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LC-MS/MS Techniques for Estimation of Antiretroviral Drug in Biological Matrices: A Review

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ABSTRACT

HIV/AIDS has remained a major global health challenge since its emergence in the 1980s. Antiretroviral therapy (ART), especially combination ART (cART) and highly active ART (HAART), has greatly improved disease management. As a result, fixed-dose combinations (FDCs) containing multiple antiretroviral (ARV) drugs are now commonly used. Accurate quantification of ARV drugs in biological samples is therefore essential for therapeutic drug monitoring, pharmacokinetic and pharmacodynamic studies, bioequivalence assessment, and drug development. HPLC and LC–MS/MS are commonly used analytical techniques for ARV bioanalysis. Due to its high sensitivity, selectivity, and ability to analyze complex biological matrices such as plasma, urine, saliva, and dried blood spots, LC–MS/MS has become the preferred analytical platform. Sample preparation is critical for improving analytical performance and common techniques include protein precipitation, liquid–liquid extraction and solid-phase extraction.

This review summarizes recent advances in LC–MS/MS methods for quantifying ARV drugs and their metabolites in biological matrices. It highlights chromatographic conditions, sample extraction techniques and internal standard selection for both single- and multi-ARV drug analyses, as well as method validation strategies based on established guidelines. Finally, the review outlines current bioanalytical trends and offers insights for developing robust and reliable bioanalytical methods for ARV drug analysis.

Keywords: *ARV drugs, biological matrix, LC-MS/MS, Method development, Validation*

1. INTRODUCTION

AIDS (Acquired Immuno Deficiency Syndrome) is a serious disease caused by HIV, which weakens the immune system and shows flu like symptoms at stage 1 [1]**.** HIV require lifelong antiretroviral therapy (ART). ARV drugs are classified into six categories based on their mechanism of action: Nucleoside/Nucleotide reverse transcriptase Inhibitors (NRTIs or NtRTIs), Non-nucleoside reverse transcriptase Inhibitors (NNRTIs), Protease Inhibitors (PIs), Fusion Inhibitors, Entry Inhibitors and Integrase strand transfer Inhibitors [2]. Current international guidelines recommend a daily combination of three or more antiretroviral drugs in fixed dose combination (FDC), known as Highly Active Antiretroviral Therapy (HAART) for treatment of HIV which reduces illness and deathrate [3,4].

Quantitative analysis of antiretroviral (ARV) drugs in biological matrices is crucial for determining bioavailability, drug metabolism, and pharmacokinetic parameters, including absorption, distribution, metabolism, and excretion (ADME). These drugs inhibit HIV by targeting various stages of its life cycle, such as viral replication and maturation [5-7]. Antiretroviral drug development has progressed through the introduction of new formulations and the identification of novel drug classes, addressing challenges such as drug toxicities and resistance [8]. Early antiretroviral therapy regimens were complex, requiring multiple drugs taken at different times throughout the day. However, advances in drug development have since simplified treatment, and many patients can now manage HIV with a

single once-daily pill [9]. In drug bioanalysis for preclinical and clinical studies, high-performance liquid chromatography (HPLC) and liquid chromatography with tandem mass spectrometry (LC-MS/MS) are common techniques. In bioanalysis, drugs and their metabolites are extracted from biological matrices using techniques like solid-phase extraction, liquid-liquid extraction and protein precipitation. The high sensitivity of LC-MS/MS then allows for the investigation of drug deposition within these biological matrices [10].

2. BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION

Developing and validating bioanalytical methods is critical in the pharmaceutical industry, particularly for antiretroviral drug development. These methods detect, identify, quantify, and characterize drugs, and their accuracy and reliability ensure the final product meets quality standards for purity, potency, safety, and efficacy [11]. Bioanalytical methods are developed to create a set of procedures for determining the concentration of unknown or novel compounds within a biological matrix. Analytical methods consider multiple factors, including the analyte's physical and chemical properties, concentration, matrix sample, instrumentation and the analysis's cost, speed and time [12].

2.1 Sample Collection

The biological matrix containing the analyte is typically blood, plasma, urine, or serum. Blood is usually collected from human volunteers via vein puncture, using a hypodermic syringe to draw 5-7 ml. The venous blood is then transferred into tubes containing an anticoagulant, such as ethylenediaminetetraacetic acid (EDTA) or heparin. Plasma is subsequently obtained by centrifugation at 4000 rpm for 15 minutes, with approximately 30-50% of the initial volume being collected [13].

