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Impurity Profiling of Paracetamol and Orphenadrine Citrate Fixed-Dose Tablets by a Stability-Indicating HPLC Method

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Abstract

A novel, sensitive, and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous determination of impurities and degradation products in a fixed-dose combination tablet containing paracetamol (500 mg) and orphenadrine citrate (30 mg). The method enabled effective separation and quantification of eight specified impurities, including *p-aminophenol*, *p-nitrophenol*, *p-chloroacetanilide*, *diphenhydramine hydrochloride*, *orphenadrine related compounds B and C*, *2-methylbenzhydrol*, and *2-methylbenzophenone*. Chromatographic separation was achieved using a Waters SunFire C18 column (250 × 4.6 mm, 5 µm) with gradient elution comprising phosphate buffer (pH 3.0) and acetonitrile–water (90:10, v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 220 nm. The method was validated in accordance with ICH Q2(R1) guidelines, demonstrating acceptable specificity, linearity, accuracy, precision, and robustness. Forced degradation studies under hydrolytic, oxidative, photolytic, and thermal conditions confirmed the stability-indicating capability of the method, with all degradation products well resolved from the active pharmaceutical ingredients and their known impurities. The limits of quantitation for all impurities ranged from 0.01% to 0.10% relative to their respective active concentrations. Minor intentional variations in chromatographic conditions did not significantly affect method performance, confirming robustness. The validated method is suitable for routine quality control analysis and stability studies of paracetamol and orphenadrine citrate fixed-dose combination tab-

lets.

Keywords

Paracetamol, Acetaminophen, Analgesic, Antipyretic, Orphenadrine Citrate, HPLC, Method Validation, Stability-Indicating, Impurity Profiling, ICH Guidelines

1. Introduction

Paracetamol (acetaminophen) is one of the most extensively used analgesic and antipyretic agents worldwide and is included on the World Health Organization's Model List of Essential Medicines [1], **Figure 1** [2]. Owing to its proven efficacy, favorable safety profile at therapeutic doses, and wide patient acceptability, paracetamol is routinely employed in the management of mild to moderate pain and fever across all age groups, including pediatric, geriatric, and pregnant populations [2] [3]. It plays a pivotal role in global healthcare as a first-line therapy for pain associated with musculoskeletal disorders, postoperative recovery, headache, and osteoarthritis. The widespread use of paracetamol, both as a single agent and in fixed-dose combination products, underscores the importance of maintaining stringent quality standards to ensure patient safety [4] [5]. Given its high global consumption and chronic use in certain patient populations, effective control of paracetamol-related impurities and degradation products is essential to safeguard therapeutic efficacy and minimize potential toxicological risks.

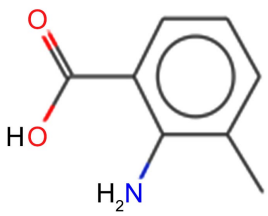

Figure 1. 2-Amino-3-methylbenzoic acid, Chemical Formula: $C_8H_9NO_2$, Molecular weight: 151.1626.

Orphenadrine citrate is a centrally acting anticholinergic muscle relaxant widely used in the management of painful musculoskeletal conditions associated with acute muscle spasms [6], **Figure 2** [6]. It exerts its therapeutic effect by modulating central nervous system pathways involved in muscle tone and pain perception, while also possessing mild analgesic properties [3] [6]. Orphenadrine citrate is frequently prescribed as an adjunct to analgesics to enhance pain relief and improve functional outcomes in conditions such as strains, sprains, and postoperative muscle spasm. Although used at relatively low doses, its clinical importance lies in its ability to improve patient mobility and quality of life, particularly in acute and subacute musculoskeletal disorders. Given its

global use in fixed-dose combination products, stringent control of orphenadrine-related impurities and degradation products is essential to ensure consistent safety, efficacy, and regulatory compliance throughout the product lifecycle [4] [5].

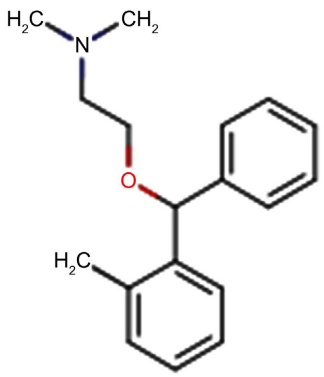

Figure 2. 2-Hydroxy-1,2,3-propantricarbonsäure-N,N-dimethyl-2-[(2-methylphenyl) (phenyl)methoxy]ethanamin (1:1), Chemical Formula: $C_{18}H_{23}NO$, Molecular weight: 269.3813.

