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REVIEW

Advanced LC-MS/HRMS Strategies and Relative Response Factor Approaches for Impurity Profiling of Asthma Inhalation Drugs: A Critical Review

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Asthma inhalation drug products deliver low doses directly to the lungs, demanding stringent control of impurities and degradation products. The chemical diversity of inhaled corticosteroids (ICS), β2-agonists (SABA/LABA) and muscarinic antagonists (LAMA), together with complex formulation/device systems (DPI, MDI, nebulizers), creates analytical challenges not fully addressed by general impurity profiling strategies. This review critically examines advanced LC-MS/HRMS strategies and relative response factor (RRF) approaches for impurity profiling and quantitation of asthma inhalation drugs and drug products, with emphasis on forced degradation design, inhalation-specific impurity risks and trace-level quantitation when impurity standards are unavailable. Literature on forced degradation, LC-MS/HRMS impurity identification, stability-indicating method development and RRF estimation for asthma inhalation APIs and combinations was assessed. Representative workflows and case studies were extracted, compared and synthesised to provide practical guidance for analytical development and quality control. HRMS-based workflows (QTOF and Orbitrap) enable high-confidence structural elucidation of unknown degradants, including oxidation, hydrolysis, photolysis and rearrangement products. Inhalation-specific risks include propellant/excipient interactions, humidity-driven solid-state transformations, extractables/leachables and container-closure induced degradation. RRF approaches using UV, CAD/ELS, CLND, NMR and MS-based normalisation strategies support impurity quantitation in the absence of reference standards, but require careful validation and uncertainty management. A targeted impurity profiling strategy for asthma inhalation products should integrate inhalation-relevant forced degradation design with orthogonal analytical workflows, including LC-MS/HRMS for identification and robust RRF-based quantitation frameworks. Future improvements are expected from predictive response modelling, standard-free quantitation and systematic integration of device formulation API degradation knowledge into analytical lifecycle management.

Keywords: Asthma inhalation, Impurity profiling, Forced degradation, LC-MS, HRMS, Relative response factor.

INTRODUCTION

Impurity profiling is a fundamental element of pharmaceutical analysis, supporting both product quality and patient safety by ensuring that impurities and degradation products are controlled within acceptable limits [1,2]. In asthma therapy, inhalation products present a unique analytical landscape, for example, delivered doses are low (often micrograms), exposure is localised to lung tissues and formulation/device design strongly influences stability and impurity formation pathways [3-5]. Consequently, impurity profiling for asthma inhalation drugs cannot be approached as a direct extension of oral solid dosage form strategies.

A key analytical challenge is the identification and quantitation of trace-level impurities. Many degradation products are formed at very low levels and reference standards for each impurity are often unavailable. These constraints necessitate the combined use of advanced analytical instrumentation, including high-resolution mass spectrometry and pragmatic quantitative approaches such as relative response factor (RRF) estimation [6-12].

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This review focuses on the intersection of three themes relevant to asthma inhalation products *viz*. (i) inhalation specific impurity risks and degradation mechanisms; (ii) advanced LC-MS/HRMS workflows for impurity identification and structure elucidation and (iii) RRF strategies for impurity quantitation when impurity standards are not available. The discussion covers inhaled corticosteroids (ICS), bronchodilators (SABA/LABA), muscarinic antagonists (LAMA) and widely used combination inhalers, with representative case studies and practical guidance for method development and validation [13-31].

Methodology: This review was conducted using a systematic approach to identify and synthesize literature relevant to impurity profiling in asthma inhalation drugs. The search strategy followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [32,33].

Search strategy and data sources: Searches were conducted across PubMed, Science Direct, Scopus and Google Scholar (2000-2024) using keywords: ("Asthma" OR "Inhalation") and ("Impurity Profiling" OR "Degradation") AND ("LC-MS/MS" OR "HRMS") and ("Relative Response Factor" OR "RRF").

Study selection: The initial search yielded 215 records. Following duplicate removal and title/abstract screening, 85 articles were assessed for eligibility. Ultimately, 65 primary research and review papers were included. Fig. 1 illustrates the PRISMA 2020 flow diagram for the literature selection process, including a total of 65 studies.

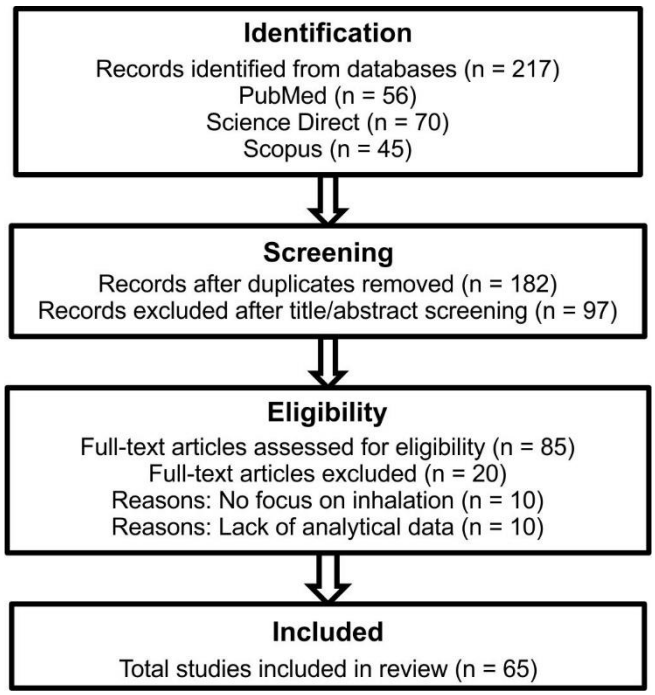

Fig. 1. PRISMA 2020 flow diagram illustrating the literature search, screening and inclusion process for the current review

Asthma inhalation drugs and formulation platforms

Therapeutic classes and representative APIs: Asthma pharmacotherapy commonly includes inhaled corticosteroids (ICS) as anti-inflammatory agents and bronchodilators such as β2 agonists and muscarinic antagonists. Table-1 summarizes the list of major asthma inhalation APIs and marketed combination products relevant to impurity profiling and forced degradation.

