RESEARCH PAPER

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Hyoscine Butylbromide and Paracetamol in Pharmaceutical Dosage Form

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Received: 30th May, 2026; **Revised:** 10th June, 2026; **Accepted:** 15th June, 2026; **Available Online:** 18th June, 2026

ABSTRACT

Background

A simple reversed phase high-performance liquid chromatographic (RP-HPLC) approach has been developed and validated to enable the simultaneous quantification of hyoscine butylbromide and paracetamol in tablet dose form.

Materials and Methods

Chromatographic analysis was performed on a C18 column (250×4.6 mm) at a flow rate of 0.8 ml/min using methanol (50:50) and 0.2% TEA (triethylamine) at pH 6.0 as the mobile phase. UV detection was performed at 210 nm.

Results

The approach's robustness, linearity, specificity, accuracy, and precision were all confirmed. The retention times for paracetamol and hyoscine butylbromide were 4.3 and 5.9 minutes respectively.

Conclusion

The developed RP-HPLC method was found to be simple, accurate, precise, and robust for the simultaneous estimation of hyoscine butylbromide and paracetamol in pharmaceutical dosage forms, and can be effectively used for routine quality control analysis.

Keywords: Validation, simultaneous estimation, RP-HPLC, hyoscine butylbromide, paracetamol.

How to cite this article: Tathode VS, Shetkar B, Satpute K, Narale PS, Chavan RL, Sonkambale AG, Mantri AS, Khadakumarge KAC. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Hyoscine Butylbromide and Paracetamol in Pharmaceutical Dosage Form. Int J Drug Deliv Technol. 2026;16(62s):27-32.
DOI: 10.25258/ijddt.16.62s.4

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) is a separation process that employs a liquid in both mobile and stationary phases. Because the RP-HPLC approach is simple, fast, and accurate for calculating tablet dose.[1]

Analytical Development: It comprises selecting the appropriate analytical methods, equipment, and processes for a certain drug or formulation. Strong emphasis on creating techniques for quantifying excipients, contaminants, and components in pharmaceuticals.[2,3]The methodical process of choosing and refining techniques to quantify particular characteristics of a medicinal ingredient or product is known as analytical method development. These techniques need to be robust, sensitive, and particular in order to provide precise measurements that stay within reasonable bounds.[4]

This process includes developing and refining methodologies for analyzing pharmaceutical formulations and compounds.

Importance: The development and verification of analytical techniques are essential for ensuring the safety, effectiveness, and uniformity of pharmaceutical products.

Regulatory agencies require validated analytical methods for product approval and quality control. These processes have a significant impact on pharmaceutical quality assurance and adherence to Good Manufacturing Practices (GMP). Validating an analytical procedure's suitability for its intended purpose is its main objective.

Hyoscine Butylbromide falls within the category of antispasmodic medications. It is used to relieve

smooth muscle spasms (cramps) in the stomach, intestines, bladder, and urethra. Hyoscine reduces spasms by relaxing smooth muscles within these organs by blocking muscarinic M2 and M3 receptors, reducing smooth muscle contractions and secretion in the gastrointestinal (GI) tract. It is chemically known as (1S,3s,5R,6R,7S,8r)-6,7-epoxy-8-butyl-3-[(S)-tropoyloxy] tropanium bromide.[5]

Paracetamol is an analgesic drug also called as acetaminophen. The prevailing understanding is that paracetamol inhibits cyclooxygenase (COX) enzymes by metabolizing their peroxidase function. However, the specific COX isoform (COX-1, COX-2, or COX-3) responsible for its action remains uncertain. It is chemically known as N-(4-hydroxyphenyl) acetamide.[6,7] (Figure 1)

There are tablet dosage forms of the paracetamol and hyoscine butylbromide combination available on the market. The tablet is used to relieve spasmodic pain. It helps with sudden, cramp-like pain in the stomach or intestines, as well as functional issues in the biliary and urinary systems. For example, it can be beneficial for conditions like dysmenorrhea (painful menstrual cramps). The literature contains few methods for simultaneously determining paracetamol and hyoscine butylbromide in pharmaceutical dose forms.

MATERIALS AND METHOD

Instrumentation

The HPLC that used was an Agilent 1260 Infinity II make equipped with a quaternary gradient pump, solvent mixing module and PDA detector with Open Lab CDS 2.7 software. An analytical column called Agilent 5TC C_{18} (250 mm × 4.6 mm) was used for the chromatographic separation and quantification, which were kept at room temperature. Digital Analytical Balance (CA-123) Contech, Ultrasonic Cleaner by Toshcon, and Digital pH Meter (EQ 611) Equiptronics were used.

