ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 18, Issue, 02, pp.36180-36183, February, 2026
DOI: https://doi.org/10.24941/ijcr.50119.02.2026

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

STABILITY INDICATING LC-MS/MS METHODS FOR DIRECT ACTING ANTIVIRALS USED IN HEPATITIS C THERAPY: A COMPREHENSIVE REVIEW

Aditi Dhananjaybhai Raval[1] and Dr. Pareshkumar Narayan Bhai Patel[2]

[1]Assistant professor, Gokul Pharmacy College, Gokul global University, Siddhpur, Gujarat, India, 384151;
[2]Principal, Gokul Pharmacy College, Gokul Global University, Siddhpur, Gujarat, India, 384151

ARTICLE INFO

Article History:
Received 25th November, 2025
Received in revised form
20th December, 2025
Accepted 18th January, 2026
Published online 27th February, 2026

Keywords:

Hepatitis C virus; Direct-acting antivirals; LC-MS/MS; Stability-indicating method; Forced degradation; ICH guidelines.

Corresponding author:
Dhananjaybhai Raval

ABSTRACT

Hepatitis C virus (HCV) infection continues to pose a significant global health burden, often leading to chronic liver diseases if inadequately treated. The introduction of direct-acting antivirals (DAAs) such as Ledipasvir, Sofosbuvir, and Voxilaprevir has markedly improved therapeutic outcomes. With the growing clinical use of fixed-dose combinations, the need for sensitive and stability-indicating analytical methods has become increasingly important. This review critically evaluates reported analytical methods for the estimation of HCV DAAs, with a particular emphasis on stability-indicating liquid chromatography-tandem mass spectrometry (LC-MS/MS) approaches. A comprehensive literature survey of peer-reviewed articles and regulatory guidelines published between 2014 and 2023 was conducted. Reported UV, HPLC, and LC-MS/MS methods were systematically analyzed with respect to sensitivity, specificity, stability-indicating capability, and regulatory compliance as per ICH guidelines. Conventional UV and HPLC methods were found to be suitable mainly for routine quality control but exhibited limited capability for degradation product detection. In contrast, LC-MS/MS methods demonstrated superior sensitivity, selectivity, and applicability for multi-drug analysis and forced degradation studies. However, only a limited number of reports addressed comprehensive stability-indicating analysis of all three DAAs within a single method. LC-MS/MS represents a robust and regulatory-preferred analytical platform for the stability-indicating evaluation of HCV DAAs. Further research focusing on comprehensive multi-drug stability studies is warranted to support quality control and regulatory submissions.

Citation: Aditi Dhananjaybhai Raval and Dr. Pareshkumar Narayan Bhai Patel. 2026. "Stability Indicating LC-MS/MS Methods for Direct Acting Antivirals Used in Hepatitis C Therapy: A Comprehensive Review.". ***International Journal of Current Research, 18, (02), 36180-36183.***

INTRODUCTION

Hepatitis C virus (HCV) infection remains a major global health concern, affecting millions of individuals worldwide and contributing significantly to liver-related morbidity and mortality. Chronic HCV infection may progress to cirrhosis, hepatic failure, and hepatocellular carcinoma. The advent of direct-acting antivirals (DAAs) has revolutionized HCV therapy by offering high sustained virological response rates, shorter treatment durations, and improved patient compliance(1). Among these, Ledipasvir, Sofosbuvir, and Voxilaprevir are widely used either as dual or triple fixed-dose combinations for treating multiple HCV genotypes. The increased clinical use of these combinations necessitates robust analytical methods capable of simultaneous estimation and stability evaluation to ensure drug quality, safety, and efficacy throughout the product lifecycle (2).

Direct-Acting Antivirals Used in HCV Therapy: Direct-acting antivirals target specific viral proteins involved in HCV replication, thereby interrupting the viral life cycle. Their use in combination therapy minimizes resistance development and enhances therapeutic efficacy (3).

