Research Article

A Comprehensive Review of Analytical Methods for Linaclotide: From Conventional Chromatography to QbD-Based Approaches

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Abstract

Linaclotide is a disulfide-rich therapeutic peptide whose analytical characterization requires attention to chemical purity, structural integrity and degradation. Conventional reversed-phase high-performance liquid chromatography (RP-HPLC) supports quantitative determination, while high-resolution mass spectrometry and ion mobility extend the characterization of peptide-related impurities and conformational heterogeneity. This narrative review evaluates representative linaclotide-specific studies and distinguishes established analytical evidence from proposed analytical Quality-by-Design (AQbD) strategies. The discussion integrates intended use, chromatographic selectivity, stability-indicating capability, analytical validation, risk assessment, design of experiments and lifecycle management in the context of ICH Q14 and Q2(R2). Environmental sustainability is considered as a complementary objective rather than a substitute for analytical performance. An integrated conceptual framework is proposed for future method development. The available evidence supports complementary analytical platforms rather than a universal procedure; further comparative studies are needed to establish robust, transferable and environmentally responsible linaclotide methods.

Keywords: Linaclotide; RP-HPLC; Peptide Impurities; Stability-Indicating Analysis; Analytical Quality-by-Design; Analytical Validation; Green Analytical Chemistry.

1. Introduction

Therapeutic peptides present analytical questions that extend beyond the determination of drug content. Sequence integrity, chemical modifications, disulfide connectivity and conformational heterogeneity may affect product quality. Linaclotide, a synthetic 14-residue peptide with three disulfide bonds, provides a useful example of these challenges. Its pharmacological activity is mediated by guanylate cyclase-C in the gastrointestinal tract, with negligible systemic exposure at recommended clinical doses [1]. Consequently, pharmaceutical quality assessment is a more directly relevant analytical focus than conventional systemic pharmacokinetic measurement.

Published studies include conventional stability-indicating chromatography, chromatographic purification, degradation-product characterization and related-peptide impurity analysis [2–5]. These investigations answer distinct questions and should not be ranked solely by retention time or detector sensitivity. The emergence of ion mobility investigations adds another dimension to structural assessment [6]. This review examines how these developments can inform a purpose-led AQbD framework without implying that a comprehensive linaclotide-specific design region has already been established.

1.1. Analytical Quality As A Multidimensional Concept

A useful distinction is between the quantity of the intended molecular species and the quality of the material represented by that quantity. An assay result expressed as a percentage of label claim answers a different question from an impurity profile expressed relative to the main peptide peak. Neither measurement alone establishes that the expected sequence, disulfide topology and biological response are preserved. A review of linaclotide therefore needs to evaluate methods by the decisions they support rather than by the number of analytical instruments mentioned. The studies considered here illustrate a progression in the information available from separation, mass-selective detection and mobility-resolved characterization [2–6].

1.2. Rationale And Objectives

The objective of this review is to connect these complementary measurements with the principles of analytical procedure development. Particular emphasis is placed on the distinction between a documented analytical capability and a proposed improvement. This is important when assessing claims of stability-indicating performance, robustness and environmental benefit. A method that has demonstrated reliable routine assay performance may remain the preferred quality-control procedure even if an advanced technique is available for investigating unusual impurity profiles.

2. Scope And Evidence Approach

This is a critical narrative review, not a systematic review or meta-analysis. Representative sources were identified through targeted web searches for linaclotide chromatography, degradation, related peptide impurities, ion mobility and analytical procedure development, supplemented by regulatory guidance and established greenness literature. Sources available through November 2026 were considered. The evidence is organized by intended analytical use. No exhaustive database screening, duplicate review or formal risk-of-bias assessment was performed. Selected primary studies are distinguished from general methodological principles and proposed future approaches. Numerical comparisons are avoided where complete experimental details have not been independently checked.

2.1 Evidence Selection And Interpretation

The evidence is organized around the intended measurement: quantitative assay, purification, degradation-product characterization, related-peptide determination and conformational investigation. Representative publications are discussed in relation to their reported scope. No pooled estimate of analytical performance is calculated because the studies do not share a common matrix, measurement objective or experimental comparator. The review does not claim a reproducible systematic search, exhaustive screening or formal risk-of-bias assessment. Where full numerical validation data are unavailable, the discussion remains qualitative.