The quality, safety, and efficacy of pharmaceutical products are critically dependent on effective control of impurities, which may originate from raw materials, manufacturing processes, or degradation pathways during storage [4] [5] [7]. Impurities present even at low levels can affect drug stability and pose potential safety risks, making their monitoring an essential component of quality control and regulatory compliance.

For paracetamol, the principal process-related and degradation impurities include p-aminophenol, p-nitrophenol, and p-chloroacetanilide. Similarly, orphenadrine citrate is associated with several specified related substances, such as diphenhydramine hydrochloride, orphenadrine related compounds B and C, 2-methylbenzhydrol, and 2-methylbenzophenone. The simultaneous presence of these impurities in a combination drug product presents significant analytical challenges.

Although individual analytical methods for paracetamol and orphenadrine citrate impurities have been reported, there is a clear need for a single, robust, and stability-indicating analytical method capable of simultaneously determining all associated impurities in the fixed-dose combination tablet [8]-[10]. Such a method would improve analytical efficiency while ensuring consistent quality control throughout the product's lifecycle.

This study describes the development and comprehensive validation of a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the determination of related substances in paracetamol (500 mg) and orphenadrine citrate (30 mg) tablets. The method was validated in accordance with International Council for Harmonization (ICH) guidelines Q2(R1) and Q3B(R2) [4] [5], covering specificity, linearity, accuracy, precision, limits of detection and quantitation, robustness, and solution stability.

2. Materials and Methods

2.1. Chemicals and Reagents

Standards: Paracetamol USP (99.9% potency), Orphenadrine Citrate USP (99.9% potency), and reference standards for all related impurities (p-Aminophenol, p-Nitrophenol, p-Chloroacetanilide, Diphenhydramine HCl, Orphenadrine Related Compound C, Orphenadrine Related Compound B, 2-Methylbenzhydrol, 2-Methylbenzophenone) were obtained from Sigma-Aldrich.

Samples: Tablets marketed product were used for validation.

Reagents: Potassium dihydrogen phosphate (HPLC grade), orthophosphoric acid (ACS grade), acetonitrile (HPLC grade), and Milli-Q water were used. All other chemicals were of analytical grade.

Placebo: A placebo mixture mimicking the tablet composition (containing microcrystalline cellulose, sodium starch glycolate, magnesium stearate, colloidal silicon dioxide, and coloring agents) was prepared in-house.

2.2. Chromatographic Conditions

The HPLC system (Agilent/Waters) equipped with a photodiode array (PDA) detector was used. The optimized chromatographic parameters are summarized in Table 1.

Table 1. Optimized chromatographic conditions.

Parameter	Condition
Column	Waters SunFire C18, 250 × 4.6 mm, 5 µm (or) equivalents
Detector Wavelength	220 nm (UV/PDA)
Flow Rate	1.0 mL/minute
Column Temperature	30˚C
Injection Volume	10 µL
Run Time	50 minutes
Diluent	Water: Acetonitrile (95:5) % v/v
Mobile Phase A	0.02 M Potassium dihydrogen phosphate buffer, pH 3.0 (adjusted with orthophosphoric acid)
Mobile Phase B	Acetonitrile: Water (90:10) % v/v

Gradient Program

Time (minutes)	Mobile Phase A (%)	Mobile Phase B (%)
0.01	100	0
4.0	100	0
25.0	50	50
29.0	36	64
41.0	36	64
45.0	100	0
50.0	100	0

2.3. Preparation of Solutions

Standard Stock Solutions: Paracetamol (200 µg/mL) and Orphenadrine Citrate (150 µg/mL) were prepared separately in the diluent.

Impurity and Degradant Determination (IDD) Standard Solution: A mixture containing 10 µg/mL of Paracetamol and 1.5 µg/mL of Orphenadrine Citrate was prepared by diluting the stock solutions with diluent.

