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Simultaneous determination of pinaverium bromide, medazepam, and their related impurities in combined dosage form by UPLC–QDA

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Abstract: A rapid ultra-performance liquid chromatographic method coupled with a quadrupole detector (UPLC–QDA) was developed and validated for the simultaneous determination of pinaverium bromide, medazepam, and their related substances in a newly developed fixed-dose pharmaceutical formulation. Chromatographic separation was achieved on an Acquity BEH C18 column (100 × 2.1 mm, 1.7 µm) using a gradient elution of 25 mM ammonium formate buffer (pH 3.5) and acetonitrile at a flow rate of 0.6 mL/min and a column temperature of 45 °C. The total run time was 11 min, providing complete resolution of all analytes without interference. Linearity was demonstrated ($R^2 \geq 0.999$) within the ranges of 2–400 ng/mL for medazepam, 10–2000 ng/mL for pinaverium bromide, 5–1000 ng/mL for RS1 (medazepam impurity), and 10–2000 ng/mL for RS3–RS6 (pinaverium bromide impurities). The limits of detection (LOD) and quantitation (LOQ) were 0.17 and 0.50 ng/mL for medazepam and 0.43 and 1.30 ng/mL for pinaverium bromide, respectively, while LOD and LOQ values for related substances ranged between 0.22–0.91 ng/mL and 0.66–2.70 ng/mL. The method was validated in accordance with the ICH Q2(R2) guideline. The validated method was successfully applied for the simultaneous quantification of pinaverium bromide, medazepam, and their respective impurities (RS1–RS6) in commercial film-coated tablets (50 mg/10 mg), demonstrating its applicability for routine quality control and stability testing of fixed-dose combination formulations.

Keywords: Pinaverium bromide; medazepam; related impurities; UPLC–QDA; method validation.

1. Introduction

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders and occurs in the absence of any underlying disease. This syndrome is a symptom-based clinical condition characterized by abdominal pain or discomfort accompanied by changes in bowel habits, such as constipation, diarrhea, or alternating episodes of both [1]. Since no specific biomarker has been identified, there is no definitive diagnostic test for IBS; therefore, the disease is diagnosed based on the patient's symptoms and clinical evaluation findings [2]. IBS affects more than 10% of the adult population worldwide. In routine clinical practice, the diagnosis is usually based on characteristic symptoms. Research studies are mostly limited to a small panel of laboratory tests that help exclude organic diseases presenting with similar symptoms, such as inflammatory bowel disease or celiac disease [3]. Although

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there is no direct mortality rate associated with IBS, it is considered a significant health problem due to its negative impact on quality of life and its high prevalence, affecting a large number of individuals [4]. Both pharmacological and non-pharmacological approaches can be applied in the treatment of IBS. Among non-pharmacological treatments, lifestyle modifications play a key role. Regular exercise, adequate sleep, stress reduction, and the development of healthy eating habits form the foundation of therapy. In addition, recent studies have shown that a gluten-free diet and diets low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) may help alleviate symptoms in some patients. Pharmacological treatments are selected according to the predominant type of symptoms. In diarrhea-predominant IBS cases, peripheral μ-opioid receptor agonist loperamide, antispasmodic agents, antidepressants, serotonin 5-HT_3 antagonists, and the gut-specific antibiotic rifaximin may be used. These drugs contribute to treatment by regulating bowel motility, reducing pain, and improving the patient's quality of life [5]. Pinaverium bromide is a calcium channel antagonist specific to the gastrointestinal system that exerts a selective spasmolytic effect on intestinal smooth muscles. It is widely used in the treatment of IBS and is effective in relieving abdominal pain and discomfort. Its action occurs through two mechanisms: first, by blocking voltage-dependent L-type calcium channels, it prevents the entry of calcium into smooth muscle cells; second, it inhibits the effects of contraction-inducing hormones and mediators such as cholecystokinin, gastrin, and substance P. In this way, it reduces intestinal contractions and alleviates IBS-related pain and discomfort [6]. Benzodiazepines are a class of drugs that act on the central nervous system and have a wide range of therapeutic uses. Chemically, they consist of a seven-membered diazepine ring fused to a benzene ring. This structure imparts hypnotic, anxiolytic, anticonvulsant, muscle relaxant, and sedative properties to the drugs. Benzodiazepines are commonly used in the treatment of conditions such as epilepsy, panic disorder, insomnia, anxiety, and muscle spasms. Medazepam is a benzodiazepine belonging to this group and is metabolized in the body to active metabolites such as N-desmethyldiazepam and subsequently oxazepam [7].

