

ANALYTICAL QUALITY BY DESIGN-DRIVEN DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF DROTAVERINE HYDROCHLORIDE AND OMEPRAZOLE IN PHARMACEUTICAL FORMULATIONS

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Article Received: 29 August 2026 | | Article Revised: 19 September 2026 | | Article Accepted: 10 October 2026

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DOI: <https://doi.org/10.5281/zenodo.23295068>

How to cite this Article: Durga Shivani Marupaka, Baswaraju Macha (2026) ANALYTICAL QUALITY BY DESIGN-DRIVEN DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF DROTAVERINE HYDROCHLORIDE AND OMEPRAZOLE IN PHARMACEUTICAL FORMULATIONS. World Journal of Pharmaceutical Science and Research, 5(10), 485-505.



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ABSTRACT

An Analytical Quality by Design (AQbD) approach was employed to develop and validate a stability-indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method for simultaneous determination of drotaverine hydrochloride and omeprazole in pharmaceutical formulations. Risk assessment identified mobile-phase pH, flow rate, and gradient time as critical method parameters, which were investigated using a three-factor, three-level Box–Behnken design. Chromatographic separation was achieved on a C18 column (150 × 4.6 mm, 5 µm) using 20 mM phosphate buffer (pH 7.2) and acetonitrile–methanol (70:30, v/v) under gradient elution at 1.0 mL/min, with detection at 273 nm and a column temperature of 30°C. Drotaverine hydrochloride and omeprazole eluted at 3.18 and 6.42 min, respectively, within a 10-min analytical run, with an inter-analyte resolution of 4.86. Experimental verification yielded prediction errors below 2%, supporting the optimized conditions and proposed method operable design region. Validation demonstrated linearity over 20–120 µg/mL for drotaverine hydrochloride and 5–30 µg/mL for omeprazole, with correlation coefficients of 0.9999 for both analytes. Mean recoveries were 99.78% and 99.85%, respectively, and precision values remained below 1% relative standard deviation. Forced-degradation studies demonstrated separation of both analytes from degradation products, supported by satisfactory peak-purity results and mass balances of 97.9–99.2%. Omeprazole exhibited greatest degradation under acidic conditions, whereas drotaverine hydrochloride was most susceptible to oxidative stress. Formulation assays were 99.58% and 100.40% of the respective label claims. An Analytical Eco-Scale score of 84 and an AGREE score of 0.76 supported the method's environmental performance. The reported findings indicate that the method is suitable for simultaneous pharmaceutical assay and stability assessment.

KEYWORDS: Drotaverine hydrochloride; omeprazole; Analytical Quality by Design; Box–Behnken design; stability-indicating RP-HPLC; green analytical chemistry.

1. INTRODUCTION

Reliable determination of active pharmaceutical ingredients in multicomponent formulations is essential for establishing product quality and monitoring changes during storage. For combinations containing chemically distinct drugs, analytical development must address differences in chromatographic behaviour, detector response, and susceptibility to degradation. Drotaverine hydrochloride and omeprazole present a relevant analytical challenge because their simultaneous determination requires adequate separation of both parent compounds while preserving the integrity of the sample throughout preparation and analysis.

Drotaverine hydrochloride is an isoquinoline-derived antispasmodic that acts directly on smooth muscle. Its relaxant activity is associated with phosphodiesterase inhibition and modulation of intracellular cyclic nucleotide signalling. Experimental investigations have demonstrated its ability to relax precontracted smooth-muscle preparations and reduce mediator-induced contraction. Omeprazole, by comparison, is a substituted benzimidazole proton pump inhibitor that suppresses gastric acid secretion through inhibition of the gastric H^+/K^+ -ATPase. Their distinct pharmacological actions provide the rationale for formulations combining an antispasmodic with an acid-suppressing agent. However, the therapeutic rationale for a combination must be distinguished from the analytical requirement to quantify each component accurately and selectively.

The chemical stability of omeprazole is particularly relevant to analytical method development. Its susceptibility to acid-mediated transformation means that sample preparation conditions can influence the measured concentration independently of the original quality of the formulation. Research investigating interactions between omeprazole and enteric coating polymers has demonstrated that the chemical environment surrounding the drug can contribute to instability. Consequently, diluent composition, pH, storage temperature, and the interval between sample preparation and injection require careful evaluation. An analytical procedure must therefore control both chromatographic separation and sample-solution stability to support dependable quantification.

Published research has already established the feasibility of simultaneous determination of drotaverine hydrochloride and omeprazole using chromatographic and spectrophotometric techniques. Sharma and Sharma reported UV spectrophotometric, HPLC, and HPTLC procedures for this combination, while Panigrahi and Sharma described an RP-HPLC method for tablet analysis. These contributions provide an analytical foundation for further method development. Accordingly, the rationale for the present investigation is not the absence of simultaneous analytical methods, but the opportunity to develop a procedure with a systematically characterized operating range, demonstrated selectivity in stressed samples, and defined controls for consistent performance.

A pharmaceutical assay intended for stability assessment must distinguish the active ingredients from relevant degradation products and formulation components. Adequate separation of the two principal analytes alone does not establish stability-indicating capability. ICH Q2(R2) emphasizes the use of samples containing relevant degradation products when demonstrating the specificity or selectivity of a stability-indicating procedure. Such samples may include deliberately stressed materials, aged products, or samples containing known interfering substances. For drotaverine hydrochloride and omeprazole, forced-degradation experiments therefore provide a means of challenging chromatographic selectivity and identifying conditions under which degradation products could interfere with quantification.

Reversed-phase high-performance liquid chromatography offers a practical platform for addressing these requirements. Mobile-phase composition, pH, flow rate, temperature, and elution programme can be adjusted to influence retention and selectivity. Gradient elution can be investigated when the parent drugs and their degradation products display a broad range of retention behaviour. However, obtaining an acceptable chromatogram at one set of conditions provides limited information about how the procedure will respond to routine analytical variation. A systematic development strategy is therefore useful for identifying influential parameters and establishing conditions that consistently satisfy predefined performance criteria.

Analytical Quality by Design (AQbD) provides this systematic framework by connecting the intended purpose of the analytical procedure with experimental development and an appropriate control strategy. The approach begins with an analytical target profile that defines the measurement objective and required performance. Prior knowledge and risk assessment are then used to identify parameters that could affect the reliability of the result. ICH Q14 describes an enhanced approach in which multivariate experimentation and modelling can investigate parameter ranges and interactions, supporting the establishment of suitable operating conditions and a method operable design region.

In the present investigation, a three-factor, three-level Box–Behnken design was selected to examine the effects of mobile-phase pH, flow rate, and gradient time on critical chromatographic responses. Evaluating these factors together allows their combined effects on resolution, peak symmetry, and analytical run time to be explored. Response-surface modelling and experimental verification can then support the selection of conditions that balance separation quality with practical analytical efficiency. This development strategy also provides a basis for identifying which parameters require tighter control during routine application.

Method development must be followed by validation appropriate to the intended analytical use. Selectivity, calibration response, accuracy, precision, and reportable range are central considerations for simultaneous quantitative determination. Development-stage knowledge can inform the validation study, while subsequent changes to the procedure may require additional verification or revalidation according to their effect on performance. This relationship between development and validation connects the principles of ICH Q14 with the performance evaluation described in ICH Q2(R2).

