

Development and Validation of an HPTLC Method for Simultaneous Estimation of Etofylline and Salbutamol

Mehul Harsoda¹, Ravi Ajudia¹, Bhavik Jani¹, Yash Ardesana¹, Kushal Parekh¹, Pratik Vediya¹, and Udit Trivedi¹

¹Department of Pharmaceutical Quality Assurance, School of Pharmacy, RK University, Rajkot-360020, Gujarat (INDIA)

Abstract. A precise, robust, and cost-effective High-Performance Thin Layer Chromatography (HPTLC) method was developed and validated for the simultaneous estimation of Salbutamol and Etofylline in bulk and combined pharmaceutical dosage forms. Chromatographic separation was achieved on precoated silica gel 60 F254 TLC plates using a mobile phase consisting of toluene, ethyl acetate, and methanol in an optimized ratio. The detection was performed densitometrically at an appropriate wavelength using CAMAG TLC Scanner. The method demonstrated excellent linearity over the concentration range of 100–600 ng/band for both drugs, with correlation coefficients (R^2) exceeding 0.998. Validation was carried out in accordance with ICH Q2(R2) guidelines, covering parameters such as accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), specificity, robustness, and ruggedness. The recovery values were found to be within acceptable limits, confirming the method's accuracy. The proposed HPTLC method can be effectively applied for routine quality control and analysis of Salbutamol and Etofylline in combined pharmaceutical formulations, offering advantages such as reduced solvent consumption, lower cost, and faster analysis time compared to conventional methods.

1 Introduction

The combination of Etofylline and Salbutamol is widely used in the treatment of bronchial asthma, chronic obstructive pulmonary disease (COPD), and other respiratory conditions involving airway obstruction. The synergistic pharmacological effects of these two drugs make the combination particularly effective: Etofylline, a xanthine derivative, acts as a bronchodilator by relaxing bronchial smooth muscles and improving pulmonary function, while Salbutamol, a selective β_2 -adrenergic agonist, offers rapid relief by inducing smooth muscle relaxation in the airways [1-3].

Due to the growing market of fixed-dose combinations (FDCs) of bronchodilators, there is a crucial need for simple, economical, and reliable analytical methods that can quantify both drugs simultaneously in quality control and routine analysis. Conventional

techniques like HPLC and UV-spectrophotometry, though widely used, often require extensive sample preparation, high solvent consumption, and longer analysis times[4-6].

High-Performance Thin Layer Chromatography (HPTLC) has emerged as an efficient and cost-effective alternative, offering high throughput, minimal solvent usage, and the capability for simultaneous multi-sample analysis. It is especially advantageous in developing countries and small- to medium-scale laboratories due to its affordability and speed.

Despite several reports on individual estimations, limited HPTLC methods have been reported for the simultaneous estimation of Etofylline and Salbutamol in combined dosage forms. Therefore, the objective of the present study is to develop and validate a novel, accurate, precise, and specific HPTLC method for the simultaneous estimation of Etofylline and Salbutamol in bulk and pharmaceutical formulations, in accordance with ICH Q2(R2) guidelines [7-10].

2 Introduction

Fixed-dose combinations (FDCs) of Etofylline and Salbutamol are commonly prescribed to manage obstructive airway diseases due to their complementary mechanisms of action. As their use increases in clinical settings, so does the necessity for efficient analytical methods that ensure the quality, safety, and efficacy of such formulations [11-13].

Several analytical techniques—such as UV spectrophotometry and HPLC—have been previously employed for the estimation of these drugs. However, these methods are often associated with challenges such as longer run times, higher operational costs, greater solvent consumption, and limited sample throughput, making them less favourable for routine quality control, especially in resource-constrained environments [14-17].