In the literature, various HPLC [8-12] and LC-MS/MS [13-18] methods have been reported for the analysis of Pinaverium bromide and medazepam from pharmaceutical formulation and biological matrices. However, to the best of our knowledge, no analytical method has been reported for the simultaneous determination of these two compounds. Therefore, the aim of this study is to develop and validate a simple, rapid, and reliable ultra-high-performance liquid chromatography (UPLC) method with a QDA detector for the simultaneous analysis of Pinaverium bromide and Medazepam and their related substances in pharmaceutical formulations. This developed method offers a novel analytical approach that can be applied for routine quality control purposes.

Pinaverium bromide and medazepam are combined in a newly developed fixed-dose tablet formulation intended to improve therapeutic compliance in patients with irritable bowel syndrome accompanied by anxiety-related symptoms. From an analytical perspective, the simultaneous determination of both active ingredients and their related substances within a single chromatographic run is particularly advantageous during formulation development and routine quality control. A single-method approach enables comprehensive impurity profiling within the same matrix, reduces analysis time and solvent consumption, and minimizes potential variability associated with multiple independent assays. Therefore, the development of a unified analytical method is well aligned with the practical requirements of combined dosage form evaluation.

2. Experimental

2.1. Materials

Pinaverium–Medazepam 50 mg/10 mg film-coated tablets were manufactured at the R&D Center of Abdi İbrahim Pharmaceuticals (Istanbul, Turkey). Working standards of pinaverium Bromide, medazepam, and their related substances were obtained from Signa S.A. (Mexico) and Cambrex Profarmaco (Italy), which serve as active pharmaceutical ingredient (API) suppliers. All chemicals and solvents used were of analytical reagent grade. Acetonitrile was supplied by J.T. Baker (USA), and ammonium formate was obtained from Sigma-Aldrich (USA).

2.2. Standard Solutions

Stock solutions of Pinaverium Bromide (1000 µg/mL) and Medazepam (200 µg/mL) were prepared in a diluent consisting of water and acetonitrile (50:50, v/v). A stock solution of the related substance RS1 (Medazepam impurity) and individual stock solutions of RS3–RS6 (Pinaverium Bromide impurities) were prepared at a concentration of 100 µg/mL in the same diluent. Working standard solutions were obtained by serial dilution of the stock solutions with the mobile phase to cover the calibration ranges of each analyte: 2–400 ng/mL for Medazepam, 10–2000 ng/mL for Pinaverium Bromide, 5–1000 ng/mL for RS1, and 10–2000 ng/mL for RS3–RS6. Calibration curves were constructed using six concentration levels corresponding to 1%, 5%, 20%, 80%, 100%, and 200% of the target concentrations. All standard and impurity solutions were found to be stable for at least 24 hours at room temperature.

2.3. UPLC-QDA Conditions

Analyses were performed on a Waters Acquity UPLC system equipped with a quaternary pump, autosampler, column oven, and a QDA mass detector operating in positive electrospray ionization (ESI^+) mode. Data acquisition and processing were carried out using Empower 3 software (Waters, USA). Chromatographic separation was achieved on an Acquity BEH C18 column (100 × 2.1 mm, 1.7 µm) maintained at 45 °C. The mobile phase consisted of Solvent A (25 mM ammonium formate buffer, pH 3.5) and Solvent B (acetonitrile), applied in gradient elution mode at a flow rate of 0.6 mL/min according to the program shown in Table 1. The injection volume was 5 µL, and the column temperature was kept constant at 45 °C.

Table 1. Optimized Gradient Program for the UPLC–QDA Analysis

Time (min)	%A	%B	Flow rate (mL/min)
0.0	80	20	0.6
2.0	60	40	0.6
4.0	40	60	0.6
6.0	20	80	0.6
8.0	20	80	0.6
9.0	80	20	0.6
11.0	80	20	0.6