Formulation platforms: DPI, MDI and nebulisers: Dry powder inhalers (DPIs) are solid-state systems often involving lactose carriers and are strongly influenced by humidity, particle engineering and physical stability. Metered dose inhalers (MDIs) are pressurised systems using propellants and stability may be impacted by propellant chemistry, valve elastomers, canister materials and solvent systems. Nebuliser

TABLE-1
ASTHMA INHALATION APIS AND COMBINATION PRODUCTS RELEVANT TO IMPURITY PROFILING, FORCED DEGRADATION (FD) AND LC-MS/HRMS ANALYSIS

Therapeutic class	API/combination	Common inhalation platform(s)	Key impurity/degradation risks (typical)	Recommended analytical approach (best practice)
ICS	Budesonide	DPI, MDI, Nebuliser suspension	Oxidation, epimerisation/isomerisation, thermal degradants [34]	SI-UHPLC-PDA + LC-HRMS (QTOF/Orbitrap)
ICS	Fluticasone propionate	DPI, MDI	Ester-related hydrolysis, oxidative degradants, photolysis [35]	UHPLC-PDA + LC-MS/MS + HRMS confirmation
ICS	Fluticasone furoate	DPI	Ester cleavage, photolysis, low-level degradants [36]	UHPLC-PDA + LC-HRMS non-target screening
ICS	Beclometasone dipropionate	MDI	Ester hydrolysis (mono-/di-de-esterified forms), oxidation [37]	SI-RP-HPLC-PDA + LC-MS/MS
ICS	Mometasone furoate	DPI, MDI	Oxidation, hydrolysis, photodegradation [38]	FD + UHPLC-PDA + LC-MS
ICS	Ciclesonide	MDI	Hydrolysis, oxidative degradants, thermal stress products [39]	SI-HPLC + LC-MS for degradant ID
ICS	Hydrocortisone (less common inhaled)	Nebuliser (rare)	Hydrolysis/oxidation	HPLC-UV + LC-MS/MS (targeted)
SABA	Salbutamol (Albuterol)	MDI, Nebuliser solution	Oxidation, related substances, excipient interactions [40]	HILIC-LC/RP-LC + MS/MS (matrix-effect control)
SABA	Levosalbutamol	Nebuliser, MDI	Similar to salbutamol; stereochemical purity	Chiral LC (if needed) + LC-MS
SABA	Terbutaline	DPI/Neb (limited)	Oxidation, process impurities	RP-HPLC + LC-MS/MS

SAMA	Ipratropium bromide	MDI, Nebuliser	Hydrolysis, quaternary ammonium related substances	RP-LC + MS/MS + ion-pair caution
LABA	Formoterol fumarate	DPI, MDI	Oxidation, related substances, trace impurities [41]	SI-UHPLC-PDA + LC-MS/MS
LABA	Salmeterol xinafoate	DPI	Oxidation, salt-related impurities, long retention/adsorption [42]	UHPLC-PDA + LC-MS/MS (carryover control)
LABA	Vilanterol trifenatate	DPI	Trace degradants, oxidative stress sensitivity [43]	UHPLC-PDA + LC-HRMS (accurate mass)
LABA	Indacaterol maleate/acetate	DPI	Oxidation, process impurities	UHPLC-PDA + LC-MS/MS
LABA	Olodaterol (asthma/COPD overlap)	Respimat/inhalation solution	Oxidation, photolysis	LC-MS/MS + HRMS confirmation
LAMA	Tiotropium bromide	DPI	Hydrolysis (ester-like cleavage), related substances [44]	SI-RP-HPLC + LC-MS/MS
LAMA	Glycopyrronium bromide	DPI	Multiple related impurities, degradation under stress [45]	UHPLC-PDA + LC-MS/MS
LAMA	Umeclidinium bromide	DPI	Oxidation, hydrolysis, trace impurities	UHPLC-PDA + LC-HRMS
LAMA	Aclidinium bromide	DPI	Hydrolysis-sensitive; rapid degradation possible	Fast UHPLC + LC-MS/MS
LTRA (oral add-on)	Montelukast sodium (not inhaled)	Tablet (asthma add-on)	Photodegradation, oxidation	HPLC-PDA + LC-MS (supporting comparison)
Methylxanthine (oral)	Theophylline (not inhaled)	Tablet (older therapy)	Oxidation, related impurities	HPLC-UV + LC-MS
ICS/LABA combo	Budesonide/Formoterol	DPI, MDI	Dual-API impurity overlap; degradant co-elution [46]	SI-UHPLC-PDA + LC-MS/MS
ICS/LABA combo	Fluticasone propionate/Salmeterol	DPI	Peak overlap; FD complexity [47]	UHPLC-PDA + LC-HRMS (unknowns)
ICS/LABA combo	Fluticasone furoate/Vilanterol	DPI	Trace-level degradants; multi-API quantitation [48]	UHPLC-PDA + LC-HRMS + RRF strategy
ICS/LABA combo	Beclometasone/Formoterol	MDI	Ester hydrolysis + amine oxidation; formulation interactions	SI-RP-HPLC + LC-MS/MS
ICS/LABA combo	Mometasone/Formoterol	DPI/MDI	Multiple stress degradants; separation challenges	UHPLC-PDA + LC-MS/MS
LABA/LAMA combo	Indacaterol/Glycopyrronium	DPI	Dual-API impurity mapping; trace impurities	UHPLC-PDA + LC-MS/MS
LABA/LAMA combo	Vilanterol/Umeclidinium	DPI	Multi-component profiling; unknown impurity screening	UHPLC-PDA + LC-HRMS
LABA/LAMA combo	Olodaterol/Tiotropium	Inhalation solution	Oxidation + hydrolysis; matrix effects	LC-MS/MS + stability-indicating method
Triple therapy	Budesonide/glycopyrronium/ formoterol	DPI, MDI	Highly complex impurity quantitation; RRF need [49]	UHPLC-PDA + LC-HRMS + RRF validation
Triple therapy	Beclometasone/Formoterol/ glycopyrronium	MDI	Co-elution + stress degradants across 3 APIs	UHPLC-PDA + LC-MS/MS + HRMS
Triple therapy	Fluticasone furoate/vilanterol/ umeclidinium	DPI	Very low-dose impurities; unknown degradants	UHPLC-HRMS workflow + suspect screening
Rescue combo (rare)	Salbutamol/Ipratropium	Nebuliser/MDI	Mixed polarity; matrix interferences	HILIC/RP dual method + LC-MS/MS
Nebuliser steroid	Budesonide (neb suspension)	Nebuliser	Suspended particle stability; oxidative impurities	LC-MS/MS + particle/physical stability link
Nebuliser bronchodilator	Salbutamol (solution)	Nebuliser	Oxidation + preservative interactions	LC-UV + LC-MS/MS

formulations may be solutions or suspensions and are influenced by microbial control, oxidation and container interactions [50-54]. The analytical consequences include low dose quantitation demands, matrix complexity and the need to monitor both API-related substances and formulation/device derived impurities such as extractables and leachables [51-54].

