Pharmacology & Toxicology Research
https://acadpub.com/ptr

From Targeted LC-MS/MS to High-Resolution Mass Spectrometry: Emerging Analytical Strategies for the Characterization of Antiviral and Antibacterial Drugs, Impurities and Degradation Products: A Critical Review

Niloufer Tasnim Khazi, Kumara Swamy Gandla

Department of Pharmacy, Chaitanya Deemed to be University, Gandipet, Hyderabad 500075, Telangana, India

About the Article

Open Access

Review Article

How to Cite:
Khazi NT, Gandla KS. From targeted LC-MS/MS to high-resolution mass spectrometry: emerging analytical strategies for the characterization of antiviral and antibacterial drugs, impurities and degradation products: A critical review. *Pharmacol Toxicol Res.* 2026;2:41-54.

Keywords:
Antibacterial drugs, antiviral drugs, degradation products, forced degradation, high-resolution mass spectrometry, impurities, LC-MS/MS, orbitrap, pharmaceutical analysis, QTOF, UHPLC, UPLC-MS/MS

Corresponding author:
Kumara Swamy Gandla,
Department of Pharmacy, Chaitanya Deemed to be University, Gandipet, Hyderabad 500075, Telangana, India

ABSTRACT

Antiviral and antibacterial medicines present a particularly broad analytical challenge. The same laboratory may need to quantify a known impurity at trace levels, investigate an unexpected peak arising during stability studies and distinguish a genuine degradation product from an adduct, isomer, or source-generated fragment. Targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS), particularly triple-quadrupole multiple-reaction monitoring (MRM), remains highly effective for the sensitive and selective quantification of predefined analytes. In contrast, high-resolution mass spectrometry (HRMS) provides complementary capabilities through accurate-mass full-scan measurements and product-ion data, which can be interrogated when the composition of an impurity is not known in advance. This review examines how these complementary capabilities can be integrated into the analysis of antiviral and antibacterial drug substances and pharmaceutical products. Recent studies published between 2022 and 2026 are considered alongside established analytical principles, with particular emphasis on forced degradation, impurity origin, chromatographic selectivity, ionization behaviour, fragmentation evidence, isomeric ambiguity, matrix effects and the level of evidence required to support structural assignments. Recent examples in antiviral drug analysis include Maillard-reaction impurities of acyclic nucleoside analogues, forced-degradation products of darunavir, impurities of peramivir, bictegravir-related substances and degradation products of umifenovir. An example from antibacterial drug analysis is the LC-HRMS/MS characterization of dalbavancin degradation products. Collectively, these studies demonstrate that accurate-mass measurements are most informative when interpreted in conjunction with retention behaviour, dependence on stress conditions, isotope patterns, product-ion shifts and , where necessary, reference standards or orthogonal structural characterization techniques. Accordingly, this review considers targeted LC-MS/MS and LC-HRMS as complementary components of a fit-for-purpose analytical workflow rather than as competing platforms. The current principles outlined in ICH Q2(R2) and Q14 are also considered in the context of analytical procedure performance and lifecycle development. Emerging approaches, including data-independent acquisition, ion mobility spectrometry and computational spectral annotation, are discussed cautiously as complementary tools that may strengthen, rather than replace, expert interpretation. Overall, the available evidence supports an integrated analytical strategy in which HRMS is primarily employed to elucidate chemical identity and targeted LC-MS/MS is applied when sensitive, selective and robust routine quantification is required.

INTRODUCTION

The analytical complexity of anti-infective medicines is not determined solely by the number of compounds requiring measurement. Rather, it arises from the broad range of chemical behaviours represented within the same therapeutic

ACADEMIA Publications

field[1-3]. Nucleoside analogues may be highly polar, protease inhibitors are often larger and more lipophilic, fluoroquinolones contain multiple ionizable centres; beta-lactams may undergo degradation during sample handling; and macrolide or glycopeptide antibiotics can produce complex adduct and fragment patterns. Consequently, an analytical method that performs well for one drug class may be unsuitable for another. More importantly, a minor chromatographic peak cannot be interpreted solely on the basis of its abundance. Its origin may be associated with synthesis, formulation, storage, sample preparation, the chromatographic system, or the ion source itself.

LC-MS has therefore become important not only for assay and bioanalytical applications but also for the investigation of impurities and degradation products[1-3]. Triple-quadrupole LC-MS/MS is particularly powerful when the analytical question is predefined, as a precursor ion and one or more product ions can be monitored with high selectivity, enabling sensitive and quantitative determination of known compounds. However, this targeted acquisition strategy also represents its principal limitation. Unexpected degradants or process-related species may remain undetected when they are not included in the predefined transition list. The increasing application of accurate-mass instrumentation has addressed part of this limitation by providing access to full-scan data, isotope patterns and high-resolution product-ion spectra during impurity investigations.

Recent studies of anti-infective medicines illustrate this evolution in analytical practice. Research published since 2022 has employed mass spectrometry to investigate Maillard-reaction impurities in acyclic nucleoside antivirals, isolate and characterize darunavir degradation products, identify impurities of peramivir and bictegravir and characterize degradation products of the lipoglycopeptide dalbavancin[4-8]. A 2026 study of umifenovir further demonstrates that LC-HRMS continues to be an active and relevant approach for investigating antiviral stability. Although, these studies differ in instrumentation, analytical objectives and experimental depth, collectively they support an important analytical principle: molecular mass alone rarely provides sufficient evidence to resolve the chemically relevant question. Reliable structural interpretation is achieved by integrating chromatographic behaviour, accurate mass, fragmentation patterns, stress-response behaviour and orthogonal confirmation.

The central argument of this review is therefore deliberately focused. Targeted LC-MS/MS and LC-HRMS should be selected according to the specific analytical question and, where appropriate, applied sequentially[2,3,9]. HRMS is particularly well suited to impurity discovery and the generation of structural hypotheses, whereas targeted LC-MS/MS is generally more appropriate for routine quantitative control once the analyte has been adequately defined. This complementary division of analytical roles is particularly relevant to antiviral and antibacterial drug products because their impurity profiles may comprise process-related impurities, degradation products, excipient-derived reaction products and closely related structural or positional isomers.

The discussion is organized around the evidence required to progress from an observed LC-MS feature to a scientifically defensible analytical conclusion. It first examines the capabilities and limitations of targeted and high-resolution MS platforms, followed by consideration of the chromatographic and ionization factors that influence the generation and interpretation of spectral evidence. Drug-class-specific examples are then discussed in conjunction with forced-degradation strategies, structural confirmation approaches, quantitative translation and regulatory lifecycle considerations. This organization is consistent with the science- and risk-based principles for analytical procedure development described in ICH Q14 and the performance-oriented validation framework established in ICH Q2(R2)[10,11].

Scope and literature-review strategy: This review is a critical narrative assessment of LC-MS/MS and LC-HRMS approaches applied to antiviral and antibacterial drug substances, drug products, impurities and degradation products. The literature base was updated with particular emphasis on publications from 2022 through 2026, while earlier studies were retained where they provide foundational analytical concepts or methodological principles[1-3,12-14]. Literature searches incorporated terms such as LC-MS/MS, LC-HRMS, QTOF, Orbitrap, antiviral, antibacterial, antibiotic, impurity, degradation product, forced degradation and fragmentation. Priority was given to peer-reviewed studies providing interpretable evidence regarding impurity origin, structural characterization, stress behaviour, or translation of qualitative findings into quantitative analysis. The review is not intended to constitute a systematic review and does not claim exhaustive coverage of every drug-specific analytical method. Instead, recent examples are used to identify recurring analytical challenges and transferable methodological solutions.