2.2 Biological Sample Preparation

Sample preparation is a critical step in bioanalysis because it isolates target analytes from biological matrices and transfers them into a solvent compatible with chromatographic analysis. This step is often challenging due to the inherent complexity of biological samples. As demand increases for higher sensitivity, accuracy, and rapid analysis of complex biological fluids, efficient sample preparation techniques have become even more crucial.

[14]. Effective sample preparation isolates the target analyte from complex biological matrices. This is achieved by removing interfering substances while minimizing analyte loss [15,16]. Solid-phase extraction, protein precipitation and liquid–liquid extraction are widely reported techniques for preparing biological matrices for the analysis of antiretroviral (ARV) drugs. The chosen extraction method influences recovery, matrix effects, sensitivity, and reproducibility [17].

Solid-phase extraction (SPE):

Solid Phase Extraction is ideally suited for bioanalysis due to its high reproducibility and ability to produce cleaner extracts. For multi-drug quantification in plasma, SPE is often preferred. However, SPE can be expensive, as it requires an HLB cartridge for each sample analysis, as well as an SPE processor during sample processing [18].

Protein precipitation (PP):

Protein precipitation is simple, rapid and cost-effective extraction method. Generally, acetonitrile or methanol are used as a solvent for PP.

Liquid–liquid extraction (LLE):

LLE is effective for hydrophobic analytes. To maximize recovery while minimizing co-extraction of matrix components, it is important to select the solvent carefully.

2.3 Chromatographic Techniques

Efficient separation and accurate quantification of active pharmaceutical substances (APS) require optimized chromatographic conditions. Method success depends greatly on column selection and mobile phase composition [19].

Column Choice:

The selection of an appropriate stationary phase can significantly improve the efficiency of method development. Column selection depends on the polarity, molecular weight, and sample matrix of the analytes. Generally, C18 (octadecyl silane) or C8 (octyl silane) reversed-phase columns are used to separate ARVs. Column dimensions, such as length and particle size, affect resolution and sensitivity. Employing a shorter column with a suitable stationary phase can reduce the run time [20].

Mobile Phase Composition:

The composition of the mobile phase is critical in chromatographic analysis, as different solvent combinations can significantly influence peak shape, retention time, and selectivity. The mobile phase can be delivered using either isocratic or gradient elution. For separating multiple drugs and their metabolites with varying polarities, gradient elution is generally the preferred technique [21-22].

2.4 Bioanalytical Method Validation

After a method is developed, it must be validated according to international guidelines to confirm its suitability, accuracy, and reliability for quantifying the analyte in the chosen biological matrix. This validation also ensures the method can be effectively applied to real patient samples [23,24]. Analytical methods used to quantify each analyte (drug or metabolite) require specific validation criteria. Bioanalytical method validation, performed in accordance with International Council for Harmonisation (ICH) guidelines [26,].

3. DEVELOPED BIOANALYTICAL METHODS

Several LC-MS/MS methods have been reported for analyzing ARV drugs in biological matrices. Most use a mobile phase of acetonitrile and buffer, with either isocratic or gradient elution, depending on the number of ARV drugs being analyzed. Prior to injection, sample preparation techniques such as liquid-liquid extraction, solid-phase extraction, or protein precipitation are used to extract the analytes from the biological matrix. The reported methods are validated according to ICH/FDA guidelines and demonstrate good performance.

Fayet *et al*. developed a sensitive LC–MS/MS method for the simultaneous determination of raltegravir, maraviroc, darunavir, and etravirine in human plasma. The method used electrospray ionization and tandem mass spectrometric detection, with validation across clinically relevant concentration ranges. Extraction recoveries exceeded 91%, and the method demonstrated acceptable accuracy and precision. The assay was considered suitable for therapeutic drug monitoring and the assessment of antiretroviral exposure in patients receiving combination therapy [27]. Rezk *et al*. developed and validated a liquid chromatography–electrospray ionization mass spectrometry method for simultaneous determination of etravirine, darunavir, and ritonavir in human plasma. The method employed liquid–liquid extraction and a short chromatographic separation. It demonstrated accuracy ranging from 88% to 106% and intra- and interday precision below 7%. The assay was evaluated using clinical samples and external quality-assurance samples, supporting its applicability to clinical pharmacology studies and therapeutic drug monitoring [28].