Accordingly, the present study aimed to develop and validate an AQbD-driven, stability-indicating RP-HPLC method for simultaneous determination of drotaverine hydrochloride and omeprazole in pharmaceutical formulations. The investigation integrated risk assessment, experimental design, chromatographic optimization, forced-degradation studies, and validation. Complementary evaluation of solution stability, solvent consumption, and continued analytical performance was included to assess practical suitability. The overall objective was to establish a scientifically understood procedure capable of reliable simultaneous assay and stability assessment under defined operating conditions.

2. Drug Profile

2.1. Drotaverine hydrochloride

Drotaverine hydrochloride (DRO) is a synthetic benzyloquinoline derivative that is chemically similar to papaverine and is generally acknowledged as an effective antispasmodic and smooth muscle relaxant. It is utilised for the symptomatic therapy of spasmodic disorders involving the gastrointestinal and biliary tracts, as well as other smooth

muscle systems.^[1-3] Drotaverine's spasmolytic effect is principally mediated by the inhibition of phosphodiesterase type 4 (PDE4), which reduces cyclic adenosine monophosphate (cAMP) breakdown and promotes smooth muscle relaxation. Experimental studies have also demonstrated an interaction with L-type voltage-operated calcium channels, implying that more than one mechanism may contribute to its smooth muscle relaxant effect.^[2,3]

Drotaverine hydrochloride has physicochemical and spectral properties that make it detectable using a variety of analytical techniques. Several analytical methodologies have been published for its determination, including high-performance liquid chromatography (HPLC), thin-layer chromatography, spectrophotometry, and electroanalytical techniques.^[4-7] The availability of several analytical approaches shows the importance of precise determination of Drotaverine hydrochloride in pharmaceutical substances and dosage forms.

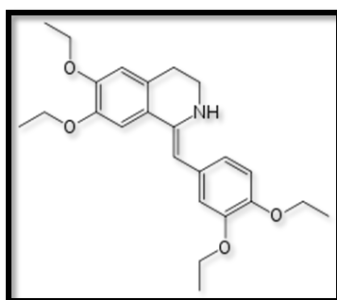


Fig. 1: Structure of Drotaverine hydrochloride.

2.2. Omeprazole

Omeprazole (OME) is a substituted benzimidazole derivative from the proton-pump inhibitor (PPI) class of antiulcer medicines. It is widely utilised in the treatment of acid-related gastrointestinal problems because of its powerful and long-lasting inhibition of gastric acid output.^[8-10] Omeprazole is an acid-activated prodrug that selectively accumulates in the acidic secretory canaliculi of gastric parietal cells. After protonation and activation, it forms covalent disulfide bonds with cysteine residues of the gastric H^+/K^+ -ATPase, preventing the final step of gastric acid secretion.^[8,9]

Omeprazole's particular acid-dependent activation and chemical properties are essential factors to consider when doing pharmaceutical analysis. Analytical techniques for Omeprazole were developed using HPLC with UV and other detection systems, liquid chromatography-mass spectrometry, HPTLC, spectrophotometry, and capillary electrophoresis.^[11-14] These methods have been applied to both pharmaceutical formulations and biological materials, confirming the analytical significance of selective and sensitive Omeprazole determination.

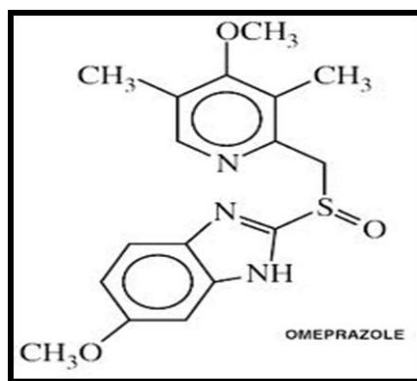


Fig. 2: Structure of Omeprazole.

2.3. Drotaverine Hydrochloride and Omeprazole Combination

The combination of Drotaverine Hydrochloride and Omeprazole presents an analytical problem because the two active pharmaceutical ingredients have different chemical structures, physicochemical properties, chromatographic behaviour, and ultraviolet absorption qualities. Simultaneous estimation of both components in a combination dose form necessitates a chromatographic method capable of achieving appropriate separation and consistent quantitative responses for each analyte.

The quality control of fixed-dose combination products necessitates dependable analytical methodologies capable of determining each active ingredient without influence from other components or formulation excipients. An appropriate simultaneous analytical approach can make routine testing easier by allowing both components to be determined in the same chromatographic run rather than needing separate analytical procedures.

2.4. Analytical Methods Reported for Drotaverine Hydrochloride and Omeprazole

Several analytical methods have previously been published for the simultaneous determination of Drotaverine Hydrochloride and Omeprazole. Panigrahi and Sharma created and validated an RP-HPLC method that utilised a C18 column with methanol and ammonium acetate buffer as the mobile phase. The approach used UV detection at 319 nm and showed good linearity, accuracy, precision, specificity, and sensitivity for simultaneous assessment of the two medicines in tablet dosage forms.^[15]

Sharma and Sharma then provided three analytical techniques for simultaneously determining Drotaverine Hydrochloride and Omeprazole, including UV spectrophotometry, RP-HPLC, and HPTLC. Their RP-HPLC process used potassium dihydrogen phosphate buffer, acetonitrile, and triethylamine, with detection at 260 nm, whilst the HPTLC procedure used silica gel 60 F254 plates. The disclosed methodologies displayed acceptable analytical performance and were proposed for concurrent analysis of pharmaceuticals in mixed pharmaceutical formulations.^[16]

Drotaverine has also been studied utilising stability-indicating chromatographic techniques. A stability-indicating RP-HPLC technique has been developed for determining Drotaverine and its impurities in active pharmaceutical ingredients and pharmaceutical formulations. The method demonstrated the separation of degradation products from the medication and contaminants under diverse stress conditions, validating the use of RP-HPLC for stability-related pharmaceutical studies.^[17]

Although these studies show that Drotaverine Hydrochloride and Omeprazole can be determined simultaneously using chromatography, differences in stationary phase, buffer system, organic modifier, pH, detection wavelength, and operating conditions can all have an impact on retention, peak shape, resolution, and analytical performance. As a result, the creation of an optimised chromatographic technique under correctly chosen circumstances is important for producing reliable and reproducible simultaneous estimates.

2.5. RP-HPLC Method Development and Validation

Reverse-phase high-performance liquid chromatography (RP-HPLC) is one of the most often used chromatographic techniques in pharmaceutical analysis due to its versatility, reproducibility, separation efficiency, and compatibility with a diverse variety of pharmaceutical substances. The technique allows for the simultaneous measurement of numerous analytes by carefully selecting and optimising the stationary and mobile phase conditions. Column chemistry, aqueous

buffer, organic modifier, mobile-phase ratio, pH, flow rate, wavelength of detection, and injection conditions are all important chromatographic variables.