Throughout this review, structural identification is considered a graded conclusion rather than a binary designation. Accurate-mass measurement can constrain the possible elemental composition of an unknown species but cannot, by itself, establish molecular connectivity or stereochemistry[1,13]. Product-ion data provide additional structural information; however, positional and stereochemical isomers may remain indistinguishable. For impurities that have implications for specification setting, safety assessment, or mechanistic interpretation, a higher level of structural evidence may therefore be required, including coelution with an authentic reference standard,

isolation followed by NMR analysis, chemical synthesis, or application of another orthogonal analytical technique. This distinction is particularly important because the high information density of modern HRMS datasets may otherwise encourage a degree of structural certainty that is not fully supported by the available experimental evidence.

Analytical continuum: From targeted LC-MS/MS to LC-HRMS

Targeted triple-quadrupole LC-MS/MS: A triple-quadrupole mass spectrometer is optimized for hypothesis-driven analytical measurement. The first quadrupole selects the precursor ion, the collision cell generates product ions and the third quadrupole filters one or more diagnostic product ions. In multiple-reaction monitoring (MRM), the selected precursor/product-ion pair serves as a highly selective analytical coordinate. This configuration is particularly well suited to the validated quantification of known active pharmaceutical ingredients, metabolites, genotoxic impurities, degradation products, or process-related impurities when authentic or well-characterized reference materials are available[2,3,9].

The principal strengths of triple-quadrupole LC-MS/MS include high sensitivity, excellent selectivity, a broad linear dynamic range, relatively straightforward quantitative validation and well-established workflows for internal-standard correction[2,3,15]. Its principal limitation, however, is the intentionally narrow nature of the acquisition strategy. A compound that does not correspond to a programmed precursor/product-ion transition may remain analytically undetected. Although, scheduled MRM facilitates greater multiplexing and more efficient use of the instrument, it remains dependent on prior knowledge of retention times and appropriate transitions.

QTOF and Orbitrap HRMS: HRMS substantially increases the information content of an LC-MS experiment. QTOF instruments combine quadrupole-based precursor-ion selection with time-of-flight accurate-mass measurement, whereas Orbitrap analyzers determine the mass-to-charge ratio (m/z) from ion oscillation frequencies within an electrostatic field. Both platforms can provide accurate precursor and product-ion masses that support constrained elemental-composition assignment when calibration accuracy, isotope patterns, adduct states and chemical plausibility are considered. Fourier-transform-based platforms can provide very high resolving power, facilitating the discrimination of near-isobaric signals and more confident elemental-formula filtering[2,3,9].

For impurity discovery, the major advantage of HRMS is the availability of full-scan data. Stress-treated samples can be compared with unstressed controls without relying on a predefined transition list[1,2,9]. Features that increase following acid, base, oxidative, thermal, humidity, or photolytic stress can consequently be identified as potential degradation products. Accurate-mass differences may suggest chemical transformations such as oxidation, dealkylation, hydrolysis, dehydration, decarboxylation, or adduct formation. However, such assignments should be regarded as structural hypotheses until they are supported by product-ion fragmentation data and evaluated within the relevant chemical context.

Why the platforms are complementary?: As summarized in Fig. 1 and Table 1, the two platforms address different stages of the same analytical problem. HRMS is particularly valuable when the objective is to determine what chemical species have emerged or changed in a stressed or otherwise altered sample. In contrast, targeted LC-MS/MS becomes particularly advantageous once a critical species has been adequately characterized and must be quantified reproducibly across routine samples. The transition between these analytical stages should therefore be determined by the intended analytical purpose rather than by instrument availability.

Table 1: Fit-for-purpose comparison of targeted LC-MS/MS and LC-HRMS

Attribute	Triple-quadrupole LC–MS/MS	QTOF-HRMS	Orbitrap-HRMS	Implication for impurity control
Primary strength	Targeted quantification	Accurate-mass screening +MS/MS	High resolving power +accurate mass	Use discovery and control as linked stages
Typical acquisition	MRM/SRM	Full scan, DDA/DIA, targeted MS/MS	Full scan, ddMS²/DIA/PRM	Match acquisition to analytical question
Unknown detection	Limited	Strong	Strong	HRMS preferred for unexpected impurities
Quantitative sensitivity	Excellent	Good to excellent	Good to excellent	QQQ remains highly efficient for routine trace quantification
Retrospective analysis	Very limited	Strong	Strong	HRMS data can be re-mined when new impurity hypotheses arise
Structural confidence	Transition-specific, limited for unknowns	Accurate precursor +fragments	Accurate precursor +fragments	Neither replaces standards/NMR for definitive isomer assignment
Method transfer	Mature and focused	Data-rich, platform -dependent	Data-rich, platform -dependent	Targeted methods often easier for QC deployment

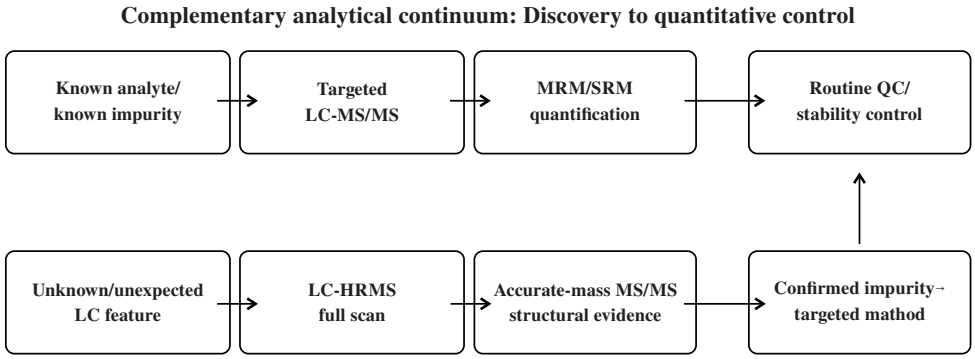

Fig. 1: Complementary use of LC-HRMS for investigation and targeted LC-MS/MS for quantitative control

A full-scan feature supported only by tentative structural evidence should not automatically be converted into a quantitative target. Conversely, an impurity that has been adequately characterized does not necessarily require continued full-scan acquisition during routine quality control. Thus, targeted LC-MS/MS and LC-HRMS are best regarded as complementary components of an analytical continuum, with each platform selected according to the level of structural information and quantitative assurance required.

LC front end: Chromatography determines the quality of the MS answer: Mass spectrometric resolving power cannot fully compensate for inadequate chromatographic separation. Isomeric impurities may produce identical or highly similar precursor and product ions, while co-elution can exacerbate ion suppression. In addition, in-source fragments may be incorrectly interpreted as distinct degradation products when their chromatographic relationships with the parent compound are not considered. Consequently, UHPLC/UPLC remains a critical component of the impurity characterization workflow[1,2].

Reversed-phase C18 chemistry is a widely used starting point; however, anti-infective drugs often require alternative chromatographic approaches[1,2]. Polar nucleos(t)ide analogues may exhibit weak retention under conventional reversed-phase conditions and may therefore benefit from polar-embedded phases, hydrophilic interaction liquid chromatography (HILIC), or mixed-mode separation strategies. Basic protease inhibitors may exhibit peak tailing because of interactions with residual silanol groups. Fluoroquinolones can interact with metallic surfaces and exhibit strongly pH-dependent retention because of their multiple ionizable functional groups. Large glycopeptide molecules may require wider-pore stationary phases and careful control of column temperature.

Mobile phases used for MS detection should be volatile and compatible with the selected ionization process[1,2]. Formic acid, acetic acid, ammonium formate and ammonium acetate are commonly employed additives. Nonvolatile phosphate buffers are generally unsuitable for direct coupling to LC-MS interfaces because of their potential to accumulate within the ion source. Strong ion-pairing reagents can improve the retention of highly polar analytes but may contaminate the ion source and suppress ionization. When their use is unavoidable, dedicated fluidic components and rigorous post-analysis washing procedures should be considered.