2.2 Criteria For Critical Comparison

A meaningful comparison requires information about the sample matrix, analytical platform, relevant interfering species, calibration model, reportable range, precision, accuracy and total analytical cycle. For structural methods, the confidence of the identification must also be considered. A mass assignment, fragmentation-supported structural proposal and independently confirmed topology are different levels of evidence. The distinction is retained throughout this review to prevent the interpretation of a reported instrumental capability as proof of complete pharmaceutical characterization.

3. Molecular Characteristics And Analytical Significance

Linaclotide has a molecular mass of approximately 1526.8 Da and the sequence CCEYCCNPACTGCY. Its three intramolecular disulfide bridges contribute to its constrained structure. Removal of the terminal tyrosine yields an active metabolite. Chemical identity, purity and biological activity are distinct attributes; a result establishing one does not automatically establish the others [1,4]. Table 1 outlines the analytical implications.

3.1 Molecular Identity And Topology

The sequence of linaclotide contains six cysteine residues, which participate in three disulfide linkages. Alternative pairings can preserve the elemental composition while changing connectivity. Consequently, a measurement of intact molecular mass can support identity but cannot independently exclude every topological isomer. Chromatographic retention may provide additional discrimination, but an apparently single peak is only evidence of homogeneity within the resolving capability of the selected procedure. The analytical significance of these features is illustrated by studies involving structural mass spectrometry and ion mobility [4,6].

3.2 Matrix-Dependent Interpretation

Drug substance and finished product represent different analytical matrices. In the former, the predominant concern may be distinction between the intended peptide and closely related manufacturing impurities. In the latter, formulation components and the representativeness of the analytical sample also become important. The same response factor and recovery cannot automatically be assumed in both settings. Accordingly, evidence obtained with reference material must be distinguished from evidence obtained in representative product matrices.

Table 1. Molecular characteristics and associated analytical questions.

Characteristic	Analytical implication
14 amino acid residues	Sequence-related impurities and fragments
Three disulfide bonds	Connectivity and structural integrity
Approximate mass 1526.8 Da	Intact mass and related species
Local gastrointestinal activity	Limited relevance of routine systemic exposure measurement
Active metabolite	Distinction between intact peptide and metabolite where relevant

4. Conventional Chromatographic Approaches

4.1 RP-HPLC And Ultraviolet Detection

Reversed-phase chromatography is an established analytical platform for peptides. Retention and resolution depend on peptide interactions with the stationary phase and the mobile-phase environment. UV detection is accessible for routine quality control, but structurally related species may exhibit similar spectra. Diode-array detection can add spectral information; it does not establish the absence of co-eluting peptide impurities. Pyla and colleagues reported a stability-indicating RP-HPLC procedure for linaclotide [2]. Its contribution is evidence of chromatographic assay feasibility, rather than definitive structural identification of all degradation products.

4.1.1 Interpretation Of Chromatographic Specificity

The conventional stability-indicating study [2] is valuable as evidence that chromatographic separation can support a quantitative measurement in the presence of degradation-related peaks. Its principal limitation is that the chromatographic signal alone does not establish the structure of those peaks. Spectral agreement across a peak may strengthen confidence in its purity, but closely related peptides can have similar absorbance characteristics. This is a reason to consider orthogonal evidence when the nature of an impurity is important, not a reason to disregard the utility of routine ultraviolet detection.

4.1.2 Quantitative Interpretation

An assay and a related-substance procedure can use similar instruments while requiring different evidence. Assay performance is usually interpreted near the intended concentration of the main component; impurity measurement concerns much smaller responses in the presence of that component. The precision and calibration demonstrated for the main peptide cannot be extrapolated to every minor species. Differences in detector response between related peptides can also limit the interpretation of normalized peak areas as absolute impurity concentrations.

4.2 Analytical Versus Preparative Chromatography

Zhu and colleagues investigated analytical and preparative chromatography for linaclotide [3]. Purification prioritizes recovery and isolation of the intended peptide, whereas quantitative analysis prioritizes reliable measurement and selectivity. Successful preparative separation cannot substitute for validation of a finished-product assay. Likewise, a method developed for drug substance may require separate evidence of suitability for a capsule matrix.