Ledipasvir: Ledipasvir is an NS5A inhibitor that interferes with viral RNA replication and virion assembly. It is commonly administered in combination with Sofosbuvir. From an analytical perspective, Ledipasvir presents challenges due to its poor aqueous solubility and susceptibility to oxidative and acidic degradation, highlighting the need for sensitive stability-indicating methods(4).

Sofosbuvir: Sofosbuvir is a nucleotide analogue prodrug that inhibits the NS5B RNA-dependent RNA polymerase. It undergoes chemical degradation under acidic and oxidative stress conditions, making it essential to employ analytical techniques capable of detecting low-level degradation products in both bulk and formulated forms(5).

Voxilaprevir: Voxilaprevir is an NS3/4A protease inhibitor primarily used in triple-drug regimens for patients with prior treatment failure(6). Its complex molecular structure and co-formulation with other antivirals increase analytical complexity, necessitating highly selective methods such as LC-MS/MS for accurate quantification and stability assessment (7).

Need for Stability-Indicating Analytical Methods: Stability-indicating analytical methods are essential to evaluate the behavior of pharmaceutical products under various environmental stress conditions. According to ICH Q1A(R2) guidelines, drugs must be

Table 1. Comparative Profile of Ledipasvir, Sofosbuvir, and Voxilaprevir with LC-MS/MS Considerations

Parameter	Ledipasvir	Sofosbuvir	Voxilaprevir
Drug class	Direct-acting antiviral (DAA)	Direct-acting antiviral (DAA)	Direct-acting antiviral (DAA)
Target protein	NS5A	NS5B RNA-dependent RNA polymerase	NS3/4A protease
Mechanism of action	Inhibits NS5A, blocking viral replication and assembly	Nucleotide analogue inhibiting NS5B, leading to RNA chain termination	Inhibits NS3/4A protease, preventing viral polyprotein processing
Therapeutic role	Viral replication suppression	Backbone antiviral agent	Resistance-barrier agent in salvage therapy
HCV genotype coverage	1, 4, 5, 6	Pan-genotypic (1-6)	Pan-genotypic (1-6)
Dose (in FDC)	90 mg once daily	400 mg once daily	100 mg once daily
Major degradation pathways	Hydrolytic and oxidative degradation	Hydrolytic cleavage of phosphoramidate moiety	Oxidative and photolytic degradation
Stability-indicating LC-MS/MS relevance	LC-MS/MS enables sensitive separation of intact drug from NS5A-related degradation products under stress conditions	LC-MS/MS is essential for distinguishing parent drug from active/inactive metabolites and degradation products	LC-MS/MS allows selective detection of protease inhibitor and its oxidative degradants with high specificity
Typical ionization mode	ESI positive	ESI positive	ESI positive
Analytical significance	Requires high sensitivity due to strong protein binding	Metabolite profiling demands high selectivity	Complex structure necessitates robust stability-indicating method
Regulatory importance	Supports forced degradation and stability studies as per ICH guidelines	Ensures accurate quantification during shelf-life studies	Confirms method specificity in presence of degradants

Table 2. Typical LC-MS/MS Instrumentation Used for Stability-Indicating Analysis of Direct-Acting Antivirals

Instrument Component	Specification / Description	Relevance to Stability-Indicating Analysis
Liquid chromatography system	UHPLC/HPLC equipped with quaternary pump, autosampler, column oven	Ensures high-resolution separation of parent drugs from degradation products
Analytical column	C18 reverse-phase column (e.g., 50-150 mm × 2.1 mm, 1.7-5 µm)	Provides adequate retention and peak shape for DAAs and degradants
Mobile phase	Volatile buffers (e.g., 0.1% formic acid or ammonium formate) with acetonitrile or methanol	MS-compatible; enhances ionization efficiency
Ionization source	Electrospray ionization (ESI), positive mode	Suitable for polar to moderately lipophilic antiviral agents
Mass analyzer	Triple quadrupole (QqQ) mass spectrometer	Enables selective and sensitive MRM transitions
Detection mode	Multiple Reaction Monitoring (MRM)	Allows specific quantification in presence of degradation products
Collision gas	Nitrogen or argon	Facilitates controlled fragmentation for product ion formation
Data acquisition software	Vendor-specific (e.g., Analyst®, MassLynx®, Xcalibur®)	Supports method validation, forced degradation, and stability studies
Autosampler temperature control	4-10 °C (optional)	Prevents autosampler-induced degradation during analysis
System compliance	ICH Q1A(R2), Q2(R1) compatible	Required for regulatory stability-indicating methods