Chromatographic selectivity should be deliberately challenged during impurity characterization[1,2]. A method that produces a single apparently pure chromatographic peak may nevertheless conceal co-eluting isomers or degradation products. Orthogonal stationary phases, controlled changes in mobile-phase pH, temperature variations and alternative organic modifiers can therefore be applied strategically during characterization, even when the final routine quality-control method employs a simpler chromatographic configuration.

Ionization and fragmentation as structural evidence

Electrospray ionization and adduct chemistry: Electrospray ionization (ESI) is a dominant interface for many anti-infective small molecules because it accommodates ionic and moderately polar analytes and can be readily coupled with reversed-phase or HILIC separations[2,3]. Positive-ion mode often provides favourable responses for protonatable amines, whereas negative-ion mode may be advantageous for analytes containing acidic functional groups. However, ionization polarity should be evaluated empirically rather than inferred solely from the therapeutic class. Sodium, ammonium and solvent adducts may coexist with protonated or deprotonated molecular ions, while multimeric species may also occur, particularly at elevated analyte concentrations.

A recurrent error in mass-spectral interpretation is to regard every observed m/z value as representing a unique molecular species[1,2]. Adduct deconvolution, isotope-pattern evaluation and correlation with chromatographic retention

behaviour are therefore essential for reliable interpretation. Ideally, a candidate impurity should exhibit a coherent group of related ion species that can be consistently explained by a single neutral molecular composition.

Collision-induced dissociation: Product-ion spectra are most informative when interpreted comparatively[1,12]. The spectrum of the parent drug can provide a reference fragmentation map that identifies reproducible bond cleavages, characteristic neutral losses, charge-retaining substructures and diagnostic product ions. When a degradation product retains an unchanged region of the parent molecule, the corresponding fragment ions should remain consistent, whereas fragments containing the chemically modified region should exhibit the expected mass shift. This principle of "fragment-shift logic" can provide stronger structural support than assigning a structure solely on the basis of a spectral-library match.

Collision energy should generally be evaluated across an appropriate range[1,2]. Lower collision energies may preserve precursor-related structural information, whereas higher energies can generate smaller and potentially more diagnostic substructures. Application of a single collision energy may result in insufficient fragmentation for one analyte and excessive fragmentation for another. Stepped or collision-energy-ramped acquisition is therefore particularly valuable for HRMS-based structural characterization because it can provide complementary fragmentation information across different energy regimes.

Confidence hierarchy for impurity identification: Building on previously published principles for reporting identification confidence[13], Figure 2 and table 2presents the proposed five-level confidence hierarchy for pharmaceutical impurity assignments. Level 1 requires confirmation using an authentic reference standard that demonstrates matching chromatographic retention and MS/MS behaviour under the same analytical conditions. Level 2 represents a probable structural assignment supported by accurate precursor mass, isotope-pattern consistency, diagnostic accurate-mass product ions and a chemically plausible formation pathway. Level 3 denotes a tentative candidate structure or structural class supported by partial fragmentation evidence but subject to unresolved positional or stereochemical ambiguity. Level 4 represents an assigned molecular formula or mass-difference feature for which the available evidence is insufficient to establish structural localization. Level 5 corresponds to an unresolved analytical feature for which no reliable structural assignment can be made. Explicit reporting according to this hierarchy helps prevent the common overinterpretation of a species being "detected" as equivalent to being definitively "identified."

Table 2: Proposed five-level confidence hierarchy for pharmaceutical impurity assignments

Level	Assignment	Minimum evidence	Recommended use
1	Confirmed	Reference standard; retention + MS/MS match	Regulatory/routine quantitative control
2	Probable	Accurate mass+isotope+ diagnostic fragments +plausible pathway	Characterization; prioritize standard synthesis/isolation if critical
3	Tentative structure/class	Partial fragment support; isomerism unresolved	Hypothesis generation
4	Formula/feature	Accurate mass and feature behavior only	Screening and tracking
5	Unknown	Reproducible chromatographic-MS feature	Further investigation

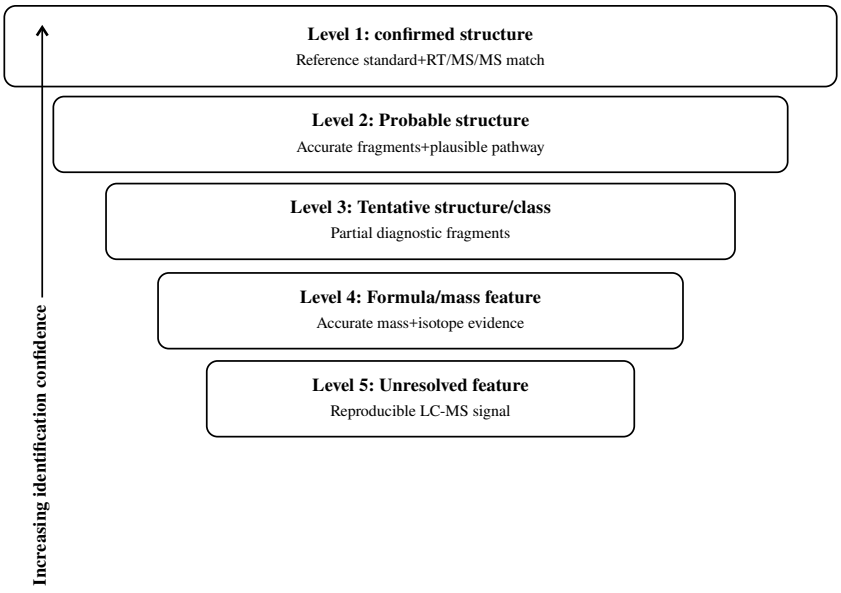

Fig. 2: Proposed confidence ladder for LC-MS-based pharmaceutical impurity identification

Antiviral drugs: Analytical opportunities and challenges

Nucleos(t)ide analogues and polymerase-directed agents: Nucleoside and nucleotide analogues frequently contain multiple heteroatoms, ionizable functional groups and highly polar structural motifs[4,12]. Consequently, their chromatographic retention can be challenging, while their fragmentation behaviour may involve glycosidic bond cleavage, loss of sugar-related moieties, phosphate-associated neutral losses in phosphorylated species and heterocycle-specific product ions. During stability investigations, hydrolysis and oxidation may generate degradation products with apparently straightforward nominal mass differences, yet their precise structural or positional identities can remain difficult to establish without detailed MS/MS analysis or orthogonal structural evidence. For these compounds, HRMS is particularly valuable when degradation pathways generate multiple isomeric products[1,2,13]. Accurate mass can constrain the possible elemental composition but chromatographic separation and diagnostic product ions are generally required to localize the site of chemical transformation. Once confirmed, critical degradation products can subsequently be monitored sensitively in stability samples using targeted MRM.

HIV protease inhibitors and integrase inhibitors: Protease inhibitors, including ritonavir-class compounds, are generally larger and more lipophilic than many nucleoside analogues and can generate extensive product-ion spectra[12]. Their multiple heteroatom-rich functional groups provide several potential protonation sites, making collision-energy-dependent fragmentation particularly important for structural interpretation. Published tandem-MS studies of HIV antivirals demonstrate that precursor/product-ion information originally developed for selected-reaction monitoring (SRM) can also facilitate the interpretation of metabolites and degradation products. This dual application illustrates a central premise of the present review: Quantitative transitions can also represent valuable structural knowledge assets.