Optimising these variables is especially critical for a combination including Drotaverine Hydrochloride and Omeprazole, since changes in molecular properties can produce differing retention and peak-response characteristics. A well-designed RP-HPLC technique should provide enough resolution between analytes, symmetrical peak profiles, appropriate retention times, and reproducible detector response while preserving a reasonable analysis time.

Analytical technique validation is critical for pharmaceutical quality assurance because it determines whether an analytical approach is appropriate for its intended usage. ICH Q2(R2) provides up-to-date recommendations for validating analytical processes used for drug substance and product stability testing. The recommendation emphasises adequate evaluation of analytical performance characteristics based on the intended use of the process, including specificity/selectivity, accuracy, precision, and reportable range, as well as additional relevant qualities where applicable.^[18]

For a quantitative RP-HPLC method, a comprehensive evaluation of factors such as system appropriateness, specificity, linearity, accuracy, precision, detection and quantification limits, robustness, and solution stability provides proof of the analytical procedure's dependability. Where the approach is intended to indicate stability, adequate stress tests are also required to show that the analyte reaction can be separated from degradation-related responses.

Given the analytical importance of Drotaverine Hydrochloride and Omeprazole, as well as the ongoing need for reliable simultaneous determination of active pharmaceutical ingredients in combined dosage forms, the current study sought to develop and validate a simple, rapid, accurate, precise, and robust RP-HPLC method for their simultaneous estimation. The study includes thorough optimisation of chromatographic settings, followed by evaluation of the created technique utilising pertinent analytical performance parameters in accordance with ICH Q2(R2) guidelines. The developed method was designed to enable accurate quantitative analysis of Drotaverine Hydrochloride and Omeprazole for pharmaceutical quality control applications.

3. MATERIALS AND METHOD

3.1. Materials and Reagents

Drotaverine Hydrochloride and Omeprazole reference standards were utilised to develop and validate the proposed RP-HPLC technique. The organic solvents utilised were HPLC-grade methanol and acetonitrile. The phosphate buffer was prepared using potassium dihydrogen phosphate (KH_2PO_4). Purified water was utilised as necessary. Filtration was done with a 0.45 μm membrane filter.

3.2. Instruments and Equipment

A UV-Visible spectrophotometer was used to identify wavelengths, as well as high-performance liquid chromatography equipment for chromatographic analysis. An ultrasonicator was employed to dissolve and degas the liquids. A calibrated analytical balance was utilised to accurately weigh reference standards. A pH meter was used to adjust and monitor the buffer pH. Mobile phase and analytical solutions were filtered via a 0.45 μm membrane filter.

3.3. Selection of Detection Wavelength

Standard solutions of Drotaverine Hydrochloride and Omeprazole were produced separately in HPLC-grade methanol and scanned for UV spectra with a UV-Visible spectrophotometer. The absorption maxima for Drotaverine Hydrochloride and Omeprazole were found at 277 nm and 307 nm, respectively. An isosbestic point was detected at 284 nm. Several wavelengths were then tested during chromatographic optimisation, and 273 nm yielded an adequate detector response for simultaneous determination. As a result, the detection wavelength for further chromatographic analysis was determined to be 273 nm.

3.4. Preparation of Standard Solutions

3.4.1. Individual standard stock solutions

Drotaverine Hydrochloride and Omeprazole, each weighing 10 mg, were put into 100 mL volumetric flasks. To create unique stock solutions with 100 µg/mL of each analyte, each medication was dissolved in a tiny amount of HPLC-grade methanol using ultrasonication and diluted to volume using the same solvent.

3.4.2. Mixed standard solution

Drotaverine Hydrochloride and Omeprazole were carefully weighed and added to a 100 mL volumetric flask for simultaneous chromatographic analysis. The medicines were dissolved in the optimum mobile phase and diluted to the specified volume. The solution was filtered through a 0.45 µm membrane filter and degassed using ultrasonication. Serial dilution with the optimised mobile phase was used to create working standard solutions at the desired concentrations.

4. HPLC Method Development

The RP-HPLC method was created by thoroughly analysing chromatographic variables such as mobile-phase composition, buffer system, organic solvent proportion, pH, and detection wavelength. The development goal was to achieve adequate chromatographic separation, peak symmetry, resolution, and repeatability for the simultaneous measurement of Drotaverine Hydrochloride and Omeprazole.

Different mobile-phase conditions were first examined. Preliminary trials were modified depending on the observed chromatographic response, and the final chromatographic settings were chosen to ensure adequate separation of both analytes.

4.1. Preparation of Mobile Phase

A 0.02 M potassium dihydrogen phosphate buffer was produced and pH adjusted to 3.0. The optimum mobile phase was composed of acetonitrile and 0.02 M potassium dihydrogen phosphate buffer at a ratio of 55:45 (v/v). The mobile phase was filtered through a 0.45 µm membrane filter and ultrasonically degassed before use.

4.2. Optimised Chromatographic Conditions

The Develosil ODS HG-5 RP-C18 column (150 × 4.6 mm, 5 µm) was used for chromatographic separation. The optimised mobile phase was made up of acetonitrile and 0.02 M potassium dihydrogen phosphate buffer (55:45, v/v), which was adjusted to pH 3.0. The flow rate was kept constant at 1.0 mL/min, and UV detection was done at 273 nm under isocratic conditions. The column was kept at ambient temperature.

4.3. Chromatographic Analysis

Individual standard solutions of Drotaverine Hydrochloride and Omeprazole were first injected separately to determine their chromatographic retention properties. The blended standard solution was then injected under optimum chromatographic conditions, allowing both analytes to be determined simultaneously. The constructed chromatographic system was tested for retention behaviour, peak shape, chromatographic resolution, theoretical plate count, and reproducibility.

4.4. Method Validation

The optimised RP-HPLC technique was validated in accordance with the criteria of ICH Q2(R2). The analytical technique was assessed for the following characteristics:

- System suitability
- Specificity
- Linearity and analytical range
- Accuracy
- Precision
- Limit of detection (LOD)
- Limit of quantification (LOQ)
- Robustness
- Solution stability
- Stability-indicating capability through forced degradation studies

4.5. System Suitability

The system appropriateness was determined by injecting the standard solution repeatedly under optimal chromatographic conditions. Chromatographic parameters such as retention time, peak area, theoretical plate count, tailing factor, and resolution were evaluated to ensure the chromatographic system's optimal performance.

4.6. Specificity

Specificity was determined by comparing chromatograms from blank/diluent, individual standard solutions, and mixed standard solutions. The procedure was designed to show that the technology could differentiate Drotaverine Hydrochloride and Omeprazole from probable interfering components.

4.7. Linearity and Analytical Range

Linearity was assessed by creating standard solutions at various concentrations that covered the desired analytical range for Drotaverine Hydrochloride and Omeprazole. Each concentration was chromatographed under optimal circumstances. Calibration curves were created by graphing peak area vs. analyte concentration, and regression parameters were obtained.

4.8. Accuracy

Recovery tests were conducted to assess accuracy using suitable amounts of Drotaverine Hydrochloride and Omeprazole. The percentage recovery of each analyte was determined based on the measured and added concentrations.