4.3 Method Comparison And Transfer

Analytical and preparative separation are linked by the same underlying separation science, but the practical outcomes are different. A purification report may establish that material can be isolated from a mixture without establishing quantitative accuracy across a pharmaceutical reportable range. Conversely, a validated analytical method may be appropriate for small-scale measurement without being efficient for preparative isolation. Published work by Zhu and colleagues [3] is therefore best interpreted in relation to the stated analytical and preparative objectives.

4.4 Limitations Of Platform-Based Claims

An instrument designation such as HPLC or UHPLC does not define the specificity of a procedure. Selectivity depends on the combined analytical system and the properties of the species under investigation. A comparison based solely on a shorter retention time overlooks the possibility of unresolved impurities, different equilibration requirements or different measurement objectives. The total analytical cycle and the quality of the resulting decision are more informative comparison criteria.

5. Advanced Analytical Characterization

5.1 LC-HRMS/MS And Related Substances

High-resolution mass spectrometry can distinguish peptide species by accurate mass and provide fragmentation evidence for structural assignments. Chaturvedi and colleagues investigated linaclotide and its degradation products using LC-HRMS/MS [4]. Their investigation illustrates the additional structural information available from mass-selective detection. A subsequent LC-HRMS investigation focused on structurally related peptide impurities [5]. Quantitative impurity assessment nevertheless requires suitable calibration, response characterization and selectivity; accurate mass alone is not sufficient to distinguish every isomer.

5.1.1 Confidence In Structural Assignments

Accurate mass is especially useful when a transformation changes the elemental composition of a peptide. However, positional isomers and changes in disulfide connectivity can remain indistinguishable by intact mass. Fragmentation information can narrow the possible structural assignments, while independent evidence may be required for definitive identification. The linaclotide degradation investigation [4] illustrates the distinction between recording a chromatographic impurity and investigating its molecular structure.

5.1.2 Quantification Of Related Peptides

The related-peptide LC-HRMS investigation [5] extends the evidence base beyond general degradation profiling to the problem of quantitative impurity measurement. Structural characterization and quantitative validation remain distinct activities: a signal that can be identified with high confidence may not be suitable for accurate measurement at every concentration. Detector response, reference-material characterization and the presence of interfering species determine the reliability of a quantitative result.

5.2 Ion Mobility And Orthogonal Techniques

Ion mobility introduces a separation dimension associated with gas-phase ion behaviour. A 2026 investigation considered conformational polymorphism in disulfide-rich peptide drugs, including linaclotide [6]. Such evidence is relevant to the limitations of conventional chromatography, but gas-phase observations should not be interpreted as direct proof of solution conformation or biological potency. Routine implementation requires appropriate reference materials, reproducibility and a justified relationship between the measured attribute and product quality.

5.2.1 Complementarity Of Mobility-Resolved Characterization

The cyclic ion mobility investigation of disulfide-rich peptides [6] provides a useful example of how additional separation dimensions can reveal heterogeneity not evident from an intact mass measurement. Mobility distributions are influenced by gas-phase ion properties, however, and should not be interpreted as a direct representation of every solution-state structure or biological property. This distinction matters for the translation of exploratory structural observations into pharmaceutical quality criteria.

5.3 UHPLC And Platform Selection

UHPLC may improve efficiency and throughput, but its benefits depend on maintaining adequate selectivity and reproducibility. A shorter separation does not necessarily resolve closely related peptide species. Transfer between platforms requires consideration of instrument dispersion, pressure, detector compatibility and the intended measurement. Table 2 and Fig 1 summarizes representative evidence rather than a head-to-head comparison.

Table 2. Representative linaclotide analytical literature.

Study	Platform	Contribution	Evidence boundary
Pyla et al. [2]	RP-HPLC	Stability-indicating assay	Does not establish structural identity of every impurity
Zhu et al. [3]	HPLC	Analysis and purification	Purification is not product-assay validation
Chaturvedi et al. [4]	LC-HRMS/MS	Degradation characterization	Not a universal routine assay
2026 impurity study [5]	LC-HRMS	Related peptide impurity	Performance is application-

		determination	specific
2026 conformation study [6]	Cyclic ion mobility-MS	Conformational assessment	Routine QC suitability requires further evidence

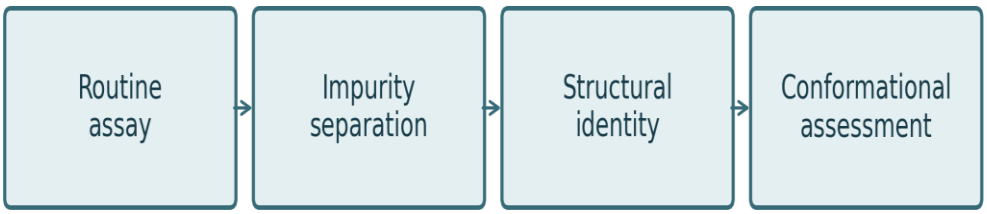

Figure 1. Complementary analytical domains for linaclotide quality assessment.