The QDA mass detector was operated in positive electrospray ionization (ESI^+) mode with a capillary voltage of 0.8 kV and a cone voltage of 15 V. The source temperature was maintained at 120 °C, and the desolvation temperature was set to 450 °C. Nitrogen was used as both nebulizing and desolvation gas, with a desolvation gas flow of 800 L/h. The scan range was set between m/z 100–800, and data were acquired in Single Ion Recording (SIR) mode at a sampling rate of 10 Hz. The dwell time for each ion was optimized to ensure maximum sensitivity while maintaining chromatographic resolution. Detection was performed in SIR mode within the 100–800 *m/z* range, monitoring the characteristic positive ions of Pinaverium Bromide *m/z* 512, Medazepam m/z 270, and their related substances: m/z 245 for RS1 (Medazepam impurity), *m/z* 228 for RS3, *m/z* 300 for RS4, *m/z* 508 for RS5, and *m/z* 361 for RS6 (Pinaverium Bromide impurities). RS3 is a brominated dimethoxybenzyl derivative consisting of a dimethoxy-substituted aromatic ring bearing bromine substituents and represents a halogenated aromatic intermediate related to the synthetic pathway of pinaverium bromide. RS4 is a quaternary ammonium morpholinium derivative containing the bicyclic terpene moiety and a dimethoxy-substituted aromatic ring, corresponding to a structurally modified synthetic intermediate of pinaverium bromide. RS5 is a quaternary ammonium bromide impurity bearing a dimethoxy-substituted aromatic ring, a morpholine moiety, and the bicyclic terpene fragment, and is associated with process-related transformations of the

parent compound. RS6 is a hydroxyethyl-substituted quaternary ammonium bromide impurity representing a sidechain modified derivative of pinaverium bromide formed during synthesis or degradation. Medazepam RS1 was identified as 2-methylamino-5-chlorobenzophenone, a known synthetic precursor and process-related impurity of medazepam.

2.4. Method Validation

The UPLC–QDA method developed for the simultaneous determination of pinaverium bromide, medazepam, and their related substances was validated in accordance with the ICH Q2(R2) guidelines [19-22]. The validation process involved the evaluation of system suitability, selectivity, accuracy, precision, linearity, sensitivity (LOD and LOQ), solution stability, and robustness to ensure the reliability and reproducibility of the proposed analytical method.

For the evaluation of system suitability, a standard mixture containing Pinaverium Bromide, Medazepam, and their related substances was prepared and injected in six replicates. System suitability was assessed by examining parameters such as the number of theoretical plates, resolution, and tailing factor values to ensure compliance with the established acceptance criteria.

Specificity refers to the ability to accurately identify the analyte in the presence of other components. The specificity of the developed method was evaluated by injecting, a placebo and a standard mixture solution.

The accuracy of the developed UPLC–QDA method was evaluated by recovery studies conducted at three concentration levels, corresponding to approximately 1%, 100%, and 200% of the target working concentration for each analyte. Known amounts of Pinaverium Bromide, Medazepam, and their related substances (RS1–RS6) were spiked into placebo matrices at the specified levels. Each concentration level was analyzed in quadruplicate on three consecutive days to assess both intra-day and inter-day accuracy and precision.

The measured concentrations were compared with the nominal concentrations, and the percent recovery and relative standard deviation (%RSD) were calculated according to the following equation:

$$\%Recovery = \frac{Recovered\ concentration}{Injected\ concentration} \times 100$$

The acceptance criterion for recovery was set within the range of 95–105% for assay levels and widened to 85–115% for related substances at low concentration levels, in accordance with commonly applied impurity evaluation practices and the principles of ICH Q2(R2).

Precision was evaluated at three concentration levels, corresponding to approximately 1%, 100%, and 200% of the target working concentration for each analyte. Known amounts of Pinaverium Bromide, Medazepam, and their related substances were prepared at the specified levels.

For intra-day (repeatability) and inter-day (intermediate precision) studies, each concentration level was analyzed six times on three consecutive days under the same chromatographic conditions. The relative standard deviation (%RSD) was calculated using the following equation:

$$\%RSD = \frac{Standard\ deviation}{Mean\ of\ measured\ concentration} \times 100$$

An acceptance criterion of %RSD $\leq$ 2.0 was applied in accordance with the ICH Q2(R2) guideline. The precision data obtained confirmed that the method provides excellent repeatability and intermediate precision across the tested range.

The limit of quantitation was established at a signal-to-noise (S/N) ratio of 10, while the limit of detection was defined at an S/N ratio of 3. The repeatability of the obtained LOD and LOQ values was assessed by performing six consecutive injections at each level, and the %RSD values were calculated.