High-Performance Thin Layer Chromatography (HPTLC) stands out as a viable alternative for the simultaneous estimation of multiple drugs, offering numerous advantages:

- Lower cost and solvent requirement
- Capability for analyzing multiple samples in parallel
- Simplicity in operation and shorter analysis time
- High accuracy and reproducibility when validated properly

In light of these advantages, there exists a critical analytical need for a validated HPTLC method that can:

- Simultaneously estimate Etofylline and Salbutamol in combined formulations
- Be adopted for routine analysis in industrial quality control laboratories
- Comply with international standards, particularly ICH Q2(R2) for validation

Thus, the present study was undertaken to address this gap by developing and validating an HPTLC method that is cost-effective, reliable, accurate, and suitable for regulatory submission and commercial application [18-21].

3 Materials and Methods

1.1 Chemicals and Reagents

- Etofylline and Salbutamol Sulfate standards were obtained as gift samples from a certified pharmaceutical manufacturer.

- Commercial tablet formulations containing the fixed-dose combination were sourced from a local pharmacy.
- Analytical-grade solvents: Methanol, Toluene, and Ethyl Acetate (Merck) were used without further purification.
- Silica gel 60 F254 precoated TLC plates (10 × 10 cm, E. Merck) were used as the stationary phase.

1.2 Instruments and Analytical Equipment

- Sample applicator: CAMAG Linomat V
- Development chamber: CAMAG Twin Trough Chamber
- Densitometric scanner: CAMAG TLC Scanner 3 with WinCATS software (version 1.4.4)
- UV Cabinet, analytical balance, ultrasonic bath, and glassware calibrated as per GLP norms

1.3 Chromatographic Conditions

Table 1. Chromatographic conditions

Parameter	Value	Parameter
Stationary phase	Silica gel 60 F254 plates	Stationary phase
Mobile phase	Toluene: Ethyl Acetate: Methanol (6:3:1, v/v/v)	Mobile phase
Chamber saturation time	20 minutes	Chamber saturation time
Development distance	80 mm	Development distance
Sample application volume	5 µL	Sample application volume
Detection wavelength	275 nm	Detection wavelength
Scanning mode	Absorbance densitometry	Scanning mode

1.4 Preparation of Standard Solutions

- Stock solutions: 10 mg each of Etofylline and Salbutamol were weighed accurately and dissolved in methanol in 10 mL volumetric flasks (concentration: 1000 µg/mL).
- Working standard solutions were prepared by serial dilution to achieve concentrations ranging from 100 to 600 ng/spot for both drugs.

1.5 Sample Preparation (Tablet Formulation)

- Twenty tablets were accurately weighed, crushed, and powdered. A quantity equivalent to the labeled dose of Etofylline and Salbutamol was transferred to a 100 mL volumetric flask with methanol.

- The solution was sonicated for 20 minutes, filtered through Whatman filter paper No. 41, and diluted to the required volume.

1.6 Method Validation (as per ICH Q2(R2))

Validation of the method was conducted according to the International Council for Harmonisation (ICH) Q2(R2) guideline for analytical procedure validation, covering the following performance characteristics:

3.6.1 Specificity

- Demonstrated by comparing chromatograms of blank matrix, placebo, standard mixture, and sample extract to confirm absence of interference at respective R_f values.

3.6.2 Linearity

- Evaluated over a range of 100–600 ng/band for each drug.
- Calibration curves were constructed (n=6 levels) by plotting peak area versus concentration.
- Linearity was confirmed with regression coefficients (R²) > 0.998.

3.6.3 Accuracy (Recovery Studies)

- Accuracy assessed via standard addition method at 80%, 100%, and 120% concentration levels.
- Percent recovery was calculated to ensure results within 98–102% range.

3.6.4 Precision

- Repeatability (intra-day) and intermediate precision (inter-day) were assessed.
- Results expressed as %RSD, with acceptable limits <2% across all levels.