Matta *et al*. developed and validated a rapid and sensitive LC–MS/MS method for the simultaneous quantification of tenofovir and lamivudine in human plasma using abacavir as an internal standard. Plasma samples were processed using solid-phase extraction, and the analytes were quantified using multiple-reaction monitoring. The method demonstrated linearity over the ranges of 5–600 ng/mL for tenofovir and 25–4000 ng/mL for lamivudine, with acceptable accuracy and precision. Its application to a pharmacokinetic study demonstrated the suitability of LC–MS/MS for the quantitative analysis of antiretroviral drugs in human plasma. This study provides a useful analytical reference for developing and validating methods for antiretroviral drug quantification [29]. Simiele *et al*. developed and validated a UPLC–MS/MS method for the simultaneous quantification of dolutegravir, elvitegravir, rilpivirine, and other antiretroviral agents in human plasma. The method enabled the simultaneous measurement of multiple antiretroviral drugs, supporting clinical pharmacology research and therapeutic drug monitoring. This study demonstrates the applicability of sensitive LC–MS/MS techniques for the quantitative analysis of antiretroviral agents in biological matrices and provides a useful reference for developing and validating methods for combination antiretroviral therapy [29].

Parsons and Marzinke developed and validated an LC–MS/MS method for multiplexed quantification of etravirine, maraviroc, raltegravir, and rilpivirine in human plasma and tissue. The study addressed the analytical requirements associated with measuring multiple antiretroviral agents in different biological matrices. Its validation approach and matrix-specific sample preparation provide useful methodological guidance for bioanalytical studies involving antiretroviral drugs [30]. Penchala *et al*. developed and validated an LC–MS/MS method for the simultaneous determination of dolutegravir, elvitegravir, and cobicistat in human plasma. Liquid–liquid extraction was performed using 100 µL of plasma, and chromatographic separation was achieved using a C18 column with a methanol–water mobile phase containing formic acid. The method was validated over a concentration range of 10–4000 ng/mL for all three analytes and was successfully applied to ongoing clinical trials. This study demonstrates the applicability of LC–MS/MS for sensitive quantification of antiretroviral drugs in human plasma [31].

Simiele *et al*. developed and validated a UPLC–MS/MS method for the simultaneous quantification of dolutegravir, elvitegravir, rilpivirine, and other antiretroviral agents in human plasma. The method enabled the simultaneous measurement of multiple antiretroviral drugs, supporting clinical pharmacology research and therapeutic drug monitoring. This study demonstrates the applicability of sensitive LC–MS/MS techniques for the quantitative analysis of antiretroviral agents in biological matrices and provides a useful reference for developing and validating methods for combination antiretroviral therapy [32]. Mwando *et al*. developed an LC–MS/MS method using multiple-reaction monitoring and solid-phase extraction for the simultaneous determination of five antiretroviral drugs in human plasma. The study evaluated selectivity, recovery, accuracy, precision, and limits of quantification. The results demonstrated the potential of SPE-based sample preparation for the bioanalysis of antiretroviral agents and provided a useful approach for developing methods for therapeutic drug monitoring [33]. Tsuchiya et al. developed and validated an LC–MS/MS method for simultaneous determination of raltegravir, dolutegravir, and elvitegravir in human plasma and cerebrospinal fluid (CSF). Only 20 µL of sample was required, and protein precipitation with acetonitrile was used for sample preparation. Chromatographic separation was achieved within five minutes. The method demonstrated acceptable accuracy and precision, with linear calibration ranges of 5–1500 ng/mL in plasma and 1–200 ng/mL in CSF. The assay provides a useful approach for monitoring integrase inhibitor concentrations in biological matrices [34]. Ocque *et al*. developed and validated an LC–MS/MS assay for the quantification of tenofovir and tenofovir alafenamide in human plasma and cerebrospinal fluid. The method supports bioanalytical measurement of tenofovir-related compounds in biological matrices and provides relevant methodological background for antiretroviral pharmacokinetic studies [35]. Bollen *et al* developed and validated a UPLC–MS/MS bioanalytical method for the simultaneous quantification of five antiretroviral drugs dolutegravir, elvitegravir, raltegravir, nevirapine, and etravirine in human plasma. Protein precipitation with methanol was used for sample preparation, and stable isotope-labelled internal standards were employed for each analyte. The method demonstrated acceptable accuracy, precision, and reproducible recovery, with a chromatographic run time of 10 minutes. The validated assay was suitable for clinical pharmacology research and therapeutic drug monitoring, demonstrating the utility of UPLC–MS/MS for reliable quantification of antiretroviral agents in biological matrices [36].

