VALIDATED RP-HPLC METHOD WITH DOE OPTIMIZATION FOR SIMULTANEOUS ESTIMATION OF XANOMELINE AND TROSPIUM CHLORIDE

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ABSTRACT

A simple, precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Xanomeline and Trospium chloride in pharmaceutical dosage forms. Chromatographic separation was achieved using a C18 column (150 × 4.6 mm, 5 µm) with an isocratic mobile phase consisting of 0.1% orthophosphoric acid buffer (pH 3.5) and methanol in the ratio of 30:70 (v/v). The mobile phase was delivered at a flow rate of 1.0 mL/min with UV detection at 232 nm.The retention times for Xanomeline and Trospium chloride were found to be 2.71 min and 3.53 min, respectively, with well-resolved and symmetrical peaks. The method was validated as per ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, robustness, and sensitivity. Linearity was observed in the concentration range of 10–50 µg/mL for Xanomeline and 4–20 µg/mL for Trospium chloride, with correlation coefficients of 0.9996 and 0.9995, respectively. Accuracy studies showed recovery between 98–100%, and precision results were within acceptable limits (%RSD < 2).

A Design of Experiments (DoE) approach was applied to optimize chromatographic conditions, demonstrating significant influence of mobile phase composition and flow rate on resolution. The developed method proved to be reliable, rapid, and suitable for routine quality control analysis of the studied drugs in bulk and pharmaceutical formulations.

Keywords: Xanomeline, Trospium chloride, RP-HPLC, Method Validation, ICH Guidelines, DoE, Pharmaceutical Analysis

INTRODUCTION

Analytical method development and validation are fundamental components of pharmaceutical research, ensuring the quality, safety, and efficacy of drug products. Among various analytical techniques, reverse-phase high-performance liquid

chromatography (RP-HPLC) has emerged as one of the most reliable and widely used methods for the quantitative estimation of pharmaceutical compounds due to its high sensitivity, reproducibility, and versatility.

RP-HPLC operates on the principle of differential partitioning of analytes between a non-polar stationary phase and a relatively polar mobile phase. The technique offers excellent resolution, shorter analysis time, and compatibility with a wide range of compounds, making it particularly suitable for simultaneous estimation of multi-component drug formulations.

Xanomeline is a muscarinic receptor agonist with selectivity towards M1 and M4 receptors, primarily investigated for the treatment of neurological disorders such as schizophrenia. Trospium chloride, on the other hand, is a quaternary ammonium antimuscarinic agent widely used in the management of overactive bladder. Recently, the combination of Xanomeline and Trospium chloride has gained significant attention as a novel therapeutic strategy, where Trospium minimizes peripheral cholinergic side effects of Xanomeline.

Despite their therapeutic importance, limited analytical methods are available for the simultaneous estimation of these drugs in combined dosage forms. The development of a validated analytical method is therefore essential for quality control and regulatory compliance.

Furthermore, traditional trial-and-error approaches in method development are time-consuming and inefficient. The application of Design of Experiments (DoE) provides a systematic and statistical approach to optimize chromatographic conditions by evaluating the interaction between critical parameters such as mobile phase composition, pH, and flow rate.

The present study aims to develop and validate a simple, accurate, and robust RP-HPLC method for the simultaneous estimation of Xanomeline and Trospium chloride in pharmaceutical dosage forms in accordance with ICH Q2(R1) guidelines, along with the application of DoE for method optimization.

METHODOLOGY

1. Chemicals and Reagents

Analytical grade Xanomeline and Trospium chloride reference standards were obtained from a certified supplier. Methanol (HPLC grade) and orthophosphoric acid were procured

from standard chemical suppliers. Ultrapure water was prepared using a Milli-Q purification system.

2. Instrumentation

The chromatographic analysis was performed using a High-Performance Liquid Chromatography system equipped with:

- Binary pump
- Autosampler
- UV detector
- Data acquisition software

Separation was carried out on a C18 column (150 mm × 4.6 mm, 5 µm particle size).

3. **Chromatographic Conditions**

Parameter	Condition
Column	C18 (150 × 4.6 mm, 5 µm)
Mobile phase	0.1% OPA buffer : Methanol (30:70 v/v)
Flow rate	1.0 mL/min
Detection wavelength	232 nm
Injection volume	10 µL
Run time	6 min
Mode	Isocratic

4. Preparation of Mobile Phase

The buffer solution was prepared by dissolving appropriate quantity of orthophosphoric acid in water and adjusting pH to 3.5. The buffer was mixed with methanol in the ratio of 30:70 (v/v), filtered through a 0.45 µm membrane filter, and degassed prior to use.