Inhalation-specific impurity sources and degradation drivers

API-driven degradation mechanisms: Across asthma inhalation APIs, common degradation pathways include hydrolysis (particularly ester-containing corticosteroids), oxidation (including peroxide-mediated and metal-catalysed), photolysis and rearrangement or isomerisation [7,34,38]. The susceptibility of corticosteroids to oxidation and ester cleavage and of amine-containing bronchodilators to oxidative or nitrosative pathways, necessitates stress testing that is tailored to chemical structure.

Formulation and device-driven impurity sources: Table-2 summarises inhalation platform-specific impurity sources and outlines analytical control strategies for DPI, MDI and nebuliser products. Inhalation products are particularly

TABLE-2
INHALATION DOSAGE-FORM AND DEVICE-SPECIFIC IMPURITY SOURCES
(DPI *vs.* MDI *vs.* NEBULISER) AND ANALYTICAL CONTROL STRATEGY

Product platform	Key formulation components	Major impurity source(s)	Practical risk to impurity profile	Recommended analytical strategy
DPI	API + lactose carrier/engineered particles	Humidity uptake, excipient interaction, solid-state transitions	Formation of new degradants, physical instability leading to chemical instability	Solid-state FD + humidity stress + UHPLC-PDA + HRMS
DPI	Multi-API blends	Content uniformity + co-elution	Overlapping impurities and inaccurate quantitation	Orthogonal LC methods + HRMS confirmation + RRF
MDI	API + propellant (HFA) + ethanol/co-solvent	Container-closure interaction, valve elastomers, canister surfaces	Catalysed oxidation, leachables interference peaks	SI-UHPLC + HRMS non-target E&L screening
MDI	Suspension MDI	Particle settling + surface chemistry	Impurity variability due to non-uniform dose	Robust sampling + method precision + FD in matrix
MDI	Solution MDI	Propellant polarity + API solubility	Higher risk of chemical degradation in solution	Stability-indicating method + stress in formulation matrix
Nebuliser solution	API in aqueous buffer + preservatives	Oxidation, microbial preservative interactions	Increased oxidative degradants	LC-MS/MS with antioxidant control studies
Nebuliser suspension	API particles + surfactants	Physical instability affecting chemical stability	Impurity increase during storage	Combined physical + chemical stability monitoring
Device materials (general)	Plastics, seals, adhesives	Extractables/leachables	False positives, toxicity concerns	LC-HRMS screening + risk assessment + confirmation
Blister packs (DPI)	Foil + polymer laminate	Moisture ingress, adhesive leachables	Stress degradants + unknown peaks	Stability + HRMS suspect screening
Cleaning/processing	Manufacturing residues	Process impurities	Unexpected impurities at trace levels	LC-HRMS + impurity trending

sensitive to packaging and device materials. For MDIs, aluminum canisters and internal coatings can influence oxidative pathways. In one example, budesonide in solution formulated MDIs formed oxidation products linked to aluminium oxide surfaces in canisters [34]. Valve components, seals and plastics may contribute leachables. New propellants and co-solvents may introduce additional impurity risks [52-54].

Forced degradation design for asthma inhalation products: Forced degradation studies support stability indicating method development by generating degradation products under controlled stress conditions. For inhalation products, forced degradation must consider both API chemistry and formulation/device context, particularly for MDIs and DPIs where the microenvironment differs significantly from conventional tablets [6-8].

Common stress conditions: Typical forced degradation conditions include acidic and basic hydrolysis, oxidation (H_2O_2 or radical initiators), thermal stress, humidity stress (for solid-state) and photolysis [2,6]. The design should avoid excessive degradation that creates artefacts; commonly targeted degradation is 5-20% to allow meaningful impurity mapping [6].

Inhalation-specific forced degradation considerations: It require stress conditions tailored to device type and formulation characteristics. As shown in Table-3, moisture-driven degradation is particularly critical for DPIs, while propellant and co-solvent effects are more relevant for MDIs and formulation buffers and preservatives influence nebuliser systems. Container-closure interactions, excipient compatibility and light exposure further contribute to impurity formation across platforms. These factors emphasise that forced degradation studies for inhalation products should incorporate solid-state humidity stress, propellant compatibility testing, extractables/leachables screening and photostability evaluation, supported by orthogonal LC and LC-HRMS methods for the comprehensive impurity identification.

Critical evaluation of food degradation in inhalation products: While standard stress testing procedures are well-documented, inhalation products require a nuanced approach to food degradation design to ensure that the resulting impurity profile is both predictive and stability-indicating without introducing analytical bias.

Avoiding overstressing and secondary artifacts: A common pitfall in food degradation studies for low-dose inha-

TABLE-3
INHALATION-SPECIFIC IMPURITY SOURCES AND ANALYTICAL CONSIDERATIONS

Source	DPI	MDI	Nebuliser	Analytical strategy
Moisture/humidity	High impact (solid-state)	Moderate	Moderate	Solid-state FD + water activity control [51]
Propellant/co-solvent	Not applicable	High	Not applicable	Targeted screening + leachables studies [52]
Container material	Blister/foil interactions	Aluminium canister/valves	Plastic bottles	E&L screening by LC-HRMS [53]
Excipient interactions	Lactose, Mg stearate	Ethanol, surfactants	Buffers, preservatives	Orthogonal LC methods + HRMS ID [54]
Light exposure	Moderate	Moderate	Moderate	Photostability per ICH + HRMS [19]

lation products is the generation of overstressing artifacts compounds that do not form under real-time or accelerated conditions. Following the Rule of 5-20%, as observed in Table-4, the objective is to generate meaningful levels of primary degradation while maintaining the integrity of the API. The risk of generating overstressing artifacts in multi-API combinations like budesonide/glycopyrronium/formoterol (BGF) must be managed to maintain mass balance [55].

Degradation kinetics and predictive stability: Unlike oral solids, the degradation kinetics in inhalation products can be non-linear due to the high surface-area-to-volume ratio of micronised particles.

