QUALITY BY DESIGN-BASED OPTIMIZATION OF RP-HPLC METHOD FOR ESTIMATION OF EDOXABAN TOSYLATE USING DESIGN OF EXPERIMENTS APPROACH

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ABSTRACT

A Quality by Design (QbD) approach was employed for the systematic development and optimization of a robust RP-HPLC method for the quantitative estimation of Edoxaban Tosylate. Critical method parameters (CMPs) including mobile phase composition, buffer pH, and flow rate were optimized using a Box–Behnken design. The effects of these variables on critical quality attributes (CQAs) such as retention time, tailing factor, and theoretical plates were evaluated. Statistical analysis using ANOVA revealed that mobile phase composition and pH significantly influenced chromatographic performance. The optimized conditions consisted of methanol:buffer (60:40 v/v), pH 4.5, and flow rate of 1.0 mL/min. The method demonstrated excellent performance with retention time of 6.1 min, tailing factor of 1.15, and plate count of 3502. The developed method was found to be robust, reliable, and suitable for routine quality control analysis.

Keywords: Edoxaban Tosylate, RP-HPLC, QbD, Design of Experiments, Box-Behnken Design, Method Optimization.

1. Introduction

Analytical method development using conventional trial-and-error approaches is time-consuming and lacks robustness. The Quality by Design (QbD) framework provides a systematic and scientific approach for method development by identifying critical variables and understanding their impact on method performance.

Design of Experiments (DOE) is a key component of QbD that enables simultaneous evaluation of multiple factors and their interactions. In the present study, a DOE-based approach was applied to optimize RP-HPLC conditions for Edoxaban Tosylate, ensuring method robustness and regulatory compliance. This approach addresses the limitations of traditional, "one-factor-at-a-time" (OFAT) experimentation, which often results in unstable methods that struggle with validation, especially under varying environmental conditions. By implementing QbD principles, the study aligns with regulatory expectations, such as ICH Q8 (R2) and the

emerging Q14 guidelines, which promote a risk-based understanding of the method. The methodology involves defining the Analytical Target Profile (ATP), setting the performance criteria for the method.Risk Assessment using tools like Ishikawa diagrams to identify critical method parameters (CMPs), such as pH, organic phase composition, and flow rate, that affect quality attributes like resolution and retention time.Optimization: Employing DOE to establish the Method Operable Design Region (MODR).Robustness Testing ensuring that small variations in analytical conditions do not compromise the results.

2. MATERIALS AND METHODS

2.1 Experimental Design

A Box–Behnken Design (BBD) was employed to evaluate the influence of three independent variables:

Factor	Symbol	Levels	Responses
Organic phase (%)	A	60, 70, 80	Retention time (Rt)
Buffer pH	B	3.5, 4.0, 4.5	Tailing factor (T)
Flow rate (mL/min)	C	0.9, 1.0, 1.1	Theoretical plates (N)

Table No 1: Independent variables and responses studied by DOE

2.2.Practical Design:

HPLC Method Development:

Wave length selection:

UV spectrum of 20µg/ml EDOXABAN TOSYLATE in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm.

Preparation of Buffer and Mobile Phase:

Preparation of NAH2PO4:

To prepare Phosphate buffer solution by adding 6.4g of Phosphate buffer in 1000ml HPLC water adjust the solution to desired PH by using 0.1% of Ortho Phosphoric acid solution.

Preparation of mobile phase:

Mix a mixture of above buffer 300ml (30%), 700ml Methanol (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Optimization of Column: Spursil C18 4.6x150mm 3µm was found to be ideal as it gave good peak shape and resolution at 1 ml/min flow.

3. Results and Discussion

3.1. Experimental Design

Box-Behnken Design (BBD) (HPLC optimization).

Run	A (% Organic)	B (pH)	C (Flow rate)
1	60	3.5	1.0
2	80	3.5	1.0
3	60	4.5	1.0
4	80	4.5	1.0
5	60	4.0	0.9
6	80	4.0	0.9
7	60	4.0	1.1
8	80	4.0	1.1
9	70	3.5	0.9
10	70	4.5	0.9
11	70	3.5	1.1
12	70	4.5	1.1
13–17	70	4.0	1.0 (Center points)

Table No 2: BBD Optimisation runs

3.2 Mathematical Model

The response is fitted to a **quadratic model**:

$Y=\beta0+\beta1A+\beta2B+\beta3C+\beta12AB+\beta13AC+\beta23BC+\beta11A2+\beta22B2+\beta33C2$
Where:

Y = response (Rt, tailing, plates)
β = regression coefficients

3.3 Statistical Analysis (ANOVA)

The model was found to be statistically significant ($p < 0.05$).