6. Stability-Indicating Methods And Impurity Profiling

Stability-indicating analysis should distinguish intact linaclotide from relevant degradation products and matrix components. Potential peptide degradation mechanisms include hydrolysis, oxidation, deamidation and changes in disulfide connectivity. Their relevance is molecule- and formulation-dependent. Table 3 presents general peptide risks rather than a list of experimentally confirmed linaclotide pathways. The structural study in reference [4] supports the value of complementary characterization when chromatographic peaks alone cannot establish identity. Chemical purity, structural identity and biological potency should be evaluated as distinct quality attributes. Where an impurity is detected, its identity and potential relevance should be considered separately from the ability of the analytical procedure to quantify it. Stability evidence should also account for possible changes during sample handling and storage.

Table 3. Potential peptide-related changes and analytical implications.

Potential change	Analytical question
Hydrolysis	Are peptide fragments distinguishable from intact material?
Oxidation or deamidation	Can chemically modified species be identified?
Disulfide alteration	Can connectivity variants be distinguished?
Conformational alteration	Is an orthogonal structural measurement warranted?

6.1 Stability-Indicating Evidence And Mass Balance

A stability-indicating procedure should be evaluated in relation to the changes it is expected to detect. Loss of the main peptide signal and growth of impurity signals need not be quantitatively equivalent when different species have different detector responses. Apparent mass balance may also be affected by unresolved species or analytical recovery. These limitations mean that a chromatographic trend is not automatically a complete description of the chemical changes in the sample. The evidence from the conventional and mass spectrometric studies should therefore be viewed as complementary [2,4].

6.2 Impurity Classification And Evidence Gaps

A useful review framework distinguishes process-related species from degradation-related species and separates both from matrix-associated analytical interference. Classification should be based on evidence, since a similar chromatographic feature may arise through different mechanisms. A general list of possible peptide degradation mechanisms should not be presented as a list of experimentally confirmed linaclotide impurities. The identity, origin and quantitative significance of a reported impurity each require their own supporting evidence.

6.3 Structural Integrity And Biological Relevance

A chemical difference does not automatically establish a difference in biological activity, and a chemically similar species is not necessarily functionally equivalent. The relationship between an observed impurity and a quality decision

depends on the relevance of the measured attribute. Biological potency, where appropriate, represents complementary evidence rather than a substitute for structural or chemical characterization. A robust quality assessment therefore considers the limitations of each measurement.

7. Analytical Quality-by-Design

7.1 Analytical Target Profile And Risk Assessment

ICH Q14 describes science- and risk-based analytical procedure development [7]. An analytical target profile (ATP) defines the intended analytical decision and performance expectations. A linaclotide assay, related-substance procedure and structural identity test require different priorities. Risk assessment can identify potential sources of variability associated with selectivity, reference materials, sample integrity, instrumentation and data interpretation. Risk rankings are preliminary judgments and require experimental confirmation.

7.1.1 Purpose-Led Analytical Target Profiles

An ATP is most useful when its objectives are expressed as measurable performance requirements linked to an intended decision. A procedure intended to quantify the principal peptide should not inherit an impurity-specific sensitivity requirement without justification. Similarly, a procedure intended to investigate topology cannot rely solely on routine assay precision as its success criterion. The proposed priorities in Table 4 are conceptual and would need to be translated into justified acceptance criteria for a specific product and analytical purpose [7].

7.1.2 Risk Assessment As A Knowledge-Building Exercise

Risk assessment should be understood as a structured evaluation of uncertainty, rather than a scoring exercise that automatically identifies critical parameters. Prior knowledge can identify plausible sources of analytical variability, while development evidence determines whether these sources meaningfully affect performance. In a peptide context, the potential for interference by related species is particularly important. A risk assessment is strengthened when the assumptions behind its conclusions are documented and revisited when new information becomes available (Table 4).