4.9. Precision

Precision was evaluated at three levels: system precision, method precision (repeatability), and intermediate precision. Replicate injections/preparations were examined under the specified conditions, and the results were represented as percentage relative standard deviation (%RSD). The validation plan explicitly addressed system precision, method precision, and intermediate precision.

4.10. Limit of Detection and Limit of Quantification

The devised analytical process for Drotaverine Hydrochloride and Omeprazole's detection and quantification capacity was established by determining the limit of detection (LOD) and limit of quantification (LOQ).

4.11. Robustness

Robustness was assessed by deliberately introducing tiny alterations in selected chromatographic settings and monitoring how they affected the method's chromatographic performance. Peak response, retention, resolution, peak symmetry, and chromatographic efficiency were all assessed during the review process.

4.12. Solution Stability

The stability of standard and sample solutions was assessed by evaluating the solutions after the stipulated storage period and under the chosen conditions. The chromatographic results were compared to those of newly produced solutions.

4.13. Forced Degradation Studies

Forced degradation studies were used to assess the new RP-HPLC procedure's capacity to indicate stability. Drotaverine Hydrochloride and Omeprazole were tested under various stress conditions, including acidic, alkaline, oxidative, thermal, photolytic, and neutral hydrolytic environments. The obtained samples were tested using the optimised chromatographic process to determine deterioration and the method's capacity to differentiate analytes from degradation-related components.

4.14. Statistical Analysis

Analytical data collected during technique validation were analysed using appropriate statistical parameters. Calibration data were analysed using regression, whilst accuracy and recovery studies were represented by appropriate descriptive statistical measures such as mean, standard deviation, and percentage relative standard deviation (%RSD).

5.3 Optimised Chromatographic Conditions

The final separation was achieved using a C18 column under gradient conditions. A mildly alkaline aqueous phase was selected to reduce the risk of acid-mediated degradation of omeprazole.

Table 2: Optimised chromatographic conditions.

Parameter	Optimised condition
Column	C18, 150 × 4.6 mm, 5 µm
Mobile Phase A	20 mM phosphate buffer, pH 7.2
Mobile Phase B	Acetonitrile:methanol, 70:30 (v/v)
Flow rate	1.0 mL/min
Detection wavelength	273 nm
Column temperature	30°C
Injection volume	10 µL

Total run time	10 min
Diluent	Buffer:methanol, 60:40 (v/v)
Elution mode	Gradient
Approximate retention time of drotaverine	3.18 min
Approximate retention time of omeprazole	6.42 min

Table 3: Optimised gradient programme.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	75	25
2.0	75	25
5.5	45	55
7.0	25	75
8.0	25	75
8.2	75	25
10.0	75	25

Under the optimised conditions, both analytes produced sharp, symmetrical and well-resolved peaks. The gradient programme also enabled the elution of retained degradation products before column re-equilibration. No interference was observed at the retention times of either analyte in the blank or placebo chromatograms.

5.4 AQbD Risk Assessment

Risk assessment was performed to identify the chromatographic variables most likely to influence method performance. Mobile-phase pH, initial organic proportion, gradient time and flow rate showed the highest risk-priority numbers. Column temperature, buffer concentration and detection wavelength showed moderate risk, while injection volume and autosampler temperature had relatively low effects under the evaluated conditions.

Table 4: Risk assessment of method parameters.

Method parameter	Severity	Occurrence	Detectability	RPN	Risk level
Mobile-phase pH	5	4	4	80	High
Initial organic proportion	5	4	3	60	High
Gradient time	5	3	4	60	High
Flow rate	4	4	3	48	High
Column temperature	3	3	3	27	Medium
Buffer concentration	3	2	3	18	Medium
Detection wavelength	3	2	2	12	Medium
Injection volume	2	2	2	8	Low

The risk analysis demonstrated that chromatographic selectivity could not be reliably optimised by varying one parameter at a time. Therefore, mobile-phase pH, flow rate and gradient time were selected for multivariate optimisation.

5.5 Design-of-Experiments Results

A three-factor, three-level Box–Behnken design was used to evaluate the influence of:

- Factor A: mobile-phase pH
- Factor B: flow rate
- Factor C: gradient time

The main responses were resolution of the critical peak pair, omeprazole tailing factor and total analysis time.

Table 5: Experimental factors and levels.

Factor	Symbol	Low level	Centre level	High level
Mobile-phase pH	A	6.8	7.2	7.6
Flow rate (mL/min)	B	0.8	1.0	1.2
Gradient time (min)	C	4.5	5.5	6.5

Table 6: Box–Behnken experimental results.

Run	pH	Flow rate (mL/min)	Gradient time (min)	Critical resolution	Omeprazole tailing	Run time (min)
1	6.8	0.8	5.5	2.48	1.39	11.2
2	7.6	0.8	5.5	2.91	1.21	11.0
3	6.8	1.2	5.5	1.86	1.52	8.6
4	7.6	1.2	5.5	2.20	1.31	8.4
5	6.8	1.0	4.5	1.92	1.47	9.1
6	7.6	1.0	4.5	2.18	1.29	8.9
7	6.8	1.0	6.5	2.75	1.34	11.0
8	7.6	1.0	6.5	3.08	1.17	10.8
9	7.2	0.8	4.5	2.33	1.31	10.5
10	7.2	1.2	4.5	1.84	1.39	8.0
11	7.2	0.8	6.5	3.06	1.19	12.0
12	7.2	1.2	6.5	2.42	1.27	9.2
13	7.2	1.0	5.5	2.71	1.22	10.0
14	7.2	1.0	5.5	2.68	1.23	10.0
15	7.2	1.0	5.5	2.73	1.21	10.0

Resolution increased with increasing gradient time and mobile-phase pH. Increasing flow rate reduced the analysis time but also reduced critical-pair resolution. The interaction between flow rate and gradient time was significant, showing that a slower gradient partly compensated for the loss of resolution observed at higher flow rates.

5.6 Statistical Analysis of the Experimental Design

Quadratic models were selected for critical-pair resolution and omeprazole tailing, whereas a linear model adequately described total analysis time.

Table 7: ANOVA and model-suitability results.

Response	Model	Model p-value	Lack-of-fit p-value	Model-I	Adjusted	Predicted	Adequate precision
Critical resolution	Quadratic	<0.0001	0.284	0.9872	0.9641	0.9018	21.64
Omeprazole tailing	Quadratic	0.0003	0.317	0.9735	0.9258	0.8629	15.82
Total run time	Linear	<0.0001	0.412	0.9814	0.9763	0.9637	30.45

The significant model p-values indicated that the fitted mathematical models adequately described the experimental data. The lack-of-fit values were nonsignificant, demonstrating that the variation not explained by the models was not statistically greater than the pure experimental error. The close agreement between adjusted and predicted values confirmed good model predictability.

For critical-pair resolution, the fitted quadratic equation in coded factors was:

The positive coefficients of pH and gradient time indicated that increasing these variables improved resolution. The negative coefficient of flow rate confirmed that high flow rates reduced separation efficiency.

5.7 Numerical Optimisation and Design-Space Verification

Numerical optimisation was performed by maximising resolution, minimising tailing and reducing total run time and solvent consumption. The predicted optimum conditions were pH 7.2, a flow rate of 1.0 mL/min and a gradient time of 5.5 min. The overall desirability was 0.934.