Drugs, chemicals and reagents

The reference standards for hyoscine butylbromide and paracetamol were obtained as gift samples from Aurochem Pharmaceuticals in Palghar, India. The Buscogast Plus tablets, marketed by Sanofi India Pvt. Ltd., claim to contain 325 mg of paracetamol and 10 mg of hyoscine butylbromide. For the HPLC analysis, we used chemicals and reagents of high purity (HPLC grade) triethylamine and orthophosphoric acid as well as isopropyl alcohol, methanol, and water supplied by Research Lab Limited in Mumbai, India. The glassware employed was Class A borosilicate glassware. Prior to injection into the HPLC system, we filtered the tablet samples, drug standard solutions, and mobile phase through a Whatman filter paper No. 41.

Preparation of Solutions:

Preparation of 0.2 %TEA- 0.2 % TEA pH 6.0 was prepared by adding 1 mL of Triethylamine in 500 mL of HPLC grade water and adjusting the pH 6.0 by using orthophosphoric acid.

Preparation of mobile phase- The mobile phase was prepared by mixing the 0.2 %TEA pH 6.0 and methanol in 50:50 (V/V) proportion and sonicated for 10 min.

Preparation of standard drug solution- Weighed out 325 mg of paracetamol and 10 mg of hyoscine butylbromide precisely, then poured the mixture into a 100 mL volumetric flask. Poured 20 mL of IPA (Isopropyl alcohol) and 80 mL of water (as a diluent) into the volumetric flask holding the paracetamol and hyoscine butylbromide. Used filter paper No. 41 to filter the mixture. Using the water, raise the final volume to the mark on the volumetric flask. 1 mL of the previously prepared solution was pipetted out and placed into a second 100 mL volumetric flask. Added water to the volumetric flask until the indicated volume is reached.

Preparation of sample solution- Determined the average weight of the 10 tablets by weighing them. Crushed the tablets to create a fine powder. Weighed an amount of tablet powder equivalent to 325 mg of paracetamol and 10 mg of hyoscine butylbromide, adding it to a 100 mL volumetric flask. To remove contaminants or solid particles, filtered the mixture through filter paper No. 41. Pipetted 1 mL of the filtered solution into a second 100 mL volumetric flask and adjusted the volume by adding water until it reached the mark. Finally, injected the solution into the HPLC apparatus for analysis.

Optimized chromatographic conditions

Mobile phase: 0.2 %TEA(Triethylamine) pH 6.0: Methanol (50:50 V/V)
Column: Agilent 5TC C_{18} (250 mm × 4.6 mm)
Flow rate: 0.8 mL/min
Detection wavelength: 210 nm
Column temperature: Room temperature
Injection volume: 50 µL
Run time: 8 min
Detector: PDA detector

Method validation

System suitability- Following protocol, a working standard solution of paracetamol and hyoscine butylbromide was created and added to the HPLC system five times. Standard Chromatograms were utilized for assessing the system appropriateness characteristics by calculating the percentage RSD of

retention time, tailing factor, theoretical plates, and peak regions from five replicate injections.

Specificity - Specificity is the ability to discern between the target analyte and other elements in the sample. The connection of the standard, sample, blank, and placebo chromatograms covered the specificity of the procedure.

Accuracy- By using the conventional addition recovery approach, the method's accuracy was estimated. To the previously studied sample solutions, a standard stock solution containing known concentrations of paracetamol and hyoscine butylbromide was added at three distinct concentrations: 80 %, 100 %, and 120 %. These options were reexamined, with three analyses of the solutions at each level. The accuracy was computed and presented as a percentage of recovery.

Precision- The degree of agreement between several measurements made from repeated samplings of the same homogenous material under specified conditions is expressed by the precision of the analytical process. Both system and method precision are examples of precision in action. Prepared 6 different sample solutions and injected them into the HPLC system. The percentage relative standard deviation (RSD) was determined for six injections.

Linearity and Range- To demonstrate the linearity of the assay procedure, injected five reference solutions containing paracetamol and hyoscine butylbromide. The range for the given combination was selected between 60 % to 140 %. The range of paracetamol was selected between 19.5 ppm to 45.5 ppm. The range of hyoscine butylbromide was selected between 0.6 ppm to 1.4 ppm. The obtained correlation coefficient was 0.99. The linearity plot was generated.

