UPLC BEH C18 (50 x 2.1)	Retention time-1.2 minutes, LOD (µg/mL)a 0.5038 LOQ (µg/mL)a 1.5113, % Recovery - 99.8 to 100.94	38

		mm, 1.7µm column in isocratic mode with flow rate 0.25 mL/min.		
A validated stability indicating Method for the determination of Emtricitabine in bulk and capsule	Rp-HPLC	M.P: Composition of buffer : acetonitrile [85:15 %(v/v)] S.P: Phenomenex - luna rp18(2),250x4.6mm, 5 µm column,	Linearity range-100-2800 ng /ml, % Recovery-98.23 to 100.61%, LOD-21.04 ng/ml, LOQ- 63.77 ng/ml	39
Stability Indicating Method for the Simultaneous Estimation of EmtricitabineTenofovirAlafenamide Bulk and their Combined Dosage Form	RP-HPLC	M.P: phosphate buffer: Acetonitrile (80:20) as mobile phase at a flow rate of 1 mL/min S.P: Inertsil ODS (4.6 × 250 mm, 5 µm)	Retention time-3.314 and 5.068, Linearity range-20-100 µg/ml for Emt,, 0.25-12.5 µg/ml for tdf, %recovery-98.86% , 99.96%	40
Development and validation of stability indicating simultaneous uv-Spectrophotometric method for determination of emtricitabine, tenofovir Disoproxilfumarate, cobicistat, and elvitegravir in pure and pharmaceutical Dosage form	uv-Spectrophotometric method (Vierordt's method)	M.P: Methanol, distilled water	Linearity range-EMT (4–24 µg/ml), TDF (10–50 µg/ml), COB (10–120 µg/ml), and ELV (2–10 µg/ml)	41

NOTE:M.P- Mobile phase, S.P-Stationary phase

Discussion:

A large range of analytical methodshas been reported for the simultaneous estimation of Emtricitabine starting from a simple simultaneous equation spectrophotometric methods to most refined HPLC method. But, Current analytical strategies or research works simply focus on altering solvents and Absorbance values in spectroscopy and retention times in the case of HPLC analysis. The HPLC method was found to be most used for Emtricitabine determination. Different Spectroscopic and Chromatographic conditions are given in the table below. But these methods lack several parameters in analysis.

Conclusion:

A wide range of techniques are available in biological samples and pharmaceutical formulations for the analysis of the drug. In the previous studies, it was revealed that in plasma, serum and urine, the HPLC methods was extensively used. HPLC with UV detection is applicable for the analysis of the drug in pharmaceuticals because it provides accurate results and low cost compared to more advanced detection techniques.

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