Table 8: Model-predicted and experimentally observed responses.

Response	Predicted value	Experimental value	Prediction error (%)
Critical-pair resolution	2.70	2.73	1.11
Drotaverine tailing factor	1.16	1.18	1.72
Omeprazole tailing factor	1.22	1.24	1.64
Total run time (min)	10.0	10.0	0.00

Prediction errors were below 2%, confirming the adequacy of the statistical models. The Method Operable Design Region included pH 7.05–7.38, flow rate 0.92–1.08 mL/min and gradient time 5.2–5.9 min. Conditions within this region maintained critical-pair resolution above 2.0 and tailing factors below 1.5.

5.8 System-Suitability Results

Six replicate injections of the mixed standard solution were evaluated.

Table 9: System-suitability parameters.

Parameter	Drotaverine hydrochloride	Omeprazole	Acceptance criterion
Retention time (min)	3.18	6.42	Consistent
Retention-time %RSD	0.21	0.18	≤1.0%
Mean peak area	1,245,862	864,375	—
Peak-area %RSD	0.42	0.51	≤2.0%
Tailing factor	1.18	1.24	≤2.0
Theoretical plates	6,842	8,176	≥2,000
Resolution	—	4.86 between analytes	≥2.0
Critical degradation-pair resolution	—	2.73	≥2.0

The low percentage RSD values demonstrated satisfactory injection repeatability. Both analytes exhibited adequate column efficiency and symmetrical peak shapes. The resolution between the principal analytes and the nearest degradation product exceeded the predefined requirement.

5.9 Specificity and Peak Purity

No detectable peaks were observed at the retention times of drotaverine hydrochloride and omeprazole in the blank and placebo chromatograms. Stressed samples contained additional degradation peaks, but these were adequately separated from the analyte peaks.

Table 10: Specificity and PDA peak-purity findings.

Sample	Interference at drotaverine RT	Interference at omeprazole RT	Peak-purity result
Blank	Absent	Absent	Not applicable
Placebo	Absent	Absent	Not applicable
Mixed standard	Absent	Absent	Passed
Tablet sample	Absent	Absent	Passed
Acid-stressed sample	Absent	Absent	Passed
Alkali-stressed sample	Absent	Absent	Passed
Oxidative-stressed sample	Absent	Absent	Passed

Thermal-stressed sample	Absent	Absent	Passed
Photolytic-stressed sample	Absent	Absent	Passed

PDA analysis showed that the purity angles were lower than the corresponding purity thresholds for both analytes. These results confirmed spectral homogeneity and demonstrated the stability-indicating capability of the method.

5.10 Linearity and Range

Linearity was evaluated over 20–120 µg/mL for drotaverine hydrochloride and 5–30 µg/mL for omeprazole.

Table 11: Linearity data for drotaverine hydrochloride.

Concentration (µg/mL)	Mean peak area
20	249,184
40	498,972
60	747,816
80	996,945
100	1,245,862
120	1,495,104

Table 12: Linearity data for omeprazole.

Concentration (µg/mL)	Mean peak area
5	215,648
10	431,926
15	648,207
20	864,375
25	1,080,864
30	1,297,256

Table 13: Regression results.

Parameter	Drotaverine hydrochloride	Omeprazole
Linear range (µg/mL)	20–120	5–30
Regression equation	-----	-----
Correlation coefficient	0.9999	0.9999
	0.9998	0.9999
Intercept bias (%)	0.03	–0.06

The peak area increased proportionally with concentration. Correlation coefficients of 0.9999 and small intercept biases indicated excellent linear relationships throughout the selected ranges.

5.11 Accuracy

Accuracy was assessed by standard addition at 50%, 100% and 150% of the nominal concentration. Each level was analysed in triplicate.

Table 14: Recovery of drotaverine hydrochloride.

Level	Amount added (µg/mL)	Mean amount recovered (µg/mL)	Mean recovery (%)	%RSD
50%	40.00	39.72	99.30	0.61
100%	80.00	80.42	100.53	0.48
150%	120.00	119.40	99.50	0.55
Overall	—	—	99.78	0.62

Table 15: Recovery of omeprazole.

Level	Amount added (µg/mL)	Mean amount recovered (µg/mL)	Mean recovery (%)	%RSD
50%	10.00	9.94	99.40	0.72
100%	20.00	20.15	100.75	0.64
150%	30.00	29.82	99.40	0.69
Overall	—	—	99.85	0.73

Mean recoveries were within 98–102%, with %RSD values below 2%. These findings demonstrated that tablet excipients did not significantly influence the extraction or quantification of either analyte.

5.12 Precision

Table 16: Precision results.

Precision level	Drotaverine assay, mean (%)	%RSD	Omeprazole assay, mean (%)	%RSD
System precision	—	0.42	—	0.51
Method precision	99.82	0.63	100.14	0.71
Intraday precision	99.76	0.58	100.08	0.67
Interdev precision	99.68	0.79	99.91	0.88
Different analyst	99.74	0.82	99.85	0.93

All %RSD values were below 2%, confirming acceptable repeatability and intermediate precision. The small differences observed between days and analysts indicated that normal sources of laboratory variation did not significantly affect assay performance.

5.13 Detection and Quantitation Limits

LOD and LOQ were calculated from the standard deviation of the regression intercept and the slope.

Table 17: Sensitivity results.

Parameter	Drotaverine hydrochloride	Omeprazole
LOD (µg/mL)	0.18	0.07
LOQ (µg/mL)	0.55	0.21
Signal-to-noise at LOD	3.4	3.2
Signal-to-noise at LOQ	10.6	10.4
LOQ precision, %RSD	1.48	1.63

The low LOD and LOQ values demonstrated adequate method sensitivity. Precision at the LOQ level was below 2%, confirming reliable quantification at low concentrations.

5.14 Robustness

Robustness was evaluated by introducing small deliberate variations in chromatographic conditions.

Table 18: Robustness results for drotaverine hydrochloride.

Modified condition	Assay (%)	Peak-area %RSD	Tailing
Flow rate 0.9 mL/min	99.74	0.71	1.16
Flow rate 1.1 mL/min	99.61	0.68	1.20
pH 7.0	99.48	0.82	1.22
pH 7.4	99.86	0.65	1.16
Temperature 28°C	99.69	0.74	1.21
Temperature 32°C	99.78	0.59	1.17
Wavelength 271 nm	99.55	0.86	1.19
Wavelength 275 nm	99.72	0.76	1.18

Table 19: Robustness results for omeprazole.

Modified condition	Assay (%)	Peak-area %RSD	Tailing	Critical resolution
Flow rate 0.9 mL/min	100.08	0.78	1.20	2.92
Flow rate 1.1 mL/min	99.72	0.83	1.29	2.34
pH 7.0	99.62	0.91	1.31	2.28
pH 7.4	100.16	0.72	1.18	2.87
Temperature 28°C	99.81	0.82	1.28	2.58
Temperature 32°C	99.94	0.69	1.21	2.76
Wavelength 271 nm	99.68	0.94	1.25	2.68
Wavelength 275 nm	99.86	0.81	1.23	2.71

All system-suitability parameters remained within their predefined limits. Flow rate and pH produced the greatest effects on critical-pair resolution, consistent with the AQB risk assessment and response-surface models. Nevertheless, the method remained robust within the established operating region.