3.6.5 Detection Limit (LOD) and Quantitation Limit (LOQ)

- Estimated based on the standard deviation of response and slope using the calibration curve:

$$\text{LOD} = 3.3 \times (\sigma/S)$$

$$\text{LOQ} = 10 \times (\sigma/S)$$

where σ = standard deviation, S = slope of calibration curve

3.6.6 Robustness

- Method robustness was tested by introducing deliberate changes in parameters such as:
 - o Slight variations in mobile phase composition ($\pm 2\%$)
 - o Development distance (± 5 mm)
 - o Detection wavelength (± 2 nm)
- No significant effect on peak area or R_f values was observed.

3.6.7 Ruggedness

- Assessed by performing analysis on different days and by different analysts to ensure reproducibility.

2 Results and Discussion

2.1 Method Development

The HPTLC method was optimized to achieve efficient separation of Etofylline and Salbutamol. The mobile phase composed of Toluene: Ethyl Acetate: Methanol (6:3:1, v/v/v) yielded sharp, symmetric, and well-resolved peaks with distinct Rf values:

- **Salbutamol:** ~0.30
- **Etofylline:** ~0.54

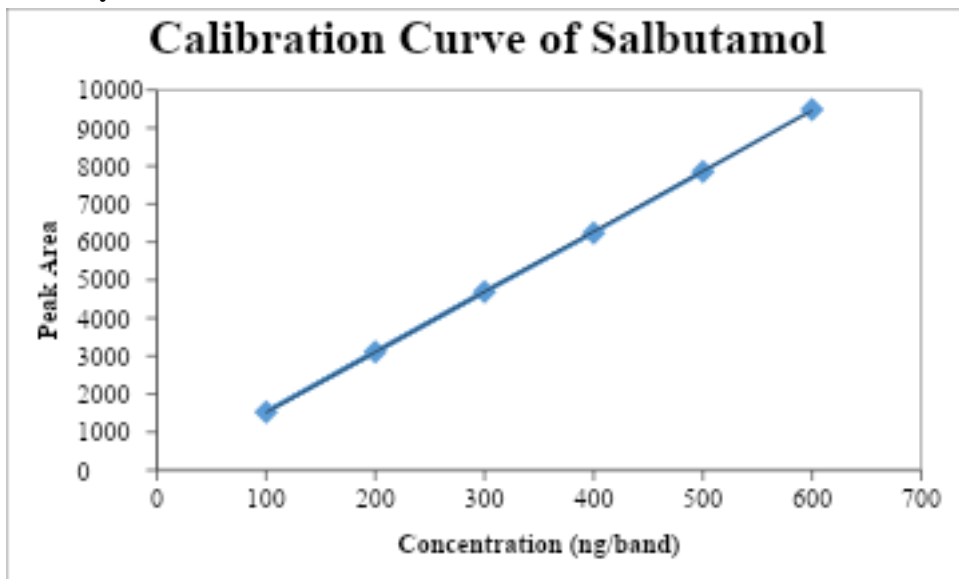


Fig. 1. Calibration curve for Etofylline

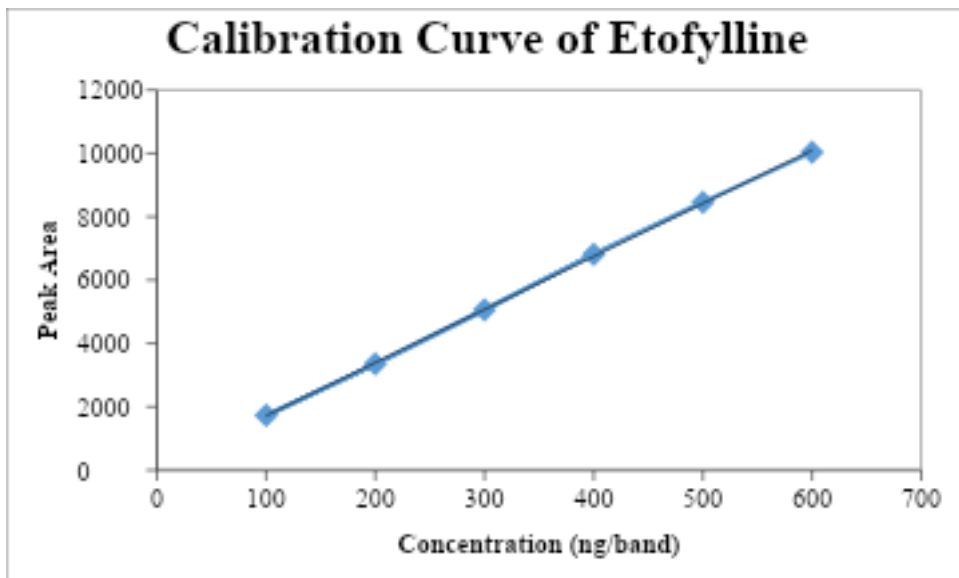


Fig. 2. Calibration curve for Salbutamol

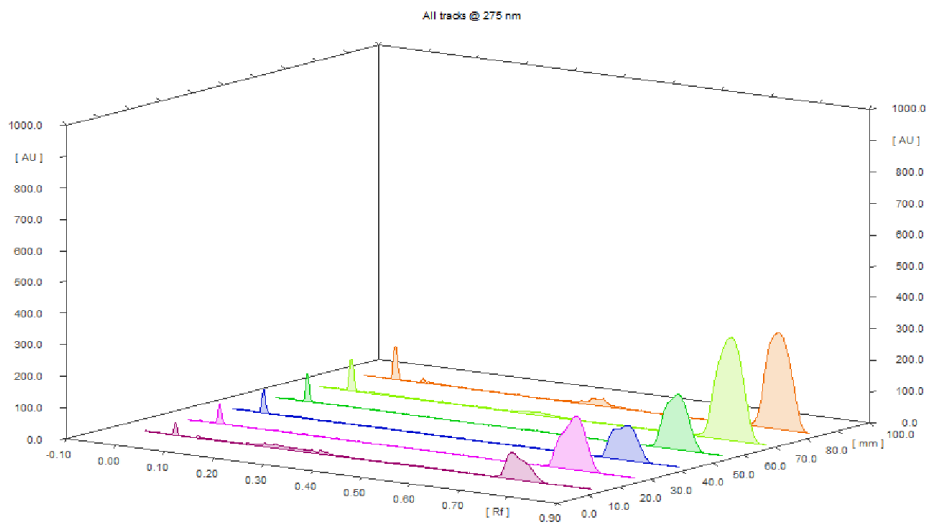


