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Matrix-Effect Evaluation and Stability-Indicating Performance of a UPLC-MS/MS Method for Extended-Release and Delayed-Release Oral Drug Formulation

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Abstract

Extended-release and delayed-release oral formulations are designed to modify the presentation of an active pharmaceutical ingredient (API) within the gastrointestinal tract and commonly contain complex combinations of polymers, coating materials, fillers, binders, lubricants and other excipients. These formulation characteristics can create significant challenges during mass-spectrometric analysis, particularly through matrix-induced ion suppression or ion enhancement. The present study describes a proposed UPLC-MS/MS analytical strategy for the quantitative analysis of selected APIs in extended-release and delayed-release oral pharmaceutical formulations, with particular emphasis on matrix-effect evaluation, mitigation and stability-indicating capability. The analytical workflow comprised formulation characterisation, sample-preparation optimisation, reversed-phase UPLC separation, electrospray ionisation, tandem mass spectrometric detection, matrix-factor determination, internal-standard normalisation and forced-degradation studies. Matrix effects were evaluated using neat standards, post-extraction spiked samples and, where appropriate, post-column infusion. Forced degradation was performed under acidic, alkaline, oxidative, thermal, photolytic and humidity-related conditions to investigate separation of the intact API from degradation-related components. The proposed validation framework included specificity/selectivity, linearity, range, accuracy, precision, lower-range performance, robustness and solution stability. Illustrative numerical values are included in this manuscript solely to demonstrate the expected presentation of experimental data and must be replaced with experimentally generated results before publication. The study provides a practical framework for evaluating whether UPLC-MS/MS can deliver reliable quantitative performance in complex modified-release oral formulations while maintaining sufficient selectivity for impurity and degradation-product monitoring.

Keywords: UPLC-MS/MS, extended-release formulation, delayed-release formulation, matrix effect, ion suppression, ion enhancement, MRM, forced degradation, stability-indicating method, pharmaceutical analysis

1. Introduction

Modified-release oral dosage forms are developed to alter the rate, location or duration of drug release within the gastrointestinal tract. Extended-release systems can employ hydrophilic or hydrophobic polymers to regulate drug diffusion, swelling and erosion, whereas delayed-release systems may incorporate pH-responsive coatings designed to resist the gastric environment and release the drug after passage into the intestine.

Such formulation strategies provide therapeutic and manufacturing advantages but increase analytical complexity. The analytical sample does not contain only the API. It may also contain polymeric excipients, coating materials, fillers, binders, disintegrants, lubricants and other formulation components. These substances can affect extraction, chromatographic behaviour and mass-spectrometric ionisation.

The influence of formulation composition on drug-release behaviour has been demonstrated in studies of extended-release matrix systems, where polymer type, excipient

composition and processing variables can alter release characteristics. Consequently, analytical methods for modified-release formulations should be sufficiently selective to distinguish the API from formulation-related components.

UPLC-MS/MS provides two complementary analytical dimensions. UPLC contributes efficient chromatographic separation, while tandem mass spectrometry provides mass-based selectivity. MRM detection can further improve selectivity by monitoring characteristic precursor-to-product-ion transitions.

1.2 Rationale

The present paper focuses specifically on the relationship between formulation complexity, matrix effects and quantitative reliability. Rather than considering matrix effects as a secondary validation parameter, the study treats them as an integral part of method development.

The objectives

- To develop a selective UPLC-MS/MS method for selected modified-release oral formulations;
- To quantify API response in the presence of formulation matrix;
- To determine matrix factors across independent formulation batches;
- To evaluate ion suppression and enhancement;
- To assess the effectiveness of sample-cleanup and internal-standard strategies;
- To investigate API degradation under forced-stress conditions; and
- To establish whether the developed method can function as a stability-indicating procedure.

2. Materials and Methods

2.1 Formulation Selection

Two representative formulation categories were considered:

- Delayed-release/enteric-coated dosage forms; and
- Extended-release matrix dosage forms.

Table 1: Formulation characteristics

Formulation	Dosage form	API strength	Release design	Principal analytical challenge
F1	Enteric-coated tablet	40 mg	Delayed release	Coating and excipient matrix
F2	Extended-release tablet	100 mg	Extended release	Polymer-rich matrix
F3	Extended-release capsule	75 mg	Extended release	Capsule and multiparticulate excipients

The exact products, API identities and strengths should be replaced with the formulations actually investigated.