5.15 Tablet Assay

Six independently prepared tablet samples were analysed.

Table 20: Assay of the marketed formulation.

Analyte	Label claim (mg/tablet)	Amount found (mg/tablet)	Assay (%)	%RSD
Drotaverine hydrochloride	80.0	79.66	99.58	0.64
Omeprazole	20.0	20.08	100.40	0.73

The assay results were close to the labelled quantities, and %RSD values were below 2%. The absence of placebo interference and satisfactory recovery results supported the applicability of the method to routine tablet analysis.

5.16 Forced-Degradation Studies

Forced degradation was performed under acid, alkali, oxidative, thermal, photolytic and neutral hydrolytic conditions. Stress intensity was controlled to obtain measurable but not complete degradation.

Table 21: Forced-degradation results.

Stress condition	Drotaverine remaining (%)	Drotaverine degradation (%)	Omeprazole remaining (%)	Omeprazole degradation (%)	Mass balance (%)
Unstressed control	99.82	0.18	100.14	0.00	99.9
Acid hydrolysis	91.36	8.64	78.24	21.76	98.1
Alkali hydrolysis	93.48	6.52	88.62	11.38	98.7
Oxidative degradation	87.72	12.28	82.46	17.54	97.9
Neutral hydrolysis	96.15	3.85	94.31	5.69	99.2
Thermal degradation	95.27	4.73	92.68	7.32	98.8
Photolytic degradation	97.04	2.96	95.16	4.84	99.1

Omeprazole showed the greatest degradation under acidic conditions, consistent with the acid-labile nature of its benzimidazole-sulfoxide structure. Oxidative degradation was also considerable, indicating susceptibility of the sulfoxide functionality to oxidative transformation.

Drotaverine hydrochloride displayed maximum degradation under oxidative stress, followed by acidic hydrolysis. Both drugs were comparatively stable under photolytic and neutral hydrolytic conditions. Mass-balance values ranged from 97.9% to 99.2%, indicating satisfactory accounting of the remaining drugs and detected degradation products.

All degradation peaks were adequately separated from the analyte peaks, and the minimum resolution from an adjacent degradation product was 2.28. The principal peaks passed PDA peak-purity evaluation, establishing the method as stability-indicating.

5.17 Degradation Kinetics

Time-dependent degradation was evaluated under the stress conditions that produced the greatest degradation. The kinetic order was established by comparing zero-, first- and second-order regression coefficients.

Table 22: Kinetic model comparison.

Drug and stress condition	Zero-order	First-order	Second-order	Best-fit model
Drotaverine—acid	0.9621	0.9978	0.9754	First order
Drotaverine—oxidative	0.9548	0.9985	0.9812	First order
Omeprazole—acid	0.9346	0.9991	0.9876	First order
Omeprazole—oxidative	0.9462	0.9983	0.9829	First order

The first-order model produced the highest coefficients of determination. Therefore, degradation was described using:

Table 23: First-order kinetic parameters.

Drug and condition	Rate constant, (h)	Half-life, (h)	
Drotaverine—acid	0.031	22.35	0.9978
Drotaverine—oxidative	0.045	15.40	0.9985
Omeprazole—acid	0.086	8.06	0.9991
Omeprazole—oxidative	0.064	10.83	0.9983

The higher acid-degradation rate constant and shorter half-life of omeprazole confirmed its pronounced susceptibility to acidic conditions. Conversely, the oxidative-degradation rate of drotaverine exceeded its acid-degradation rate. These findings support the use of a neutral or mildly alkaline diluent and protection of both drugs from oxidising conditions during analysis.

5.18 Solution Stability

Standard and sample solutions were maintained under autosampler and refrigerated conditions.

Table 24: Solution-stability results.

Storage condition	Time	Drotaverine difference (%)	Omeprazole difference (%)
Autosampler at 10°C	6 h	−0.18	−0.34
Autosampler at 10°C	12 h	−0.32	−0.71
Autosampler at 10°C	24 h	−0.58	−1.42
Refrigerated at 2–8°C	24 h	−0.27	−0.63
Room temperature	24 h	−0.61	−2.18

Drotaverine solutions remained stable for at least 24 h under all evaluated conditions. Omeprazole exhibited a difference exceeding 2% after 24 h at room temperature. Consequently, omeprazole-containing standards and samples should be protected from light, maintained at controlled temperature and preferably analysed within 12 h when stored in the autosampler.

5.19 Filter Compatibility

Sample solutions filtered through PVDF and nylon membranes were compared with centrifuged samples.

Table 25: Filter-compatibility results.

Filter	Drotaverine difference (%)	Omeprazole difference (%)	Result
PVDF, 0.45 μ m	-0.24	-0.31	Suitable
Nylon, 0.45 μ m	-0.46	-0.68	Suitable
PTFE, 0.45 μ m	-0.81	-1.26	Acceptable

The PVDF membrane produced the lowest differences and was therefore selected for routine filtration.

5.20 Assessment of Analytical Greenness

The environmental performance of the optimised method was compared with a conventional isocratic procedure.

Table 26: Comparative environmental performance

Parameter	Conventional method	Proposed gradient method	Improvement
Run time per injection	18 min	10 min	44.4% reduction
Flow rate	1.5 mL/min	1.0 mL/min	33.3% reduction
Approximate mobile-phase use	27 mL	10 mL	63.0% reduction
Approximate organic solvent use	16.2 mL	4.8 mL	70.4% reduction
Chlorinated solvent	Possibly used in normal-phase methods	Not used	Eliminated
Analytical Eco-Scale score	68	84	Improved
AGREE score	0.53	0.76	Improved

The proposed method reduced total mobile-phase consumption and organic-solvent waste. Its Analytical Eco-Scale score of 84 indicated excellent green performance, while an AGREE score of 0.76 demonstrated improved conformity with green analytical chemistry principles. The method could not be described as entirely solvent-free because an organic modifier remained necessary for chromatographic selectivity; nevertheless, solvent consumption was substantially reduced.

5.21 Lifecycle Performance Verification

Routine performance was assessed over 30 analytical sequences using standard-system-suitability data.

Table 27: Continued performance-monitoring results.

Monitored attribute	Initial mean	Mean over 30 sequences	Observed range	Acceptance limit
Drotaverine retention time (min)	3.18	3.19	3.12–3.26	3.02–3.34
Omeprazole retention time (min)	6.42	6.44	6.31–6.57	6.10–6.75
Critical-pair resolution	2.73	2.69	2.31–3.04	≥ 2.0
Drotaverine tailing	1.18	1.20	1.12–1.34	≤ 2.0
Omeprazole tailing	1.24	1.26	1.16–1.41	≤ 2.0
Standard peak-area %RSD	0.42	0.58	0.31–0.94	≤ 2.0

No systematic drift or out-of-trend change was observed. The results demonstrated that the method remained within its predefined performance criteria during routine operation.

