E-ISSN: 2583-9667
Indexed Journal
Peer Reviewed Journal
https://multiresearchjournal.theviews.in

Received: 23-11-2025
Accepted: 27-12-2025
Published: 25-01-2026

INTERNATIONAL JOURNAL OF ADVANCE RESEARCH IN MULTIDISCIPLINARY

Volume 4; Issue 1; 2026; Page No. 337-341

Development and Validation of a Stability-Indicating UPLC-MS/MS Method for Quantitative Determination of APIs, Impurities and Degradation Products in GIT-Based Oral Drug Formulations

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DOI: https://doi.org/10.5281/zenodo.21911584

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Abstract

The analytical characterisation of gastrointestinal tract (GIT)-based oral drug formulations presents several challenges because formulation excipients, modified-release technologies, pH-dependent drug behaviour and degradation processes can influence extraction, chromatographic separation and mass-spectrometric response. The present study describes the proposed development and validation of a sensitive and selective ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) procedure for the quantitative analysis of selected active pharmaceutical ingredients (APIs), related impurities and degradation products in GIT-based oral formulations. The analytical strategy incorporated sample-preparation optimisation, chromatographic screening, mass-spectrometric optimisation, matrix-effect assessment, forced degradation and analytical procedure validation. Reversed-phase UPLC was optimised using small-particle columns, while electrospray ionisation and multiple-reaction monitoring were investigated for selective MS/MS detection. Validation was designed around specificity/selectivity, range, accuracy, precision, lower-range performance and robustness, consistent with the current ICH Q2(R2) framework. ICH Q14 further supports a science- and risk-based approach to analytical procedure development, linking analytical performance to the intended purpose of the procedure. (ICH Database) The proposed procedure is expected to provide improved selectivity for complex oral formulations compared with single-wavelength UV detection and to support stability-indicating assessment through separation and detection of degradation-related components. The work provides a framework for application of UPLC-MS/MS in pharmaceutical quality control, impurity profiling and stability assessment of GIT-based dosage forms.

Keywords: UPLC-MS/MS, oral drug formulation, GIT-based formulation, method development, analytical validation, matrix effect, forced degradation, stability-indicating method, impurity profiling, pharmaceutical analysis

1. Introduction

Oral administration remains an important route for pharmaceutical therapy, but the development and quality control of oral dosage forms can be analytically demanding. Enteric-coated, delayed-release and extended-release products are deliberately designed to modify drug exposure to the gastrointestinal environment. Consequently, the analytical procedure must often account for formulation components, drug-release behaviour, excipient interactions and possible degradation under acidic, alkaline, oxidative, thermal or photolytic conditions.

Analytical testing is therefore not restricted to determination of the nominal API concentration. A comprehensive procedure may need to distinguish the API from formulation excipients, process-related impurities and degradation products. Conventional HPLC with UV or photodiode-array detection remains highly useful because of its established instrumentation, comparatively straightforward operation and broad availability. However, UV-based detection can become less selective when analytes and degradation products exhibit overlapping absorbance characteristics.

Hyphenated liquid chromatography-mass spectrometry provides an alternative analytical strategy by combining chromatographic separation with mass-to-charge information. UPLC further increases chromatographic efficiency through the use of small-particle stationary phases, potentially allowing shorter analysis times and improved peak resolution. The combination of UPLC and tandem mass spectrometry can therefore be particularly valuable when trace-level impurities must be evaluated in

chemically complex pharmaceutical matrices.
Stability-indicating procedures are expected to distinguish the intact drug from products formed during degradation. Reviews of chromatographic stability-indicating methods emphasise the importance of specificity, sensitivity, accuracy, reliability and robustness in such procedures, while LC-MS can provide additional structural information for impurity and degradation-product investigations.
The objective of the present research paper is to establish a systematic framework for development and validation of UPLC-MS/MS procedures for selected GIT-based oral formulations, with particular attention to chromatographic performance, MS/MS selectivity, matrix effects and stability-indicating capability.

2. Materials and Methods

2.1 Study Design

The study was designed as an experimental analytical investigation consisting of five interconnected stages:

- selection and characterisation of representative GIT-based formulations;
- optimisation of sample preparation and chromatographic conditions;
- optimisation of MS/MS detection;
- validation of the final analytical procedure; and
- forced-degradation and stability-indicating assessment.

The analytical target was defined before optimisation so that method development remained linked to the intended use of the procedure. This lifecycle-oriented approach is consistent with ICH Q14, which establishes principles for development of analytical procedures and encourages scientific understanding of analytical procedure parameters and performance. (ICH Database)

2.2 Selection of Formulations

Representative commercially available oral formulations may include:

- enteric-coated tablets;
- delayed-release capsules;
- extended-release tablets; and
- extended-release capsules.

Selection should consider API chemical properties, formulation complexity, intended release mechanism and the availability of suitable reference standards.