Integrase inhibitors and combination products introduce additional analytical complexity because simultaneous assays may need to resolve multiple active pharmaceutical ingredients, excipient-related interferences and structurally related impurities[2,7]. The HRMS can assist in determining whether an emerging chromatographic feature is derived from the drug molecule, whereas a targeted multi-analyte method can subsequently be developed once the critical impurities have been characterized.

Direct-acting antivirals and combination therapies: Direct-acting antivirals used for the treatment of hepatitis viruses often possess relatively high molecular weights, multiple stereocenters and diverse functional groups[12,16]. Their degradation can produce closely related products whose chromatographic and mass-spectral discrimination may be challenging. Combination therapies further expand the analytical search space because one active component may influence the stability, extraction efficiency, or ionization response of another. A robust impurity-characterization program should therefore, where feasible, incorporate individually stressed components, stressed placebo, stressed combination samples and appropriate blank controls.

Recent reviews of antiviral impurity analysis confirm the continuing importance of impurity control within this therapeutic area[16], while broader analytical reviews demonstrate that antiviral drug analysis encompasses a range of techniques, including UV spectrophotometry, HPLC, UPLC and LC-MS/MS. Accordingly, the value of a contemporary review lies not simply in cataloguing existing analytical methods but in explaining how targeted and high-resolution MS approaches can be integrated to support structural characterization and regulatory decision-making. The literature also reveals a recurring ambiguity in the use of the term "identified by LC-MS," which can encompass substantially different levels of structural certainty[1,13]. An observed molecular ion consistent with a proposed elemental formula does not provide the same level of evidence as a structure supported by diagnostic product ions and an authentic reference standard. Similarly, such evidence is distinct from structural confirmation based on isolation followed by NMR analysis. Recent studies are particularly informative when they explicitly distinguish among these levels of evidence. For future investigations of antiviral impurities, reporting the confidence level associated with each proposed structure would improve comparability of findings across laboratories, analytical platforms and study designs.

Recent publications provide valuable case studies because they encompass structurally diverse antiviral compounds and varying levels of structural evidence[4-7,17]. Lai et al.[4] investigated Maillard-reaction products formed between lactose and five acyclic nucleoside antiviral drugs and reported previously uncharacterized impurities supported by purification and mass-spectrometric fragmentation data. During the same period, Modini and co-workers isolated forced-degradation products of darunavir and integrated UPLC-MS, HRMS and NMR analyses, demonstrating the increased confidence obtained when accurate-mass assignments are followed by isolation and orthogonal spectroscopic characterization. Research on peramivir published in 2023 combined forced-degradation studies with chromatographic separation and mass-spectrometric confirmation, whereas a 2023 investigation of bictegravir employed LC-MS to identify degradation-related species followed by development of a quantitative related-substances method. A 2026 study of umifenovir further extends this analytical approach by coupling

chromatographic separation with LC-HRMS for the characterization of stress-induced degradation impurities. Although these studies differ substantially in experimental design, instrumentation and depth of structural characterization, collectively they demonstrate a broader transition from simple molecular-mass confirmation toward integrated evidence chains linking stress conditions, chromatographic behaviour, accurate-mass data, fragmentation patterns and orthogonal structural evidence.

Point-wise review of recent antiviral impurity studies (2022-2026): Recent work on umifenovir[17] employed chromatographic separation coupled with LC-HRMS to characterize impurities generated under stress-degradation conditions. The study reflects the continuing shift toward accurate-mass analytical workflows for the characterization and assignment of antiviral degradation products. It also highlights the importance of distinguishing tentative MS-based structural proposals from assignments supported by stronger orthogonal evidence.

Ramakrishnan et al.[16] critically reviewed impurity analysis in antiviral drugs and emphasized the pharmaceutical importance of identifying, monitoring and controlling trace impurities generated during synthesis, formulation and storage. Their review supports the need for analytical strategies that integrate sensitive chromatographic separation with structurally informative detection techniques.

Kumar et al.[7] reported the determination and quantification of related substances and degradation products of bictegravir using an experimentally optimized HPLC method supported by mass spectrometric analysis. Their study is particularly relevant from a method-development perspective because it integrates chromatographic optimization with the assessment of impurities and degradation products, rather than treating MS-based characterization as an isolated analytical step.

Alumuri et al.[6] investigated the degradation of the neuraminidase inhibitor peramivir under acid, alkaline, oxidative, thermal and photolytic stress conditions and employed HPLC-MS to support the identification of the resulting impurities. Their findings reinforce the importance of stress-specific degradation studies for elucidating degradation pathways and establishing stability-indicating analytical strategies for antiviral compounds.

Modini et al.[5] investigated forced-degradation products of darunavir using a complementary analytical strategy incorporating UPLC-MS, preparative HPLC, HRMS, NMR and FT-IR. Their approach illustrates an important principle in pharmaceutical degradation research: LC-MS can facilitate the detection, localization and prioritization of degradation products, whereas orthogonal spectroscopic techniques may be necessary to achieve unequivocal structural assignment.

Lai et al.[4] investigated Maillard-reaction impurities associated with five acyclic nucleoside antiviral drugs. Their study combined impurity purification with mass-spectrometric characterization and demonstrated that formulation- or process-related reactions can generate structurally significant impurities that may not be adequately characterized by assay measurements alone. This work is particularly relevant to the present review because it demonstrates the value of MS-based structural evidence in comprehensive antiviral impurity profiling.

Antibacterial drugs: Class-specific considerations: The class-specific relationships considered in this section are summarized in Fig. 3.

β-lactams: β-Lactam antibiotics are intrinsically susceptible to hydrolysis because of the chemical reactivity associated with their strained β-lactam ring[1]. Consequently, sample-preparation conditions, diluent pH, autosampler temperature and analysis time can alter the impurity profile during

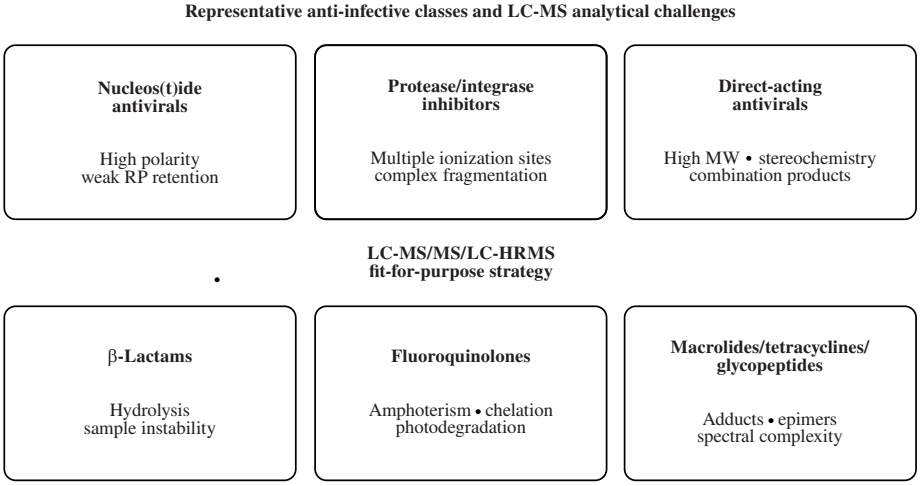

Fig. 3: Representative antiviral and antibacterial classes and their dominant LC-MS analytical challenges

measurement. An apparent "sample impurity" may therefore be generated during or after sample preparation rather than being present in the original material. The stability of analytical solutions should consequently be explicitly demonstrated, while rapid UHPLC separations can minimize residence time under potentially degradative conditions.