Fig. 3. All Chromatograms (Assay + Linearity) of Etofylline & Salbutamol

These values showed good separation and no overlapping or interference, even in the presence of excipients and degradation products.

2.2 Linearity

The method exhibited excellent linearity for both drugs in the range of 100–600 ng/band. Calibration plots showed correlation coefficients greater than 0.998, confirming a direct relationship between concentration and peak area.

Table 2. Linearity data

Concentration (ng/band)	Peak Area – Salbutamol	Peak Area – Etofylline
100	1532	1721
200	3108	3345
300	4689	5062
400	6243	6820
500	7854	8451
600	9480	10034

2.3 Accuracy (Recovery Studies)

Accuracy was determined via recovery studies using the standard addition method at 80%, 100%, and 120% of target concentration. The % recovery ranged from 99.31% to 100.65%, demonstrating excellent accuracy.

Table 3. Accuracy data

Level (%)	Drug	Amount Added (ng)	Amount Recovered (ng)	Recovery (%)	%RSD
80	Salbutamol	160	159.4	99.63	0.74
100	Salbutamol	200	201.3	100.65	0.62
120	Salbutamol	240	238.5	99.37	0.88
80	Etofylline	160	158.9	99.31	0.79
100	Etofylline	200	199.8	99.90	0.65
120	Etofylline	240	241.2	100.50	0.52

2.4 4.4 Precision

Both intra-day and inter-day precision showed %RSD values below 1.5%, indicating excellent method precision.