In DPI systems: Solid-state transformations and humidity driven kinetics are paramount. As summarised in Table-4, humidity stress is essential for hygroscopic blends like fluticasone furoate/vilanterol because moisture can catalyze local hydrolysis at the micro-scale before any bulk degradation is observed [56].

In MDI systems: Kinetics are often influenced by the canister surface and propellant polarity. Stress testing must evaluate the catalytic effect of metal canisters on oxidation pathways, which can differ significantly from the degradation observed in aqueous solution.

Formulation-specific food degradation design: As detailed in Table-4, the choice of stressor must align with the specific chemistry of the asthma API and its delivery matrix.

Oxidative pathways: Inhalation APIs like budesonide or umeclidinium are highly sensitive to oxidation. Using HRMS confirmation (QTOF/Orbitrap) allows for the differentiation between hydroxy/oxo derivatives and actual rearrangement isomers, which is critical for ensuring that the analytical method is truly stability-indicating [42,57].

Co-elution in multi-API systems: A critical analytical challenge in triple combinations (*e.g.* BGF) is the overlap of degradants from different APIs. This review advocates for the use of orthogonal LC-HRMS and peak purity checks to ensure that co-eluting degradants are not misidentified, a risk is heightened by the ultra-low concentration of the formoterol component relative to the budesonide matrix [55].

TABLE-4
FORCED DEGRADATION DESIGN AND MAJOR DEGRADATION PATHWAYS REPORTED/EXPECTED FOR ASTHMA INHALATION APIS (WITH LC-MS/HRMS CONFIRMATION STRATEGY)

API/combination	Stress condition(s) (typical)	Major degradation pathway(s)	Expected/common degradant types	Best LC-MS/HRMS confirmation approach
Budesonide	Oxidation, thermal, photolysis	Oxidation, rearrangement/ isomerisation	Hydroxy/oxo derivatives, epimers	UHPLC-PDA + LC-QTOF/Orbitrap full scan + ddMS2
Fluticasone propionate	Base hydrolysis, oxidation, photolysis	Ester cleavage, oxidation	De-esterified products, sulfoxides/oxidised forms	UHPLC-PDA + LC-MS/MS targeted + HRMS for unknowns
Fluticasone furoate	Acid/base hydrolysis, photolysis	Ester cleavage, photodegradation	Hydrolysed acid/alcohol forms, photoproducts	UHPLC-HRMS + MS/MS library matching + accurate mass
Beclometasone dipropionate	Hydrolysis, oxidation	Ester hydrolysis (stepwise)	Mono-de-esterified + fully de-esterified impurities	SI-HPLC-PDA + LC-MS/MS confirmation
Mometasone furoate	Acid/base/oxidation/UV	Hydrolysis + oxidation	Multiple degradants, oxidised products	UHPLC-PDA + LC-MS/MS structural fragments
Ciclesonide	Acid/base/thermal/photo	Hydrolysis + oxidation	New degradants + related impurities	RP-HPLC + LC-MS/MS (product ion scans)
Salbutamol	Oxidation, thermal	Oxidation	Quinone-like oxidised species, related substances	HILIC/RP-LC + LC-MS/MS (matrix-effect control)
Formoterol	Oxidation, photolysis	Oxidation/nitrosation risk	N-oxide, oxidative degradants	UHPLC-PDA + LC-MS/MS + nitrosamine screening if needed
Salmeterol	Oxidation, thermal	Oxidation	Hydroxy/oxo products, related substances	UHPLC-PDA + LC-MS/MS (carryover check)
Vilanterol	Oxidation, hydrolysis, photo	Oxidation + degradation	Multiple degradants, trace impurities	UHPLC + HRMS full scan + MS/MS elucidation
Indacaterol	Oxidation, photo	Oxidation	Oxidised impurities	UHPLC-PDA + LC-MS/MS
Tiotropium	Hydrolysis, oxidation	Hydrolysis/cleavage	Hydrolysed impurities, process impurities	UHPLC-PDA + LC-MS/MS
Glycopyrronium	Hydrolysis, oxidation	Degradation + related substances	Multiple impurities, oxidation products	UHPLC-PDA + LC-MS/MS + RRF strategy
Umeclidinium	Oxidation/hydrolysis	Oxidation	Oxidised degradants	UHPLC-PDA + LC-HRMS
Budesonide/ formoterol	Oxidation + thermal + humidity	Mixed multi-API degradation	Co-eluting degradants across both APIs	Orthogonal LC + HRMS + peak purity check
Fluticasone/ salmeterol	Hydrolysis + oxidation	Multi-component degradation	Overlapping impurities	UHPLC-PDA + LC-MS/MS + HRMS for unknown peaks
FF/Vilanterol	Hydrolysis + photo	Multi-API degradation	Trace degradants	UHPLC-HRMS + suspect screening + RRF
BUD/GLY/FOF (Triple)	Oxidation + thermal + photo	Highly complex degradation	Many trace degradants, co-elution	2D-LC (optional) + UHPLC-HRMS + RRF validation

Table-4 indicates a mismatch between broadly applied ICH stress conditions and the delivery platform dependent chemical sensitivity of asthma APIs. For instance, the transition from solution-phase stress to solid-state humidity stress for DPIs (*e.g.*, fluticasone furoate) highlights that 'purity' in inhalation is not just a chemical attribute but a physical one. Analysts must prioritize humidity-driven hydrolysis over thermal stress, as the high surface area of micronised particles in DPIs creates micro-environments where degradation kinetics bypass traditional Arrhenius predictions.

MDI systems: stress testing may require evaluation in propellant matrices and container closure systems, as degradation may be catalysed by canister surfaces or influenced by propellant polarity [34,52].

DPI systems: humidity stress and solid-state transformations are essential due to hygroscopic excipients and powder surface phenomena [51].

Combination inhalers: Multi-API systems require careful stress design because degradation products may overlap and interact and analytical separation must be demonstrated [46-49].

Critical framework for advancing inhalation impurity strategies: While existing literature extensively documents standard LC-MS and RRF protocols, this review proposes a specialised "Inhalation Integrated Risk Framework" (IIRF) to move the field from reactive testing to proactive safety design. This framework addresses the limitations of oral-centric ICH guidelines by integrating three novel analytical dimensions:

Local-to-systemic decision tree: Current practices treat all impurities equally if they fall below ICH thresholds. A critical decision tree is proposed where impurities are first screened for localised pulmonary reactivity before systemic toxicological assessment.

