Heterocyclic Letters
Vol. 14| No.3|583-589|May-July|2024
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

DEVELOPMENT OF NOVEL BIOANALYTICAL METHOD FOR QUANTIFICATION OF FOSTEMSAVIR BY LC/MS IN HUMAN PLASMA

Dommeti Bharathi,[a] S. V. S. Sumaltha,[a] Ramana Tamminana,[b] Sapavat Madhavi,[c] and Rudraraju Ramesh Raju[a*]

[a] *Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, A.P., India -522 510.*
[b] *Department of Chemistry, VIT-AP University, Inavolu, Beside AP Secretariat, AmaravatiAP-522237, India. E-mail: rtamminana17@gmail.com*
[c] *Department of Chemistry, VSR Government Degree College, Movva, Krishna District, 521135.*
[1]***Email:*** *rudrarajurameshraju786@gmail.com.*

ABSTRACT:
Generally, we have required LC (liquid chromatography) and mass spectrometry for developing method and quantification of human plasma. Combination of solvents (CH_3CN : MeOH (80:20)) was used for the extraction in these equipment's. In this regard, samples were inserted on a Xbridge C18, 50 x 4.6 mm analytical column and mixture of methanol, acetonitrile and 1mM aqueous ammonium phosphate (25:50:25) at pH 6.1 used as mobile phase having 0.7 mL/min flow rate. Positive electrospray ionization mode was used to perform mass spectrometric detection *via* monitoring of multiple reactions. In addition, 57 eV energy was used to identify the mass resolution for FSVR (2.5 amu) and for Lopinavir (LPVR). Very fortunately, precisions and accuracies were identified in acceptable limits. In this report, we mainly would like to propose a method for identifying FSVR in human plasma using LC-MS and liquid-liquid extraction technique.

KEYWORDS: Fostemsavir; Bio-analytical; LC-MS; Human Plasma; Method validation.

INTRODUCTION:
Fostemsavir (FSVR) is the organic compound and it is generally known as pyridyl-1,2,4-triazoles.[i] It acts as HIV-1 inhibitor and investigational prodrug for patients with HIV-1/AIDS infection.[ii-iii] The drug has been approved by FDA in 2020.[iv] This drug is tested against the gp120 subunit within the HIV-1 and gp160 envelope glycoprotein.[v] Reports said that initial step in the viral cycle is facilitated by the gp120 subunit within the gp160 envelope glycoprotein.[vi-ix] Thus, gp120 subunits are considered as novel target in the treatment of HIV-1 infection. To the best of our knowledge, no report is available for quantification of FSVR in plasma using LC-MS. Therefore, herein we would like to present a bio-analytical LC-MS method for quantification of FSVR in plasma.

EXPERIMENTAL

GENERAL PROCEDURE

Preparation of standard stock and calibration solutions

Stock solution (1000 µg/mL)of FSVR standard and Lopinavir (LPVR)internal standard were prepared individually in acetonitrile, by accurately weighed 10 mg of FSVR and LPVR drugs and transferred to 25 mL volumetric flask. To this, 10 mL of acetonitrile, sonicated and adjusted the volume with acetonitrile made up to 25 mL. Solutions were stored at 2-8 ºC was used to spike plasma samples. The resulting solution having concentration of 1000µg/mL of FSVR and LPVR. Different dilutions of standard solutions were obtained thereof by dilution with acetonitrile. Solutions of FSVR and LPVR were dissolved in intact human plasma to the required concentrations; Standard stock solutions and IS spiking solutions stored in refrigerator conditions 2 - 8°C until analysis. The calibration standards and quality control (QC) samples were prepared daily in drug-free human plasma. The calibration levels of NIF were 0.5, 1, 5, 10, 15, 20 and 25ng/mL, respectively. The FSVR QC samples were 0.5 ng/mL (LQC), 10 ng/mL (MQC), and 25 ng/mL (HQC).

Plasma sample preparation

Liquid-liquid extraction was selected for the preparation of plasma samples containing FSVR and IS. Sample solutions are extracted by liquid-liquid extraction method. Liquid-liquid extraction procedure using different organic solvents like diethyl ether, methanol, chloroform, ethanol, dichloromethane and acetonitrile etc. finally acetonitrile was successfully used for extraction of both FSVR and IS. For this, 250 µL of FSVR (10 ng/mL), 250 µL of IS (50 ng/mL) and 250 µL plasma were added into labeled polypropylene tubes and vortexed briefly about 5 minutes. After vortex mixing, samples were centrifuge at 4000 rpm for approximately 5min at an ambient temperature. The organic layer was transferred to an autosampler vial, and the solvent was evaporated under a nitrogen stream for 10 min. All processing was performed in a manner that prevented exposure to light. To the dry residue, 250 µL of acetonitrile was added, and the solution was mixed for 3 min. From the resulting sample, 10 µL was injected into the column. All operations were carried out under a sodium lamp. The blank plasma solution was preparing by following the same above procedure without addition of any drug.