2.2 Sample Preparation

For each formulation, tablets were weighed individually and subsequently homogenised. Capsule contents were similarly homogenised before extraction.

A general extraction sequence consisted of

1. Accurately weighing powdered formulation;
2. adding extraction solvent;
3. vortex mixing;
4. sonication;
5. centrifugation;
6. collection of the supernatant;
7. dilution to analytical concentration;
8. membrane filtration; and
9. transfer to autosampler vials.

Sample preparation was optimised by comparing extraction solvents, extraction time, dilution factor and filtration conditions.

Table 2: Sample-preparation optimisation

Extraction condition	Recovery (%)	Matrix factor (%)	% RSD
Methanol	94.8	84.6	3.21
Acetonitrile	96.2	88.4	2.54
Methanol: water 80:20	98.1	92.6	1.47
Methanol: water 70:30	97.4	94.1	1.61

The mixed aqueous-organic extraction condition would therefore be considered preferable in this example because it provides a favourable balance between recovery, matrix cleanliness and reproducibility.

3. UPLC Method Development

3.1 Chromatographic Conditions

Reversed-phase C18 and phenyl-hexyl columns were screened. The mobile phase consisted of an aqueous volatile component and an organic modifier, with pH adjusted where appropriate.

Table 3: Illustrative column-screening results

Column	Retention time (min)	Resolution	Tailing factor	Plates
C18, 1.7 µm	2.74	2.12	1.09	12,430
C18, 1.9 µm	3.16	1.86	1.13	10,960
Phenyl-hexyl, 1.7 µm	3.04	2.41	1.06	13,180

The phenyl-hexyl column produced the highest example resolution, whereas the C18 column provided shorter retention. The final selection should therefore depend on the intended analytical target, including separation from impurities and degradation products.

3.2 Optimisation of pH and Organic Modifier

Mobile-phase pH can influence retention and ionisation of ionisable APIs. Several pH conditions should therefore be investigated rather than assuming a single universally optimal value.

Table 4: pH optimisation

pH	Retention time (min)	Peak area	Tailing factor	Resolution
2.5	2.61	845,210	1.18	1.72
3.0	2.78	912,460	1.10	2.04
3.5	2.91	938,720	1.07	2.31
4.0	3.08	901,330	1.14	1.96

4. Mass-Spectrometric Optimisation

Electrospray ionisation was evaluated in positive and negative modes. The mode producing the most stable and selective response was selected for subsequent optimisation.

Precursor-ion selection was performed from full-scan spectra, followed by product-ion scanning. Collision energy and cone voltage were then optimised.

Table 5: Illustrative MS/MS optimisation

Parameter	Tested condition	Selected condition
Ionisation	ESI+/ESI−	ESI+
Precursor ion	m/z 301.2	m/z 301.2
Product ion	m/z 183.1	m/z 183.1
Cone voltage	20–35 V	28 V
Collision energy	10–30 eV	22 eV
Source temperature	100–150 °C	120 °C
Desolvation temperature	350–500 °C	450 °C

MRM transitions should be confirmed experimentally for each API. They should not be transferred from another compound or publication without verification.

5. Matrix-Effect Evaluation

5.1 Principle

Matrix effects arise when components other than the analyte alter the efficiency of ion formation. Depending on their nature and concentration, co-eluting components may suppress or enhance ionisation.

A basic matrix factor can be expressed as

MF (%) = [Peak response in post-extraction spiked matrix / Peak response in neat standard] × 100

A value of 100% represents no apparent matrix effect. Values below 100% indicate suppression, whereas values above 100% indicate enhancement.

Because matrix effects can vary between formulation batches, multiple independent batches should be assessed whenever possible.

5.2 Matrix Factor

Table 6: Illustrative batch-to-batch matrix effects

Formulation batch	Neat-standard response	Post-extraction response	Matrix factor (%)	Effect
F1-B1	1,000,000	926,000	92.6	Suppression
F1-B2	1,000,000	914,000	91.4	Suppression
F1-B3	1,000,000	938,000	93.8	Suppression
F2-B1	1,000,000	957,000	95.7	Mild suppression
F2-B2	1,000,000	963,000	96.3	Mild suppression
F2-B3	1,000,000	951,000	95.1	Mild suppression

The example results indicate relatively consistent suppression across batches rather than highly variable enhancement.

5.3 Internal-Standard Normalisation

An isotopically labelled internal standard is generally advantageous where available because it can compensate for some variation in extraction and ionisation behaviour.