Standard solution:

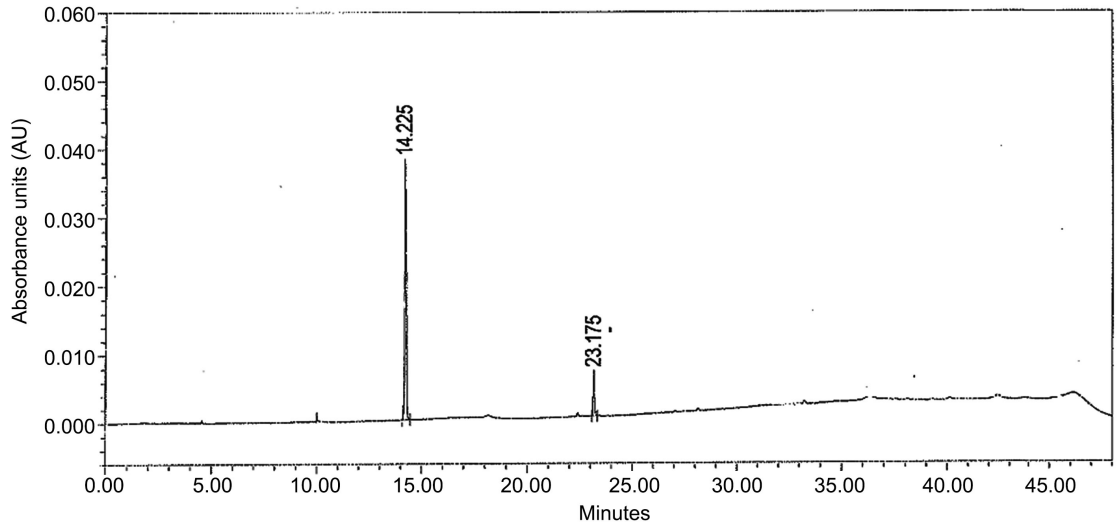

Figure 3. Standard solution of paracetamol and orphenadrine-reference chromatogram.

Standard solution of Paracetamol and Orphenadrine reference chromatogram obtained using the developed RP-HPLC method in accordance with ICH Q2(R1) validation principles [4], as shown in **Figure 3**.

System Suitability Solution: Powdered tablet equivalent to 500 mg Paracetamol and 30 mg Orphenadrine Citrate was stressed with 2 mL of 1N HCl at 90˚C for 2 hours, neutralized, and diluted to a concentration of 50 µg/mL Paracetamol and 3 µg/mL Orphenadrine Citrate.

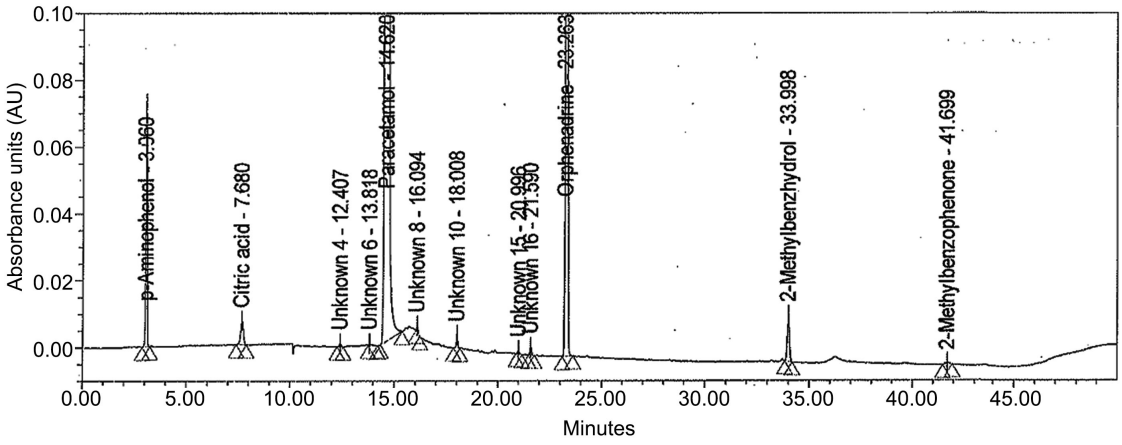

Figure 4. System suitability solution-reference chromatogram.

System suitability solution reference chromatogram demonstrating chromatographic performance in accordance with USP <621> Chromatography and ICH Q2(R1) [4] [7], as shown in **Figure 4**.