The HRMS characterization can be valuable for distinguishing hydrolytic products from secondary degradation or rearrangement products[1]. However, mechanistic plausibility remains essential for reliable structural interpretation. A mass increase consistent with water addition is insufficient to establish a unique molecular structure. Product-ion fragmentation and for critical impurities, isolation or comparison with an authentic reference standard may therefore be required to support a definitive assignment.

Fluoroquinolones: Fluoroquinolones contain both acidic and basic functional groups and can exhibit complex, pH-dependent chromatographic behaviour[1,2]. Chelation with trace metals may affect analyte recovery and chromatographic peak shape. Their aromatic and conjugated structural systems also make photodegradation analytically relevant. Accurate-mass product-ion spectra can assist in localizing structural modifications involving piperazinyl and related side chains, whereas targeted LC-MS/MS is well suited to trace-level quantification once diagnostically appropriate transitions have been established.

Macrolides and tetracyclines: Macrolides are high-molecular-weight, polyfunctional molecules that can generate abundant adducts and dehydration-related ions during electrospray ionization[1,2]. Tetracyclines contain multiple ionizable and metal-chelating functional groups and may undergo epimerization in addition to conventional degradation reactions. For both drug classes, reliable interpretation benefits from adequate chromatographic resolution of isomeric species and careful optimization of source conditions to minimize in-source transformations. Apparent degradation products that increase with increasing cone or source voltage should therefore initially be considered potential source-generated fragments until their chromatographic independence from the parent compound has been demonstrated.

Glycopeptides and lipoglycopeptides: Large glycopeptide antibacterials can generate information-rich yet analytically complex mass spectra because glycosidic, peptide-related and side-chain cleavages may occur simultaneously. Dalbavancin provides a recent example of this analytical challenge. Paritala et al[8]. separated the parent compound, its homologues and stress-generated products and employed LC-HRMS/MS to characterize seven degradation products formed under acidic, basic and oxidative conditions. Their study is particularly instructive because isomeric degradation products were observed under basic stress, demonstrating a limitation of accurate mass that is directly relevant to pharmaceutical impurity characterization. For such structurally complex compounds, HRMS is valuable for constraining the range of possible structures; however, chromatographic behaviour, diagnostic fragmentation and mechanistic plausibility remain essential for distinguishing closely related degradation products.

Selected recent antibacterial and broader impurity literature (2024-2026): Cordeiro et al.[18] reviewed impurities in active pharmaceutical ingredients and finished pharmaceutical products from a broader pharmaceutical-quality perspective. Their analysis provides important context for the present anti-infective-focused review by emphasizing that impurity origin, toxicological relevance and analytical control should be considered collectively rather than treating impurity detection as an endpoint in itself.

Rambhad et al.[19] reviewed the application of LC-MS to impurity profiling of drug substances and pharmaceutical products. Although, their review extends beyond anti-infective medicines, it is highly relevant to the present discussion because it situates targeted and high-resolution LC-MS approaches within contemporary pharmaceutical impurity-control practices and highlights the increasing analytical importance of hyphenated mass-spectrometric techniques.

Paritala et al.[8] characterized degradation products of the lipoglycopeptide dalbavancin using LC and LC-HRMS/MS following acidic, basic and oxidative stress. Seven degradation products were reported, including isomeric products generated under basic conditions and fragmentation pathways were evaluated to support structural interpretation. This study provides a strong antibacterial example of how HRMS/MS can extend a stability-indicating LC method beyond detection of additional chromatographic peaks toward mechanistic characterization of degradation products.

Forced degradation as an information-generation experiment: Forced degradation should not be regarded merely as a regulatory requirement or as a means of generating additional chromatographic peaks. When appropriately designed, stress studies constitute controlled chemical experiments that reveal the chemical vulnerability profile of a drug molecule[14]. Acidic and basic hydrolysis, oxidation, thermal exposure, photolysis and humidity stress can be selected according to the structural and chemical risks associated with the drug. The objective should be to generate interpretable degradation products without causing indiscriminate or excessive molecular destruction.

For LC-MS-based characterization, stress studies should incorporate time-course sampling together with appropriate

unstressed controls[1,14,20,21]. Time-course experiments can help distinguish primary from secondary degradation products. A primary degradation product may increase at early time points and subsequently decrease as secondary products emerge. Such temporal relationships provide valuable supporting evidence for proposed degradation pathways and structural assignments. Placebo and excipient controls can further assist in identifying adducts or formulation-derived reaction products. Where practical, mass-balance assessment and orthogonal detection should also be incorporated because differences in ionization efficiency between the parent compound and its degradation products may otherwise lead to misleading conclusions based solely on MS response.

A stability-indicating chromatographic method should demonstrate adequate separation of degradation products for its intended analytical purpose[10,11,14]. The HRMS can reveal potentially hidden co-elution by extracting exact-mass ion chromatograms from a single UV-detected peak. However, such findings should prompt chromatographic optimization when quantitative specificity is required, rather than being used to justify unresolved separation in routine quality-control analysis.

A proposed integrated LC-MS/MS/HRMS workflow:

- **Step 1:** Define the analytical target profile. Specify whether the analytical objective is impurity discovery, structural characterization, trace-level quantification, release testing, stability testing, or investigation of an out-of-specification result. The required sensitivity, selectivity and decision limits should be defined according to the intended analytical use[10,11]
- **Step 2:** Establish a chemically informative stress set. Generate unstressed, stressed, placebo-stressed and blank samples, as appropriate. Incorporate time-course sampling and avoid excessive degradation that could obscure interpretable primary pathways[14]
- **Step 3:** Develop MS-compatible chromatography. Prioritize chromatographic selectivity, adequate peak shape and orthogonality. Systematically evaluate relevant parameters, including mobile-phase pH, stationary-phase chemistry, organic modifier and column temperature[1,2].
- **Step 4:** Acquire full-scan HRMS data. Select the appropriate ionization polarity and acquire calibrated accurate-mass data with sufficient isotope information and dynamic range. Pooled or stressed quality-control injections should be included, where appropriate, to evaluate analytical repeatability[20,21]
- **Step 5:** Detect differential features. Compare stressed and control samples using retention time, accurate mass and changes in signal intensity. Background signals, solvent-related features and system-derived components should be evaluated and excluded where appropriate[20,21]
- **Step 6:** Acquire MS/MS evidence. Apply appropriate fragmentation strategies, including data-dependent acquisition (DDA), targeted MS/MS, parallel reaction monitoring (PRM), or data-independent acquisition (DIA). Stepped collision energies can provide complementary fragmentation information and the resulting product-ion spectra should be compared with those of the parent drug[1,2]
- **Step 7:** Rank structural confidence. Integrate elemental composition, isotope-pattern consistency, mass-defect changes, diagnostic product ions, chromatographic retention behaviour and chemically plausible degradation mechanisms. Positional and stereochemical isomers should not be assigned beyond the level supported by the available evidence[13]
- **Step 8:** Confirm critical impurities. Where toxicological or regulatory significance warrants a higher level of structural certainty, confirmation should involve authentic reference standards, co-injection or spiking experiments, chemical synthesis, isolation followed by NMR, or other orthogonal spectroscopic techniques[1,13]
- **Step 9:** Translate findings to targeted LC-MS/MS. Once critical impurities have been adequately characterized, select stable and specific precursor/product-ion transitions. Optimize collision energy and internal-standardization procedures and establish appropriate calibration, sensitivity, selectivity and carryover controls[2,15]
- **Step 10:** Validate and manage the analytical lifecycle. Validate the analytical procedure according to its intended use and maintain appropriate knowledge and control of critical analytical procedure parameters throughout the method lifecycle, consistent with the principles described in ICH Q2(R2) and Q14[10,11]

Quantification: Translating discovery into a routine MRM method: Once an impurity has been structurally characterized and determined to be analytically or pharmaceutically relevant, the analytical objective shifts from discovery to reliable quantitative measurement. The MRM transition should ideally employ a precursor ion that is sufficiently abundant and chemically representative of the analyte, together with a product ion that is both intense and structurally diagnostic. Where adequate sensitivity permits, a second qualifier transition can provide additional confirmation of analyte identity. Transition-ion ratios, retention time and chromatographic peak shape can further enhance analytical specificity[2,10].