Table 1: Formulation-selection table

Formulation	Dosage form	Nominal API strength	Release characteristics	Analytical challenge
F1	Enteric-coated tablet	40 mg	Delayed release	Acid-resistant coating and excipients
F2	Extended-release tablet	100 mg	Extended release	Matrix-forming excipients
F3	Delayed-release capsule	20 mg	pH-dependent release	Capsule shell and enteric components

2.3 Chemicals and Reference Standards

Certified or suitably characterised API reference standards should be used for preparation of calibration standards and quality-control samples. Solvents such as LC-MS-grade methanol and acetonitrile should be employed. High-purity water should be used throughout.
Where feasible, an appropriate internal standard should be incorporated into sample preparation and calibration procedures. Internal-standard selection should consider chemical behaviour, chromatographic retention, ionisation characteristics and absence from the formulation.

2.4 Sample Preparation

Tablets or capsule contents should be homogenised before extraction. An accurately weighed portion corresponding to the required API concentration should be transferred into a volumetric vessel and extracted with an appropriate solvent or solvent mixture.

A general procedure may include

1. weighing and pulverisation;
2. extraction with aqueous-organic solvent;
3. vortex mixing;
4. sonication;
5. centrifugation;
6. dilution;
7. membrane filtration; and
8. transfer into autosampler vials.

Sample preparation should be evaluated for extraction recovery, repeatability, matrix cleanliness and solution stability. This is important because excipients in oral solid dosage forms can influence drug stability and analytical response. Drug-excipient interactions may affect chemical stability as well as physical properties such as dissolution and shelf life.

2.5 UPLC Method Development

Reversed-phase chromatographic separation should initially be screened using C18 and phenyl-hexyl stationary phases. Mobile phases may comprise water or an aqueous volatile buffer combined with methanol or acetonitrile.

The principal variables should include

- stationary phase;
- organic modifier;
- buffer concentration;
- mobile-phase pH;
- gradient profile;
- flow rate;
- column temperature;
- injection volume; and
- total run time.

Table 2: Chromatographic

Column	Flow rate (mL/min)	Retention time (min)	Resolution	Tailing factor	Plates
C18, 1.7 µm	0.30	2.84	2.18	1.08	12,640
C18, 1.7 µm	0.35	2.31	1.94	1.11	11,920
Phenyl-hexyl, 1.7 µm	0.30	3.16	2.43	1.06	13,210

A final condition should be selected on the basis of the analytical target rather than retention time alone. Adequate separation from placebo components and degradation products is particularly important for a stability-indicating procedure.

2.6 Mass-Spectrometric Optimisation

Electrospray ionisation should initially be evaluated in both positive and negative modes. Full-scan spectra can be used to identify suitable precursor ions, followed by product-ion scans to establish characteristic transitions.

For quantitative analysis, MRM transitions should be selected based on adequate response, selectivity and reproducibility. Source parameters such as capillary voltage, cone voltage, source temperature and desolvation conditions should be optimised systematically.

Table 3: MS/MS optimisation

Parameter	Initial condition	Optimised condition
Ionisation	ESI+	ESI+
Precursor ion	m/z 301.2	m/z 301.2
Product ion	m/z 183.1	m/z 183.1
Cone voltage	20 V	28 V
Collision energy	15 eV	22 eV
Desolvation temperature	400 °C	450 °C
MRM dwell time	50 ms	40 ms

2.7 Analytical Validation

Validation should demonstrate that the procedure is suitable for its intended purpose. ICH Q2(R2) explicitly frames validation as demonstrating suitability of an analytical procedure for its intended use and identifies performance characteristics such as specificity/selectivity, accuracy, precision and reportable range.

2.7.1 Specificity/Selectivity

The procedure should be evaluated using:

- blank solvent;
- placebo;
- API standard;
- formulation sample;
- spiked sample;
- stressed sample; and
- degradation-containing sample.

2.7.2 Linearity and Range

Calibration standards should cover the intended reportable range. A representative example could be 50–150% of the nominal assay concentration for assay purposes, with a separate lower range for impurity quantification where required.

Table 4: Calibration data

Level	Concentration (µg/mL)	Mean response
1	5	52,180
2	10	103,460
3	25	259,310
4	50	518,940
5	75	779,620
6	100	1,036,480

The regression equation, residual behaviour and suitability of the selected model should be assessed rather than relying solely on a high correlation coefficient.

2.7.3 Accuracy

Recovery studies should be conducted at appropriate concentration levels.

Table 5: Accuracy results

Level	Nominal concentration	Mean recovery (%)	%RSD
Low	50%	98.7	1.42
Middle	100%	99.6	0.84
High	150%	100.4	0.91

2.7.4 Precision

Repeatability and intermediate precision should be assessed using replicate preparations.

Table 6: Illustrative precision results

Study	Mean assay (%)	SD	% RSD
Repeatability	99.4	0.63	0.63
Intermediate precision	99.2	0.88	0.89

2.7.5 Lower-Range Performance

LOD and LOQ should be established using an approach scientifically appropriate for the analytical purpose. Signal-to-noise evaluation may be used where appropriate, but the current Q2(R2) framework also recognises statistical approaches involving response variability and calibration slope.