Sample Solution: 20 tablets were powdered. An amount equivalent to 500 mg Paracetamol and 30 mg Orphenadrine Citrate was accurately weighed, transferred to a 100 mL volumetric flask, sonicated with ~70 mL diluent for 15 minutes, and diluted to volume with diluent. The solution was filtered through a 0.45 µm GxF/GHP filter.

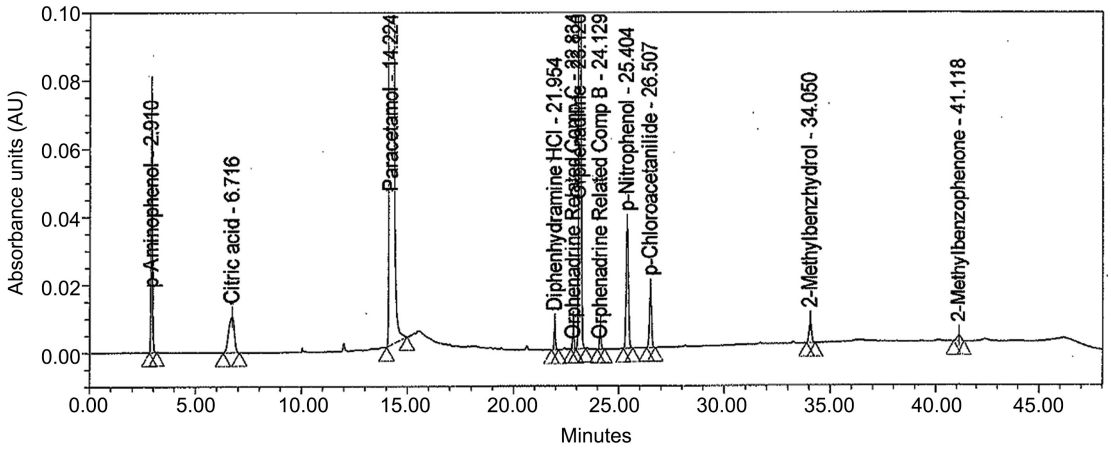

Figure 5. Spiked sample solution with all impurities-Reference Chromatogram.

Chromatogram of the spiked sample solution containing all specified impurities demonstrating the specificity of the developed stability-indicating RP-HPLC method [4] [5], as shown in **Figure 5**.

Impurity of Known and Unknown impurities calculated using as below formula.

$$\frac{\text{A idd}}{\text{A std}} \times \frac{\text{W std*}}{100} \times \frac{5}{100} \times \frac{100}{\text{Wspl}} \times \frac{\text{Avt}}{\text{Dosage}} \times \frac{1}{\text{RRF}} \times 100 = \%\ \text{of known impurity}$$

$$\frac{\text{A unk}}{\text{A std}} \times \frac{\text{W std*}}{100} \times \frac{5}{100} \times \frac{100}{\text{Wspl}} \times \frac{\text{Avt}}{\text{Dosage}} \times 100 = \%\ \text{of unknown impurity}$$

2.4. Method Validation

The International Council for Harmonization (ICH) guideline Q2(R1), "Validation of Analytical Procedures: Text and Methodology," provides a harmonized framework for validating analytical method to ensure their reliability, accuracy, and suitability for intended use throughout the pharmaceutical products lifecycle [4]. This analytical method was developed and validated for the identification and quantification of impurities in the finished drug products of Paracetamol and Orphenadrine citrate fixed dose tablets in accordance with ICH Q2(R1) and ICH Q3B (R2) [4] [5].

Specificity and Forced Degradation Studies

The specificity of the method was demonstrated by evaluating potential inter-

ference from the diluent and placebo at the retention times of all target analytes. No interfering peaks were observed, confirming that the method is selective for the active pharmaceutical ingredients, their impurities, and degradation products [7] [8].

Forced degradation studies were conducted to establish the stability-indicating capability of the method. The drug product was subjected to stress conditions including acid hydrolysis (1N HCl at 90˚C), alkali hydrolysis (1N NaOH at 90˚C), oxidative degradation (3% hydrogen peroxide), thermal stress (60˚C), and photolytic stress in accordance with ICH Q1B guidelines. Under all stress conditions, degradation product was adequately resolved from the main analyte peaks.

Peak purity analysis was performed using a photodiode array (PDA) detector, confirming the absence of co-eluting peaks with the active components. Mass balance, expressed as the sum of assay values and total degradation products, was calculated for each stressed sample and found to be within acceptable limits.