Table 3. Comparative Summary of Reported Analytical Methods for Direct-Acting Antivirals Used in HCV Therapy [22]

S. No.	Drug(s) Analyzed	Analytical Technique	Matrix	Stability-Indicating Capability	Major Findings	Key Limitations
1	Ledipasvir, Sofosbuvir	UV Spectrophotometry	Bulk, tablets	No	Simple and economical method for routine analysis	Poor specificity; unsuitable for degradation studies
2	Ledipasvir, Sofosbuvir	RP-HPLC	Bulk, dosage forms	Partial	Good linearity and precision as per ICH	Limited sensitivity for degradants
3	Sofosbuvir	HPLC	Bulk drug	Yes	Identified degradation under acidic and oxidative stress	Single-drug focus
4	Voxilaprevir	RP-HPLC	Bulk drug	Yes	Evaluated forced degradation behavior	Not applicable to combination products
5	Ledipasvir, Sofosbuvir	LC-MS/MS	Plasma	No	High sensitivity and selectivity for bioanalysis	Lacks stability indication
6	Sofosbuvir, Voxilaprevir	LC-MS/MS	Plasma	No	Suitable for pharmacokinetic studies	Not validated for dosage forms
7	Sofosbuvir, Velpatasvir, Voxilaprevir	LC-MS/MS	Plasma	Partial	Simultaneous estimation achieved	Limited forced degradation data
8	Individual DAAs	LC-MS/MS	Bulk drug	Yes	Detailed degradation profiling	Absence of multi-drug assessment

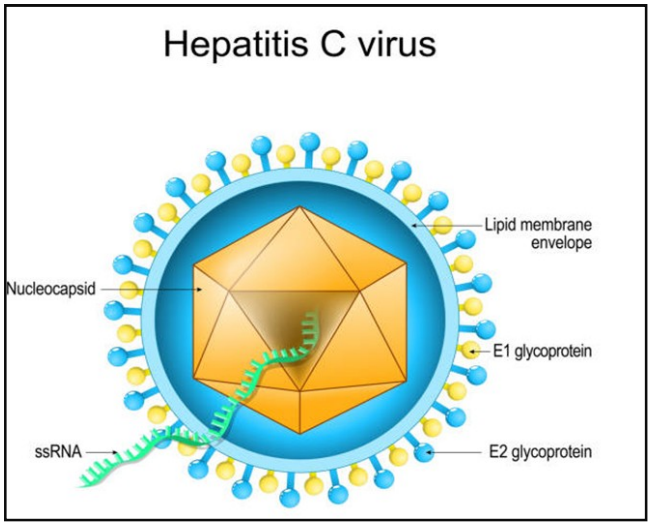

Figure 1. Hepatitis C virus (HCV)

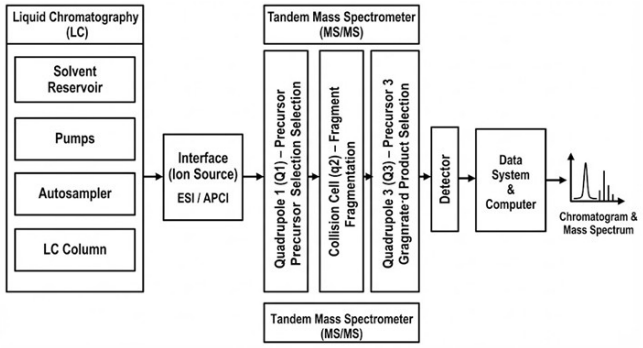

Figure 2. LC–MS/MS (Liquid Chromatography with Tandem Mass Spectrometry)