Stable-isotope-labeled internal standards are preferred when available because they can compensate for variations arising from extraction losses, matrix effects and instrumental response[15]. For pharmaceutical drug-product assays, structurally related analogues may be used when

isotope-labeled standards are unavailable; however, equivalent recovery and ionization behaviour should not be assumed without experimental verification. The calibration range should be established according to the relevant specification, reporting threshold, or intended quantitative range rather than being determined solely by the instrumental capability.

Carryover requires particular attention when the Active Pharmaceutical Ingredient (API) is present at concentrations several orders of magnitude higher than the impurity of interest. Source contamination, autosampler carryover and detector saturation can compromise the accuracy and reliability of trace-level impurity measurements[2,3]. Dilution integrity, optimized needle-wash procedures and strategically positioned blank injections should therefore be incorporated into method development and routine analytical sequences.

Matrix effects and sample preparation: Matrix effects, although frequently emphasized in bioanalytical applications, are equally relevant to complex pharmaceutical formulations. Excipients, surfactants, counterions and co-eluting degradation products can suppress or enhance electrospray ionization and consequently alter analytical response[15]. Established approaches for evaluating or mitigating these effects include post-column infusion, post-extraction spiking, matrix-matched calibration and the use of stable-isotope-labeled internal standards. A clean chromatographic baseline does not, by itself, demonstrate the absence of ion suppression or enhancement.

Sample preparation should be sufficiently simple to minimize analyte loss while providing adequate protection for the LC-MS system[2,15]. Simple dilution may be sufficient for pharmaceutical tablets containing readily soluble active ingredients, whereas protein precipitation, liquid-liquid extraction, or solid-phase extraction may be necessary for biological matrices. During impurity characterization, however, extensive sample cleanup may unintentionally remove or reduce the recovery of previously unknown species. Discovery workflows should therefore, where feasible, compare minimally processed extracts with more extensively cleaned extracts to assess the potential loss of relevant impurities.

Data-dependent, data-independent and targeted HRMS acquisition: Data-dependent acquisition (DDA) selects precursor ions according to their signal intensity or predefined inclusion and exclusion criteria. This approach generates readily interpretable precursor-specific product-ion spectra but may fail to adequately characterize low-abundance features when highly abundant ions repeatedly occupy the available acquisition duty cycle. Data-independent acquisition (DIA) fragments broader precursor-ion windows and can provide more comprehensive coverage, although the resulting datasets require more computationally demanding spectral deconvolution. Parallel reaction monitoring (PRM) combines targeted precursor selection with high-resolution product-ion detection and can therefore provide a useful bridge between discovery and structural confirmation[2,3,9].

For pharmaceutical impurity profiling, no single acquisition mode is universally optimal[2,3,21]. A practical strategy may involve initial full-scan screening, followed by inclusion-list DDA or targeted MS/MS acquisition for candidate impurities, with DIA employed when sample quantity is limited or broad fragmentation coverage is required. Acquisition parameters should also be designed in relation to chromatographic peak width, because excessive spectral complexity or insufficient acquisition speed can reduce the number of data points collected across narrow UHPLC peaks and consequently compromise quantitative reliability.

Regulatory and lifecycle perspective: ICH Q2(R2) extends analytical validation principles to accommodate a broader range of analytical technologies, whereas ICH Q14 establishes a science- and risk-based framework for analytical procedure development and introduces the analytical target profile as a technology-independent statement of the intended purpose and performance requirements[10,11]. These principles are highly compatible with LC-MS-based analytical workflows. The analytical target should therefore be defined before determining whether a triple-quadrupole mass spectrometer, QTOF, Orbitrap, or an orthogonal analytical technique is most appropriate.

A discovery-oriented HRMS procedure used to characterize an unknown impurity does not necessarily require the same validation strategy as a routine quantitative release-testing method. Conversely, once an LC-MS/MS procedure is used to control a specification, relevant performance characteristics, including specificity/selectivity, accuracy, precision, range, quantitation capability and robustness, should be established according to the intended analytical use[10,11]. The analytical control strategy should therefore clearly distinguish among exploratory characterization, confirmatory structural identification and validated quantitative determination.

Impurity control also intersects with broader ICH guidance addressing organic impurities and mutagenic or genotoxic risk[22-24]. Analytical sensitivity should consequently be determined by the applicable reporting, identification, qualification, or toxicological threshold rather than by instrument sensitivity alone. The analytical procedure should be sufficiently sensitive and selective to support the relevant regulatory and scientific decision-making requirements. Common failure modes in LC-MS impurity characterization is presented in Table 3.

Table 3: Common failure modes in LC-MS impurity characterization

Failure mode	Why it is problematic	Corrective strategy
Structure assigned from exact mass alone	Many isomers share a formula	Require diagnostic MS/MS, pathway evidence and, where critical, standard/NMR confirmation
In-source fragment called a degradant	Source energy can create product-like ions	Check chromatographic independence and source- voltage dependence
Adduct treated as separate impurity	Na /NH /solvent adducts inflate feature count	Adduct grouping and neutral-mass reconciliation
DDA misses low-level impurity	Top-N selection favors abundant ions	Use inclusion lists, targeted MS/MS, PRM or DIA
Co-elution ignored because HRMS resolves m/z	Isomers can share exact mass and fragments	Improve chromatography or use orthogonal separation/ion mobility
Parent API overload masks impurity	Dynamic range and ion suppression problems	Dilution, divert valve, alternate injection, targeted enrichment
No stressed blank/placebo	System and excipient features misassigned	Run matched controls
Only one stress time point	Primary/secondary relationships lost	Use time-course stress design

Emerging technologies

Ion mobility and collision cross section: Ion mobility spectrometry introduces an additional gas-phase separation dimension based on the size, shape and charge of ions. For isomeric or conformationally distinct impurities, ion mobility behaviour and Collision Cross Section (CCS) measurements can provide orthogonal structural evidence beyond mass-to-charge ratio (m/z) and chromatographic retention time[25]. The principal value of ion mobility in pharmaceutical analysis is therefore not simply the generation of an additional analytical parameter but its potential to resolve species that are insufficiently differentiated by LC-MS alone and to establish reproducible physicochemical descriptors for future comparison and identification.

In-silico fragmentation and spectral libraries: Rule-based and machine-learning-based fragmentation tools can assist in ranking candidate structures and predicting plausible product ions[13,21]. Their role, however, should remain one of decision support rather than autonomous structural confirmation. Pharmaceutical impurities present a particular challenge because the actual degradation or process-related product may not be represented in publicly available spectral libraries. Parent-drug-aware transformation strategies, which first predict chemically plausible structural modifications and subsequently evaluate the observed fragmentation behaviour, may therefore provide greater utility than unconstrained database searching.

Artificial intelligence and automated interpretation: The major bottleneck in contemporary HRMS-based impurity characterization is increasingly computational rather than instrumental. Modern HRMS platforms can generate substantially more candidate features and mass spectra than can be manually evaluated within a practical analytical workflow[3,21]. Artificial intelligence (AI)-assisted approaches may facilitate prioritization of stress-responsive features, recognition of adduct groups, prediction of plausible transformation pathways, ranking of elemental-formula candidates and identification of spectra requiring expert review. Recent developments in untargeted LC-HRMS have also explored the prediction of eluting features based on chromatographic sequence context, illustrating the expanding role of machine-learning approaches in both data acquisition and spectral annotation. Nevertheless, application within regulated pharmaceutical analysis will require transparent evidence trails, version-controlled computational models, predefined acceptance criteria and human-review checkpoints to ensure traceability and scientific accountability.

