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A CRITICAL REVIEW OF ANALYTICAL AND BIO-ANALYTICAL METHODS FOR THE ESTIMATION OF ANTI-HIV DRUG BICTEGRAVIR.

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ABSTRACT

Bictegravir is an antiviral drug of the 'Integrase Strand Transfer Inhibitor' class that was structurally derived from an earlier compound Dolutegravir. The drug has demonstrated favorable outcomes in managing HIV-1 and HIV-2 infections and has received authorization for HIV-1 monotherapy in conjunction with two other antiretroviral medications in a single tablet.[1] Bictegravir is a recommended treatment for patients who have not undergone antiretroviral therapy and are diagnosed with HIV-1 infection. This is an enzyme encoded by the HIV-1 virus that is essential for the virus to replicate. The prevention of the integration of HIV-1 into the host DNA is achieved through the inhibition of the integrase enzyme. This action effectively blocks the conversion of the HIV-1 provirus and impedes the progression of the virus.[2] [3] Although this drug is used extensively, only limited studies have been conducted. This article is intended to highlight the analytical and bio-analytical methods such as UV, HPLC, LC-MS/MS, UHPLC-MS has been used for the estimation of Bictegravir as individual as well as in pharmaceutical dosage form in combination with other drugs. Many analytical and bio-analytical methods have been reported for the estimation of Bictegravir individually as well as in combination.

KEYWORDS: HIV Infection, UV-Spectrophotometry, EMT, TAF, RP-HPLC, LC-MS/MS, UHPLC-MS/MS, Mobile Phase, Column, Wavelength, Flow rate, m/z ratio, EMT, TAF.

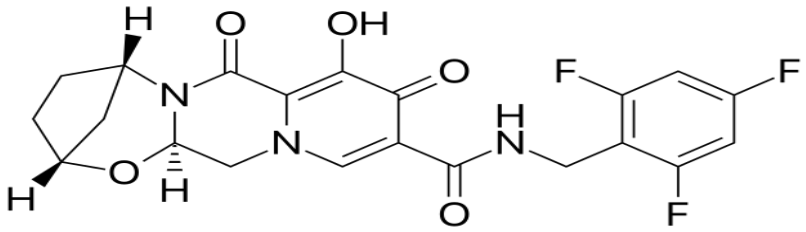
INTRODUCTION TO DRUG PROFILE

Bictegravir

Integrase strand transfer inhibitors (INSTIs) are a novel category of antiretroviral drugs that have been approved for the management of HIV-1 infection. These drugs function by obstructing the strand transfer phase of viral DNA integration into the host genome, thereby hindering HIV-1 replication.[2]

Bictegravir, a newly developed integrase strand transfer inhibitor (INSTI), is currently undergoing clinical trials for registration. It is being tested in combination with Tenofovir Alafenamide (TAF) and Emtricitabine (EMT) in a single tablet formulation for the treatment of HIV-1 infection. Bictegravir is an 'Integrase strand transfer inhibitor' with a strong in vitro barrier, highly resistible to significant interactions of the drug, acts selectively against infection of HIV. It interacts with the active site of HIV integrase, thereby inhibiting the replication of HIV. Bictegravir along with two other anti-retroviral derivatives commercially available as "BIKTARVY" has proven to act selectively against HIV-1. Based on the US Food and Drug Administration (FDA), it is proven that the 'Bictegravir' has been authorized as a daily-once fixed-dose regimen to treat HIV-1 infection. Bictegravir is developed by Gilead Sciences Inc. for the treatment of HIV-1 and has been recently approved by the US Food and Drug Administration (FDA). Bictegravir has improved resistance and safety profile compared with other INSTIs. The use of a single tablet containing three anti-retroviral agents has been authorized for the treatment of HIV-1 as a monotherapy.[4]