Robustness- The robustness of an analytical procedure refers to its ability to remain unaffected by small, deliberate variations in method parameters. It serves as an indicator of reliability during routine use. To assess robustness, the assay is performed by intentionally altering parameters, such as flow rate, mobile phase ratio, pH, wavelength detection, and temperature. The goal is to ensure that the results remain consistent despite these parameter changes. And the percentage RSD is calculated.

Solution stability- To demonstrate the stability of the solutions during analysis, standard and sample solutions were examined for 24 hours at room temperature.

RESULT AND DISCUSSION

Method development and optimization

The main goal of this study is to create a reliable method for separating and quantifying the studied drugs without any prior steps. To achieve this, we developed a simple, sensitive, and accurate isocratic high-performance liquid chromatography (HPLC) method. Our focus was on simultaneously determining paracetamol and hyoscine butylbromide in a pharmaceutical formulation. By adopting this method, we aimed to make more efficient the analysis process while maintaining precision and reliability. We opted for reversed-phase separation over normal-phase separation.

Chromatographic separation was achieved by using the C_{18} column. At first various mobile phases were tried like water: acetonitrile, 0.2 % TEA: acetonitrile, phosphate buffer: acetonitrile in which 0.2 % TEA and methanol in the proportion of 50:50 V/V showed two peaks and complete separation of paracetamol and hyoscine butylbromide at given ratio of mobile phase. The detection of the two peaks was carried out at different wavelengths like 242 nm, 257 nm but a good peak response was shown at 210 nm. Also, different pH values were tested for better resolution of two peaks in which pH 6.0 gave the best results in the resolution of paracetamol and hyoscine butylbromide.

The optimized chromatographic conditions for the better separation of paracetamol and hyoscine butylbromide were using the column Agilent 5TC C_{18} (250 × 4.6 mm, 4 µm) with the mobile phase 0.2 % TEA pH 6.0 and methanol in the ratio of 50:50 V/V at a flow rate of 0.8 ml/min at detection wavelength 210 nm. The retention time for paracetamol and hyoscine butylbromide were 4.3 and 5.9 respectively in a continuous rutime of 8 minutes. (Figure 2)

Method Validation

After the method development, the method was validated as per the guidelines given in the ICH (Q2) section.
System suitability parameters meet the acceptance criteria, the % RSD was found to be 1.9 and 1.8 for paracetamol and hyoscine butylbromide respectively at a peak resolution factor of 7.75. When conducting an HPLC analysis or any analytical testing, ensure that the system is suitable. (Table I)

Table I: System suitability of paracetamol and hyoscine butylbromide

Sr.no	Surface area of Paracetamol	Retention time Paracetamol	The surface area of Hyoscine butylbromide	Retention time Hyoscine butylbromide
1	6492.44	4.34	62.25	5.99
2	6231.48	4.31	64.79	5.89
3	6448.68	4.33	62.63	5.91
4	6321.56	4.31	61.49	5.89
5	6533.17	4.30	63.76	5.91
Average	6445.283		63.6192	
SD	123.8438		1.1545	
%RSD	1.921		1.815	
Tailing Factor	1.26		1.57	

The chromatogram of paracetamol and hyoscine butylbromide sample solution is similar to the chromatogram of paracetamol and hyoscine butylbromide standard solution. The gap does not affect the retention time of the main peak. The RSD of the method precision is 1.98 % & 1.67 % for paracetamol and hyoscine butylbromide respectively. Results of repeated studies showed that the cure rate ranged from 95 % to 103 %. The choice of this method is indicated by the fact that excipients do not interfere with the analysis of the analytes. The % RSD value of method precision is <2 %, indicating that this method has good precision in the simultaneous analysis of paracetamol and hyoscine butylbromide. Therefore, the HPLC method for the determination of paracetamol and hyoscine butylbromide is precise. (Table II)

Table II: Method precision of paracetamol

Sample no	Area of Paracetamol	% Assay of Paracetamol	Area of Hyoscine butylbromide	% Assay of Hyoscine butylbromide
1	6576.502	103.65	62.181	99.28
2	6480.674	102.25	62.433	99.79
3	6463.348	98.64	65.73	101.63
4	6669.084	103.90	64.783	102.18
5	6623.254	101.50	66.155	102.71
6	6570.259	100.28	67.02	103.63
	Average	101.70	Average	101.54
	SD	2.02	SD	1.69
	%RSD	1.98	%RSD	1.67

The method demonstrated linearity for both paracetamol and hyoscine butylbromide within the tested ranges (19.5 ppm to 45.5 ppm for paracetamol and 0.6 ppm to 1.4 ppm for hyoscine butylbromide). The correlation coefficient (r^2) was 0.99 for both compounds, confirming the method's suitability. Overall, the method exhibited linearity across the specified range of 60% to 140%. (Table III) (Figures 3 and 4)