Table 4. Application-specific analytical priorities.

Intended use	Priority	Candidate platform
Drug substance assay	Accuracy and precision	RP-HPLC-UV
Finished product assay	Matrix specificity	HPLC/UHPLC
Related substances	Selectivity and quantification capability	HPLC or LC-HRMS
Structural identity	Sequence/connectivity evidence	Orthogonal MS characterization
Conformational assessment	Reproducible structural discrimination	Ion mobility-MS

7.2 Design Of Experiments And Operating Region

Design of experiments (DoE) provides a framework for evaluating interactions among analytical variables and for assessing the robustness of a procedure. Screening and response-surface designs serve different purposes. Model quality should be judged through predictive performance, residual analysis and confirmation evidence, not solely through a high coefficient of determination. A method operable design region (MODR) requires demonstrated acceptable performance throughout a justified region; a statistical optimum alone does not establish an MODR. The framework in Figure 2 is conceptual and is not presented as a validated linaclotide method.

7.2.1 Statistical Models And Predictive Evidence

A statistical model is informative only within the domain supported by the experimental evidence. An apparently strong goodness-of-fit statistic does not by itself establish predictive performance, particularly near the edges of the investigated domain. Model uncertainty and confirmation evidence are essential to the interpretation of multivariate optimization. A method operable design region should be distinguished from a single selected operating condition, and neither should be claimed as established for linaclotide without corresponding experimental evidence [7].

7.2.2 Multiple Analytical Objectives

Analytical development can involve competing objectives, including impurity discrimination, quantitative reliability and operational efficiency. Combining responses into a single optimization criterion may obscure failure to satisfy an

essential performance requirement. A more defensible interpretation distinguishes mandatory analytical criteria from preferences among otherwise acceptable alternatives. In this review, AQbD is proposed as an organizing framework for future research rather than as a claim that a particular optimization has already been performed.



Figure 2. AQbD framework for linaclotide.

7.3 Control Strategy And Lifecycle

Development knowledge should inform routine system suitability, reference-material management, data review, method transfer and change assessment. An enhanced development approach can provide a stronger understanding of parameter relationships, but a minimal approach may remain suitable for a well-understood, low-risk analytical procedure. The extent of development should be proportionate to the intended decision and complexity.

7.4 Knowledge Management And Analytical Change

The value of enhanced analytical development extends beyond the initial selection of a method. Documented knowledge about influential factors, uncertainty and performance boundaries can inform the assessment of changes during routine use. The relevance of that knowledge depends on whether the new conditions are represented by the original evidence. A procedure transferred between laboratories may require additional evidence even when the original development was extensive. Q14 provides a framework for considering such lifecycle issues [7].

8. Conventional Versus Qbd-Based Development

Neither approach guarantees superior performance. An AQbD claim should be supported by the quality of the evidence and the resulting control strategy, not merely the use of statistical terminology (Table 5).

Table 5. Comparison of analytical development approaches.

Dimension	Conventional	Enhanced AQbD
Objective	Method requirements	Explicit performance objectives
Experimentation	Often sequential	Structured evaluation of interactions
Robustness	Demonstrated for intended use	Integrated with development knowledge
Operating ranges	Justified set points or ranges	Evidence-supported multivariate region where warranted
Lifecycle	Documented change control	Risk-based use of development knowledge

8.1 Proportionality Of Analytical Development

Conventional development is not intrinsically inadequate. When the analytical problem is well understood and the procedure is fit for purpose, a minimal development approach can be appropriate. Enhanced development is most informative where uncertainty about interactions or performance boundaries is consequential. For linaclotide, the extent of development should reflect the complexity of the measurement, particularly when structural variants or low-level impurities are relevant. This proportional approach avoids presenting AQbD as a mandatory label for every acceptable procedure [7].

9. Validation And Regulatory Considerations

ICH Q2(R2) provides validation principles for analytical procedures within its scope, while ICH Q14 describes development and lifecycle considerations [7,8]. The two guidelines support complementary activities. Validation characteristics should be selected according to the intended use, including specificity, accuracy, precision, reportable range and detection or quantification capability where relevant. Robustness evidence generated during development

can inform validation and routine control. ICH M10 is relevant when a bioanalytical procedure supports regulated study-sample measurement [9]; it is not a replacement for pharmaceutical quality-control validation.