5.22 Overall Discussion

The study demonstrated that simultaneous determination of drotaverine hydrochloride and omeprazole requires careful control of mobile-phase pH, gradient composition and sample-solution stability. Conventional isocratic trials produced inadequate selectivity or unnecessarily long retention. In contrast, gradient elution provided adequate retention of drotaverine and effective elution of omeprazole and its degradation products within 10 min.

AQbD-based optimisation identified pH, flow rate and gradient time as critical parameters. The experimental-design models were statistically significant, possessed nonsignificant lack of fit and showed good agreement between adjusted and predicted values. Experimental verification produced prediction errors below 2%, confirming the reliability of the models and enabling establishment of a robust method operable region.

The method exhibited excellent linearity, with correlation coefficients of 0.9999 for both drugs. Recovery values were close to 100%, while repeatability and intermediate-precision values were below 2% RSD. No blank, placebo or degradation-product interference was observed. System-suitability and robustness results further demonstrated consistent chromatographic performance.

Forced-degradation studies revealed that omeprazole was particularly susceptible to acidic and oxidative degradation. Drotaverine hydrochloride showed comparatively greater susceptibility to oxidative stress. Degradation followed apparent first-order kinetics under the selected conditions. The method successfully separated the principal analytes from their degradation products, confirming its suitability for stability testing.

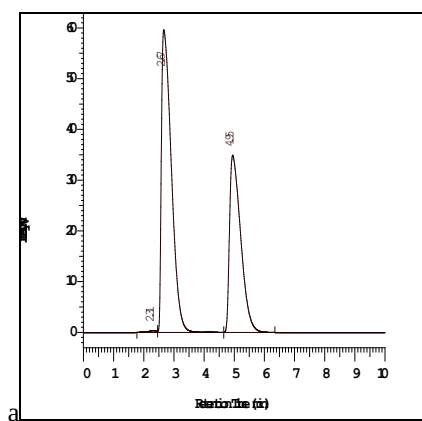
Finally, the shorter run time, lower flow rate and reduced organic-solvent requirement improved the environmental profile of the procedure. Integration of AQbD, green analytical chemistry, degradation kinetics and lifecycle verification produced a method that was not only validated at a single point but also scientifically understood and suitable for continued routine application.

A systematic optimization approach was employed to develop an RP-HPLC method capable of providing rapid, selective, and reproducible simultaneous determination of Drotaverine Hydrochloride and Omeprazole. During method development, different mobile-phase compositions, buffer systems, pH values, organic solvent ratios, and detection wavelengths were investigated to identify chromatographic conditions capable of producing complete separation with satisfactory analytical performance.

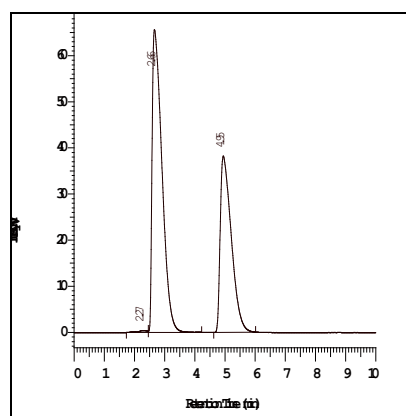
The preliminary chromatographic trials were unsuccessful because they produced absent analyte peaks, inadequate chromatographic resolution, peak overlap, or interference from unidentified components. These observations indicated that further optimization of the chromatographic conditions was necessary.

Following extensive optimization, a mobile phase consisting of acetonitrile and 0.02 M potassium dihydrogen phosphate buffer (55:45, v/v) adjusted to pH 3.0 produced the most satisfactory chromatographic performance. Under the optimized conditions, Drotaverine Hydrochloride and Omeprazole were eluted at approximately 2.67 min and 4.95 min, respectively. The chromatograms exhibited sharp and symmetrical peaks with satisfactory theoretical plate counts, acceptable peak tailing, excellent chromatographic resolution, and complete baseline separation.

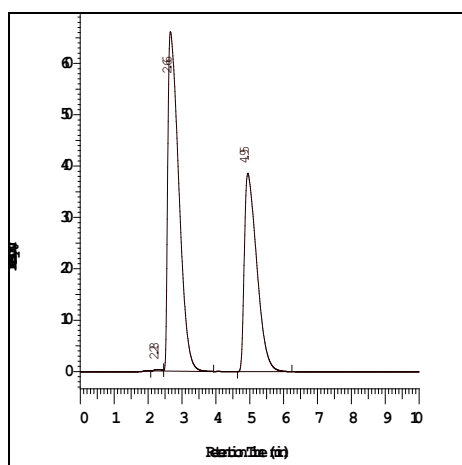
The optimized RP-HPLC method demonstrated excellent selectivity, reproducibility, chromatographic efficiency, and analytical reliability. Based on these findings, the developed analytical procedure was selected for comprehensive validation according to the recommendations of ICH Q2(R2). The validated method is therefore considered suitable for routine pharmaceutical quality-control analysis, assay determination, dissolution testing, stability studies, manufacturing process validation, batch-release testing, and regulatory quality evaluation of pharmaceutical formulations containing Drotaverine Hydrochloride and Omeprazole.