Concept: Even if an impurity is below the systemic identification threshold (*e.g.* 0.1%), if it possesses structural alerts for pulmonary irritation (*e.g.* specific aldehydes or reactive esters common in MDI propellants), it must undergo mandatory qualification.

Advancement: This shifts the industry standard from the "dose-dependent identification" to "tissue-specific risk identification".

Analytical quality by design (AQbD) for ultra-low doses: A methodological shift from the traditional validation to an AQbD-based lifecycle approach specifically for DPIs is also proposed.

Concept: Instead of static RRF values, dynamic RRF monitoring is proposed throughout the product lifecycle. Because the lactose-to-API ratio in DPIs can exceed 99:1, the minor shifts in instrument sensitivity or matrix interference can lead to a 20-30% error in trace-level quantification.

Advancement: Implementing an Analytical Target Profile (ATP) that accounts for matrix-induced suppression in HRMS ensures that trace-level actually remains safe-level.

Integration of new modalities: Beyond small molecules: A critical gap in existing reviews is the omission of inhaled biologics and RNA. Thus, incorporation of functional purity markers is recommended for the impurity profiling of next-generation inhaled therapies.

Concept: For inhaled RNA, the "impurity" is not just a chemical degradant but the loss of circularisation efficiency or secondary structure.

Advancement: Incorporating in-cell NMR as an orthogonal method in early-stage inhalation development can help evaluate the interaction of "biophysical impurities" with lung surfactant is not addressed by standard LC–MS workflows, which is essential.

LC-MS/HRMS workflows for impurity profiling

LC method development for impurity separation: The stability-indicating LC methods for inhalation products must separate API, process-related impurities, degradation products and excipient/device-related peaks. Gradient UHPLC with C_{18} or phenyl columns is commonly used for corticosteroids, while polar bronchodilators may require alternative stationary phases or ion-pairing considerations [13-18].

Critical evaluation of HRMS platforms and analytical constraints: While HRMS is indispensable for structural elucidation, its application to inhalation drug products involves managing specific analytical limitations related to mass accuracy, matrix effects and acquisition logic.

The trade-off between resolving power and acquisition speed in inhalation profiling is a critical hardware consideration [12,19]. While Orbitrap systems provide ultra-high resolution, QTOF platforms allow for faster duty cycles necessary for narrow UHPLC peaks [12].

Mass accuracy and resolving power constraints: The reliability of elemental composition assignment depends on both mass accuracy and resolving power. For complex inhalation matrices, a mass accuracy of < 2 ppm is typically required to minimize the number of potential molecular formulas. However, mass accuracy can drift during long UHPLC runs if internal calibration (lock-mass) is not utilised. Furthermore, the resolving power must be sufficient to distinguish between isobaric impurities. For instance, in triple-combination therapies (BGF), high resolution (*e.g.* >70,000 FWHM) is necessary to separate the isotopic envelopes of sulfur-containing degradants from other organic impurities that might otherwise co-elute or overlap in the mass spectrum [42,57].

Ion suppression and matrix effects: Inhalation products, particularly DPIs, present a significant challenge due to the high ratio of carrier excipients (*e.g.* lactose). During electrospray ionisation (ESI), these matrix components can cause ion suppression, where the ionisation efficiency of trace-level impurities is reduced in the presence of more abundant species.

Critical impact: Suppression can lead to the under-quantification of impurities if RRF-based methods are used without considering matrix effects.

Mitigation: As observed by Liigand *et al.* [20], the degree of suppression is highly dependent on the mobile phase composition. This review advocates for the use of diverted flow (sending early-eluting lactose peaks to waste) and the evaluation of matrix effects during method validation to ensure that "microgram-level" impurities remain detectable.

Ionisation mode suitability and acquisition strategies: The choice of ionisation mode and data acquisition strategy is a critical decision point in the impurity workflow:

Polarity switching: Most corticosteroids used in asthma (*e.g.* budesonide, fluticasone) ionize efficiently in positive ESI mode (forming $[M+H]^+$ or $[M+Na]^+$ adducts). However, certain acidic degradants may only be visible in Negative ESI mode. This review suggests that for "unknown" screening, dual-polarity acquisition is mandatory to ensure no degradants are missed.

Data-Dependent (DDA) *vs.* Data-Independent Acquisition (DIA): DDA is superior for targeted structural elucidation as it provides "clean" MS/MS spectra for the most abundant ions. DIA (*e.g.* all-ions fragmentation) is more effective for non-targeted screening of trace impurities, as it captures fragmentation data for all ions simultaneously. This ensures that even low-level impurities, which might not reach the intensity threshold to trigger a DDA event, are still recorded for retrospective analysis [19].

As shown in Table-5, the transition from guidance-based to decision-based HRMS selection is a prerequisite for robust inhalation drug development. The primary analytical risk in these products is not a lack of sensitivity, but the complexity of the data produced by ultra-trace analytes. For example, selecting a DIA approach (DIA-HRMS) over DDA is often necessary for DPIs because the intense signal from the lactose carrier can suppress or mask the precursor ion of a degradant, preventing the instrument from triggering a fragmentation event in standard DDA modes.

Case studies: Asthma inhalation drugs and impurity profiling: This section outlines representative LC-MS/HRMS approaches used for impurity profiling of inhalation drug products.

Budesonide solution MDI: A thermal food degradation strategy combined with LC-QTOFMS and HPLC-UV enabled impurity profiling of budesonide solution-formulated MDI. Ten impurities were identified and classified as process- or degradation-derived [34]. Oxidation products were linked to aluminium oxide catalysed aerobic oxidation in aluminium canisters [34].

Mometasone furoate: Forced degradation studies of mometasone furoate under acid/base/oxidative/thermal/photolytic stress have been used to develop stability-indicating RP-HPLC methods and confirm degradants by LC-MS [38].

Ciclesonide: Validated forced degradation studies for ciclesonide have demonstrated the formation of new impurities under multiple stress conditions, with LC-MS supporting identification and method specificity [39].

Fluticasone furoate/vilanterol DPI: Impurity profiling of Fluticasone furoate/vilanterol combinations has required robust chromatographic separation and detection of trace impurities. Stability-indicating HPLC-PDA methods have been developed and applied for impurity quantitation in DPIs [48].

Budesonide/glycopyrronium/gormoterol triple combination: Triple therapy inhalers introduce analytical complexity due to co-eluting impurities and multi-API quantitation requirements. Stability-indicating methods with RRF estimation have been proposed for this combination [49].