Table 7: Effect of internal-standard correction

Batch	Uncorrected response variability (%RSD)	Corrected variability (% RSD)
F1	6.21	2.14
F2	5.74	1.96
F3	7.03	2.38
Overall	6.33	2.16

The example demonstrates how internal-standard normalisation may improve response reproducibility. Actual effectiveness must be established experimentally.

6. Forced-Degradation Studies

Forced degradation was conducted under controlled conditions representing major degradation pathways relevant to pharmaceutical stability assessment.

The objective was not to produce maximum degradation but to determine whether the analytical procedure could distinguish the intact API from degradation-related components.

6.1 Acid and Alkaline Stress

Table 8: Illustrative hydrolytic degradation

Condition	Exposure	API remaining (%)	Total degradation (%)	Additional peaks
Control	—	99.5	0.5	0
Acidic	1 h	95.2	4.8	2
Acidic	2 h	91.7	8.3	3
Alkaline	1 h	93.6	6.4	2
Alkaline	2 h	88.9	11.1	4

The actual degradation pathway should not be assigned solely from retention time. Structural interpretation requires appropriate mass-spectral and, where necessary, orthogonal evidence.

6.2 Oxidative Stress

Table 9: Oxidative degradation

Oxidative condition	API remaining (%)	Degradation (%)	Major degradation peaks
Control	99.5	0.5	0
Mild oxidation	97.4	2.6	1
Moderate oxidation	94.8	5.2	2
Strong oxidation	90.6	9.4	3

6.3 Thermal and Photolytic Stress

Table 10: Illustrative thermal and photolytic degradation

Stress condition	API remaining (%)	Degradation (%)	Peaks detected
Control	99.5	0.5	0
Thermal	98.1	1.9	1
Thermal severe	94.7	5.3	2
Photolytic	96.8	3.2	2
Photolytic severe	92.5	7.5	3

7. Stability-Indicating Assessment

A method can be considered stability-indicating only when its analytical performance demonstrates adequate ability to distinguish the API from relevant degradation products and other components.

Table 11: Stability-indicating evaluation

Stress condition	API RT (min)	Nearest degradation RT (min)	Resolution	API peak integrity	Assessment
Acid	2.91	2.18	2.14	Maintained	Suitable
Base	2.91	2.36	1.92	Maintained	Suitable
Oxidative	2.91	3.47	2.26	Maintained	Suitable
Thermal	2.91	2.11	2.05	Maintained	Suitable
Photolytic	2.91	3.62	2.31	Maintained	Suitable
Humidity	2.91	2.29	1.88	Maintained	Suitable

Peak-purity assessment and mass-spectral information should be incorporated where available. If degradation products are not fully characterised, they should be reported as observed degradation-related components or tentative assignments rather than definitively identified structures.

8. Validation

8.1 Specificity

Specificity should be evaluated using blank, placebo, standard, formulation and stressed samples. No significant interference should be observed at the API MRM transition.

Table 12: Illustrative specificity results

Sample	API response	Interference at API RT	Result
Blank	Not detected	<0.5%	Pass
Placebo	Not detected	<0.7%	Pass
Standard	1,025,430	None	Pass
Formulation	1,018,760	<0.8%	Pass
Stressed sample	986,420	<1.0%	Pass

8.2 Linearity

Table 13: Illustrative calibration data

Concentration (µg/mL)	Mean response
2.5	26,180
5	51,920
10	103,870
25	259,460
50	518,720
75	779,160
100	1,037,840

$R^2 = 0.9993$

The residual distribution should also be examined to determine whether the selected calibration model is appropriate.

8.3 Accuracy and Precision

Table 14: Illustrative accuracy

Level	Mean recovery (%)	% RSD
50%	98.8	1.52
100%	99.7	0.86
150%	100.5	0.93

Table 15: Illustrative precision

Precision experiment	Mean assay (%)	% RSD
Repeatability	99.4	0.71
Analyst 1	99.3	0.82
Analyst 2	99.1	0.95
Different day	99.2	1.02

8.4 LOD and LOQ

Lower-range limits should be established using an approach appropriate for the intended analytical application. Signal-to-noise can be used where scientifically justified, but statistical approaches based on response variability and calibration slope should also be considered.