RESULTS AND DISCUSSION:

Method development

Initially the dug extraction was carried out with liquid – liquid extraction method using different organic solvents. Among all studied solvents combination of solvents acetonitrile and methanol in 80: 20 ratio was found suitable for precipitation of the protein material in plasma. The mixture of methanol, acetonitrile and 1mM aqueous ammonium phosphate by taking the ratio of 25:50:25 (v/v) mixtures and flow rate (0.7 mL/min) was found to be effective condition in terms of the

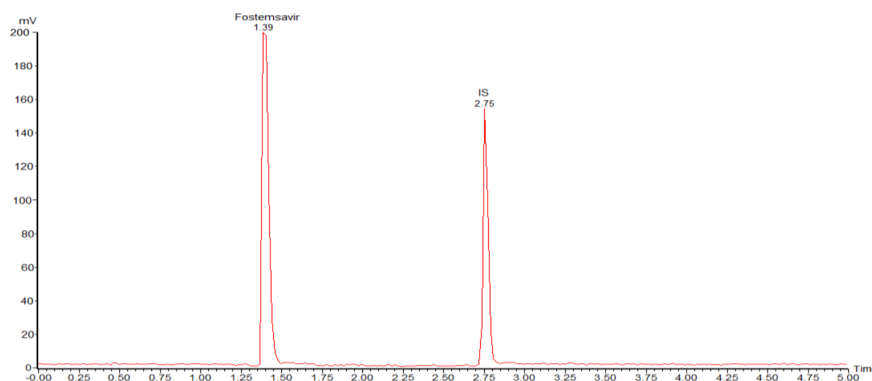

Fig.1: standard chromatogram of FSVR and LPVR IS

sensitivity and peak shape of the compound FSVR and IS (Fig 1). Chromatographic separation was optimized in various columns like Zodiac C18 (100 x 4.6, 5µm), Intertsil ODS=2VV (100 x 4.6, 5µm), during the method development. Optimized chromatographic separation was achieved at room temperature and the column C18 having measurement 50 mm x 4.6 mm. In order to continue the optimization of chromatographic conditions, we have increased the analyte response time. In this connection, mobile phase flow rate (0.7 ml/min) continued for 5 minutes to evaluate for both drugs. They found the standard retention time of FSVR and IS as 1.39 and 2.75 min respectively.

The mass spectrometer is running positive mode using electron spray ionization source and nitrogen gas (300 psi) as carrier with 5 L/min flow rate at 370 °C. It has 40-100 amu mass range to set up in multitudinous reaction monitoring (MRM) in the triple quadrupole analyzer. In addition, 57eV was used to identify the mass resolution for FSVR (2.5 amu) and 155 eV was utilized for LPVR IS. The results are shown in below figures.