5. Preparation of Standard Solution

Accurately weighed quantities of Xanomeline and Trospium chloride were transferred into a volumetric flask and dissolved in mobile phase to obtain stock solutions. Working standard solutions were prepared by appropriate dilution to obtain concentrations within the linearity range.

6. Method Optimization Using DoE

A Box-Behnken design was employed to evaluate the effect of critical chromatographic parameters:

- % Methanol in mobile phase
- Flow rate
- Buffer pH

Responses such as resolution and retention time were analyzed using statistical software. Response surface methodology was used to determine optimal conditions.

7. Method Validation (ICH Q2(R1))

The method was validated for the following parameters:

✓ Specificity

Evaluated by analyzing blank, standard, and sample solutions to ensure no interference.

✓ Linearity

Assessed by plotting concentration vs peak area over the range:

✓ Accuracy

Determined by recovery studies at 80%, 100%, and 120% levels.

✓ Precision

- Repeatability (intra-day)
- Intermediate precision (inter-day)

✓ LOD and LOQ

Calculated based on standard deviation and slope of calibration curve.

✓ Robustness

Evaluated by small deliberate variations in:

- Flow rate (±0.2 mL/min)
- Mobile phase composition (±5%)
- pH (±0.2)

✓ System Suitability

Parameters such as retention time, tailing factor, theoretical plates, and resolution were evaluated.

RESULTS AND DISCUSSION

1. Method Development and Optimization

A reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed for the simultaneous estimation of Xanomeline and Trospium

chloride. Various chromatographic conditions were initially evaluated, including different mobile phase compositions, pH levels, and flow rates, to achieve optimal separation with acceptable system suitability parameters.

The optimized chromatographic conditions consisted of a C18 column (150 × 4.6 mm, 5 µm) with a mobile phase comprising 0.1% orthophosphoric acid buffer (pH 3.5) and methanol in the ratio of 30:70 (v/v), delivered at a flow rate of 1.0 mL/min. Detection was carried out at 232 nm.

Under these conditions, well-resolved peaks were obtained with retention times of 2.71 min for Xanomeline and 3.53 min for Trospium chloride, indicating efficient separation within a short runtime.

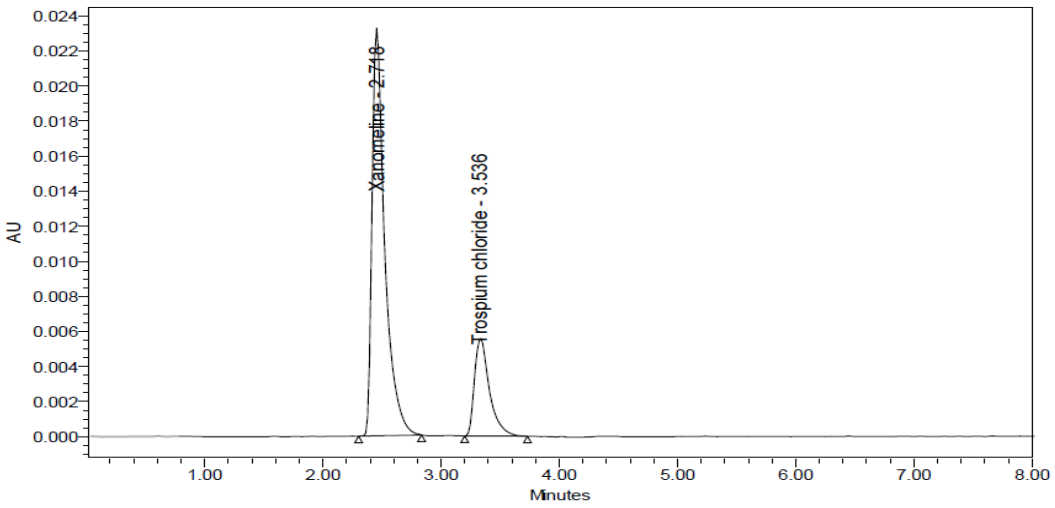

Figure 1: Chromatogram for Xanomeline & Trospium Chloride

3. System Suitability

The system suitability results complied with ICH recommendations, demonstrating that the chromatographic system was appropriate for analysis.

Parameter	Xanomeline	Trospium Chloride
Retention Time (min)	2.71	3.53
Theoretical Plates (N)	4200	5100
Tailing Factor	1.21	1.18

Resolution	-	3.2

Table No 1 System Suitability Results

4. Linearity

The method exhibited excellent linearity over the selected concentration ranges:

- Xanomeline: 10–50 µg/mL
- Trospium chloride: 4–20 µg/mL

Correlation coefficients were found to be:

- Xanomeline: 0.9996
- Trospium chloride: 0.9995

5. Design of Experiments (DoE) Analysis

A Box-Behnken design was applied to evaluate the influence of critical factors such as % methanol, flow rate, and pH on chromatographic responses.