Bictegravir is a recommended treatment option for patients who have not undergone antiretroviral therapy and are diagnosed with HIV-1 infection. Bictegravir is an effective treatment option for patients with HIV-1 infection who have achieved virological suppression (HIV-1 RNA <50 c/mL) after being on a regular antiretroviral regimen for at least three months. This medication should only be prescribed to patients who have not experienced treatment failure and who do not have any known factors associated with resistance to the individual components of the drug. It is administered in combination with Tenofovir and Emtricitabine.[5]



(Structure of Bictegravir)

- **Chemical Name:** 2,5- Methanopyrido[1',2':4,5] pyrazino [2,1-b][1,3]oxazepine-10-carboxamide,2,3,4,5,7,9,13,13a-octahydro-8-hydroxy-7,9-dioxo-N-[(2,4,6-trifluorophenyl)methyl]-, sodium salt (1:1)
- **Molecular Formula:** $C_{21}H_{17}F_3N_3NaO_5$.
- **Molecular Weight:** 471.4 g/mol.
- **Solubility:** 0.1 mg/mL in water at 20°C.

Mechanism of Action: Bictegravir acts by inhibiting the HIV-1 integrase enzyme, which is necessary for HIV virus replication. Bictegravir is an integrase strand transfer inhibitor that prevents the replication of the virus. Additionally, it prevents the HIV-1 provirus from growing and spreading.[5]

Methods

The estimation of Bictegravir in Pharmaceutical Formulations & Biological Matrices follows the parameters as per Guidelines for Method development & Validation. Various instrumental methods like Spectrophotometric and Chromatographic methods are employed for the analysis of Bictegravir. Literature suggests that most of the studies were conducted by the help of High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-MS/MS (LC-MS/MS) methods and some studies included Ultra Performance Liquid Chromatography (UPLC-MS/MS), Gas Chromatographic (GC) and UV-Visible Spectrophotometric methods. This review also focuses quantification of Bictegravir in various matrices. By the analytical methods, information on the safety, efficacy, quality, & stability of the drugs in pharmaceutical industries is obtained. By the Bio-Analytical methods, qualitative and quantitative estimation of analytes in the biological samples are obtained.

Sample Preparation

Sample preparation plays a major role in the estimation of analyte of interest (Bictegravir) present in Biological matrices. It can be done by one of these several different methods, such

as, Protein Precipitation, Solid phase extraction and Liquid-liquid extraction. Sample preparation provides efficient separation of analytes from biological matrices and helps in removing unwanted substances which can cause interferences during analysis. Proper sample preparation is essential to obtain accurate and precise results in analytical methods, as it removes interfering substances and concentrates the target analytes for detection. There are several reasons why sample preparation is important in analytical method development.

- **Elimination of interfering substances:** Samples often contain matrix components or impurities that can interfere with the analysis, leading to inaccurate or imprecise results. Sample preparation techniques such as filtration, extraction, or purification can remove these interfering substances and improve the accuracy and precision of the analytical method.

- **Concentration of analytes:** Some analytical methods require a minimum concentration of analytes for detection. Sample preparation can be used to concentrate the analytes, increasing their concentration and improving the sensitivity of the analytical method.

- **Protection of analytical instruments:** Some samples may contain substances that can damage analytical instruments or reduce their lifespan. Sample preparation can remove these substances or protect the analytical instruments from damage, increasing their lifespan and reducing the need for costly repairs.

- **Reproducibility:** Proper sample preparation techniques can ensure the reproducibility of the results, even when different analysts or instruments are used for the analysis.[6]

INSTRUMENTATION

For the estimation of Bictegravir in Bulk drugs, Pharmaceutical dosage form as well as in Biological Matrix, researchers have used various instruments such as, UV-visible Spectrophotometer, High-Performance Liquid Chromatography (HPLC), Ultra-Performance Liquid Chromatography (UPLC), Liquid Chromatography-MS/MS (LC-MS/MS).