RRF strategies for impurity quantitation

RRF concept and practical requirements: When impurity standards are not available, quantitation often relies on RRFs. The RRF approach relates the impurity response to the API response under identical detection conditions, enabling approximate quantitation [58,59].

UV-based RRF: UV-based RRF estimation uses calibration slopes of impurity and API when both standards are available. In practice, for unknown degradants without standards, analysts may assume RRF = 1.0, but this introduces uncertainty, especially for structurally dissimilar impurities [58,59].

Universal detectors and orthogonal approaches: Orthogonal approaches using charged aerosol detection (CAD) provide a more uniform response for non-volatile analytes where UV chromophores may be absent [24,25]. NMR has also been used to determine RRFs without isolated impurity standards by providing molar quantitation based on proton signal integration [58].

Table-6 critically compares RRF estimation strategies for trace impurity quantitation including detector-specific strengths, limitations and recommended validation controls. The synthesis of RRF strategies in Table-6 highlights a significant regulatory risk: the RRF = 1.0 assumption. However, even a minor deviation in chromophore response can result in a 30-50% under-reporting of degradants. For inhalation products, where

TABLE-5
DECISION MATRIX FOR HRMS WORKFLOW SELECTION IN INHALATION IMPURITY PROFILING

Analytical objective	Recommended HRMS strategy	Instrument selection	Critical decision factor	Advantage for inhalation products
Non-targeted screening (NTS)	Data-independent acquisition (DIA/all-ions)	Q-TOF or orbitrap	Need for retrospective data: Captures fragmentation for all ions without intensity thresholds.	Ensures trace degradants in high-lactose matrices are not missed by DDA.
Structural elucidation of known unknowns	Data-dependent acquisition (DDA/ddMS2)	Orbitrap (ultra-high resolution)	Need for resolution: High FWHM (>100k) to distinguish isobaric impurities.	Essential for multi-API combinations (*e.g.*, BGF) with overlapping isotopic envelopes.
Trace quantitation & confirmation	Targeted MRM/PRM	Triple quadrupole or Q-orbitrap	Need for sensitivity: Lowest possible LOD/LOQ for safety-critical contaminants.	Ideal for monitoring known genotoxic impurities or nitrosamines at ppm levels.
Leachables & extractables (E&L)	Suspect screening (library matching)	HRMS with curated databases	Need for efficiency: Rapid matching against polymer/propellant additives.	Faster identification of device-related impurities *vs.* manual elucidation.
Isomer/epimer differentiation	Ion mobility-HRMS (IM-MS)	IM-QTOF	Need for orthogonality: Separation of species with identical mass but different shapes.	Critical for chiral APIs like Budesonide (epimer A/B) or Formoterol isomers.

TABLE-6
CRITICAL EVALUATION OF RRF ESTIMATION STRATEGIES AND REGULATORY VALIDATION CONTROLS

RRF strategy	Principal detector	How RRF is obtained	Critical strength	Technical limitation	Recommended validation/statistical control
True RRF (slope ratio)	UV/PDA	Ratio of calibration slopes (Impurity/API) using isolated standards.	Gold standard; highest quantitative accuracy for known impurities.	Requires high-purity standards (>95%) for each impurity.	RSD of slopes <5.0%; intercept bias check; verify stability in diluent.
Response factor normalisation	LC-MS (ESI)	Comparison of MS response of impurity *vs.* parent API or surrogate.	Extremely sensitive for trace-level (<0.05%) inhalation impurities.	Ionisation efficiency varies wildly with mobile phase composition.	Matrix effect evaluation; use of internal standards; ±20% uncertainty margin [20].
Universal detection (CAD/ELS)	CAD/ELSD	Mass-based response independent of chromophores.	Uniform response for non-volatile analytes; excellent for E&L.	Incompatible with volatile mobile phases; nonlinear at low concentrations.	Comparative RRF check against UV for chromophoric peaks; LOD/LOQ validation.
qNMR-derived RRF	High-field NMR	Molar quantification *via* proton signal integration.	Absolute quantification without isolated impurity standards.	Low sensitivity; requires high sample concentration (~mg/mL).	Signal-to-noise check; verification of peak overlap in proton spectra.
Predictive RRF (p-RRF)	HRMS + QSAR	Theoretical modeling based on molecular descriptors.	Allows early-stage assessment of "unknowns" during FD studies.	High uncertainty (15-30%); dependent on model training sets.	Verification against surrogate standards with similar structural motifs.
RRF = 1.0 assumption	UV/PDA	Direct area normalisation to API response.	Simple; no additional experimental effort required.	High risk of bias (under-reporting); structurally dependent.	Only acceptable for early screening; mandatory structural alert check.

dose accuracy is highly sensitive, this review recommends a mandatory "RRF robustness check" during method validation. If a degradant exhibits ionisation or UV-absorption characteristics different from the API, a surrogate standard or qNMR-derived correction factor should be applied to avoid underestimation and false acceptance of potentially toxic impurities.

MS-based RRF and response normalisation: The MS response depends strongly on ionisation efficiency and matrix effects. For MS-based quantitation, internal standards (including isotopically labelled standards) provide the most robust approach. However, in impurity profiling workflows, surrogate standards and response normalisation strategies are sometimes used when labelled standards are unavailable [9,10].

Quantitative analysis of RRF variability and statistical error: While RRFs are essential for quantifying impurities without reference standards, their application is governed by mathematical relationships that, if ignored, lead to significant reporting errors. The RRF of an impurity ($_i$) relative to the API ($_a$) is defined as:

$$RRF = \left[\frac{Slope_{impurity}}{Slope_{API}}\right] = \left[\frac{Area_i / Conc_i}{Area_a / Conc_a}\right]$$

Mathematical treatment of response variability: The reliability of an RRF depends on the linearity and intercept of the response curves. A critical but often overlooked factor is the y-intercept bias. If the API and impurity exhibit significantly different intercepts in their calibration models, the RRF will be concentration-dependent rather than a constant.

For inhalation products where impurities are measured at trace levels (near the LOQ), the relative error (Er) in impurity concentration (Ci) resulting from an incorrect RRF assumption (using $RRF_{assumed}$ instead of true RRF_{true}) can be expressed as:

$$\text{Relative error (Er, \%)} = \frac{RRF_{true} - RRF_{assumed}}{RRF_{assumed}} \times 100$$

Impact of incorrect RRF assumptions: A quantitative example: In inhalation drug development, analysts often default to an RRF of 1.0 when a standard is unavailable. Consider a degradant of Vilanterol that has lost a chromophore, resulting in a true UV-RRF of 0.5.