The linearity of the developed UPLC–QDA method was evaluated across six concentration levels covering the entire calibration range of each analyte. Calibration curves were established within the ranges of 10–2000 ng/mL for Pinaverium Bromide, 2–400 ng/mL for Medazepam, 5–1000 ng/mL for RS1 (Medazepam impurity), 10–2000 ng/mL for RS3, 10–2000 ng/mL for RS4, 10–2000 ng/mL for RS5, and 10–2000 ng/mL for RS6 (RS3–RS6 being Pinaverium Bromide impurities). Three independent calibration

curves were constructed for each analyte by plotting peak area versus nominal concentration, using least-squares linear regression. The correlation coefficient (R^2), slope, intercept, and residual plots were examined to verify the linear relationship and response consistency within the tested concentration ranges.

To evaluate the stability of the method, freshly prepared standard solutions and samples obtained from tablets were stored at room temperature for 24 hours and subsequently injected. The peak areas of the analytes were compared to assess the stability of the solutions.

Robustness reflects the reliability of an analytical method when minor, but deliberate variations are introduced into the experimental conditions. In this study, method robustness was evaluated by varying critical chromatographic parameters by approximately ±10% from their nominal values. The flow rate was adjusted to 0.54 mL/min and 0.66 mL/min, while the column temperature varied to 40.5 °C and 49.5 °C, keeping all other chromatographic conditions constant. The influence of these deliberate changes on retention time, peak area, resolution, and quantification of all analytes and related substances (RS1–RS6) was examined, confirming that small operational variations did not significantly affect the analytical performance of the method.

2.5. Sample Preparation

Twenty tablets, each containing 50 mg of Pinaverium Bromide and 10 mg of Medazepam, were accurately weighed, finely powdered, and used for analysis. The investigated product was Pinaverium Bromide / Medazepam film-coated tablets (Abdi İbrahim Pharmaceuticals, Türkiye). An accurately weighed portion of the powdered sample, equivalent to the average tablet weight, was transferred into a 100 mL volumetric flask containing approximately 90 mL of diluent (water:acetonitrile, 50:50, v/v). The mixture was sonicated for 30 min to ensure complete dissolution, allowed to cool to room temperature, diluted to volume with the same diluent, and filtered through a 0.45 µm PTFE membrane filter. The stock tablet solution obtained contained approximately 500 µg/mL Pinaverium Bromide and 100 µg/mL Medazepam. This stock solution was used directly for impurity analysis. For assay determination, an intermediate dilution of the stock solution was first prepared, followed by a second dilution with the mobile phase to obtain the final analytical solutions containing approximately 500 ng/mL Pinaverium Bromide and 200 ng/mL Medazepam. All sample solutions were freshly prepared, filtered through a 0.45 µm PTFE membrane prior to injection, and analyzed using the UPLC system.

2.6. Uncertainty Assessment

The principal contributors to measurement uncertainty in the validated method were assessed in line with the EURACHEM/CITAC recommendations. Factors related to reference standard purity, sample preparation (including weighing), calibration model parameters, recovery performance, and repeatability were identified as the dominant sources. The overall standard uncertainty was then estimated by combining these components through the root-sum-of-squares approach, as expressed by the following equation:

$$u = \sqrt{(u_{\text{standard}})^2+(u_{\text{weighing}})^2\,(u_{\text{calibration}})^2+(u_{\text{recovery}})^2+(u_{\text{repeatability}})^2}$$

In this context, u_{standard} represents the uncertainty associated with the certified purity of the reference material, whereas u_{weighing} reflects the contribution from the mass measurement step. The term $u_{\text{calibration}}$ accounts for the uncertainty propagated from the calibration model—particularly the slope—while u_{recovery} captures the variability observed in the recovery experiments. Finally, $u_{\text{repeatability}}$ quantifies the within-run precision of the analytical procedure. The expanded uncertainty (U) at the 95% confidence level was subsequently obtained by multiplying the combined standard uncertainty by a coverage factor of k = 2, as shown in the following expression:

$$U = k.u$$

Here, k denotes the coverage factor, set to 2 to approximate a 95% confidence level. The resulting expanded uncertainty (U) remains traceable to SI units through the certified purity of the reference standard and the calibration status of the analytical balance, ensuring metrological integrity of the reported measurements. The uncertainty budget was constructed in accordance with the principles outlined in the EURACHEM/CITAC Guide [21-24], and the full numerical evaluation is presented in the Supplementary Information.