Table 4. Precision data

Drug	Repeatability (%RSD)	Intermediate Precision (%RSD)
Salbutamol	1.1	1.4
Etofylline	1.2	1.5

2.5 LOD and LOQ

Sensitivity of the method was established through LOD and LOQ calculations based on the standard deviation of response and slope of the calibration curve.

Table 5. LOD and LOQ data

Drug	LOD (ng/band)	LOQ (ng/band)
Salbutamol	15.8	47.9
Etofylline	18.6	56.4

2.6 Specificity

No interference was observed at the R_f values of Salbutamol or Etofylline from excipients or placebo. Peak purity analysis confirmed the specificity of the method.

2.7 Robustness and Ruggedness

Deliberate variations in mobile phase composition, development distance, and detection wavelength produced no significant changes in peak area or R_f, confirming robustness.

Ruggedness results were consistent across analysts and days, confirming the method's reproducibility.

2.8 Assay of Marketed Formulation

The validated HPTLC method was successfully applied for the quantitative estimation of Salbutamol and Etofylline in a commercially available fixed-dose tablet formulation. Each formulation claimed to contain 2 mg of Salbutamol and 77 mg of Etofylline per tablet. Sample solutions were prepared as per the method described, and analysis was conducted in triplicate. The results are summarized below:

Table 6. Assay data

Drug	Label Claim (mg/tablet)	Amount Found (mg/tablet)	% Assay	%RSD
Salbutamol	2.00	2.04	102.00	1.12
Etofylline	77.00	76.35	99.55	0.96

The results demonstrate that both drugs were estimated within the acceptable limits (95–105%) defined by pharmacopoeial standards. The low %RSD values indicate the precision and repeatability of the method for commercial product analysis.

3 Conclusion

The present study successfully developed and validated a High-Performance Thin Layer Chromatography (HPTLC) method for the simultaneous estimation of Etofylline and Salbutamol in combined pharmaceutical formulations. The method employed a mobile phase of Toluene: Ethyl Acetate: Methanol (6:3:1, v/v/v) and demonstrated well-resolved, sharp, and reproducible peaks at distinct R_f values. Validation was performed in accordance with the updated ICH Q2(R2) guidelines and confirmed the method's compliance across all critical parameters, including linearity, accuracy, precision, specificity, robustness, ruggedness, LOD, and LOQ. The method was also successfully applied to the analysis of marketed formulations, confirming its suitability for routine quality control. Its simplicity, low solvent consumption, cost-effectiveness, and compliance with international standards make this HPTLC method an ideal candidate for analytical applications in the pharmaceutical industry.

Table 7. Comparison Table with Other Methods

Method	Solvent Use	Time	Cost	LOD/LOQ	Applicability
HPLC	High	Medium	High	Lower	High-end labs
UV	Low	Low	Low	Moderate	Limited to single drugs
HPTLC (Performed)	Low	Fast	Low	Suitable	Ideal for QC & industry

4 Future Aspects

This work lays a strong foundation for further extensions of the method, including:

- **Stability-Indicating Studies:** Applying forced degradation under ICH Q1A conditions to assess drug stability.
- **Bioanalytical Adaptation:** With enhanced sensitivity, the method may be adapted for plasma or urine analysis.
- **Green Chemistry Optimization:** Exploring eco-friendly solvent systems to reduce environmental impact.
- **Scale-up for Industrial QC Labs:** Implementing high-throughput sample screening in batch release processes.
- **Regulatory Submissions:** With multi-lab validation, this method may support ANDA or dossier filings for combination respiratory therapies.