Key Findings:

- % Methanol had the most significant positive effect on resolution
- Flow rate showed a negative impact on retention time and resolution

pH moderately influenced peak shape and separation

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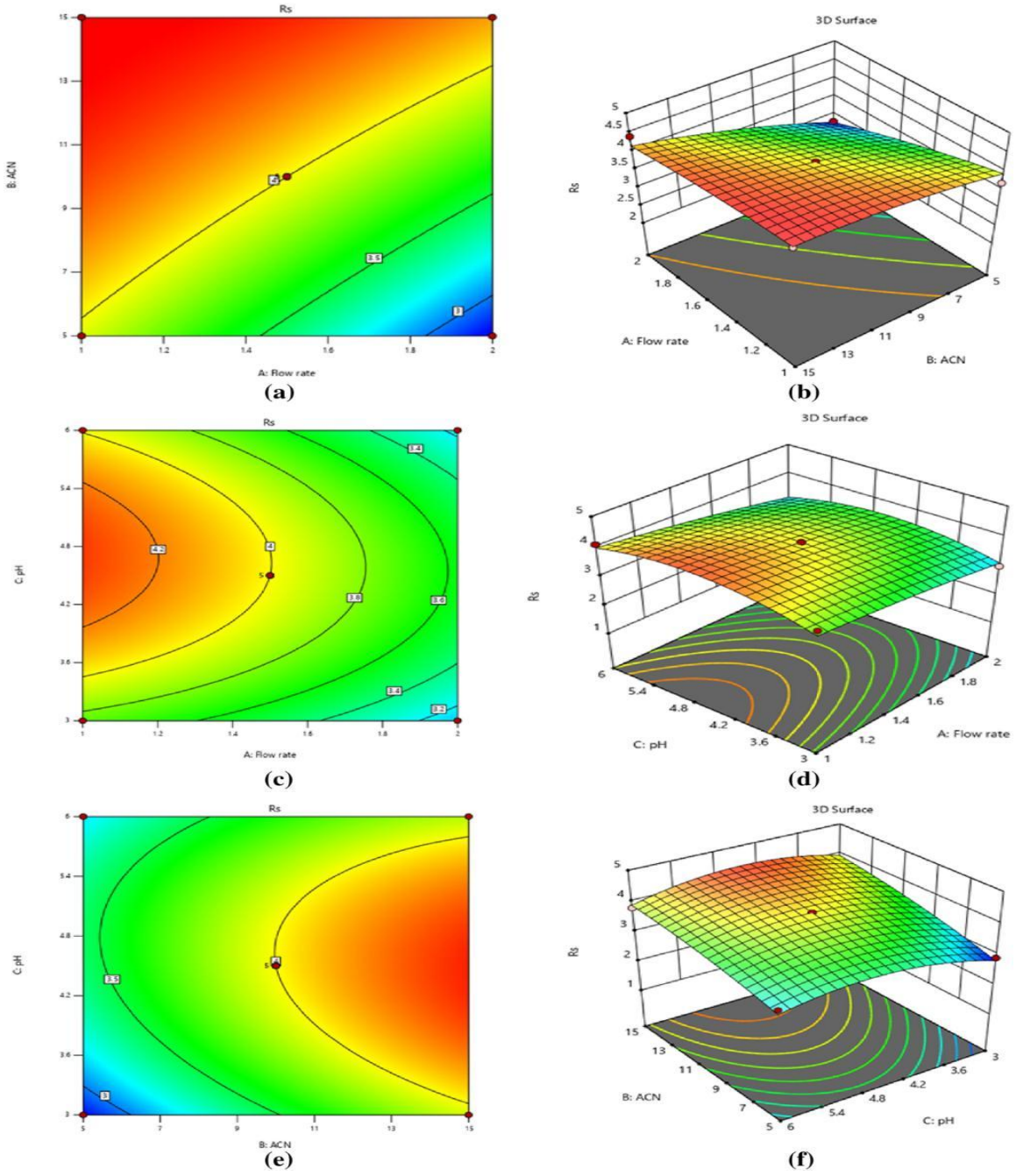

Figure 2: The response surface plots indicated that optimal conditions were achieved