Scenario: If the true concentration is 0.2% w/w, but an RRF of 1.0 is assumed, the calculated concentration would be:

$$C_{calc} = \frac{Area_i}{Area_a \times RRF_{assumed}} = 0.1\%\ w/w$$

Thus, the impurity is under-reported by 50%, potentially falling below the ICH identification threshold (*e.g.* 0.15%) and evading necessary toxicological qualification despite being present at a level of concern for pulmonary safety.

Statistical error analysis and acceptance criteria: To mitigate these risks, this review proposes a rigorous statistical framework for RRF validation in inhalation filings.

RRF robustness: Statistical evaluation should include the relative standard deviation (RSD) of RRFs determined across different days and instrument setups. An RSD of >10% indicates that the RRF is not robust enough for regulatory submission [56].

Standard uncertainty (u): The uncertainty in the calculated impurity concentration should be derived from the combined uncertainties of the API response, the impurity area and the RRF itself. Following Liigand *et al.* [20], a reporting strategy using the upper confidence limit of impurity concentration is recommended when RRF values are obtained through predictive modeling (p-RRF), thereby ensuring a safety-focused approach for the respiratory route.

Quantitative uncertainty in RRF estimation is highly dependent on mobile phase composition and ionisation efficiency [20]. Using predictive modeling (p-RRF) introduces a calculated error margin of 15-30% [21], necessitating a conservative ±20% safety buffer for inhaled contaminants [20,55].

Comparative analysis of high-resolution platforms and RRF reliability

Performance benchmarking: QTOF *vs.* orbitrap in inhalation profiling: While both quadrupole time-of-flight (QTOF) and orbitrap systems are pivotal for HRMS workflows, their operational differences significantly impact the detection of trace-level impurities in the complex inhalation matrices such as lactose carriers or propellant-based aerosols.

Resolution and mass accuracy: Orbitrap systems typically offer higher resolving power (up to 500,000 FWHM), which is essential for resolving isobaric impurities in multi-API combinations (*e.g.* indacaterol/glycopyrronium/mometasone) [57]. QTOF systems, while generally offering lower resolution, provide faster acquisition rates, making them more suitable for high-throughput screening and complex gradient elution profiles where narrow chromatographic peaks are encountered [42].

Dynamic range and sensitivity: For the ultra-low doses typical of inhaled steroids, dynamic range is critical. Orbitraps provide excellent intra-scan dynamic range, facilitating the detection of trace degradants in the presence of high concentration API. Conversely, QTOF systems excel in duty cycle and "all-ions" MS/MS fragmentation, which is advantageous for non-targeted "unknown" identification in products like fluticasone furoate combinations [19,56].

Uncertainty and reliability of RRF-based quantification: The use of relative response factors (RRFs) is a standard regulatory practice when impurity standards are unavailable, particularly in multi-component DPIs [56]. However, their application in inhalation products introduces specific uncertainties that require critical evaluation:

Solvent and mobile phase dependency: RRF values are not absolute constants and are highly sensitive to pH and organic modifier concentrations. In inhalation profiling, where complex gradients are used to separate hydrophobic APIs from hydrophilic degradants, an RRF determined at one point in the gradient may not be accurate if the impurity elutes in a significantly different mobile phase composition [20].

Detector response linearity: The reliability of RRFs decreases at the trace levels (LOQ) required for inhalation safety. Studies on indacaterol and mometasone combinations highlight the need for validated RRFs to prevent impurity under estimation, with a ±20% uncertainty margin proposed as a threshold for RRF reliability [57].

Predictive RRF (p-RRF) limitations: Molecular descriptor based predictive RRFs (p-RRFs), derived from QSAR or related models, provide a useful approach for early-stage development but are associated with inherent uncertainties of ~15-30% [20,21]. Therefore, a conservative reporting strategy is recommended, whereby impurities quantified using uncertain RRF values for inhaled products are reported at their upper estimated concentrations to ensure safety at the pulmonary site.

Analytical method validation considerations: The validation of impurity methods for inhalation products must address specificity, sensitivity, accuracy, precision, linearity and robustness. Low-dose inhalation products require low LOQs and high confidence in impurity detection [2,6].

Future trends and research opportunities: Future directions include the (i) development of predictive ionisation response models for MS-based quantitation; (ii) implementation of standard-free quantitation strategies using HRMS and qNMR; (iii) improved integration of extractables and leachables assessment with impurity profiling, and (iv) adoption of digital analytical workflows with automated degradant annotation to enhance efficiency and reliability in impurity characterization [60-64]. To support the practical implementation, Table-7 presents an end-to-end workflow for impurity profiling and trace quantitation in asthma inhalation drug substances and products.

Proposal of critical framework: Inhalation integrated risk matrix: To move beyond conventional analytical compilations, this review introduces an inhalation integrated risk framework (IIRF) that shifts impurity assessment from identification toward risk-based prioritization, with particular emphasis on pulmonary-specific safety considerations not fully addressed by general ICH Q3A/B guidelines.

Route-specific identification decision tree: A shift from purely concentration-based thresholds to structural alert-based identification is proposed. In this framework, impurities in inhaled products are not disregarded solely based on low levels (*e.g.* < 0.1%); instead, any unknown species should be screened by HRMS for structural alerts linked to pulmonary sensitisation, such as isocyanates or reactive aldehydes, irrespective of concentration.

Methodological advance: This integrates toxicological predictive modelling directly into the analytical LC-MS workflow.