UV-Visible Spectrophotometry, is a very commonly used analytical technique for the estimation of drugs, which involves the Absorption Maxima method, the higher the efficiency of a molecule in absorbing light of a specific wavelength, the larger the magnitude of light absorption. Based on this principle, Beer-Lambert law can be derived.[7]

HPLC, nowadays, performs an important role in the field of analysis for the separation of various substances from mixture of substances. HPLC refers to 'High Performance Liquid Chromatography', which is an analytical technique for separating, identifying and quantifying each component in a mixture based on a solid stationary phase and liquid mobile phase. There are two most popular types of HPLC-Normal Phase & Reversed Phase. In Normal Phase the column is packed with relatively non-polar particles and in Reversed Phase the column is packed with relatively polar particles.[8], [9]

LC-MS/MS method is a robust analytical approach that combines Liquid Chromatography with Mass Spectrometry in a tandem fashion. This technique facilitates the generation, separation, and characterization of ionized molecules. LC-MS covers a broad range of application areas. The mass spectrometer is an analytical instrument that has been specifically engineered to segregate ions in the gaseous phase based on their m/z (mass to charge ratio) value. Mass Spectrometry involves the separation of charged species which are produced by a variety of ionization methods in LC-MS. These include.

- Electrospray Ionization (EI)
- Atmospheric Pressure Chemical Ionization (APCI)

In all cases the charged species are produced as gas phase ions under atmospheric conditions. The process of segregating ions in the gaseous phase is accomplished through the utilization of electrical and/or magnetic fields in the mass spectrometer to differentiate between ions.[10] ,[11],[12]

UPLC-MS

Ultra-high-performance liquid-chromatography (UHPLC) covers liquid chromatography separations implementing columns enclosing particles smaller than the 2.5–5µm sizes typically used in high-performance liquid chromatography (HPLC). UHPLC works on the same assumption as that of HPLC and of which governing principle is that, as column packing particle size decrease, efficiency and thus resolution accretion**.** The utilization of UPLC-MS has been shown to enhance the performance of analytical methods in terms of speed, resolution, and sensitivity. This represents a novel and sophisticated classification of the high-performance liquid chromatography (HPLC) technique, which retains the fundamental principles and methodology while exhibiting enhanced chromatographic efficiency.[13]

REPORTED METHODS FOR ANALYSIS OF BICTEGRAVIR

Analytical Methods

UV Method

Sl No.	AUTHORS AND YEAR	SPECTROSCOPIC CONDITIONS		VALIDATION PARAMETERS				
		DILUENT	λmax (nm)	LINEARITY	Regression Line Equation Slope (m)	Intercept (c)	Correlation Coefficient (r2)	% RSD
1	Pavankumar Gangavarapu [2020][14]	Methanol	340	5-25 µg/ml	0.0092	0.0344	0.9981	1.91

HPLC Methods

Sl. No.	Authors And Year	Chromatographic Conditions				Validation Parameters			
		Eluent	Column	Detector	Rt (Min)	Linearity	Lod	Loq	Recovery
1	Nagaraju Pappula (2021)[15]	0.01N Na2 HPO4 + Acetonitrile [50:50, v/v]	Agilent C18	UV Detection at 272 nm	2.2(EMT), 2.8(BIC), 3.2(TAF)	25-150µg/ml(EMT), 6.25-37.5µg/ml(BIC), 3.125-18.75µg/ml(TAF)	0.99µg/ml (EMT), 0.30 µg/ml (BIC), 0.10 µg/ml (TAF)	3.0µg/ml (EMT), 0.91µg/ml (BIC), 0.30 µg/ml (TAF)	100.24% (EMT), 100.30% (BIC), 100.09% (TAF)
2	Tej Kumar Kokkirala (2019)[16]	0.1% Ortho Phosphoric acid + Acetonitrile [50:50,v/v]	Denali C18 column	UV Detection at 272 nm	2.303 (EMT), 3.219 (BIC), 3.754 (TAF)	50-300µg/ml(EMT), 12.5-75µg/ml(BIC), 6.25- 37.5µg/ml (TAF)	1.04 µg/ml(EMT), 0.62 µg/ml(BIC), 0.70 µg/ml (TAF)	3.14µg/ml (EMT), 1.89µg/ml (BIC), 2.13µg/ml (TAF)	99.89% (EMT), 100.65% (BIC), 100.38% (TAF)
3	Tanuja ATTALURI (2020)\[17]	0.2% Triethylamine buffer + Methanol [40:60, v/v]	Inertsil Octylde cylsilyl	UV Detection at 260	2.805 (EMT), 5.998	100-500 µg/mL (EMT), 25-125 µg/mL (BIC), 12.5-62.5 µg/mL (TAF)	1.05 µg/ml(EMT), 2.7µg/ml(BIC)	3.30µg/ml (EMT), 8.78µg/ml (BIC), 4.61µg/ml (TAF)	100.48% (EMT), 99.97% (BIC), 99.82% TAF)