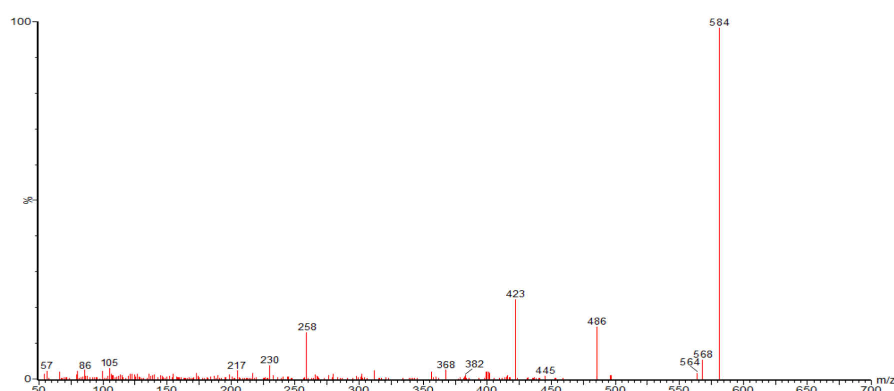

Fig.2: Mass spectra of FSVR

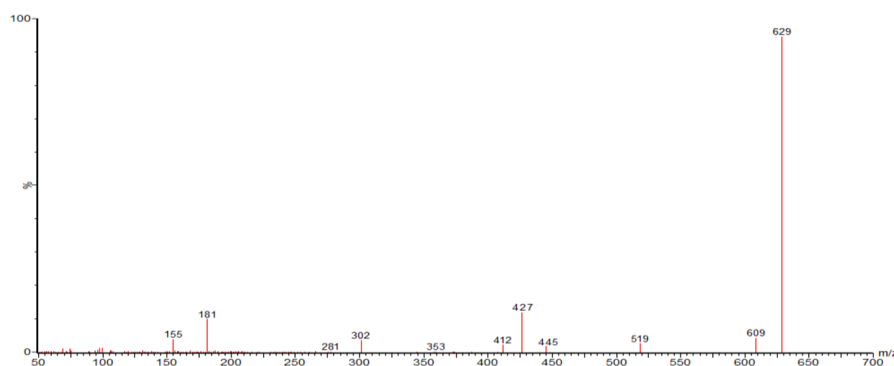

Fig.3: Mass spectra of LPVR internal standard

Method validation

The standard chromatogram achieved with optimized conditions and we found symmetrical peaks, acceptable retention. We have studied the selectivity of the samples using analyte and internal standard, and we have identified no remarkable interference from any endogenous plasma components. Initially, we got the calibration with linear curve noting the concentration range of 0.5 to 25 ng/mL which we shown in below figure 4. Here, x and y represents the ratio of the analyte and IS peak area and IS concentration, respectively. This is very sensitive method to determine the FSVR in plasma which can used for pharmacokinetic analysis.

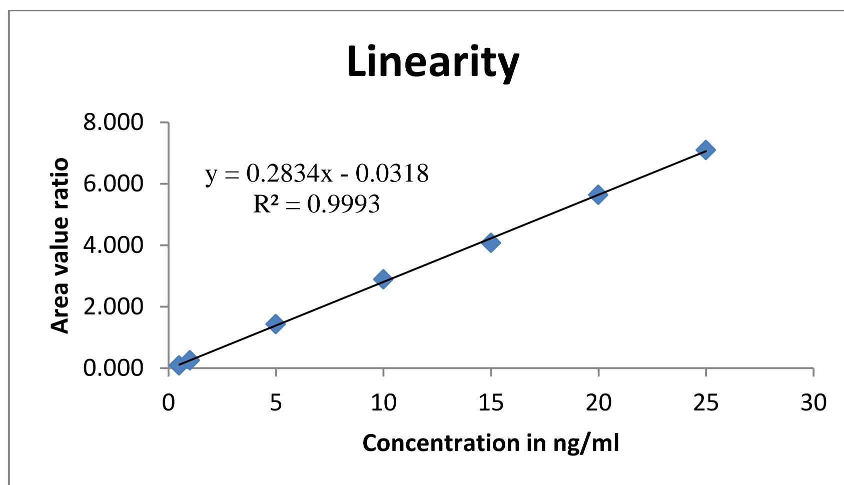

Fig. 4: Calibration curve of FSVR and LPVR IS

The QC samples at three concentration levels (HQC, MQC, LQC) were analyzed to demonstrate the other validation parameters and stability studies. Precision of the method was passed by the intra and inter-batch variations at three QC level concentrations (0.5, 10 and 25 ng/mL) in six replicates. The precision deviation values for intra-batch and inter-batch are all within 15 % of the relative standard deviation (RSD) at each quality control level. Intra-batch and intra batch precision and accuracy of the method for FSVR are summarized in Tables 1 and 2. The intra-batch % CV was less than 1.25% and the accuracy ranged from 98.1 to 101.02% at HQC, % CV was less than 2.0% and the accuracy ranged from 95.8 to 101.44% at MQC, % CV was less than 1.5% and the accuracy ranged from 98.4 to 100.9 % at LQC. Inter-batch % CV was less than 1.2% and the accuracy ranged from 98.6 to 100.6% at HQC, % CV was less than 1.2% and the accuracy ranged from 98.2 to 101.24% at MQC, % CV was less than 1.5% and the accuracy ranged from 98.88 to 100.96 % at LQCThese results indicate that on both the occasions (intra-and inter-day) results were found to be within the accepted limits and proves the adequate reliability and reproducibility of this method within the analytical range.

Results of extraction efficiency measured for FSVR was consistent. The recovery following the sample preparationusing Liquid-Liquid extraction method was calculated bycomparing the peak area ratios of FSVR in plasma samples with the peak area ratios of solvent samples and was estimated at control levels. The recovery of FSVR was determined at three different concentrations 0.5, 10 and 25ng/mL and found the recovery rangeof FSVR was found 97-88 to 98.62 % for HQC, 96.71 to 97.7 % for MQC and 90.09 to 94.31 % for LQC % respectively.