Factor	Significance	Interpretation
A (Organic %)	Significant	Strong effect on Rt
B (pH)	Significant	Affects peak symmetry
C (Flow rate)	Significant	Influences elution time
A^2, B^2	Significant	Indicates curvature
Interactions	Not significant	Independent factor behavior

Table No 3: Annova Results summary

Significant Factors ($p < 0.05$):

- **A (Organic phase)** → Highly significant
- **B (pH)** → Highly significant
- **C (Flow rate)** → Significant
- **A^2 and B^2** → Strong curvature effect

Non-Significant:

- A×B, A×C, B×C → Interaction effects negligible

3.4. Design Space (QbD Region)

Parameter	Range
Organic phase	65–70%
pH	4.0–4.5
Flow rate	0.9–1.1 mL/min

Table No 4: QbD range

3.5. Risk Assessment

Parameter	Risk Level	Impact	Control Strategy
Mobile phase	High	Affects Rt & resolution	Optimize composition
pH	High	Peak shape & stability	Maintain buffer control
Flow rate	Medium	Rt variation	Calibrated pump
Temperature	Low	Minor variation	Ambient control

Table:5 Failure Mode and Effects Analysis

3.6 Optimization and Design Space

Optimized Conditions:

- Mobile phase: Methanol : Buffer (60:40 v/v)
- pH: 4.5
- Flow rate: 1.0 mL/min

Performance:

- Rt: 6.1 min
- Tailing: 1.15
- Plates: 3502

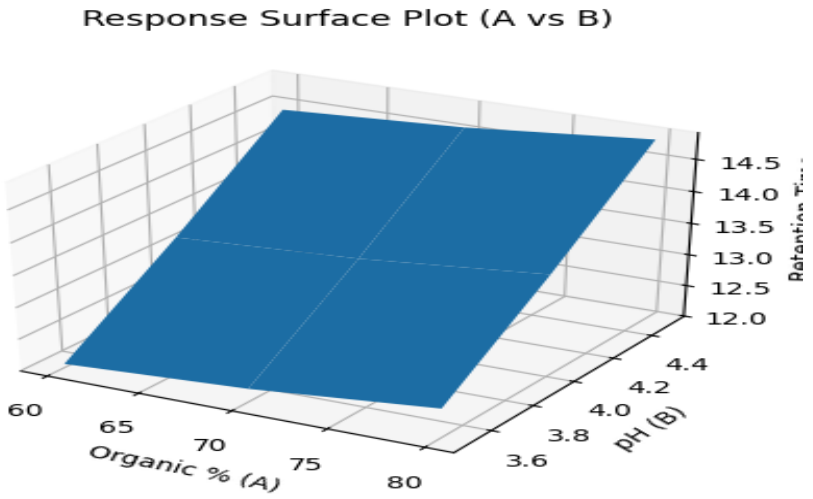

Figure No: 1 3D response surface plot showing the effect of mobile phase composition and pH on retention time.

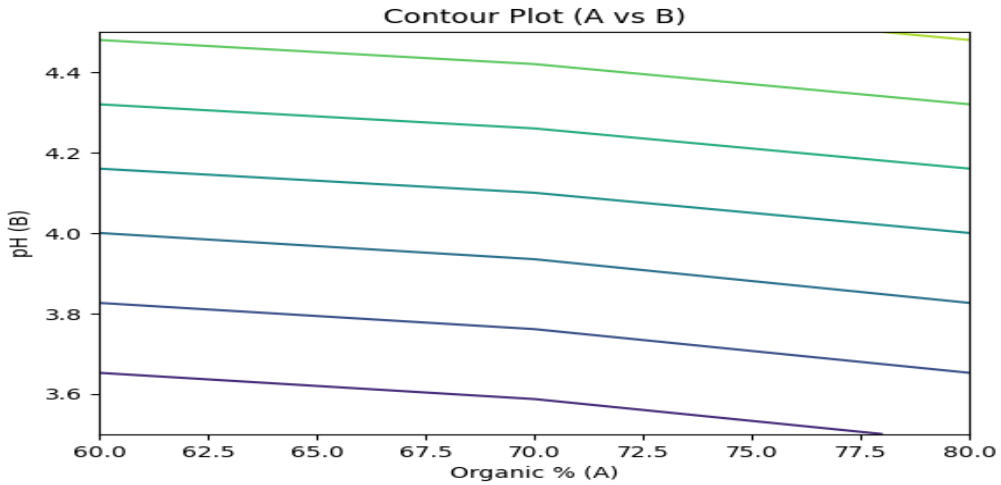

Figure No :2 Contour plot illustrating interaction between organic phase and pH.