			C18	nm	(BIC), 4.537 (TAF)		, 1.35 µg/ml (TAF)		
4	R. Meenakshi et al. (2018)[18]	Potassium Dihydrogen Orthophosphate + Methanol and Water [70:30, v/v]	Inertsil 30V C18	UV Detection at 265 nm	6.278 (EMT), 10.487 (BIC), 8.439 (TAF)	160µg-480µg/ml (EMT), 40µg-120µg/ml (BIC), 20µg-60µg/ml (TAF)	0.2 µg/ml (EMT), 0.5µg/ml (BIC), 0.0025µg/ml (TAF)	0.6 µg/ml (EMT), 1.5µg/ml (BIC), 0.0075µg/ml (TAF)	
5	Vendra Sri Surya Deepthi et al. (2019)[19]	0.01N KH2PO4 + Acetonitrile [58:42, v/v]	BDS C8	UV Detection at 272 nm	2.229 (EMT), 2.958 (BIC), 3.568 (TAF)	50-300µg/ml (EMT), 12.5-75µg/ml (BIC), 6.25-37.5µg/ml (TAF)	1.40µg/ml (EMT), 0.12µg/ml (BIC), 0.15µg/ml (TAF)	4.24 µg/ml (EMT), 0.36µg/ml (BIC), 0.47µg/ml (TAF)	100.17% (EMT), 100.14% (BIC), 100.17% TAF)

BIO-ANALYTICAL METHODS

LC-MS/MS Methods

Sl No.	AUTHORS AND YEAR	CHROMATOGRAPHIC CONDITIONS				VALIDATION PARAMETERS			
		ELUENT	COLUMN	DETECTOR	RT (min)	LINEARITY	LLOQ	RECOVERY	MATRICES
1	Raju et al. [2018][20]	Methanol + 0.1% formic acid in water [85:15, v/v]	Zorbax C18	Tandem Mass- Triple Quadrupole	1.5 (BIC),	10-3000 ng/mL (EMT, 5-1500 ng/mL (BIC), 5-1500 ng/mL(TAF)	10 ng/mL (EMT), 5 ng/mL (BIC), 5 ng/mL (TAF)	78.5 % (EMT), 77.6 % (BIC), 50.2 % (TAF)	Human Plasma
2	Tanuja et al. [2022][21]	Acetonitrile + 0.1%Formic acid in water [70:30, v/v]	Zorbax XDB C18	Tandem Mass- Triple Quadrupole	1.05 (BIC),	2-500 ng/mL (EMT), 2-500 ng/mL (BIC), 2-500 ng/mL(TAF)	2 ng/mL (EMT), 2 ng/mL (BIC), 2 ng/mL (TAF)	94.95% (EMT), 81.23% (BIC), 87.76% (TAF)	Human Plasma
3	Junichi MASUDA, et al.[22]	Ammonium Formate - Water + Ammonium Formate - Methanol	InertSustain C18	MS/MS - ESI	3.82 (BIC)	0.5- 1250 ng/ml	0.5ng/ml(BIC)	72.2%(BIC)	Human Plasma