9.1 Validation Of Assay And Impurity Procedures

Validation is a demonstration of fitness for a defined purpose and should not be interpreted as a universal certification of an instrument or a method. An assay validation may support quantitative determination near the product specification, whereas an impurity procedure requires evidence appropriate to its lower measurement range and potential interference from the principal component. Q2(R2) provides a framework for identifying relevant performance characteristics [8].

9.2 Regulatory Scope And Biological Matrices

The intended application determines which regulatory framework is relevant. Pharmaceutical release and stability procedures fall within the quality-control context addressed by Q2(R2) and Q14, while regulated bioanalytical study-sample measurements are addressed by M10 [7–9]. The negligible systemic exposure described for linaclotide limits the relevance of routine plasma measurement to the quality-control questions emphasized in this review [1]. A biological measurement, if scientifically justified, should not be assumed valid on the basis of a pharmaceutical assay.

9.3 Lifecycle Evidence And Method Transfer

Changes in analytical systems or reference materials can affect the performance of a procedure after its original validation. The evidence required to evaluate such changes depends on the potential impact on the intended analytical result. For a peptide impurity procedure, equivalent measurement of the main component may not establish equivalent discrimination of minor species. Lifecycle monitoring and documented change assessment therefore complement initial validation [7,8].

Table 6. Validation considerations by intended use.

Characteristic	Relevance
Specificity	Distinction from relevant interfering species
Accuracy and precision	Reliability of quantitative results
Reportable range	Performance across intended measurement interval
Detection/quantification limits	Relevant to low-level impurity measurement
Robustness	Performance under relevant variation
Sample integrity	Reliability across the intended analytical workflow

10. Green Analytical Chemistry

Environmental evaluation should encompass the complete analytical workflow, including solvent consumption, consumables, waste, instrument use and repeat analysis. A shorter retention time does not establish a smaller environmental footprint. AGREE and GAPI provide structured but different assessments of greenness [10,11]. Their outputs should not be directly ranked against one another. Analytical fitness remains a prerequisite: an environmentally preferable procedure that cannot meet its intended performance is not an adequate substitute. Future linaclotide studies should report raw resource-use data alongside any greenness metric.

10.1 Defining The Environmental Boundary

A comparison of environmental performance is meaningful only if the workflow boundary is stated. Solvent consumption during the analytical separation represents one component of the total burden. Sample preparation, equilibration, cleaning, instrument time and repeat measurements can also contribute. The environmental advantage of a shorter chromatographic separation is uncertain if the complete cycle and reliability of the resulting measurement have not been evaluated.

10.2 Interpretation Of Greenness Metrics

AGREE and GAPI provide different representations of the environmental characteristics of analytical procedures [10,11]. Neither establishes chemical specificity, structural identity or quantitative reliability. A procedure should first meet essential analytical performance requirements before environmental comparisons are interpreted as grounds for

selection. Missing inventory information should be reported rather than replaced by unsupported assumptions or numerical scores [11].

Table 7. Environmental assessment approaches.

Tool	Focus	Interpretive limitation
AGREE	Twelve green analytical chemistry principles	Depends on inputs and weighting
GAPI	Workflow-level pictorial assessment	Not a directly comparable numerical score
Resource inventory	Solvent, waste, time and consumables	Requires consistent system boundaries

11. Critical Synthesis And Limitations

The selected literature supports a transition from quantitative chromatography towards complementary structural characterization. Conventional HPLC remains useful for routine assay, whereas high-resolution MS provides information about molecular composition and degradation. Ion mobility may address additional structural questions. These methods are not interchangeable, and direct comparisons require equivalent analytical objectives, matrices and performance criteria. The evidence reviewed here does not establish a complete experimentally verified AQbD framework for linaclotide. This observation is limited to the selected literature and is not proof that no other study exists. The narrative search and incomplete access to some full experimental reports limit quantitative comparison.

11.1 Evidence Hierarchy And Comparative Limitations

The literature reviewed here is heterogeneous in analytical purpose. The conventional assay study [2], purification investigation [3], degradation characterization [4], related-peptide quantification [5] and mobility-resolved study [6] establish different capabilities. A fair comparison therefore emphasizes the information generated and the limitations of that information. A single ranking of these procedures would imply a common objective that the source studies do not share.