Table 16: Lower-range performance

Parameter	Example value
LOD	0.012 µg/mL
LOQ	0.040 µg/mL
LOQ accuracy	98.1%
LOQ precision	3.41% RSD

8.5 Robustness

Table 17: Illustrative robustness study

Variable	Nominal	Low condition	High condition	Maximum % RSD
Flow rate	0.30 mL/min	0.27	0.33	1.18
Column temperature	35 °C	32 °C	38 °C	1.24
Organic phase	60%	58%	62%	1.43
Ph	3.5	3.3	3.7	1.67
Injection volume	2 µL	1.8	2.2	1.11

9. Discussion

The central analytical issue addressed in this study is the potential influence of formulation matrix on quantitative MS response. Extended-release formulations can contain substantial quantities of polymers and other excipients, and delayed-release products may contain additional coating materials. These components may influence extraction and can potentially co-elute with the API.

The illustrative matrix-factor results demonstrate moderate suppression rather than enhancement. Importantly, matrix effects should not be judged solely by whether the factor is

close to 100%. Their consistency across independent batches and their impact on quantitative accuracy are also relevant.

Chromatographic optimisation provides one important means of reducing matrix effects. If matrix components elute simultaneously with the API, they may compete for ionisation or otherwise alter the MS response. Improved chromatographic separation can therefore reduce the amount of matrix reaching the ion source at the analyte retention time. Published work comparing HPLC-MS/MS and UPLC-MS/MS similarly identifies co-eluting matrix components as a major source of suppression or enhancement.

Sample preparation provides a second mitigation strategy. A cleaner extract may reduce the concentration of non-volatile excipients entering the MS system. However, excessive cleanup can reduce analyte recovery. Consequently, the optimal procedure should balance extraction recovery, matrix cleanliness, precision and operational simplicity.

Internal-standard normalisation represents another useful strategy. The illustrative results show reduced variability following correction. However, an internal standard should not be regarded as a substitute for appropriate chromatographic separation and sample preparation.

The forced-degradation experiments further demonstrate the importance of stability-indicating assessment. Published pharmaceutical studies have successfully used UPLC procedures for stability-indicating analysis of APIs and impurities in dosage forms, including methods incorporating hydrolytic, oxidative, photolytic and thermal stress testing.

For modified-release formulations, the stability problem may extend beyond chemical degradation of the API. Formulation polymers and coating materials can themselves undergo changes that affect drug release. Photostability studies of extended-release matrix formulations, for example, have investigated light-induced changes in formulation components and their consequences for release behaviour.

10. Comparison with Conventional HPLC

Where a conventional HPLC-UV/PDA method is available, comparison should be performed using experimentally matched samples.

Table 18: Illustrative comparison

Parameter	HPLC-UV/PDA	UPLC-MS/MS
Run time	12.0 min	5.0 min
LOD	0.080 µg/mL	0.012 µg/mL
LOQ	0.250 µg/mL	0.040 µg/mL
API specificity	Moderate–high	High
Matrix-effect assessment	Limited	Directly assessable
Low-level impurity detection	Moderate	High
Solvent consumption/run	6.0 mL	1.5 mL
Instrument complexity	Lower	Higher
Capital cost	Lower	Higher

The principal advantage of UPLC-MS/MS is not simply faster chromatography. Its major analytical benefit is the additional selectivity provided by mass transitions. Nevertheless, higher instrument cost, maintenance requirements and the need for skilled personnel may limit implementation in laboratories where routine HPLC is already sufficient.

Recent pharmaceutical research continues to demonstrate the value of UPLC for rapid and stability-indicating analysis, while comparative work between conventional HPLC and UPLC illustrates differences in efficiency and analysis time.

11. Conclusion

The proposed UPLC-MS/MS procedure provides a comprehensive analytical framework for modified-release oral pharmaceutical formulations in which matrix complexity, low-level impurities and degradation products may compromise conventional analytical measurements.

The study places particular emphasis on matrix-effect evaluation rather than treating it as a secondary analytical characteristic. Assessment of neat standards, post-extraction spiked samples and independent formulation batches can establish whether ion suppression or enhancement is present and whether it is sufficiently consistent for reliable quantification.

The integration of sample-preparation optimisation, chromatographic separation, MRM detection and internal-standard correction provides multiple levels of control over analytical variability. Forced-degradation experiments further establish whether the method can separate the API from degradation-related components.

The ultimate suitability of the method should be determined from experimentally generated validation data. When the procedure demonstrates adequate specificity, range, accuracy, precision, robustness and stability-indicating capability for its intended purpose, UPLC-MS/MS can serve as a powerful analytical approach for quality control and stability assessment of complex GIT-based oral formulations.

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