References

1. ICH, ICH harmonised guideline Q2(R2): Validation of analytical procedures, International Council for Harmonisation (2023). <https://www.ich.org>
2. B. Srivastava, D. Jain, Development and validation of HPTLC method for simultaneous estimation of salbutamol and etofylline in combined dosage form, *J. Chem. Pharm. Res.*, 3(4), 147–152 (2011).
3. N. Kamble, S. Raut, R. Kharat, HPTLC method development and validation: A review, *J. Drug Deliv. Ther.*, 10(3), 136–144 (2020). <https://doi.org/10.22270/jddt.v10i3-s.4054>
4. V.K. Gupta, R. Jain, Simultaneous estimation of salbutamol sulphate and theophylline in tablets using RP-HPLC and HPTLC methods, *J. Pharm. Biomed. Anal.*, 48(3), 835–838 (2009). <https://doi.org/10.1016/j.jpba.2008.07.019>
5. R.B. Patel, J.K. Patel, Development and validation of a stability-indicating HPTLC method for the simultaneous estimation of drugs in a combined formulation, *Indian J. Pharm. Sci.*, 74(6), 519–523 (2012). <https://doi.org/10.4103/0250-474X.110616>
6. H.A. Pawar, P.R. Joshi, HPTLC: A versatile technique for qualitative and quantitative analysis, *Int. J. Pharm. Sci. Res.*, 6(2), 408–415 (2015).
7. S.A. Desai, P. Loya, Development and validation of HPTLC method for estimation of etofylline in bulk and dosage form, *Int. J. Pharm. Sci. Rev. Res.*, 25(1), 133–136 (2014).
8. Y. Agrawal, K. Pundarikakshudu, Validated HPTLC method for estimation of salbutamol in tablets, *Indian Drugs*, 48(8), 48–51 (2011).
9. S. Ahuja, HPTLC in pharmaceutical analysis, in *Handbook of Modern Pharmaceutical Analysis*, S. Ahuja, S. Scypinski (Eds.), Academic Press, pp. 109–137 (2007). <https://doi.org/10.1016/B978-012369521-5/50005-7>
10. Y.S. Jaiswal, A.K. Sighai, Validated HPTLC method for the quantitation of etofylline in bulk and marketed tablets, *Asian J. Chem.*, 22(6), 4503–4507 (2010).
11. R.K. Goyal, P.L. Sharma, *Practical HPTLC applications in pharmaceutical analysis*, CBS Publishers, New Delhi (2006).
12. Sharma, R. Jain, Validation of analytical methods for pharmaceutical analysis, *Asian J. Res. Chem.*, 4(1), 8–11 (2011).
13. E. Reich, A. Schibli, *High-performance thin-layer chromatography for the analysis of medicinal plants*, Thieme Medical Publishers, Stuttgart (2006).

14. A.K. Srivastava, R. Sinha, Analytical method development and validation: A concise review, *J. Anal. Pharm. Res.*, 7(4), 405–412 (2018). <https://doi.org/10.15406/japlr.2018.07.00253>
15. Indian Pharmacopoeia Commission, *Indian Pharmacopoeia*, Vol. I & II, Ministry of Health and Family Welfare, Government of India (2018).
16. S. Bhattacharya, Recent advances in HPTLC methods for drug analysis, *Int. J. Pharm. Sci. Res.*, 6(6), 2383–2392 (2015).
17. European Medicines Agency, Guideline on bioanalytical method validation, EMA (2015). <https://www.ema.europa.eu>
18. Sethia, K. Patidar, HPTLC: A modern analytical technique, *Asian J. Pharm. Anal.*, 7(3), 157–161 (2017).
19. D.R. Mundhada, M. Dhore, HPTLC method development and validation for simultaneous estimation of salbutamol and ambroxol in syrup, *Int. J. Pharm. Qual. Assur.*, 4(2), 45–50 (2013).
20. N. Mishra, A. Nair, Role of ICH guidelines in analytical method validation, *J. Pharm. Sci. Innov.*, 3(2), 92–98 (2014).
21. Y.N. Ardesana, R. Ajudia, D. Patel, N. Srinivas, *E3S Web of Conferences* (2025).