Table 1: Results of ruggedness study

S.NO	HQC at 25ng/ml		MQC at 10ng/ml		LQC at 0.5ng/ml	
	Ratio of Peak area of standard/IS	% Drug estimated	Ratio of Peak area of standard/ IS	% Drug estimated	Ratio of Peak area of standard/ IS	% Drug estimated
1.	7.057	99.418	2.876	99.770	0.085	99.384
2.	7.031	99.049	2.869	99.535	0.085	99.890
3.	7.025	98.962	2.866	99.438	0.085	99.781
4.	7.046	99.267	2.801	97.174	0.086	100.591
5.	6.969	98.178	2.853	98.986	0.085	99.931

6.	6.905	97.269	2.856	99.079	0.086	101.264
% CV	0.85	0.85	0.95	0.95	0.67	0.67

The ruggedness of the proposed methods was studied by changing the analyst, column etc and observed that there are no significant changes in the area percentage.The recovery range of ruggedness study was observed from 97.6 to 99.5 % for HQC, 97.1 to 99.77 % for MQC and 99.38 to 101.26 % for LQC respectively. Results of ruggedness study were presented in table 1. The robustness of the methods was studied by changing the experimental conditions (mobile phase pH, flow rate, and composition) and change in the chromatogram was observed.

Table 2: Results of Long-term Stability:

S.NO	HQC at 25ng/ml		MQC at 10ng/ml		LQC at 0.5ng/ml	
	Amount of Drug estimated	% Drug estimated	Amount of Drug estimated	% Drug estimated	Amount of Drug estimated	% Drug estimated
1.	24.837	99.347	9.804	98.037	0.497	99.396
2.	24.645	98.582	9.961	99.605	0.506	101.286
3.	24.310	97.239	9.791	97.908	0.497	99.454
4.	24.834	99.337	10.017	100.170	0.507	101.331
5.	25.123	100.490	9.977	99.771	0.506	101.266
6.	24.597	98.389	9.950	99.496	0.499	99.724
% CV	1.11	1.11	0.96	0.96	0.97	0.97

Stability (freeze-thaw, bench top, long term)

Stability data provides the idea about reliable stability of FSVR in tested conditions. Results of long term, short term and freeze-thaw stability at all QC concentrations are presented in table 2, 3, 4. Quantification of the FSVR in plasma subjectedto 3 freeze-thaw cycles (-30 °C)showed the stability of the analyte even after 48 hours and the % accuracy was between 97.1 to 101.4 % of the theoretical values. The bench top (short term) stability performed at ambient temperature was found stable up to 24 hours and the average % recovery found between 97.1 to 101.26 %. In addition, long term stock solution stability was performed for 3 months and % recovery of FSVR was ranged between 97.16 to 101.33 %. These results confirmed the stability of FSVR in human plasma for at least 90 days at -70°C.

Table 3: Results of Short-term Stability:

S.NO	HQC at 25ng/ml		MQC at 10ng/ml		LQC at 0.5ng/ml	
	Amount of Drug estimate d	% Drug estimated	Amount of Drug estimated	% Drug estimated	Amount of Drug estimated	% Drug estimated
1.	24.444	97.775	9.976	99.758	0.501	100.102
2.	25.212	100.846	9.954	99.537	0.488	97.554
3.	24.779	99.118	10.064	100.644	0.495	99.082
4.	24.861	99.442	9.979	99.793	0.486	97.147
5.	25.141	100.563	9.899	98.992	0.505	101.064
6.	24.857	99.428	9.960	99.605	0.505	101.009
% CV	**1.11**	**1.11**	**0.54**	**0.54**	**1.71**	**1.71**

Table 4: Results of Freez thaw Stability:

S.NO	HQC at 25ng/ml		MQC at 10ng/ml		LQC at 0.5ng/ml	
	Amount of Drug estimated	**% Drug estimated**	**Amount of Drug estimated**	**% Drug estimated**	**Amount of Drug estimated**	**% Drug estimated**
1.	24.846	99.385	10.025	100.247	0.504	100.724
2.	24.832	99.329	9.913	99.127	0.501	100.231
3.	24.525	98.101	9.816	98.160	0.507	101.364
4.	24.660	98.640	10.133	101.332	0.485	97.067
5.	24.581	98.325	10.104	101.040	0.507	101.476
6.	24.544	98.175	10.100	101.002	0.492	98.451
% CV	**0.58**	**0.58**	**1.26**	**1.26**	**1.77**	**1.77**

In summary, a novel bioanalytical method has been successfully developed using a single column and liquid extraction and one-step LC-MS assay to detect FSVR from plasma sample. There are only HPLC methods[x-xi] and spectroscopic method,[xii] have been reported for quantification of FSVR and no bioanalytical method was reported yet, hence the present study is a novel approach. The proposed method isreproducible, with excellent recovery and stability results. This validated assay can be used to evaluate plasma drug concentrations in a sensitiveandreproducible manner, with good consistency and precision.