4	PRATHIPATI ET AL.[23]	Acetonitrile + Water with 0.1% Formic acid [80:20, v/v]	Kinetex EVO C18	Q Trap MS	0.92 (BIC)	1– 10,000 ng/mL	1 ng/ml(BIC)	98.64% (BIC)	Human Plasma

UHPLC-MS/MS Methods

Sl No.	AUTHORS AND YEAR	CHROMATOGRAPHIC CONDITIONS				VALIDATION PARAMETERS			
		ELUENT	COLUMN	DETECTOR	RT (min)	LINEARITY	LOD LOG	RECOVERY	MATRICES
1	COURLET ET AL.[24]	H2O with 0.1% FA + ACN with 0.1% FA	Xselect HSS T3	MS/MS – ESI	2.16	25-10000 ng/mL	LLOQ-30ng/ml	Not Mentioned	Human Plasma
2	H. Gouget, G. Noé, A. Barrail-Tran et al.[25]	0.1% (v/v)formic acid in water + Acetonitrile	Acclaim TM RSLC 120 C18	TSQ ALTIS MS (ESI)	3.54	10-5000ng/ml	LLOQ-10ng/ml	Not Mentioned	Human Plasma

REULTS AND DISCUSSION

A thorough and complete study of all the available analytical and bio-analytical methods for the estimation of the anti-retroviral analogue- Bictegravir in Pharmaceutical formulations as well as Biological sample is done.

Among all the methods, such as, UV Spectrophotometry, HPLC, LC-MS/MS, UPLC-MS/MS; HPLC was most frequently used for the determination of Bictegravir in pharmaceutical dosage form and biological samples due to their fast and rapid quantification aspects. The most frequently used detection method was UV detection in a wavelength ranging from 260 to 272nm, and the most commonly used Mobile Phase were Methanol, Acetonitrile, Phosphate Buffer: and Stationary Phase used were C18 Reverse Phase column.
In HPLC, the Retention Time, given by all authors were within the range of 0.92 to 10.48mins, and the LOD, LOQ and Linearity 0.12 to 2.7 µg/ml, 0.36 to 8.78µg/ml and 6.25 to 125 µg/ml, thus, it provides effective quantification.

Bio-analytical techniques involve the utilization of diverse biological fluids such as blood, urine, and serum. However, the majority of researchers have employed human plasma as their preferred sample. LC-MS/MS and UPLC-MS/MS is the most commonly utilized method for bio-analytical estimation due to its efficacy. The C-18 (RP Column) and Acetonitrile: Ammonium Formate Buffer: Water (in varying Proportions) are the most frequently utilized Stationary and Mobile phases in LC-MS/MS. The Electro-spray Ionization Technique coupled with Tandem Mass Spectrometer is a frequently employed method for detection.

The authors of various studies have reported the retention time of Bictegravir in LC-MS/MS and UPLC-MS/MS to be between 0.92 and 3.28 minutes. The Linearity, Lower Limit of Quantification (LLOQ), and Recovery of Bictegravir in LC-MS/MS and UPLC-MS/MS have been observed to be in the ranges of 1 to 10000 nanograms per milliliter, 0.5 to 30 nanograms per milliliter, and 50.02 to 98.64 percent, respectively. Therefore, this demonstrates that the aforementioned technique offers a proficient measurement of Bictegravir in biological specimens.