Courlet *et al*. developed and validated a multiplex ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method for monitoring bictegravir, cabotegravir, doravirine, and rilpivirine concentrations in human plasma. Plasma samples were prepared using a simple protein-precipitation procedure, followed by chromatographic separation and tandem mass spectrometric detection. Stable isotope-labelled internal standards were used to improve analytical reliability. The method was validated for accuracy, precision, and clinically relevant concentration ranges and demonstrated suitability for therapeutic drug monitoring in people living with HIV. This study illustrates the applicability of UHPLC–MS/MS for sensitive and reliable quantification of antiretroviral agents in human plasma [37].

Gouget *et al.* developed and validated a rapid UPLC–MS/MS method for the simultaneous quantification of 14 antiretroviral drugs and two pharmacokinetic boosters in human plasma. Sample preparation involved protein precipitation using acetonitrile containing labelled internal standards, and chromatographic separation was achieved within six minutes. The method was validated according to European Medicines Agency guidelines, demonstrating acceptable precision and accuracy across the evaluated concentration ranges. The assay was suitable for therapeutic drug monitoring and clinical pharmacology research in people living with HIV [38].

4. DISCUSSION

This review highlights current developed methods and provides guidance for future method development to support accurate and reliable therapeutic drug monitoring in clinical and research study. The various strategies highlight the methodological advancements, analytical challenges and validation approaches employed in LC–MS/MS-based bioanalysis, involved in therapeutic drug monitoring and pharmacokinetic studies of ARV drugs.

5. CONCLUSION

LC–MS/MS is the preferred platform for quantifying antiretroviral (ARV) drugs in complex biological matrices due to its high sensitivity, specificity, and robustness. As the gold standard for pharmaceutical analysis, it supports pharmacokinetic studies, therapeutic drug monitoring, and drug development. This article discusses the development and validation of bioanalytical methods for ARV

drugs, including chromatographic method development, sample pretreatment, extraction techniques, and validation parameters. Overall, LC–MS/MS has significantly advanced bioanalysis, particularly in the accurate quantification of antiretroviral drugs.

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6. REFERENCES

1. Cohen MS, Shaw GM, McMichael AJ, Haynes BF. Acute HIV-1 infection. *New England Journal of Medicine*. **2011**;364(20):1943–1954. doi: https://doi.org/10.1056/NEJMra1011874.
2. Daskapan, A., van Hateren, K., Stienstra, Y., Kosterink, J., van der Werf, T., Touw, D. et al. Development and Validation of a Bioanalytical Method for the Simultaneous Determination of 14 Antiretroviral Drugs using Liquid Chromatography-Tandem Mass Spectrometry. J Appl Bioanal. **2018**; 4:37-50.
3. Önal, A. Analysis of antiretroviral drugs in biological matrices for therapeutic drug monitoring. J Food and Drug Anal. **2006**; 14:99-199.
4. Gouget, H., Noé, G., Barrail-Tran, A., and Furlan, V. 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.**2020**; 181:113057.
5. Checa A, Oliver R, Hernández-Cassou S, Saurina J. Determination of HIV drugs in biological matrices: a review. *Anal Chim Acta*. **2009**;647(1):1–13. doi: https://doi.org/10.1016/j.aca.2009.05.028.
6. Saag, M., Gandhi, R., Hoy. J., Landovitz, R., Thompson, M., Sax, P., Smith, D.M., et al. Antiretroviral Drugs for Treatment and Prevention of HIV Infection in Adults 2020 Recommendations of the International Antiviral Society–USA Panel. J Ame Med Asso.**2020**; 324:1651-1669.
7. Shin, Y., Park, C., and Yoon C. An Overview of Human Immunodeficiency Virus-1 Antiretroviral Drugs: General Principles and Current Status. Inf. Chemother. 53:29-45.
8. Maeda K, Das D, Kobayakawa T, Tamamura H, Takeuchi H. Discovery and development of anti-HIV therapeutic agents: Progress towards improved HIV medication. *Current Topics in Medicinal Chemistry*. **2019**;19(18):1621–1649. doi: https://doi.org/10.2174/1568026619666190712204603
9. Charbe, N., Zacconi, F., Amnerkar, N., Ramesh, B., Tambuwala, M., and Climenti, E. Bio-analytical Assay Methods used in Therapeutic Drug Monitoring of Antiretroviral Drugs-A Review. Curr. Drug Ther.**2019**;14:16-57.
10. Taylor, P., Tai, C., Franklin, M., and Pillans. P. The current role of liquid chromatography-tandem mass spectrometry in therapeutic drug monitoring of immunosuppressant and antiretroviral drugs. Clin Biochem.**2011**;44:14–20.
11. Tiwari G, Tiwari R. Bioanalytical method validation: An updated review. Pharm Methods. **2010**;1(1):25–38. doi: https://doi.org/10.4103/2229-4708.72226.
12. Tijare, L., Rangari, N., and Mahajan, U. A Review on Bioanalytical Method Development and Validation. Asian J Pharm Clin Res.**2016**; 9: 6-10.
13. Neogi, S., Mehndiratta, M., Gupta, S., and Puri D. Pre-analytical phase in clinical chemistry laboratory. J Clin Sci Res.**2016**; 5:171-178.
14. Sharma MK, Dhakne P, NN S, Reddy PA, Sengupta P. Paradigm shift in the arena of sample preparation and bioanalytical approaches involving liquid chromatography mass spectroscopic technique. Anal Sci. **2019**;35(10):1069–1082. doi: https://doi.org/10.2116/analsci.19R003.