Table III: Datasheet for linearity

Concentration Paracetamol	Area of Paracetamol	Concentration of Hyoscine butylbromide	Area of Hyoscine butylbromide
0	0	0	0
19.5	3783.483	0.6	28.792
26	4594.462	0.8	42.729
32.5	5916.288	1.0	58.259
39	6808.286	1.2	71.811
45.5	7618.523	1.4	82.334

The average mean recovery for paracetamol was 100.93 %, and for hyoscine butylbromide, it was 99.29 %. The % RSD (relative standard deviation) was 1.23 % for paracetamol and 1.58 % for hyoscine butylbromide. Based on these

results, the HPLC method for determining the assay of paracetamol and hyoscine butylbromide tablets is accurate. (Table IV and V)

Table IV: Data for accuracy of paracetamol

% Level	Area	Amount mg added	Mg found	% recovery	Average	SD	%Relative standard deviation
	5218.601	260	263.145	101.20			
80%	5295.443	260	267.019	102.69	101.05	1.73	1.71
	5078.054	258	256.058	99.24			
	6341.165	325	319.749	98.38			
100%	6670.179	332	336.340	101.30	99.26	1.78	1.80
	6321.564	325	318.761	98.08			
	8123.436	400	409.619	102.40			
120%	8083.291	398	407.595	102.41	102.51	0.17	0.17
	8116.853	398.5	409.288	102.70			

Table V: Data for accuracy of hyoscine butylbromide

% Level	Area	Amount mg added	Mg found	% recovery	Average	SD	Relative standard deviation
	52.51	8.1	8.253	101.89			
80%	50.065	8.00	7.869	98.36	99.69	1.93	1.93
	50.281	8.00	7.903	98.79			
	64.26	10.00	10.10	101.007			
100%	63.081	10.00	9.91	99.15	99.60	1.25	1.25
	61.492	9.8	9.66	98.62			
	70.509	11.4	11.08	97.21			
120%	76.516	12.00	12.02	100.22	98.60	1.52	1.54
	71.969	11.5	11.31	98.36			

The % relative difference in standard solution areas was 1.88 % for paracetamol and -0.60 % for hyoscine butylbromide. The absolute difference in sample assay was 0.18 % for paracetamol and -1.15 % for hyoscine butylbromide. Consequently, both standard and sample solutions remain stable for 24 hours at room temperature. The parameter meets the acceptance criteria, with % RSD within 2.0 % for both paracetamol and hyoscine butylbromide. Consequently, the system is suitable. The difference between modified and original conditions also falls within the specified ±2.0 % range. The results demonstrate the importance of the method by showing that the peak area of the drug is not affected by small changes in mobile phase composition. (Table VI and VII)

Table VI: Robustness data for paracetamol and hyoscine butylbromide mobile phase (0.2 %TEA pH 6: Methanol 52.5:47.5) (for flow rate 0.8 ml/min)

Sr.no	Area Paracetamol	Retention time Paracetamol	Area Hyoscine butylbromide	Retention time Hyoscine butylbromide
1	6726.219	4.61	42.875	8.38
2	6583.939	4.67	42.697	8.36
3	6640.939	4.70	43.685	8.52
Average	6650.37		43.09	
SD	71.61		0.53	
%RSD	1.08		1.22	

Table VII: Robustness data for paracetamol and hyoscine butylbromide mobile phase (0.2 %TEA pH 6: Methanol 47.5:52.5) (for flow rate 0.8 ml/min)

Sr.no	Area Paracetamol	Retention time Paracetamol	Area Hyoscine butylbromide	Retention time Hyoscine butylbromide
1	6044.776	4.29	51.898	5.53
2	6068.393	4.23	51.507	5.54
3	6016.17	4.38	52.18	5.63
Average	6043.11		51.86	
SD	26.15		0.34	
%RSD	0.43		0.65	

In conclusion, we can conclude that the established validated RP HPLC method for the quantitative determination of paracetamol and hyoscine butylbromide in a formulation is accurate, precise, economical, and simple to use. The approach was verified in compliance with ICH regulations. Therefore, the method may be effectively used for the routine analysis of paracetamol and hyoscine butylbromide in tablet dosage form.

ACKNOWLEDGEMENT

The authors express their gratitude to Aurochem Laboratories, Palghar, for donating gift samples of paracetamol and hyoscine butylbromide.

Conflict of Interest

The authors affirm that they do not have any conflicting interests

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