CONCLUSION

Bictegravir proves to show a very efficient activity against HIV virus infection, the virus that causes AIDS or Acquired Immuno Deficiency Syndrome. This review provides an overview of the various techniques available for the determination of Bictegravir in both

pharmaceutical formulations and biological fluids. The results obtained from analytical techniques indicate that the HPLC method utilizing UV detection exhibited superiority over alternative methods in terms of quantifying Bictegravir in pharmaceutical formulations, whether administered alone or in combination. Similarly, the data obtained from bio-analytical techniques indicate that LC-MS/MS and UPLC-MS/MS methods were superior to alternative methods in quantifying Bictegravir in biological samples, whether administered alone or in combination. This study provides a comprehensive overview of the various analytical and bio-analytical techniques that can be employed for the quantification of Bictegravir. The information presented in this review can serve as a valuable resource for future research endeavors, particularly in the development of new methods and related areas.

REFERENCES

1. E. D. Deeks, "Bictegravir/emtricitabine/tenofovir alafenamide: A review in HIV-1 infection," *Drugs*, vol. 78, no. 17. Springer International Publishing, Nov. 20, 2018; 1817–1828, doi: 10.1007/s40265-018-1010-7.
2. M. Tsiang *et al.*, "Antiviral activity of bictegravir (GS-9883), a novel potent HIV-1 integrase strand transfer inhibitor with an improved resistance profile," *Antimicrob Agents Chemother*, Dec. 2016; 60(12): 7086–7097, , doi: 10.1128/AAC.01474-16.
3. V. Spagnuolo, A. Castagna, and A. Lazzarin, "Bictegravir," *Current Opinion in HIV and AIDS*, vol. 13, no. 4. Lippincott Williams and Wilkins, Jul. 01, 2018; 326–333. doi: 10.1097/COH.0000000000000468.
4. Nokuzola Mbhele, Benjamin Chimukangara, and Michelle Gordon, "HIV-1 integrase strand transfer inhibitors: a review of current drugs, recent advances and drug resistance," *Int J Antimicrob Agents*, vol. 57, Apr. 2021.
5. "https://go.drugbank.com/drugs/DB11799."
6. JANUSZ PAWLISZYN and HEATHER L. LORD, *HANDBOOK OF SAMPLE PREPARATION*. WILEY, 2010.
7. D. L. Pavia, G. M. Lampman, G. S. Kriz, and J. R. Vyvyan, *Introduction to spectroscopy*. Brooks/Cole, Cengage Learning, 2009.
8. L. R. Snyder, J. J. Kirkland, and J. L. Glajch, *Practical HPLC Method Development*. Hoboken, NJ, USA: John Wiley & Sons, Inc, 1997. doi: 10.1002/9781118592014.
9. Yury Timchenko, "A Brief Note on High-Performance Liquid Chromatography (HPLC)," *Journal of Environmental Analytical Chemistry*, Sep. 2021; 8: 327.