Role of LC-MS/MS in Antiviral Analysis: LC-MS/MS integrates chromatographic separation with mass-based detection, providing superior sensitivity and selectivity compared to conventional analytical techniques. The use of multiple reaction monitoring enables accurate quantification of analytes even at trace levels and facilitates identification of degradation products. These attributes make LC-MS/MS particularly suitable for multi-component antiviral formulations and stability-indicating studies required for regulatory compliance (11-14).

Review of Reported Analytical Methods: A wide range of analytical techniques has been reported for the estimation of direct-acting antivirals used in HCV therapy. Earlier investigations predominantly relied on UV spectrophotometric and reverse-phase high-performance liquid chromatographic (RP-HPLC) methods due to their simplicity and cost-effectiveness. Although these techniques are suitable for routine quality control, their application is limited when simultaneous estimation and impurity profiling are required, particularly in fixed-dose combinations (15-18). With increasing regulatory emphasis on stability-indicating capability, LC-MS/MS-based methods have gained substantial attention. These methods offer superior sensitivity, enhanced selectivity, and improved resolution of analytes from degradation products and excipients. Several studies have demonstrated the successful application of LC-MS/MS for individual or dual antiviral combinations in bulk drugs, pharmaceutical dosage forms, and biological matrices. However, only a limited number of investigations have addressed comprehensive forced degradation behavior and simultaneous estimation of Ledipasvir, Sofosbuvir, and Voxilaprevir within a single analytical framework. This highlights a significant analytical gap and justifies the need for further research in this area (19,20). "LC-MS/MS instrumentation, typically comprising a UHPLC system coupled with a triple quadrupole mass spectrometer operated in positive ESI and MRM mode, provides high sensitivity and selectivity for stability-indicating analysis of direct-acting antivirals, enabling reliable detection of drugs and their degradation products (21).

Forced Degradation and Stability Studies: Forced degradation studies are performed to assess the intrinsic stability of drug substances and formulations under stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic environments. These studies aid in identifying degradation pathways and establishing stability-indicating capability. DAAs have demonstrated variable degradation behavior depending on chemical structure and stress condition, and LC-MS/MS has proven effective in detecting and characterizing the resulting degradation products (23).

Method Validation Parameters: Method validation ensures the reliability and suitability of analytical procedures for their intended purpose. Reported LC-MS/MS methods for DAAs have demonstrated compliance with ICH Q2 guidelines, including acceptable specificity, linearity, accuracy, precision, robustness, and sensitivity. The low limits of detection and quantification achievable with LC-MS/MS further support its applicability for stability and impurity analysis (24).

Research Gaps and Future Perspectives Despite extensive research, there remains a lack of single, comprehensive LC-MS/MS methods capable of simultaneous estimation and complete stability evaluation of Ledipasvir, Sofosbuvir, and Voxilaprevir. Future studies should focus on quality-by-design-based method development, detailed degradation pathway elucidation, and application to finished dosage forms to strengthen regulatory and quality control frameworks (24,25).

CONCLUSION

The present review highlights the critical role of LC-MS/MS in the stability-indicating analysis of direct-acting antivirals used in HCV therapy. Compared to conventional analytical techniques, LC-MS/MS offers superior sensitivity, selectivity, and capability to identify degradation products, making it a preferred tool for regulatory-compliant quality control. Continued advancement in analytical methodologies will further enhance the assurance of safety, efficacy, and quality of antiviral pharmaceutical products.