Acceptable limits for the specificity via Forced Degradation:

Mass Balance	Purity angle	Purity Threshold	Purity Flag
≥95%	Purity angle must be less than the Purity threshold		No

Detection Limit (DL) and Quantitation Limit (QL):

The detection limit (DL) and quantitation limit (QL) were estimated using the signal-to-noise (S/N) ratio approach described in ICH Q2(R1) [4], where DL corresponded to an S/N ratio of approximately 3:1 and QL to an S/N ratio of approximately 10:1. The determined QL for each impurity was subsequently verified for precision by analyzing six replicate preparations (n = 6) at the QL level, demonstrating acceptable repeatability.

Linearity:

Linearity was assessed for each specified impurity as well as the active pharmaceutical ingredients across a concentration range from the quantitation limit (QL) to 140% of the respective specification level according to the ICH Q2(R1) [4]. Calibration solutions were prepared in triplicate at six concentration levels within the defined range. The linearity of response was evaluated by calculating the correlation coefficient (r^2), slope, and intercept of the calibration curves. Relative response factors (RRFs) for each impurity were also determined to ensure accurate quantification relative to the corresponding active substance [7] [8].

Accuracy:

Accuracy was evaluated by recovery studies in which known quantities of specified impurities were spiked into the placebo at three concentration levels corresponding to the quantitation limit (QL), 100%, and 140% of the specification level in accordance with ICH validation requirements [4]. Each level was analyzed in triplicate (n = 3). Percent recovery was calculated by comparing the measured impurity concentration with the theoretical spiked amount, demonstrating the accuracy of the method across the evaluated range.

Precision:

Method precision (repeatability) was evaluated by analyzing six individual unspiked sample preparations and six sample preparations spiked at 100% of the specification level, all prepared from the same batch of the drug product. The results were expressed as percentage relative standard deviation (%RSD) for the quantified impurities and active components [4].

Intermediate precision was assessed by repeating the repeatability study under varied conditions, including analysis by a different analyst, on a different day, and using a different HPLC system. The combined results demonstrated acceptable precision of the method under normal and intermediate analytical conditions.

Robustness:

The robustness of the method was evaluated by introducing deliberate and minor variations in chromatographic conditions to assess their impact on method performance. The evaluated parameters included changes in buffer pH (2.8 - 3.2), column temperature (25˚C - 35˚C), buffer concentration (±10%), and the use of an alternative C18 column (Waters Symmetry Shield RP18). The effects of these variations were assessed by monitoring system suitability parameters, including resolution, tailing factor, theoretical plates, and repeatability. The method demonstrated robustness, as no significant changes in system suitability or separation performance were observed under the tested conditions [8]-[10].

Solution Stability:

The stability of both standard and sample solutions was evaluated by analyzing the solutions after storage at room temperature and under refrigerated conditions for up to 7 days. The obtained results were compared with those from freshly prepared solutions. No significant changes in impurity levels or assay values were observed, indicating that both standard and sample solutions were stable under the evaluated storage conditions for the specified duration [4]-[8].

3. Results and Discussion

3.1. Specificity and System Suitability

The method demonstrated excellent specificity, as no interfering peaks originating from the diluent or placebo were observed at the retention times of any of the analytes (**Table 2**). All critical peak pairs were well resolved, with resolution values exceeding the predefined acceptance criteria, thereby ensuring accurate and reliable quantification of the analytes.

System suitability parameters evaluated using the standard and system suitability solutions were performed according to the requirements of USP general chapter <621> chromatography and accepted chromatographic method validation practices [7]-[9]. All critical peak pairs demonstrated resolution, confirming the suitability of the chromatographic system for routine analysis (**Table 3**).

3.2. Forced Degradation (Stability-Indicating Nature)

Forced degradation studies (**Table 4**) demonstrated that the drug product was

susceptible to degradation under acidic, alkaline, and oxidative (peroxide) stress conditions. Under all applied stress conditions, the calculated mass balance values remained within an acceptable range (97.9% - 101.7%), indicating that all major degradation products were adequately detected and quantified [4] [11].

Table 2. Retention times of active ingredients, related substances and its specification.