Non-target and suspect screening: Suspect screening provides a useful intermediate strategy between targeted analysis and fully non-targeted workflows. A suspect list may include known process-related impurities, predicted hydrolysis and oxidation products, previously reported degradation products, metabolites, potentially relevant extractables and leachables and structurally related compounds. Accurate-mass full-scan data can subsequently be screened retrospectively against an evolving knowledge base[2,9,21,26]. Non-targeted screening can then be applied to features that fall outside the predefined suspect list, thereby reducing the risk that the analytical knowledge base becomes fixed at the time of method development or validation.

Research gaps and opportunities:

- **Limited cross-class knowledge:** Anti-infective impurity investigations often remain focused on individual drug substances, while transferable fragmentation rules and reusable chemical-transformation libraries spanning multiple drug classes remain insufficiently developed.
- **Inconsistent structural-confidence reporting:** Many published studies report tentative structural assignments without applying an explicit confidence hierarchy, making it difficult to compare the strength and quality of evidence across studies.
- **Limited platform comparisons:** Systematic head-to-head comparisons of triple-quadrupole (QQQ), QTOF and Orbitrap workflows using the same pharmaceutical impurity sets remain limited
- **Limited standardization of DIA:** Data-independent acquisition (DIA) remains less standardized for pharmaceutical impurity characterization and control than it is in fields such as omits and environmental analysis

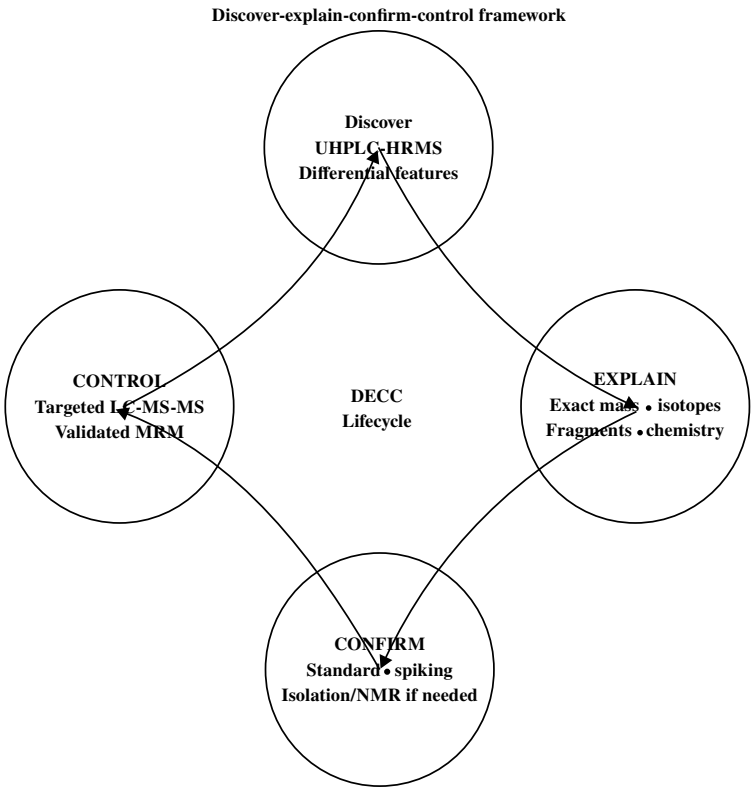

Fig. 4: Discover-explain-confirm-control (DECC) framework proposed for integrated LC-HRMS characterization and targeted LC-MS/MS control

- **Incomplete spectral-library coverage:** Publicly accessible spectral libraries provide incomplete coverage of pharmaceutical degradation products and process-related impurities, limiting the effectiveness of library-based annotation.
- **Incomplete assessment of analytical greenness:** Green analytical metrics rarely account comprehensively for the energy requirements, computational burden and data-processing demands associated with high-resolution MS in addition to solvent consumption.
- **AI validation and auditability:** AI-assisted structural annotation requires transparent uncertainty estimation, reproducibility, validation and auditability before such approaches can be routinely implemented in regulated pharmaceutical analysis
- **Ion mobility and CCS standardization:** Further research is required to develop comprehensive ion-mobility and CCS reference libraries for pharmaceutical impurities and to establish the transferability and comparability of CCS measurements across different instrument platforms, operating conditions and laboratories

A Practical workflow: Discover, explain, confirm and control: A useful framework for describing this sequence is the four-stage process presented in Fig. 4: discovery, explanation, confirmation and control. Discovery involves identifying reproducible analytical features that differ between control samples and relevant stress conditions. Explanation examines whether accurate mass, isotope-pattern consistency, chromatographic retention behaviour, fragment shifts and chemically plausible degradation pathways collectively support a coherent structural hypothesis. Confirmation increases the level of structural certainty according to the significance and risk associated with the impurity, using targeted MS/MS, authentic reference standards, spiking experiments, chemical synthesis, isolation followed by NMR analysis, or another orthogonal analytical technique when justified. Control begins only after the analytical target has been sufficiently defined and translates the resulting knowledge into a fit-for-purpose quantitative procedure, often based on targeted LC-MS/MS.

This sequence is not intended to constitute a new regulatory classification. Rather, its purpose is practical: To distinguish analytical questions that are frequently compressed into a single statement of "identification." An analytical feature may be reproducibly detected without being structu rally assigned; a chemically plausible structure may remain unsupported by definitive evidence for positional or stereochemical identity and a confirmed impurity may ultimately require a different analytical procedure for routine quantitative control. Maintaining clear distinctions among these stages facilitates more transparent communication of the evidentiary boundaries of LC-MS-based investigations.

Future directions: The near-term development of anti-infective pharmaceutical analysis is likely to favour complementary use of analytical platforms rather than reliance on a single technology[3,9]. Triple-quadrupole systems remain highly efficient for high-throughput targeted quantification, whereas HRMS provides broader chemical visibility during analytical development, stability investigations and evaluation of unexpected chromatographic features. Continued improvements in the quantitative performance of HRMS may further narrow the distinction between these platforms; nevertheless, the selection of acquisition strategy should remain primarily driven by the analytical objective.

A greater opportunity may lie in data interpretation rather than further increases in instrumental resolving power[3,21]. Future computational tools may assist with adduct grouping, prioritization of stress-responsive features, elemental-formula filtering and product-ion annotation. However, automated structural assignments should retain an auditable evidence trail that allows the underlying data and decision process to be independently evaluated. In regulated pharmaceutical analysis, transparent uncertainty estimation, reproducibility and appropriate expert review are more important than simply maximizing the number of candidate structures generated by an algorithm.

Retrospective interrogation of full-scan data may also become increasingly valuable throughout the pharmaceutical product lifecycle. When manufacturing processes, formulations, or stability profiles change, previously acquired HRMS datasets may in some circumstances be re-examined to investigate newly recognized impurities[2,9]. Such retrospective analysis offers substantial practical value but its reliability depends on the quality of the original acquisition, associated metadata, mass calibration and comparability of the samples and analytical conditions. It should therefore be regarded as a valuable complementary capability rather than a replacement for a purpose-designed analytical investigation.

CONCLUSION

The literature reviewed in this article supports a measured conclusion: targeted LC-MS/MS and LC-HRMS are most effective when their respective strengths are matched to the different stages of pharmaceutical impurity analysis. Triple-quadrupole methods provide sensitive, selective and efficient quantitative measurement of predefined analytes. HRMS provides broader chemical information when an impurity or degradation product is not known in advance. Neither platform, however, eliminates the need for adequate chromatographic selectivity, appropriate controls, scientifically justified stress studies, or careful interpretation of mass-spectral fragmentation data.