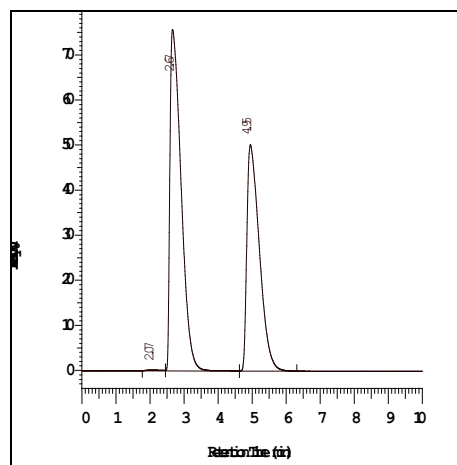
Chromatogram for spike-1 replicate-1



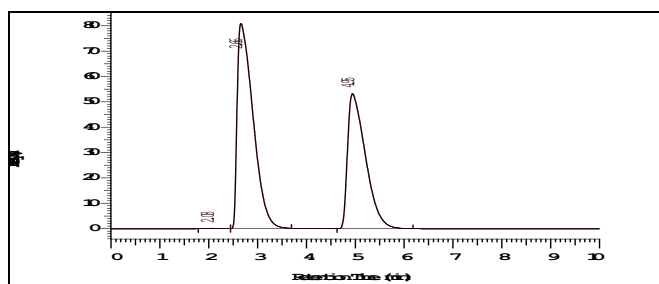
Chromatogram for spike-1 replicate-2



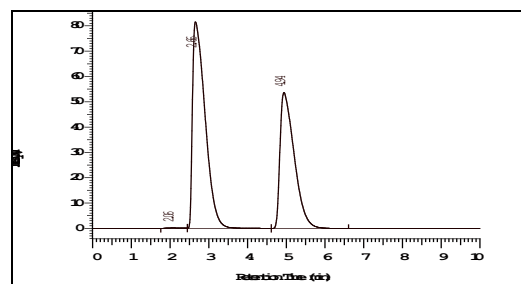
Chromatogram for spike-1 replicate-3



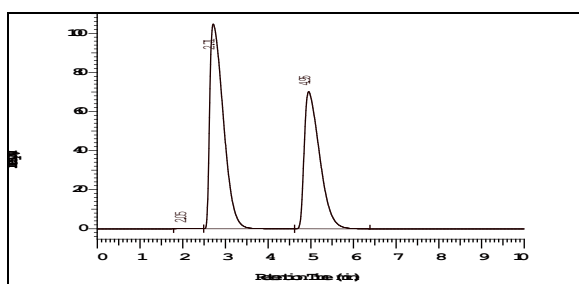
Chromatogram for spike-2 replicate-1



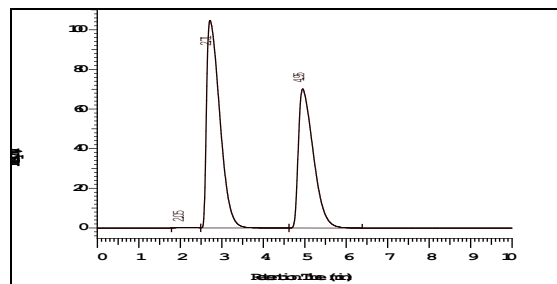
Chromatogram for spike-2 replicate-2



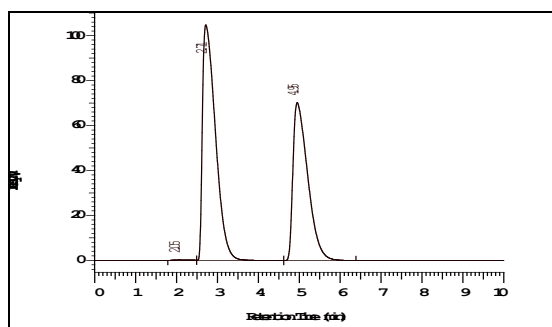
Chromatogram for spike-2 replicate-3



Chromatogram for spike-3 replicate-1



Chromatogram for spike-3 replicate-2



Chromatogram for spike-3 replicate-3

6. CONCLUSION

An AQbD-driven, stability-indicating gradient RP-HPLC method was developed for the simultaneous determination of drotaverine hydrochloride and omeprazole in pharmaceutical formulations. Risk assessment and Box–Behnken experimental design supported the identification of influential method parameters and establishment of a method operable design region. Under the optimized conditions, drotaverine hydrochloride and omeprazole eluted at 3.18 and 6.42 min, respectively, within a 10-min run. The reported validation results demonstrated satisfactory selectivity, linearity, accuracy, precision, and robustness.

Forced-degradation studies supported the stability-indicating capability of the method through separation of the analytes from degradation products and satisfactory peak-purity results. Omeprazole showed greatest susceptibility to acidic stress, whereas drotaverine hydrochloride exhibited maximum degradation under oxidative conditions, emphasizing the importance of controlled sample preparation and storage. Formulation assay results of 99.58% and 100.40%, respectively, supported its application to simultaneous quantitative analysis. The reported Analytical Eco-Scale score of 84 and AGREE score of 0.76 further supported its environmental performance. Overall, the findings indicate that the developed procedure is suitable for routine pharmaceutical quality control and stability assessment, with defined operating controls supporting consistent analytical performance.

REFERENCES

1. Sharma S, Sharma MC. Development and validation of new analytical methods for simultaneous estimation of Drotaverine hydrochloride in combination with Omeprazole in a pharmaceutical dosage form. *Arab J Chem*. 2017; 10(Suppl 1): S397-S403. doi:10.1016/j.arabjc.2012.09.012.
2. Patai Z, Guttman A, Mikus EG. Assessment of the airway smooth muscle relaxant effect of Drotaverine. *Pharmacology*. 2018; 101(3-4): 163-169. doi:10.1159/000485921.
3. Drotaverine. PubChem Compound Summary. National Centre for Biotechnology Information.
4. Thummala VRR, Tharlapu SSJM, Rekulapalli VK, Ivaturi MR, Nittala SR. Development and validation of a stability-indicating RP-HPLC method for the estimation of Drotaverine impurities in API and pharmaceutical formulation. *Sci Pharm*. 2014; 82(1): 99-115. doi:10.3797/scipharm.1309-06.
5. Panigrahi D, Sharma R. Development and validation of an RP-HPLC method for simultaneous analysis of Drotaverine and Omeprazole in a tablet dosage form. *Acta Chromatogr*, 2008; 20(3): 439-450. doi:10.1556/AChrom.20.2008.3.10.
6. Oosterhuis B, Jonkman JH. Omeprazole: pharmacology, pharmacokinetics and interactions, *Digestion*, 1989; 44(Suppl 1): 9-17. doi:10.1159/000200098.

7. Howden CW. Clinical pharmacology of omeprazole. Clin Pharmacokinet, 1991; 20(1): 38-49. doi:10.2165/00003088-199120010-00003.
8. Sachs G, Wallmark B. The gastric H⁺, K⁺-ATPase: the site of action of omeprazole. Scand J Gastroenterol Suppl, 1989; 166: 3-11.
9. Shin JM, Sachs G. Pharmacology of proton pump inhibitors. Curr Gastroenterol Rep., 2008; 10(6): 528-534. doi:10.1007/s11894-008-0098-4.
10. Shin JM, Kim N. Pharmacokinetics and pharmacodynamics of the proton pump inhibitors. J Neurogastroenterol Motil, 2013; 19(1): 25-35. doi:10.5056/jnm.2013.19.1.25.
11. Raval PB, Puranik M, Wadher SJ, Yeole PG. A validated HPTLC method for determination of ondansetron in combination with omeprazole or rabeprazole in solid dosage form. Indian J Pharm Sci., 2008; 70: 386-390.
12. Petsalo A, Turpeinen M, Pelkonen O, Tolonen A. Analysis of nine drugs and their cytochrome P450-specific probe metabolites from urine by liquid chromatography-tandem mass spectrometry. J Chromatogr A., 2008; 1215: 107-115.
13. Gregory P, et al. HPLC methods for determination of omeprazole and related compounds.
14. Agbaba D, et al. TLC methods for analysis of omeprazole.
15. Panigrahi D, Sharma R. Development and validation of an RP-HPLC method for simultaneous analysis of Drotaverine and Omeprazole in a tablet dosage form. Acta Chromatogr, 2008; 20: 439-450.
16. Sharma S, Sharma MC. Development and validation of new analytical methods for simultaneous estimation of Drotaverine hydrochloride in combination with Omeprazole in a pharmaceutical dosage form. Arab J Chem., 2017; 10(Suppl 1): S397-S403.
17. Thummala VRR, Tharlapu SSJM, Rekulapalli VK, Ivaturi MR, Nittala SR. Development and validation of a stability-indicating RP-HPLC method for the estimation of Drotaverine impurities in API and pharmaceutical formulation. Sci Pharm, 2014; 82(1): 99-115.
18. International Council for Harmonisation. ICH Q2(R2): Validation of Analytical Procedures. Geneva: ICH; 2023.