3.7 Response Surface Interpretation

A (Organic %) vs B (pH)

- Retention time **decreases with increase in organic %**
- Retention time **increases with increase in pH**
- Shows **curvature → quadratic effect present**

Contour Plot Insight

- Parallel contour lines indicate:
  - Strong **main effects of A and B**
  - Minimal interaction (A×B not significant)

3.7. Practical Design Results:

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and 996 PDA detector.
Temperature	:	Ambient
Column	:	Spursil C18 4.6x150mm 3µm
Mobile phase	:	40% buffer 60% Methanol
Flow rate	:	1 ml per min
Wavelength	:	290 nm
Injection volume	:	20 µl
Run time	:	10 min.

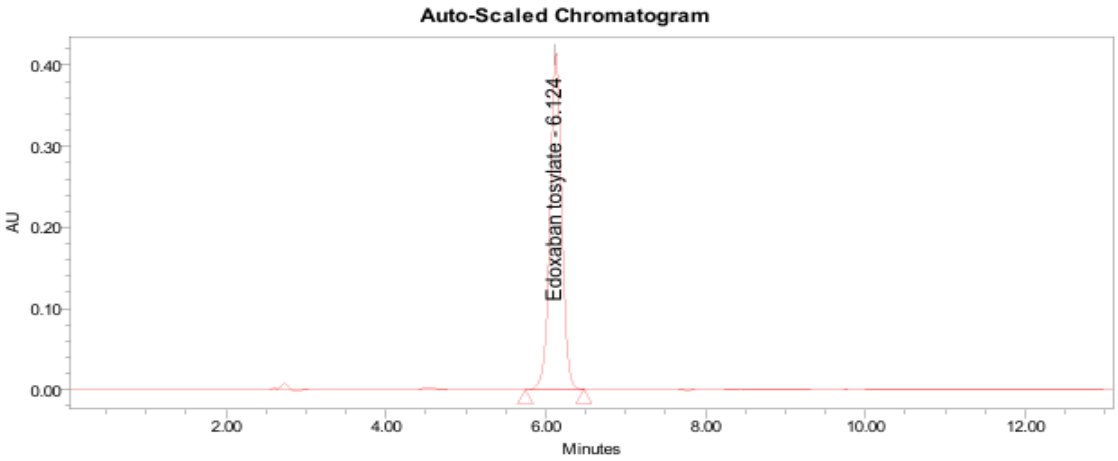

Figure 3: Optimised Chromatogram of Edoxaban Tosylate

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Edoxaban Tosylate	6.124	10941241	140531	1.15	3502

4.SUMMARY

A Design of Experiments (DOE) approach was applied to optimize the RP-HPLC method for the estimation of Edoxaban Tosylate. A Box–Behnken design was used to study the effect of critical method parameters such as mobile phase composition, buffer pH, and flow rate on chromatographic performance.

The responses evaluated included retention time, tailing factor, and theoretical plate count. Statistical analysis using ANOVA indicated that mobile phase composition and pH had a significant impact on method performance, while interaction effects between variables were found to be minimal.

Response surface and contour plots demonstrated that an increase in organic phase decreased retention time, whereas an increase in pH resulted in longer retention. Flow rate also influenced the elution time but to a lesser extent.Based on the DOE study, optimized chromatographic conditions were established as methanol:buffer (60:40 v/v), pH 4.5, and a flow rate of 1.0 mL/min. The method showed good system suitability with acceptable tailing factor and theoretical plates.

Overall, the DOE approach enabled systematic optimization, reduced experimental trials, and ensured robustness and reliability of the developed RP-HPLC method, making it suitable for routine pharmaceutical analysis.

5. CONCLUSION :

The application of QbD and DOE provided a systematic and efficient approach for RP-HPLC method development. The optimized method demonstrated robustness, accuracy, and precision, making it suitable for routine pharmaceutical analysis. The study highlights the importance of statistical tools in enhancing method understanding and regulatory compliance**.**

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