REFERENCES

1. World Health Organization. Hepatitis C: Key facts. Geneva: WHO; 2023.
2. Pawlotsky JM. Hepatitis C virus resistance to direct-acting antiviral drugs in interferon-free regimens. Gastroenterology. 2016;151(1):70-86.
3. Afdhal N, Reddy KR, Nelson DR, et al. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. N Engl J Med. 2014;370(16):1483-1493.
4. Gane EJ, Shiffman ML, Etzkorn K, et al. Efficacy of sofosbuvir, velpatasvir, and voxilaprevir for HCV genotype 1-6 infection (POLARIS-2 trial). Lancet. 2017;390(10003):59-68.
5. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability-indicating methods - A review. J Pharm Anal. 2014;4(3):159-165.
6. International Council for Harmonisation. ICH Q1A(R2): Stability testing of new drug substances and products. Geneva: ICH; 2003.
7. International Council for Harmonisation. ICH Q2(R2): Validation of analytical procedures. Geneva: ICH; 2023.
8. Niessen WMA. Liquid chromatography-mass spectrometry: General principles and instrumentation. J Chromatogr A. 2013;1000:413-436.
9. Xu RN, Fan L, Rieser MJ, El-Shourbagy TA. Recent advances in high-throughput quantitative bioanalysis by LC-MS/MS. J Pharm Biomed Anal. 2007;44(2):342-355.

10. Gupta A, Kaur R, Sharma S. Stability-indicating HPLC method for simultaneous estimation of Ledipasvir and Sofosbuvir. J Pharm Biomed Sci. 2020;10:31-36.
11. El-Bagary RI, Elkady EF, Ayoub BM. LC-MS/MS method for simultaneous determination of Ledipasvir and Sofosbuvir in plasma. Biomed Chromatogr. 2019;33(2):e4372.
12. Vuppala S, Dahiya R, Bala R. Method development and validation of sofosbuvir, velpatasvir, and voxilaprevir by LC-MS/MS. Biomed Chromatogr. 2021;35:e5098.
13. Aggarwal A, et al. Forced degradation and method validation for Voxilaprevir. Anal Chem Lett. 2021;11(4):524-532.
14. Verma S, et al. Validated stability-indicating HPLC method for Sofosbuvir and Voxilaprevir. J Pharm Res. 2021;15(3):153-160.
15. Gupta A, et al. Stability-indicating HPLC method for Ledipasvir and Sofosbuvir. Asian J Chem. 2018;30(12):2691-2696.
16. Rana A, et al. Stability testing of Sofosbuvir by HPLC and LC-MS/MS. J Pharm Biomed Anal. 2018;158:45-51.
17. Ahmed S, et al. Simultaneous HPLC determination of Ledipasvir and Sofosbuvir in pharmaceutical dosage forms. J Chromatogr B. 2017;1060:374-380.
18. Zamek-Gliszczynski MJ, et al. Pharmacokinetics of Ledipasvir and Sofosbuvir. Antimicrob Agents Chemother. 2015;59(12):7128-7136.
19. Khan M, et al. Degradation profiling of Ledipasvir using LC-MS/MS. J Anal Chem. 2022;77(6):802-812.
20. Kumar V, et al. Forced degradation and LC method development for Voxilaprevir. Curr Pharm Anal. 2022;18(4):309-317.
21. Mehta H, et al. Comparative study of HPLC and LC-MS/MS for antiviral analysis. Int J Pharm Sci Res. 2021;12(2):752-759.
22. Joshi A, et al. Stability-indicating method for combination antivirals. World J Pharm Pharm Sci. 2021;10(6):1300-1310.
23. Rajput H, et al. Method development for HCV antivirals in bulk and dosage forms. Res J Pharm Technol. 2021;14(11):5912-5918.
24. Patel N, et al. LC-MS/MS method for simultaneous quantification of direct-acting antivirals. Chromatographia. 2020;83(10):1221-1230.
25. Fernandes R, et al. RP-HPLC method validation for Sofosbuvir and Ledipasvir. Asian J Pharm. 2020;14(3):425-432.

****