CONCLUSION:

A novel LC-MS approach has been developed for the quantification of FSVR levels in human plasma. Liquid-liquid extraction using acetonitrile allowed for the extraction of FSVR and IS which is sufficient for quantitation. The proposed method was highly sensitive with good linearity range. The reported method and analyte were compared with deuterated internal standard. The method described here is fast (requires less than 2.5 min of analysis time), rugged, reproducible bioanalytical method. The developed method was found stable for 48 hours in bench top stability, 90 days for long terms stability and 48 hours for freeze thaw stability. The developed method is simple and efficient and can be recommended for use in pharmacokinetics studies as well as in the monitoring of the investigated FSVR in body fluids.

REFERENCES

i Lai Y. T.; Wang T.; O'Dell S.; Louder M. K.; Schön A. C.; Crystal S. F.; Chuang G. Y.; Druz A. L.; Bob M. K. and Peng D.; Lattice engineering enables definition of molecular features allowing for potent small-molecule inhibition of HIV-1 entry. *Nature Communications*. 2019, **10,** 47.

ii FDA Approves New HIV Treatment for Patients With Limited Treatment Options. U.S. Food and Drug Administration (Press release). 2020. Public Domain This article incorporates text from this source, which is in the public domain.

iii FDA Approves New HIV Treatment for Patients With Limited Treatment Options. U.S. Food and Drug Administration (Press release). 2020. This article incorporates text from this source, which is in the public domain.

iv ViiV Healthcare Announces US FDA Approval for Rukobia (fostemsavir), a First-in-Class Treatment for HIV in Adults With Few Treatment Options Available (Press release). ViiV Healthcare. 2020 – via Business Wire.

v New Drug Therapy Approvals 2020". U.S. Food and Drug Administration (FDA). 31 December 2020. Retrieved 17 January 2021. This article incorporates text from this source, which is in the public domain.

vi Meanwell N.A.; Krystal M.R.; Nowicka-Sans B.; Langley D.R.; Conlon D.A.; Eastgate M.D.; Grasela D.M.; Timmins P.; Wang T.; Kadow J.F.; Inhibitors of HIV-1 Attachment: The Discovery and Development of Temsavir and its Prodrug Fostemsavir; *Journal of Medicinal Chemistry*, 2018, **61,** 62-80.

vii Moore K.; Magee M.; Sevinsky H.; Chang M.; Lubin S.; Myers E.; Ackerman, P.; Llamoso C.; Methadone and buprenorphine pharmacokinetics and pharmacodynamics when coadministered with fostemsavir to opioid-dependent, human immunodeficiency virus seronegative participants; *British journal of clinical pharmacology*. 2019, **85,** 1771-1780.

viii Wang T.; Ueda Y.; Zhang Z.; Yin Z.; Matiskella J.; Pearce B.C.; Yang Z.; Zheng M.; Parker D.D.; Yamanaka G.A.; Gong Y.F.; Ho H.T.; Colonno R.J.; Langley D.R.; Lin P.F.; Meanwell N.A.; Kadow J.F.; Discovery of the Human Immunodeficiency Virus Type 1 (HIV-1) Attachment Inhibitor Temsavir and Its Phosphonooxymethyl Prodrug Fostemsavir; *Journal of Medicinal Chemistry*. 2018, **61,** 6308-6327.

ix Sundquist W.I.; Krausslich H.G.; HIV-1 assembly, budding, and maturation; *Old Spring Harbor Perspectives in Medicine*. 2012, **2,** a006924.

x Srinivasarao S.; Neelu Jain.; New Method for the Estimation and Validation of Fostemsavir and Ganciclovir by using RP-HPLC; *International journal of pharmaceutical sciences research*. 2012, **12,** 1182-1187.

xi Rashnita S.; Asha D.C.; Estimation of fostemsavir using a novel HPLC analytical method. *International Journal of Pharmaceutical Sciences and Research*. 2022, **13**, 257-265.

xii Asha D.C.; Rashnita S.; Development and validation of fostemsavir in bulk and pharmaceutical dosage form by UV spectrophotometric method; *Indo American Journal of Pharmaceutical Research*. 2021, **11,** 1665-1671.

Received on January 31, 2024.