Compound	Retention Time (minutes)	Relative Retention Time (w.r.t. Paracetamol/ Orphenadrine)	Specification as per the USP
Paracetamol Impurities			
p-Aminophenol	2.91	0.20 (w.r.t Paracetamol)	NMT 0.2%
Paracetamol	14.22	1.0	N/A
p-Nitrophenol	25.40	1.79	NMT 0.2%
p-Chloroacetanilide	26.51	1.86	NMT 0.2%
Orphenadrine Citrate Impurities			
Diphenhydramine HCl	21.95	0.95 (w.r.t Orphenadrine)	NMT 0.5%
Orphenadrine Rel. Cmpd. C	22.83	0.99	NMT 0.5%
Orphenadrine Citrate	23.12	1.0	N/A
Orphenadrine Rel. Cmpd. B	24.13	1.04	NMT 0.5%
2-Methylbenzhydrol	34.05	1.47	NMT 0.5%
2-Methylbenzophenone	41.12	1.78	NMT 0.5%

Table 3. Typical system suitability results.

Parameter	Requirement	Result
Paracetamol Peak		
USP Tailing Factor	NMT 2.0	1.1
USP Theoretical Plates	NLT 4000	>190,000
Resolution from RRT ~1.2 peak	NLT 3.0	>24.0
Orphenadrine Peak		
Resolution from 2-Methylbenzhydrol	NLT 2.0	>55.0
% RSD (Paracetamol peak area, n = 6)	NMT 10.0%	≤0.4%
% RSD (Orphenadrine peak area, n = 6)	NMT 10.0%	≤1.1%

Peak purity analysis of paracetamol and orphenadrine citrate in all stressed samples showed purity angle values lower than the corresponding purity thresholds, confirming the absence of co-eluting peaks and ensuring the purity of the active pharmaceutical ingredient peaks. Furthermore, all degradation products were well resolved from the main analytes and known impurities, thereby confirming the stability-indicating capability of the developed RP-HPLC method.

Acceptable limits for the specificity via Forced Degradation:

Mass Balance	Purity angle	Purity Threshold	Purity Flag
≥95%	Purity angle must be less than the Purity threshold		No

Table 4. Mass balance from forced degradation studies.

Stress Condition	Paracetamol			Orphenadrine		
	% Assay	% Total Degradants	% Mass Balance	% Assay	% Total Degradants	% Mass Balance
Unstressed	100.2	0.00	100.2	99.6	0.15	99.8
Photostability	99.0	0.01	99.0	98.1	0.36	98.5
Thermal	100.9	0.01	100.9	99.4	0.35	99.8
Acid (1N HCl, 90˚C)	101.2	0.18	101.4	97.3	2.07	99.4
Alkali (1N NaOH, 90˚C)	100.8	0.86	101.7	94.5	6.77	101.3
Peroxide (3% H_2O_2)	100.4	0.04	100.4	92.1	5.79	97.9

Above degradation studies found not considerable degradation of impurity though these stress conditions will be mentioned in the method.

3.3. Detection and Quantitation Limits

The DL and QL concentrations and their percentage with respect to the active ingredient concentration are summarized in Table 5. The determined QL levels were sufficiently low for all impurities, allowing for precise quantification at levels well below the specification limits (e.g., 0.2% for Paracetamol impurities and 0.5% for Orphenadrine impurities). The precision at the QL level was within acceptable limits (RSD ≤ 20%), confirming the method's sensitivity.

Table 5. Detection and quantitation limits.

Compound	DL Conc. (µg/mL)	% w.r.t. Active	QL Conc. (µg/mL)	% w.r.t. Active
p-Aminophenol	0.1	0.002	0.5	0.01
Paracetamol	0.1	0.002	0.5	0.01
p-Nitrophenol	0.1	0.002	0.5	0.01
p-Chloroacetanilide	0.1	0.002	0.5	0.01
Diphenhydramine HCl	0.12	0.04	0.24	0.08
Orphenadrine Rel. Cmpd. C	0.12	0.04	0.24	0.08
Orphenadrine Citrate	0.12	0.04	0.24	0.08
Orphenadrine Rel. Cmpd. B	0.12	0.04	0.24	0.08
2-Methylbenzhydrol	0.12	0.04	0.24	0.08
2-Methylbenzophenone	0.12	0.04	0.30	0.10

3.4. Linearity and Accuracy

The method exhibited excellent linearity for all impurities and the active ingredients over the range from QL to 140% of the specification level (Table 6) described

in ICH Q2(R1) [4] [8]-[10]. The correlation coefficients (r^2) were ≥ 0.998 for all compounds, demonstrating a strong linear relationship. The calculated Relative Response Factors (RRFs) for the impurities are crucial for accurate quantification and are presented in Table 6.