Recent studies of antiviral and antibacterial medicines further demonstrate why structural claims should be proportional to the available evidence. Accurate precursor mass combined with chemically plausible product ions may support a strong structural hypothesis but positional or stereochemical isomers may require confirmation using authentic reference standards, isolation and NMR analysis, chemical synthesis, or another orthogonal analytical technique. Similarly, an impurity initially discovered using HRMS may ultimately be controlled more efficiently using targeted LC-MS/MS or a conventional chromatographic procedure. The analytical workflow should therefore be designed around the specific scientific or regulatory decision to be made rather than around the availability of the most sophisticated instrumentation. Further progress is likely to arise from improved integration of data acquisition and interpretation, including broader application of carefully designed DIA and ion-mobility experiments, expanded spectral resources for pharmaceutical degradation products and computational tools capable of reporting confidence levels rather than merely proposing candidate identities. These approaches are promising but their adoption in routine pharmaceutical quality control will depend on reproducibility, transparent validation, appropriate uncertainty assessment and clear alignment with the intended analytical purpose. For current practice, a staged workflow that combines HRMS-based investigation with fit-for-purpose targeted quantitative analysis remains a scientifically defensible and practically applicable strategy for the characterization and control of antiviral and antibacterial impurities.

REFERENCES

1. Narayanam M, Handa T, Sharma P, Jhajra S, Muthe PK, Dappili PK et al. Critical practical aspects in the application of liquid chromatography–mass spectrometric studies for the characterization of impurities and degradation products. *J Pharm Biomed Anal.* 2014;87:191-217.
2. Beccaria M, Cabooter D. Current developments in LC-MS for pharmaceutical analysis. *Analyst.* 2020;145(4):1129-1157.
3. Khalikova M, Jireš J, Horáček O, Douša M, Kučera R, Nováková L. What is the role of current mass spectrometry in pharmaceutical analysis? *Mass Spectrom Rev.* 2023;43(3):560-609.
4. Lai X, Chen X, Li M, Zhou Y, Xia B. Purification and mass spectrometry study of Maillard reaction impurities in five acyclic nucleoside antiviral drugs. *J Pharm Biomed Anal.* [Internet]. 2022;212. Available from: https://doi.org/10.1016/j.jpba.2022.114637
5. Modini AK, Ranga M, Puppala U, Kaliyapermal M, Geereddy MKR, Samineni R et al. Identification, isolation and structural characterization of novel forced degradation products of darunavir using advanced analytical techniques like UPLC–MS, Prep-HPLC, HRMS, NMR and FT-IR spectroscopy. *Chromatographia.* 2022;86:63-78.

6. Alumuri T, Merugu KS, Namburi LAA, Kurnool A, SaravanaVadivu A, Balasubramanian S. An antiviral drug-peramivir: Degradation and identification of impurities and the endorsement of an HPLC–MS method. *J AOAC Int*. 2023;106(5):1138-1144.
7. Kumar SRJ, Rao VK, Katari NK, Jyothi NS, Kowtharapu LP. Determination and quantification of related substances and degradation products in bictegravir by full factorial design evaluated HPLC and mass spectrometry. *Anal Methods*. 2023;15(10):1274-1285.
8. Paritala ST, Sharma N, Shah RP. A comprehensive study on the identification and characterization of degradation products of lipoglycopeptide dalbavancin using LC and LC-HRMS/MS. *J Pept Sci*. [Internet]. 2024;30(10). Available from: https://doi.org/10.1002/psc.3608
9. Kaufmann A. The current role of high-resolution mass spectrometry in food analysis. *Anal Bioanal Chem*. 2011;403:1233-1249.
10. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Validation of analytical procedures. Q2(R2). ICH Harmonised Guideline. International Council for Harmonisation; 2023. https://database.ich.org/sites/default/files/ICH_Q2%28R2%29_Guideline_2023_1130.pdf
11. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Analytical procedure development. Q14. ICH Harmonised Guideline. International Council for Harmonisation; 2023. https://database.ich.org/sites/default/files/ICH_Q14_Guideline_2023_1116.pdf
12. Niessen WMA. Tandem mass spectrometry of small-molecule antiviral drugs: 1. HIV-related antivirals. *Int J Mass Spectrom*. [Internet]. 2020;455. Available from: https://doi.org/10.1016/j.ijms.2020.116370
13. Schymanski EL, Jeon J, Gulde R, Fenner K, Ruff M, Singer HP et al. Identifying small molecules via high resolution mass spectrometry: Communicating confidence. *Environ Sci Technol*. 2014;48(4):2097-2098.
14. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs-A review. *J Pharm Anal*. 2014;4(3):159-165.
15. Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS. *Anal Chem*. 2003;75(13):3019-3030.
16. Ramakrishnan B, Raju JM, Thangavelu P, Mariappan E, Natarajan J, Selvaraj J. Significance of impurity analysis in antiviral drug: A critical review. *Curr Drug Res Revs*. 2025;17(3):394-411.
17. Somkuwar K, Sabale P, Sawale V, Ikhar R, Sabale V. Identification, separation of stress degradation impurities of antiviral drug umifenovir by UFLC and their structural elucidation using hyphenated technique LC-HRMS. *Anal Chem Lett*. 2026;16(2):131-147.
18. Cordeiro CF, Franco LL, Carvalho DT, Bonfilio R. Impurities in active pharmaceutical ingredients and drug products: A critical review. *Crit Revs Anal Chem*. 2024;56(1):55-75.
19. Rambhad SR, Dave H. A review on applications of liquid chromatography coupled with mass spectrometry in impurity profiling of drug substances and drug products. *Sep Sci Plus*. [Internet]. 2025;8(6). Available from: https://doi.org/10.1002/sscp.70073
20. Broadhurst D, Goodacre R, Reinke SN, Kuligowski J, Wilson ID, Lewis MR et al. Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. *Metabolomics*. [Internet]. 2018;14. Available from: https://doi.org/10.1007/s11306-018-1367-3
21. Broeckling CD, Beger RD, Cheng LL, Cumeras R, Cuthbertson DJ, Dasari S et al. Current practices in LC-MS untargeted metabolomics: A scoping review on the use of pooled quality control samples. *Anal Chem*. 2023;95(51):18645-18654.
22. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Impurities in new drug substances. Q3A(R2). ICH Harmonised Tripartite Guideline. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2006. https://database.ich.org/sites/default/files/Q3A%28R2%29%20Guideline.pdf
23. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Impurities in new drug products. Q3B(R2). Current Step 4 version. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2006. https://database.ich.org/sites/default/files/Q3B%28R2%29%20Guideline.pdf
24. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Application of the principles of the ICH M7 guideline to calculation of compound-specific acceptable intakes. Addendum to M7(R2). Final version. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; 2023. https://database.ich.org/sites/default/files/ICH_M7%28R2%29_Addendum_Step4_2023_0216_0.pdf
25. Kartowikromo KY, Olajide OE, Hamid AM. Collision cross section measurement and prediction methods in omics. *J Mass Spectrom*. [Internet]. 2023;58(9). Available from: https://doi.org/10.1002/jms.4973
26. Xia J, Jin Y, Hong Y, Huang X, Pan J, Sun K et al. Application of advanced high resolution mass spectrometric techniques for the analysis of losartan potassium drug substance degradation products: From nontargeted to targeted screening. *J Sep Sci*. [Internet]. 2024;47(23). Available from: https://doi.org/10.1002/jssc.70027