3. Results and Discussion

3.1. Chromatographic Separation

Chromatographic conditions were optimized to ensure efficient and selective separation of Pinaverium Bromide, Medazepam, and their related substances. Different stationary phases, including C18 and C8 columns, and various mobile phase combinations containing ammonium formate or formic acid with acetonitrile or methanol were systematically investigated.

Among the tested conditions, the best chromatographic performance in terms of resolution, peak symmetry, and signal intensity was achieved using an Acquity BEH C18 column (100 × 2.1 mm, 1.7 µm) maintained at 45 °C. The mobile phase consisted of 25 mM ammonium formate buffer (pH 3.5) and acetonitrile, applied in gradient elution mode at a flow rate of 0.6 mL/min. These optimized conditions provided complete baseline separation of all analytes and their related substances within a total analysis time of 11 minutes, including column re-equilibration.

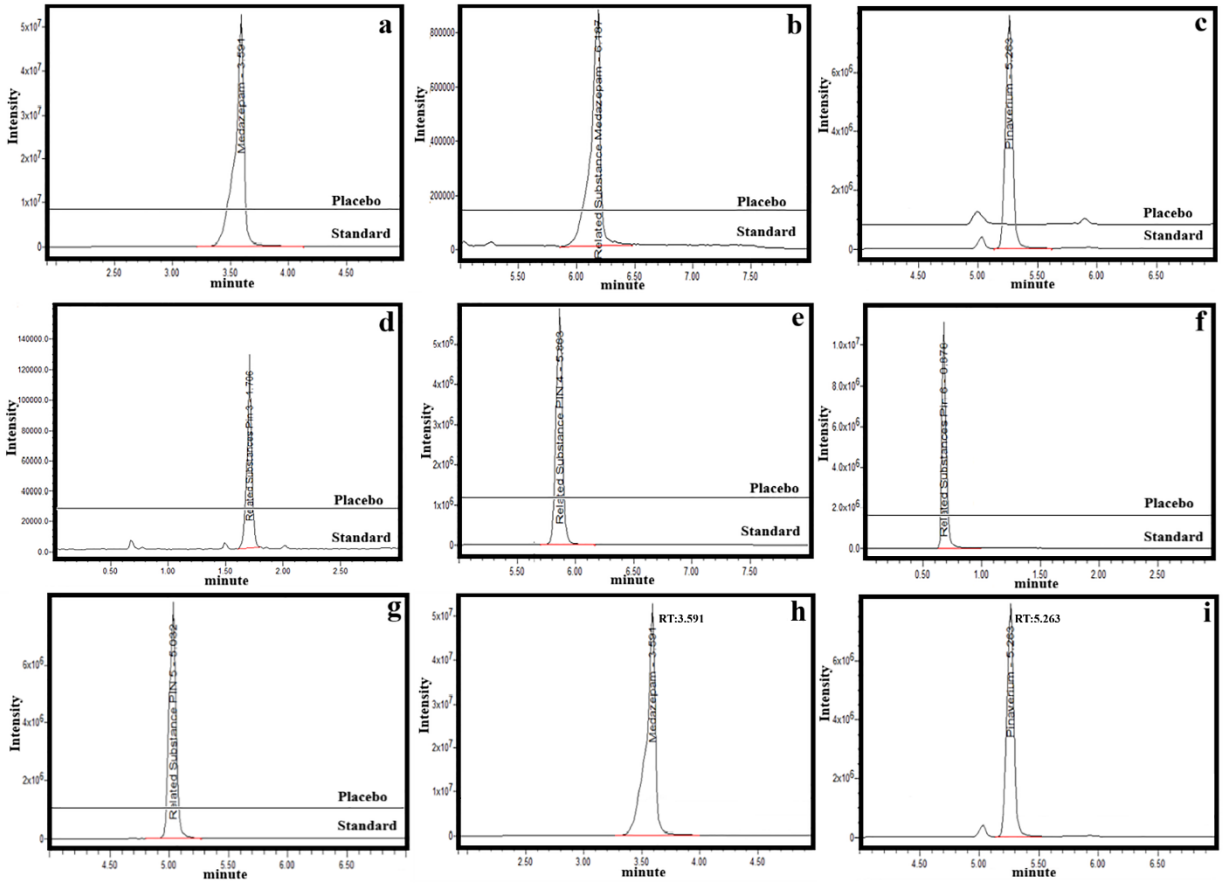

Figure 1. Representative chromatograms demonstrating the specificity of the developed UPLC–QDA method: (a) medazepam, (b) medazepam RS1, (c) pinaverium bromide, (d) pinaverium bromide RS3, (e) pinaverium bromide RS4, (f) pinaverium bromide RS5, (g) pinaverium bromide RS6, (h) medazepam in pharmaceutical formulations, and (i) pinaverium bromide in pharmaceutical formulation

3.2. Validation of the Method

The developed UPLC–QDA method was validated in accordance with the ICH Q2(R2) guideline for system suitability, specificity, linearity, accuracy, precision, robustness, sensitivity, and stability parameters.