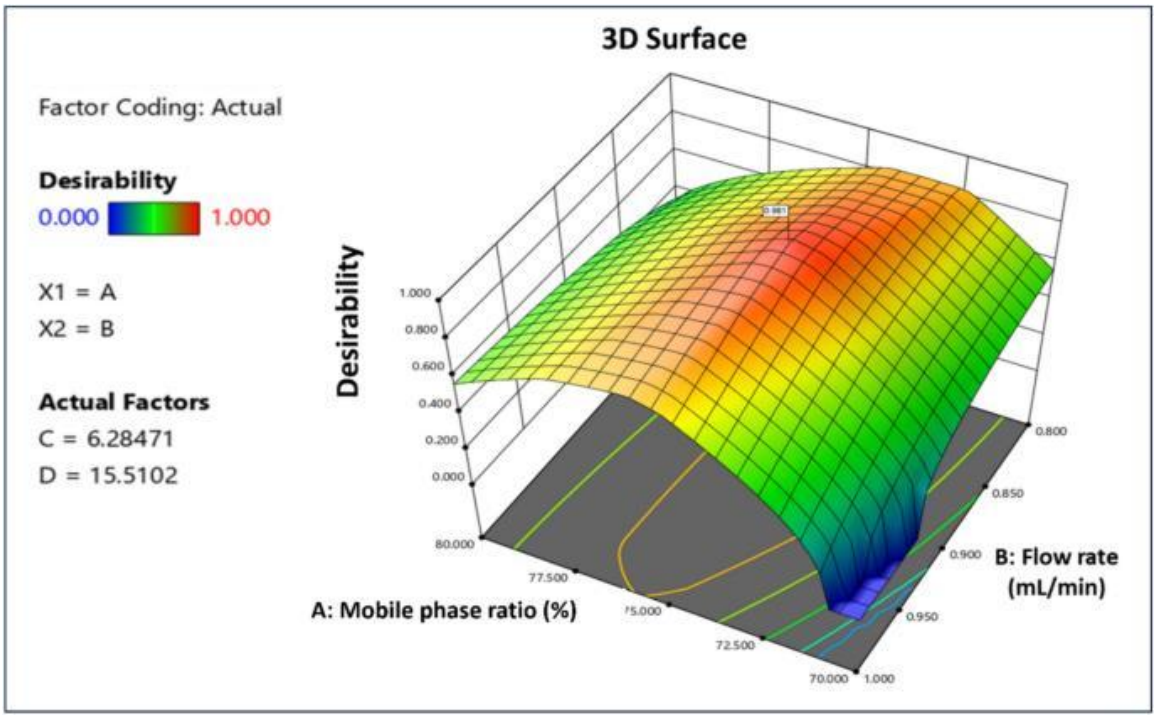

Figure 3:The contour plots indicated that optimal conditions were achieved

6. Optimised Conditions by DoE :

- **70% methanol**
- **Flow rate: 1.0 mL/min**
- **pH: 3.5**

The model showed high significance ($p < 0.0001$) with good agreement between predicted and experimental values, confirming model adequacy.

7. Overall Discussion

The developed RP-HPLC method demonstrated excellent performance in terms of specificity, accuracy, precision, linearity, and robustness. The short retention times and good resolution indicate that the method is efficient and suitable for routine quality control analysis.

The incorporation of Design of Experiments (DoE) provided a systematic approach to method optimization, reducing experimental variability and improving method reliability. The optimized method conditions were found to be consistent with experimental observations, validating the effectiveness of the statistical model.

Overall, the method is simple, rapid, cost-effective, and compliant with ICH guidelines, making it highly suitable for simultaneous estimation of Xanomeline and Trospium chloride in pharmaceutical dosage forms.

A simple, rapid, and reliable reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Xanomeline and Trospium chloride in pharmaceutical dosage forms. The optimized chromatographic conditions provided well-resolved, symmetrical peaks with short retention times, ensuring efficient analysis within a minimal runtime.

The method demonstrated excellent performance in terms of specificity, linearity, accuracy, precision, robustness, and sensitivity, complying with ICH Q2(R1) guidelines. The correlation coefficients for both analytes were found to be greater than 0.999, and recovery values ranged between 98–100%, confirming the accuracy of the method. Precision studies showed %RSD values well below 2%, indicating high reproducibility.

The application of Design of Experiments (DoE) significantly enhanced method optimization by systematically evaluating the influence of critical chromatographic variables. The statistical model confirmed that mobile phase composition and flow rate had a significant impact on resolution and retention behavior.

Overall, the developed RP-HPLC method is simple, cost-effective, robust, and suitable for routine quality control analysis of Xanomeline and Trospium chloride in bulk and pharmaceutical formulations. The integration of DoE further strengthens the reliability and scientific validity of the method, making it highly applicable for regulatory and industrial purposes.

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