15. Bylda C, Thiele R, Kobold U, Volmer DA. Recent advances in sample preparation techniques to overcome difficulties encountered during quantitative analysis of small molecules from biofluids using LC-MS/MS. Analyst. **2014**; 139:2265–2276. doi: https://doi.org/10.1039/C4AN00094C.

16. Sharma MK, Dhakne P, NN S, Reddy PA, Sengupta P. Paradigm shift in the arena of sample preparation and bioanalytical approaches involving liquid chromatography mass spectroscopic technique. Anal Sci. **2019**;35(10):1069–1082. doi: https://doi.org/10.2116/analsci.19R003.

17. Checa, A., Oliver, R., Hernández-Cassou, S., and Saurina, J., Determination of HIV drugs in biological matrices: A review. Anal. Chim. Acta.2009; 647:1-13.

18. Płotka-Wasylka J, Szczepańska N, de la Guardia M, Namieśnik J. Miniaturized solid-phase extraction techniques. TrAC Trends Anal Chem. **2015**; 73:19–38. doi: https://doi.org/10.1016/j.trac.2015.04.026.

19. Wong, A., Xiang, X., Ong, P., Mitchell, E., Syn, N., Wee, L., et al. A Review on Liquid Chromatography-Tandem Mass Spectrometry Methods for Rapid Quantification of Oncology Drugs. Pharm.**2018**; 10:1-20.

20. Wal, P., Kumar, B., Bhandari, A., Rai, A., and Wal, A. Bioanalytical Method Development – Determination of Drugs in Biological Fluids. J. Pharm. Sci. Technol. **2010**; 2:333-347.

21. Whitmire, M., Ammerman, J., De Lisio, P., Killmer, J., Kyle, D., Mainstone, E., et al. LC-MS/MS bioanalysis method development, validation, and sample analysis: points to consider when conducting nonclinical and clinical studies in accordance with current regulatory guidances. J Anal Bioanal Techniques.**2011***;* 4:1-12.

22. Schellinger AP, Carr PW. Isocratic and gradient elution chromatography: a comparison in terms of speed, retention reproducibility and quantitation. J Chromatogr A. **2006;**1109(2):253–266. doi: https://doi.org/10.1016/j.chroma.2006.01.047.

23. Viswanathan CT, Bansal S, Booth B, et al. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm Res. **2007**;24(10):1962–1973. doi: https://doi.org/10.1007/s11095-007-9291-7.

24. Hopfgartner, G. Bioanalytical method validation: How much should we do and how should we document? Anal. Bioanal. Chem.**2020**; 412:531-532.

25. Kaza, M., Karaźniewicz-Łada, K., Kosicka, K., Siemiatkowska A, and Rudzki Piotr, J. Bioanalytical method validation: new FDA guidance vs. EMA guideline. Better or worse? J. Pharm. BioMed. Anal. **2019;** 165:381-385.

26. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH M10: Bioanalytical Method Validation. Draft version, Step 2b. **2019**.

27. Fayet A, Béguin A, Zanolari B, Cruchon S, Guignard N, Telenti A, et al. A LC-tandem MS assay for the simultaneous measurement of new antiretroviral agents: raltegravir, maraviroc, darunavir, and etravirine. J Chromatogr B Analyt Technol Biomed Life Sci. **2009**;877(11–12):1057–1069. doi: https://doi.org/10.1016/j.jchromb.2009.02.057.

28. Rezk NL, White NR, Jennings SH, Kashuba ADM. A novel LC-ESI-MS method for the simultaneous determination of etravirine, darunavir and ritonavir in human blood plasma. Talanta. **2009**;79(5):1372–1378. doi: https://doi.org/10.1016/j.talanta.2009.06.005.

29. Matta MK, Burugula L, Pilli NR, Inamadugu JK, Seshagiri Rao JVLN. A novel LC-MS/MS method for simultaneous quantification of tenofovir and lamivudine in human plasma and its application to a pharmacokinetic study. Biomed Chromatogr. **2012**;26(10):1202–1209. doi: https://doi.org/10.1002/bmc.2679.

30. Parsons TL, Marzinke MA. Development and validation of a liquid chromatographic-tandem mass spectrometric method for the multiplexed quantification of etravirine, maraviroc, raltegravir, and rilpivirine in human plasma and tissue. J Pharm Biomed Anal. **2016**; 131:333–344. doi: https://doi.org/10.1016/j.jpba.2016.08.016.

31. Penchala SD, Fawcett S, Else L, Egan D, Amara A, Elliot E, et al. The development and application of a novel LC-MS/MS method for the measurement of dolutegravir, elvitegravir and cobicistat in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. **2016**; 1027:174–180. doi: https://doi.org/10.1016/j.jchromb.2016.05.040.

32. Simiele M, Ariaudo A, De Nicolò A, Favata F, Ferrante M, Carcieri C, et al. UPLC-MS/MS method for the simultaneous quantification of three new antiretroviral drugs, dolutegravir, elvitegravir and rilpivirine, and other thirteen antiretroviral agents plus cobicistat and ritonavir boosters in human plasma. J Pharm Biomed Anal. **2017**; 138:223–230. doi: https://doi.org/10.1016/j.jpba.2017.02.002.

33. Mwando E, Massele A, Sepako E, Sichilongo K. A method employing SPE, MRM LC-MS/MS and a THF–water solvent system for the simultaneous determination of five antiretroviral drugs in human blood plasma. Anal Methods. **2017**; 9:450–458. doi: https://doi.org/10.1039/C6AY02442D.

34. Tsuchiya K, Ohuchi M, Yamane N, Aikawa H, Gatanaga H, Oka S, et al. High-performance liquid chromatography-tandem mass spectrometry for simultaneous determination of raltegravir, dolutegravir and elvitegravir concentrations in human plasma and cerebrospinal fluid samples. Biomed Chromatogr. **2018**;32(2): e4058. doi: https://doi.org/10.1002/bmc.4058.

35. Ocque AJ, Hagler CE, Morse GD, Letendre SL, Ma Q. Development and validation of an LC-MS/MS assay for tenofovir and tenofovir alafenamide in human plasma and cerebrospinal fluid. J Pharm Biomed Anal. **2018**; 156:163–169. doi: https://doi.org/10.1016/j.jpba.2018.04.035.

36. Bollen PDJ, de Graaff-Teulen MJA, Schalkwijk S, van Erp NP, Burger DM. Development and validation of an UPLC-MS/MS bioanalytical method for simultaneous quantification of the antiretroviral drugs dolutegravir, elvitegravir, raltegravir, nevirapine and etravirine in human plasma. J Chromatogr B. **2019**; 1105:76–84. doi: https://doi.org/10.1016/j.jchromb.2018.12.008

37. Courlet P, Alves Saldanha S, Cavassini M, Marzolini C, Choong E, Csajka C, 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. J Mass Spectrom. **2020**;55(6): e4506. doi: https://doi.org/10.1002/jms.4506.

38. Gouget H, Noé G, Barrail-Tran A, Furlan V. 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. **2020**; 181:113057. doi: https://doi.org/10.1016/j.jpba.2019.113057.