All analytes—pinaverium bromide, medazepam, and their related substances were eluted with distinct retention times, symmetrical peaks (tailing factor < 2), and theoretical plate numbers exceeding 2000, confirming satisfactory system suitability and column efficiency.

The method exhibited excellent specificity, as chromatograms of blank and placebo solutions showed no interference at the retention regions of any analyte. Representative chromatograms of standard and sample solutions are shown in Figure 1, clearly demonstrating complete baseline resolution (resolution > 2.0) and the absence of co-eluting peaks.

The results obtained demonstrated excellent sensitivity for all analytes. The LOD and LOQ values were found to be 0.17 and 0.50 ng/mL for Medazepam, 0.55 and 1.65 ng/mL for Medazepam Impurity (RS1), 0.43 and 1.30 ng/mL for Pinaverium Bromide Standard, 0.22 and 0.66 ng/mL for RS3, 0.42 and 1.30 ng/mL for RS4, 0.39 and 1.20 ng/mL for RS5, and 0.91 and 2.70 ng/mL for RS6, respectively.

Linearity was confirmed across the studied calibration ranges with correlation coefficients (R^2) > 0.999 for all analytes, indicating a strong linear relationship between detector response and analyte concentration.

Accuracy and precision were evaluated at three concentration levels (1%, 100 %, and 200 % of target concentration). The mean recovery values were within 85–115%, and %RSD values remained below 2 %, confirming the trueness and repeatability of the method. Both intra-day and inter-day precision data demonstrated high reproducibility.

The linearity, accuracy, and precision findings demonstrated that the method provides reliable quantitative performance, as summarized in Table 2.

Table 2. The linearity, accuracy, and precision results

Compound	Linearity Range (ng/mL)	R^2	Slope	Intercept	Intra day	Inter day	%Recovery					
							Accuracy (%)			RSD (%)		
					RSD (%)		1%	100%	200%	1%	100%	200%
Medazepam	2–400	0.9999	1834.7	318.1	1.5	0.6	105.8	103.6	102.4	7.0	0.3	2.0
Medazepam RS1	5–1000	0.9999	443.4	3.77	0.1	0.5	109.6	104.0	104.2	3.9	0.4	0.6
Pinaverium Bromide	10–2000	0.9999	322.2	8.00	1.8	0.4	107.5	102.3	101.7	1.2	0.3	0.6
Pinaverium Bromide RS3	10–2000	0.9999	363.4	82.38	1.6	0.8	108.8	100.8	99.9	3.0	0.4	0.4
Pinaverium Bromide RS4	10–2000	0.9999	254.1	18.80	1.7	0.9	107.1	104.8	102.9	7.9	0.7	0.8
Pinaverium Bromide RS5	10–2000	0.9999	338.4	–17.18	1.5	0.7	104.0	101.2	103.5	1.4	0.5	2.7
Pinaverium Bromide RS6	10–2000	0.9999	282.4	86.27	1.7	0.9	106.0	99.9	102.9	1.6	0.7	0.3

Robustness testing, performed by applying deliberate 10% variations in flow rate and column temperature while maintaining all other chromatographic conditions constant, showed no meaningful

impact on retention time, resolution, or peak area, indicating strong method resilience. Additionally, the method exhibited excellent solution stability, with both standard and sample preparations remaining stable for at least 24 hours under routine laboratory conditions (Table 3). Overall, all validation parameters—including linearity, accuracy, precision, robustness, and solution stability—meet the ICH Q2(R2) acceptance criteria, confirming that the developed UPLC–QDA method is suitable for the routine quantitative determination of pinaverium bromide, medazepam, and their related impurities in combined tablet formulations.