11.2 Research Gap In Enhanced Development

The selected evidence does not demonstrate a complete linaclotide-specific AQbD package spanning an ATP, risk assessment, multivariate development, confirmed operating region and lifecycle strategy. This is an opportunity for further research, not evidence that such work has never been undertaken. A more comprehensive search and evaluation of full publications would be needed to establish the prevalence of enhanced development approaches.

11.3 Reporting Priorities

Future comparative publications would benefit from separating observed findings from proposed interpretations. In particular, chromatographic retention, total cycle time, quantitative range and structural assignment should be reported with their respective definitions. For peptide-related impurities, the basis of identification and the basis of quantification should be described separately. These distinctions would improve the interpretability of studies across different analytical platforms.

12. Future Perspectives

Research should prioritize transparent reporting of intended use, analytical performance, impurity identity and reference-material characterization. Comparative studies could establish whether complementary techniques improve quality decisions beyond conventional assay. Enhanced development approaches should document the rationale for selected performance attributes, model adequacy and the resulting control strategy. Interlaboratory evaluation and lifecycle monitoring would strengthen evidence of transferability. Environmental assessments should be integrated with performance objectives rather than added as unsupported retrospective scores. Figure 3 show these priorities.

12.1 Orthogonal Characterization

Future studies could assess the incremental value of structural methods relative to conventional chromatography. The central question is whether the additional information changes the confidence of a relevant pharmaceutical quality

decision. Such comparisons should distinguish molecular composition from topology and conformational heterogeneity. The emerging mobility-resolved evidence provides a rationale for investigating these distinctions [6].

12.2 Integrated Analytical Development And Sustainability

A future research program could evaluate analytical fitness and environmental burden together, rather than treating greenness as a post-development label. The evidence should identify the complete workflow boundary and maintain mandatory analytical criteria. Reporting both the raw resource inventory and the interpretation of the chosen metric would strengthen comparability between publications [10,11].

12.3 Reproducibility And Evidence Translation

An important longer-term question is whether the capabilities demonstrated in specialized research laboratories can be reproduced in routine pharmaceutical settings. Multi-laboratory evaluations, transparent documentation and characterized reference materials would help address this question. Predictive models and advanced structural tools should be assessed against independently supported analytical decisions rather than assumed to be superior because of their technical sophistication.

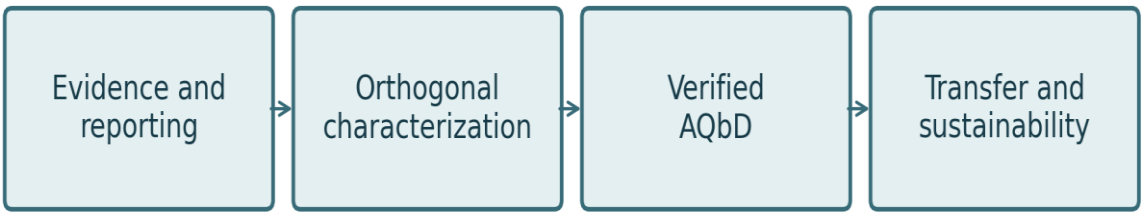

Figure 3. Research priorities for linaclotide analytical science.

13. Conclusion

Linaclotide analysis requires a purpose-led combination of quantitative chromatography and structural characterization. Published investigations demonstrate the feasibility of conventional HPLC, LC-HRMS and emerging ion mobility approaches for different analytical questions. ICH Q14 and Q2(R2) support a structured framework for method understanding, validation and lifecycle management. Future work should establish experimentally verified AQbD approaches, comparable performance evidence and transparent environmental assessments. The objective is reliable pharmaceutical quality measurement rather than adoption of a single universal analytical platform.

Abbreviations

AGREE, Analytical GREEnness; AQbD, analytical Quality-by-Design; ATP, analytical target profile; DoE, design of experiments; GAPI, Green Analytical Procedure Index; GC-C, guanylate cyclase-C; ICH, International Council for Harmonisation; LC-HRMS/MS, liquid chromatography–high-resolution tandem mass spectrometry; MODR, method operable design region; RP-HPLC, reversed-phase high-performance liquid chromatography; UHPLC, ultra-high-performance liquid chromatography.

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