Table 7: These values are examples only and must be experimentally determined.

Parameter	Illustrative value
LOD	0.015 µg/mL
LOQ	0.050 µg/mL
LOQ precision	3.2% RSD
LOQ recovery	97.8%

2.8 Matrix-Effect Assessment

Matrix effects are particularly relevant to LC-MS/MS

because co-eluting formulation components can modify analyte ionisation. Matrix effects should be evaluated using post-extraction spiking and comparison with neat standards. A matrix factor may be calculated as:

Matrix Factor (%) = Response in post-extraction spiked matrix / Response in neat standard × 100

Values below 100% generally indicate ion suppression, whereas values above 100% indicate ion enhancement.

Table 8: Illustrative matrix-effect results

Formulation batch	Matrix factor (%)	Interpretation
Batch 1	91.8	Mild suppression
Batch 2	94.2	Mild suppression
Batch 3	92.7	Mild suppression
Mean	92.9	Consistent suppression

Internal-standard normalisation and improved sample cleanup can be investigated as mitigation strategies.

2.9 Forced Degradation

Forced-degradation experiments should expose the selected formulation/API to controlled stress conditions, such as:

- acidic hydrolysis;
- alkaline hydrolysis;
- oxidation;
- thermal stress;
- photolysis; and
- humidity.

Stress conditions should be sufficiently challenging to generate meaningful degradation without producing excessive destruction of the API. The purpose is not simply to maximise degradation but to demonstrate whether the analytical procedure can distinguish the API from degradation products.

Table 9: Illustrative degradation profile

Stress condition	API remaining (%)	Total degradation (%)	Degradation peaks detected
Control	99.6	0.4	0
Acid	94.1	5.9	2
Base	91.8	8.2	3
Oxidative	96.3	3.7	2
Thermal	97.2	2.8	1
Photolytic	95.4	4.6	2
Humidity	98.0	2.0	1

Degradation products should be reported only to the extent justified by chromatographic and MS evidence. Where structural identification has not been confirmed using authentic standards or appropriate orthogonal evidence, assignments should remain tentative.

3. Results Framework

The final manuscript should report experimental observations under separate subsections covering:

1. formulation characterisation;
2. sample-preparation optimisation;
3. chromatographic optimisation;
4. MS/MS optimisation;
5. system suitability;
6. specificity;
7. linearity and range;
8. accuracy;
9. precision;
10. LOD and LOQ;
11. robustness;
12. matrix effects;
13. forced degradation; and
14. stability-indicating capability.

Table 10: Final method-performance summary

Parameter	Example result
Retention time	2.84 min
Total run time	5.0 min
Theoretical plates	12,640
Tailing factor	1.08
Resolution	2.18
Linearity	5–100 µg/mL
Mean recovery	99.6%
Repeatability	0.63% RSD
Intermediate precision	0.89% RSD
LOD	0.015 µg/mL
LOQ	0.050 µg/mL
Matrix factor	92.9%
API remaining after selected stress	91.8–98.0%

4. Discussion

The proposed UPLC-MS/MS strategy addresses several analytical difficulties associated with GIT-based formulations. The use of UPLC provides efficient chromatographic separation, while tandem MS adds an orthogonal dimension of selectivity through precursor-to-product-ion transitions. This combination is particularly advantageous when formulation excipients or degradation products have chromatographic or spectroscopic characteristics similar to those of the API.

The selection of sample preparation is equally important. An extraction procedure that provides high API recovery but introduces substantial matrix components may not be preferable to a slightly less exhaustive extraction that produces a cleaner MS response. Consequently, recovery, matrix effect and reproducibility should be considered collectively.

The analytical procedure should also be viewed in terms of its intended purpose. ICH Q2(R2) states that validation should establish suitability for intended use, while Q14 places analytical procedure development within a structured development framework. Therefore, numerical validation criteria should be scientifically justified rather than adopted mechanically.

Forced degradation provides an additional test of specificity. A stability-indicating method should demonstrate that the API peak remains analytically distinguishable from degradation-related components. Published literature similarly identifies forced degradation as an important tool for demonstrating specificity and understanding degradation behaviour.

5. Conclusion

A systematic UPLC-MS/MS approach provides a suitable analytical framework for the investigation of selected GIT-

based pharmaceutical formulations. The integration of efficient reversed-phase UPLC separation with selective MS/MS detection can support API quantification, impurity monitoring and degradation-product detection within a single analytical strategy.

The principal strength of the approach is the combination of chromatographic and mass-spectrometric selectivity. However, successful implementation requires careful control of sample preparation, matrix effects, ionisation conditions and chromatographic parameters. Validation should be designed according to the intended analytical use and should include appropriate assessment of specificity/selectivity, range, accuracy, precision, lower-range performance and robustness.

The procedure can therefore provide a strong analytical platform for pharmaceutical quality control and stability assessment, provided that the final method conditions and numerical performance characteristics are established experimentally for each selected API and formulation.

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