Table 3. Solution stability and robustness results

Compound	Solution Stability	Flow		Temperature	
	(%)	+10%	–10%	+10%	–10%
Medazepam	–0.42	–1.75	1.26	–0.46	0.39
Medazepam RS1	0.88	0.21	–0.15	0.09	0.11
Pinaverium Bromide	0.62	2.86	0.58	–0.70	0.82
Pinaverium Bromide RS3	–0.30	1.07	–1.34	0.31	–0.28
Pinaverium Bromide RS4	–1.94	–1.12	–0.73	0.73	0.63
Pinaverium Bromide RS5	0.73	0.64	0.51	–0.44	–0.38
Pinaverium Bromide RS6	–1.82	–1.68	–1.74	–0.82	–0.91

The relatively higher %RSD values observed at the lowest concentration level (~1%) are attributed to increased signal variability near the LOQ and the inherent response characteristics of single-quadrupole (QDA) detection at trace concentration ranges. At such low levels, minor fluctuations in signal-to-noise ratio and peak integration can lead to increased variability; however, the observed precision remained within acceptable limits for low-level related substance analysis and did not compromise the quantitative reliability of the method.

3.3. Application to the Analysis of Pharmaceutical Dosage Forms

The validated UPLC–QDA method was successfully applied for the simultaneous quantification of pinaverium bromide, medazepam, and their related substances in commercial film-coated tablets containing 50 mg Pinaverium Bromide and 10 mg Medazepam per unit dose.

All analytes were well resolved without any interference from excipients, confirming the specificity and selectivity of the developed method. The recovery results obtained from the commercial formulation were 99.6 % for pinaverium bromide and 99.8 % for medazepam, while all related impurities were below 0.10 % of the labeled amount.

These results demonstrate the accuracy, reproducibility, and applicability of the proposed UPLC–QDA method for the routine quality control and stability testing of fixed-dose combination products containing pinaverium bromide and medazepam.

3.4. Uncertainty Assessment

The combined and expanded uncertainty values for all analytes are summarized in Table 4, while the full calculation steps and component-wise uncertainty evaluations are provided in the Supporting Information. Among the evaluated contributors, the uncertainty contribution from the weighing step was found to be negligible compared with the other sources and therefore was excluded from the combined uncertainty calculation [22-24].

Table 4. Data of combined and expanded uncertainty

Analyte	$u_{standard}$	$u_{calibration}$	$U_{recovery}$	$u_{repeatability}$	$u_{combined}$	$U_{expanded}$ (k = 2)
Medazepam[a]	0.289	0.154	0.187	0.091	0.388	0.776
Medazepam RS1[b]	0.577	0.514	0.216	0.080	0.807	1.613
Pinaverium Bromide[c]	0.058	0.424	0.087	0.053	0.440	0.879
Pinaverium RS3[d]	0.115	0.395	0.096	0.035	0.424	0.848
Pinaverium RS4[e]	0.115	0.442	0.136	0.038	0.478	0.957
Pinaverium RS5[f]	0.115	0.402	0.121	0.034	0.437	0.873
Pinaverium RS6[g]	0.115	0.474	0.118	0.033	0.503	1.005

[a]Uncertainty for 200.17 ng/mL; 95 % confidence level; U % values reported.
[b]Uncertainty for 497.42 ng/mL; 95 % confidence level; U % values reported.
[c]Uncertainty for 1008.39 ng/mL; 95 % confidence level; U % values reported.
[d]Uncertainty for 1001.65 ng/mL; 95 % confidence level; U % values reported.
[e]Uncertainty for 1005.22 ng/mL; 95 % confidence level; U % values reported.
[f]Uncertainty for 998.50 ng/mL; 95 % confidence level; U % values reported.
[g]Uncertainty for 1001.13 ng/mL; 95 % confidence level; U % values reported.

4. Conclusions

A UPLC–QDA method was developed and validated for the simultaneous quantification of pinaverium bromide, medazepam, and their related impurities in combined pharmaceutical formulations. All validation parameters complied with the ICH Q2(R2) criteria, and measurement uncertainty assessment confirmed the reliability of the quantitative results, with low expanded uncertainty values (k = 2). The method was successfully applied to commercial products, demonstrating its suitability for the routine determination of active ingredients and trace-level impurities without interference from excipients. Overall, this approach provides a practical and efficient solution for routine quality control, release testing, and stability assessment of fixed-dose formulations by enabling simultaneous analysis within a single chromatographic run.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/ journal-of-chemical-metrology

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