10. McMaster and Marvin C., *LC/MS: A Practical User's Guide*. Wiley, 2005; doi: 10.1002/0471736589.
11. R. E. Ardrey, *Liquid chromatography-mass spectrometry : An Introduction*. J. Wiley, 2003.
12. S. Parasuraman, S. Balamurugan, S. Muralidharan, K. Jayaraj Kumar, V. Vijayan, and S. Parasuraman, "An Overview of Liquid Chromatography-Mass Spectroscopy Instrumentation," vol. 2, doi: 10.5530/phm.2014.2.2.
13. R. H. Rathod, S. R. Chaudhari, A. S. Patil, and A. A. Shirkhedkar, "Ultra-high performance liquid chromatography-MS/MS (UHPLC-MS/MS) in practice: analysis of drugs and pharmaceutical formulations," *Futur J Pharm Sci*, Dec. 2019; 5(1): doi: 10.1186/s43094-019-0007-8.
14. P. Gangavarapu, "ESTIMATION OF BICTEGRAVIR IN BULK SAMPLES BY UV-VISIBLE SPECTROPHOTOMETRIC METHOD," *World J Pharm Res*, 2020; 10: 1029–1037, doi: 10.20959/wjpr202101-19303.
15. N. Pappula and A. Dharma Teja, "METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF EMTRICITABINE, BICTEGRAVIR AND TENOFOVIR IN BULK AND TABLET DOSAGE FORM BY RP-HPLC METHOD," *Certified Journal* │ *1832 World Journal of Pharmaceutical Research SJIF Impact Factor*, 2021; 10: 1833, , doi: 10.20959/wjpr202113-22211.
16. T. K. Kokkirala and D. Suryakala, "RP-HPLC method development and validation for the estimation of Emtricitabine, Bictegravir and Tenofovir alafenamide in bulk and pharmaceutical dosage form," *Journal of Taibah University for Science*, 2019; 13(1): 1137–1146, doi: 10.1080/16583655.2019.1689601.
17. T. Attaluri, G. Seru, and S. N. M. Varanasi, "Development and validation of a stability-indicating rp-hplc method for the simultaneous estimation of bictegravir, emtricitabine, and tenofovir alafenamide fumarate," *Turk J Pharm Sci*, 2021; 18(4): 410–419, doi: 10.4274/tjps.galenos.2020.70962.
18. R. Meenakshi and R. Shyam Sunder, "Method Development and Validation by RP-HPLC for Simultaneous Estimation of Emtricitabine, Bictegravir, Tenofovir Alafenamide in Fixed Dosage Form., Indo Am," *J. P. Sci*, 2018; 07(5): [Online]. Available: http://www.iajps.com
19. Vendra Sri Surya Deepthi and Dr. Devanaboyina Narendra, "Method Development and Validation for Simultaneous Estimation of Emtricitabine, Bictegravir and Tenofovir Alafenamide by RP-HPLC," *Int. J Pharm Sci Rev Res*, Oct. 2019; 58(2): 54–59.

20. V. Appala Raju, M. Venkata Ramana, T. Shyam Principal, V. Kiran Kumar, N. Appala Raju, and N. Appala Raju Professor, "BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF BICTEGRAVIR, TENOFOVIR AND EMTRICITABINE IN HUMAN PLASMA BY LC-MS/MS," *WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES SJIF Impact Factor*, 2018; 7: 441–463, doi: 10.20959/wjpps201812-12754.
21. A. Tanuja and S. Ganapaty, "Bio-analytical Method Development and Validation for Simultaneous Determination of Bictegravir, Emtricitabine, and Tenofovir Alafenamide Fumarate in Human Plasma by LC-MS/MS," *Indian Journal of Pharmaceutical Education and Research*, Oct. 2022; 56(4): 1190–1205, , doi: 10.5530/ijper.56.4.201.
22. J. Masuda and Y. Kato, "Simultaneous Determination of Raltegravir, Dolutegravir, Elvitegravir, and Bictegravir in Human Plasma Using High-performance Liquid Chromatography-tandem Mass Spectrometry," 2020. doi: https://doi.org/10.15369/sujms.32.91.
23. P. K. Prathipati, S. Mandal, and C. J. Destache, "Development and validation of LC–MS/MS method for quantification of bictegravir in human plasma and its application to an intracellular uptake study," *Biomedical Chromatography*, Feb. 2019; 33(2): doi: 10.1002/bmc.4379.
24. P. Courlet *et al.*, "Development and validation of a multiplex UHPLC-MS/MS assay with stable isotopic internal standards for the monitoring of the plasma concentrations of the antiretroviral drugs bictegravir, cabotegravir, doravirine, and rilpivirine in people living with HIV," *Journal of Mass Spectrometry*, Jun. 2020; 55(6): doi: 10.1002/jms.4506.
25. H. Gouget, G. Noé, A. Barrail-Tran, and V. Furlan, "UPLC–MS/MS method for the simultaneous quantification of bictegravir and 13 others antiretroviral drugs plus cobicistat and ritonavir boosters in human plasma," *J Pharm Biomed Anal*, vol. 181, Mar. 2020, doi: 10.1016/j.jpba.2019.113057.