Table 6. Linearity data and relative response factors.

Compound	Concentration Range (µg/mL)	r^2	RRF
p-Aminophenol	0.54 - 15.21	0.998	1.85
Paracetamol	0.46 - 12.76	1.000	1.00
p-Nitrophenol	0.54 - 15.07	1.000	1.54
p-Chloroacetanilide	0.54 - 15.19	1.000	0.69
Diphenhydramine HCl	0.24 - 2.12	0.999	1.47
Orphenadrine Related Compound C	0.24 - 2.10	0.999	1.56
Orphenadrine Citrate	0.24 - 2.10	0.999	1.00
Orphenadrine Related Compound B	0.24 - 2.10	0.999	1.26
2-Methylbenzhydrol	0.24 - 2.11	0.998	1.89
2-Methylbenzophenone	0.30 - 2.07	0.998	1.41

Accuracy of the method was demonstrated by excellent recovery of impurities from placebo samples spiked at three concentration levels corresponding to the quantitation limit (QL), 100%, and 140% of the specification level. The average recoveries for all known impurities ranged from 87% to 105% (Table 7), which are well within the predefined acceptance criteria of 80% - 115%.

For unknown impurities purposes, recovery studies conducted using placebo spiked paracetamol and orphenadrine citrate at the unknown impurity specification level yielded recoveries between 98.4% and 104.5%, further confirming the accuracy and reliability of the method across the evaluated concentration range.

Table 7. Summary of accuracy and precision results.

Name	% Level	Avg. Recovery (%)	%RSD
p-Aminophenol	QL	96.5	2.8
	100	95.2	3.0
	140	96.7	0.2
p-Nitrophenol	QL	105.3	1.1
	100	94.1	3.2
	140	94.1	0.3
p-Chloroacetanilide	QL	98.5	2.5
	100	91.5	3.0
	140	93.2	0.2

Continued

Diphenhydramine HCl	QL	87.3	2.0
	100	97.8	1.3
	140	97.4	0.4
Orphenadrine related compound C	QL	92.5	0.4
	100	99.2	1.9
	140	98.0	0.2
Orphenadrine related compound B	QL	91.1	6.3
	100	91.8	3.5
	140	94.4	0.3
2-Methylbenzhydrol	QL	93.2	0.9
	100	103.0	6.8
	140	100.7	3.9
2-Methylbenzophenone	QL	91.7	4.7
	100	93.3	6.5
	140	91.3	3.6
Paracetamol	QL	104.5	2.0
	100	98.5	0.1
	140	98.4	1.0
Orphenadrine Citrate	QL	99.4	2.0
	100	101.0	1.2
	140	99.5	1.1

Table 8. Combined summary results (average % accuracy and %RSD) for both Repeatability (Precision) and Intermediate Precision across all eight spiked analytes.

Analyte	Repeatability (Precision)		Intermediate Precision	
	Average (%)	%RSD	Average (%)	%RSD
p-Aminophenol	103.1	0.3	90.9	3.3
p-Nitrophenol	104.8	0.2	96.2	0.6
p-Chloroacetanilide	106.2	0.1	102.0	1.2
Diphenhydramine HCl	100.5	0.4	102.2	2.8
Orphenadrine Rel. Comp. C	95.3	0.8	104.7	2.6
Orphenadrine Rel. Comp. B	97.9	1.1	102.0	2.4
2-Methylbenzhydrol	101.7	0.7	104.7	2.1
2-Methylbenzophenone	99.5	5.7	98.4	3.4

3.5. Precision and Robustness

Method precision was established through repeatability and intermediate precision studies. In unspiked sample preparations, impurity levels were below the

quantitation limit; therefore, percentage relative standard deviation (%RSD) values were not applicable. For samples spiked with impurities at the 100% specification level, the %RSD of recoveries was consistently below 1.2% for paracetamol-related impurities and below 5.7% for orphenadrine citrate–related impurities (Table 7), demonstrating excellent repeatability of the method. Results obtained during intermediate precision studies, conducted under varied analytical conditions, were comparable to those of repeatability, confirming the ruggedness of the method.