Unified RRF-HRMS workflow for ultra-low doses: The unified RRF-HRMS workflow for ultra-low dose inhalation products integrates impurity identification and quantification within a single analytical strategy. As outlined in Table-8, HRMS-based screening enables sensitive detection and structural assignment of trace impurities, while RRF estimation provides semi-quantitative evaluation when reference standards

TABLE-7
RECOMMENDED LC-MS/HRMS WORKFLOW FOR INHALATION IMPURITY PROFILING

Step	Purpose	Recommended tools
Stress design	Generate meaningful degradants	Acid/base/oxidation/thermal/photo/humidity
Screening LC	Separate impurities	UHPLC gradient, orthogonal columns
HRMS ID	Identify unknowns	QTOF/Orbitrap full scan + MS/MS
Quantitation	Estimate impurity levels	UV + RRF; CAD/qNMR; MS with IS
Reporting	Assign risk and control	Trending, uncertainty, lifecycle management

TABLE-8
RECOMMENDED END-TO-END ANALYTICAL WORKFLOW FOR IMPURITY PROFILING AND TRACE IMPURITY QUANTITATION OF ASTHMA INHALATION DRUG SUBSTANCES AND PRODUCTS USING LC-MS/HRMS AND RRF-BASED APPROACHES

Step No.	Workflow stage	Objective	Key experiments/actions	Recommended tools/techniques	Expected output/ decision points
1	Product understanding & risk assessment	Identify impurity risks specific to inhalation route	Review API structure, excipients, device/container, dose level, storage	QbD risk tools (Ishikawa/FMEA), prior knowledge	List of critical quality attributes (CQAs) and impurity risks
2	Forced degradation (FD) study design	Generate meaningful degradants for stability-indicating method	Acid/base hydrolysis, oxidation, thermal, photolysis; humidity for DPI; matrix stress for MDI	ICH-aligned stress design + inhalation-specific stress	Target 5-20% degradation without artefacts
3	Preliminary chromatographic screening	Select conditions to separate API + impurities	Column screening (C18/phenyl/HILIC), pH range, gradient optimisation	UHPLC/HPLC + PDA	Baseline separation feasibility + peak capacity
4	Stability-indicating method (SIAM) optimisation	Develop robust method for impurities	Optimise mobile phase, gradient, temp, injection, diluent, sample prep	UHPLC-PDA; optional CAD/ELSD	SI separation of API/ impurities; good peak shape and resolution
5	Peak purity and specificity confirmation	Prove method specificity under stress	Evaluate peak purity, co-elution, unknown peaks	PDA peak purity + LC-MS check	Confirm SI nature; flag unknown peaks for HRMS
6	LC-HRMS impurity identification (non-targeted)	Identify unknown impurities and degradants	Full-scan HRMS + MS/MS fragmentation; accurate mass; isotopic pattern	QTOF/Orbitrap HRMS, ddMS2/data-dependent MS/MS	Structural assignment proposal + elemental composition
7	Suspect screening & targeted confirmation	Confirm known impurities across asthma APIs	Use known impurity list and expected degradants	HRMS suspect list + targeted MS/MS	Confirm identity and reduce false positives
8	Impurity origin classification	Classify impurities as process-related, degradation, E&L	Compare FD samples *vs.* unstressed *vs*. placebo *vs*. packaging extracts	Comparative profiling + HRMS	Impurity source assignment and control strategy
9	Quantitation strategy selection	Choose quantitation route based on standard availability	Determine if impurity reference standard exists	Decision tree: UV-RRF/CAD/qNMR/MS-IS	Defined quantitation method with justification
10	RRF estimation (when standards unavailable)	Estimate impurity levels reliably	UV slope ratio (if possible), assumed RRF=1.0 (screening), CAD/CLND/ qNMR-assisted RRF	UV/PDA, CAD/ELSD, CLND, qNMR, LC-MS	RRF values with uncertainty and applicability limits
11	Method validation (ICH Q2 expectations)	Demonstrate method suitability for QC/stability	Specificity, LOQ, linearity, accuracy, precision, robustness, solution stability	ICH Q2-style validation protocol	Validated SI impurity method + reportable/quantifiable limits
12	Mass balance evaluation (FD & stability)	Confirm completeness of impurity accounting	Compare assay loss *vs*. total impurities	LC-PDA + CAD/ELSD (optional)	Improved mass balance confidence
13	Routine stability & release testing application	Apply method for DP release and stability monitoring	Analyse batches, stability timepoints, accelerated conditions	QC UHPLC-PDA + periodic HRMS	Trending of impurities; identify out-of-trend peaks
14	Change control & lifecycle management	Maintain method performance across product lifecycle	Column lot change, instrument change, formulation/device updates	Analytical lifecycle approach	Continuous verification; reduced regulatory risk
15	Regulatory reporting & toxicological assessment	Ensure compliance and patient safety	Evaluate impurity thresholds, qualification, inhalation exposure risk	ICH Q3A/Q3B/M7 alignment	Final impurity specifications + justification package

are unavailable. This combined approach is particularly valuable for complex DPI matrices such as API-lactose blends, where co-eluting low-level degradants may otherwise be under estimated. Integration of predictive RRF calculation, orthogonal detection (UV/CAD/HRMS) and validated decision criteria supports reliable quantitation, improved mass balance and risk based impurity control for ultra-low dose inhalation formulations.

Novel approach: By applying predicted p-RRFs derived from molecular descriptors during initial HRMS screening, semi-quantitative estimation of trace degradants can be achieved prior to synthesis of reference standards, enabling early safety assessment of newly identified impurities.

Life-cycle impurity mapping (LCIM): The concept of life-cycle impurity mapping is introduced, advocating the application of analytical quality by design (AQbD) to monitor the analytical target profile (ATP) of an inhaler as device components age. This critical evaluation highlights that impurity profiling must be a dynamic process where the interaction between the formulation and the device (leachables) is

re-validated at multiple time points, a practice not currently mandated by standard frameworks.

Conclusion

Impurity profiling for asthma inhalation drugs demands tailored analytical strategies that address low-dose delivery, formulation device complexity and diverse physico-chemical properties of inhaled APIs. Inhalation platforms such as DPIs, MDIs and nebuliser systems are influenced by the moisture sensitivity, excipient interactions, propellant or co-solvent effects, container–closure compatibility and extractables/leachables, all of which can alter degradation pathways and stability profiles across the product lifecycle. These platform-specific variables necessitate impurity control approaches designed specifically for inhalation products rather than adaptation from oral dosage forms. Advanced LC-MS/HRMS workflows provide high sensitivity, accurate-mass identification and structural elucidation of unknown degradants, particularly at trace levels or in co-eluting systems. High-resolution full-scan data with MS/MS fragmentation enables characterization of impurities arising from forced degradation, manufacturing processes and packaging interactions. RRF-based quantitation offers a practical approach when reference standards are unavailable, a common scenario in inhalation drug development. Integration of inhalation-relevant forced degradation design, orthogonal detection techniques and scientifically justified RRF strategies with rigorous validation supports reliable impurity profiling, accurate quantitation and regulatory-compliant stability assessment, strengthening lifecycle control and patient safety for current and next-generation asthma inhalation therapies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

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