Robustness studies demonstrated that deliberate variations in chromatographic parameters—including buffer pH (±0.2), column temperature (±5˚C), buffer concentration (±10%), and the use of a different C18 column (Waters Symmetry Shield RP18)—did not adversely affect method performance. System suitability criteria were consistently met under all evaluated conditions, confirming that the method is robust and reliable for routine quality control and stability testing.

3.6. Solution Stability

The standard solution was found to be stable for up to 7 days at both room temperature and under refrigeration, with a % relative difference from the initial solution of ≤6.2%. The sample solution was stable for up to 1 day at both storage conditions, as the observed changes in impurity levels were consistent with normal analytical variation based on scientific judgment. Beyond 1 day, an increase in unknown impurities was observed, establishing the recommended sample analysis timeframe.

4. Conclusion

A simple, robust, and stability-indicating RP-HPLC method was successfully developed and fully validated for the simultaneous determination of related substances in paracetamol and orphenadrine citrate fixed-dose combination tablets. The method demonstrated excellent specificity, accuracy, precision, linearity, robustness, and sensitivity across the required concentration ranges, meeting the validation requirements outlined in ICH Q2(R1) [4]

Forced degradation studies conducted under acidic, alkaline, oxidative, thermal, and photolytic stress conditions confirmed the stability-indicating nature of the method, with all degradation products adequately separated from the active pharmaceutical ingredients and known impurities. The observed mass balance and peak purity results further demonstrated the suitability of the method for impurity profiling and stability assessment applications [4] [5] [11].

The method exhibited satisfactory performance with respect to detection capability, quantitation of specified and unspecified impurities, recovery, repeatability, intermediate precision, and robustness, consistent with established chromatographic method development and validation practices [4] [7]-[10] [12] [13].

Owing to its reliable performance, regulatory compliance, and ability to simultaneously monitor impurities associated with both active pharmaceutical ingredi-

ents, the validated method is suitable for routine quality control testing, stability studies, and lifecycle management of paracetamol and orphenadrine citrate tablet formulations [4] [5] [7].

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] World Health Organization. WHO Model List of Essential Medicines.

[2] Brunton, L.L., Knollmann, B.C., Gilman, A. and Goodman, L.S. (2023) Goodman & Gilman's The Pharmacological Basis of Therapeutics (Fourteenth Edition). McGraw Hill.

[3] DrugBank Online. Acetaminophen Monograph. Paracetamol (Acetaminophen). https://go.drugbank.com/drugs/DB00316

[4] ICH Harmonized Guideline Q2(R1): Validation of Analytical Procedures: Text and Methodology. https://database.ich.org/sites/default/files/ICH_Q2%28R2%29_Guideline_2023_1130_ErrorCorrection_2025.pdf

[5] ICH Harmonized Guideline Q3B(R2): Impurities in New Drug Products. https://database.ich.org/sites/default/files/Q3B%28R2%29%20Guideline.pdf

[6] Orphenadrine Citrate Monograph. Orphenadrine. Orphenadrine Citrate. DrugBank. https://go.drugbank.com/salts/DBSALT000400

[7] United States Pharmacopeia USP-NF, General Chapter <621> Chromatography. ⟨621⟩ Chromatography. https://doi.usp.org/USPNF/USPNF_M99380_06_01.html

[8] Snyder, L.R., Kirkland, J.J. and Dolan, J.W. (2010) Introduction to Modern Liquid Chromatography. 3rd Edition, Wiley. https://doi.org/10.1002/9780470508183

[9] Ahuja, S. and Rasmussen, H. (2007) HPLC Method Development for Pharmaceuticals. Elsevier, Academic Press.

[10] Snyder, L.R., Kirkland, J.J. and Glajch, J.L. (1997) Practical HPLC Method Development. Wiley.

[11] ICH Q1B. Photostability Testing of New Drug Substances and Products. https://database.ich.org/sites/default/files/Q1B%20Guideline.pdf

[12] Ermer, J. and Nethercote, P.W. (2025) Method Validation in Pharmaceutical Analysis: A Guide to Best Practice (3. Auflage). Wiley-VCH.

[13] Swartz, M.E. and Krull, I.S. (2012) Handbook of Analytical Validation. CRC Press. https://doi.org/10.1201/b12039