DEVELOPMENT AND VALIDATION OF THE RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF XANOMELINE AND TROSPIUM IN TABLET AND BULK DOSAGE FORM

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Abstract : The present study focuses on the development and validation of a simple, precise, and accurate RP-HPLC method for the simultaneous estimation of Xanomeline and Trospium in bulk and tablet dosage forms. Chromatographic separation was achieved using a Phenomenex C18 column with a mobile phase comprising methanol and formic acid buffer (60:40, v/v) at a flow rate of 1 mL/min. Xanomeline and Trospium were eluted at retention times of 2.245 min and 2.295 min, respectively. The method exhibited excellent precision, with %RSD values of 0.5% for Xanomeline and 0.8% for Trospium. Accuracy was confirmed through recovery studies, yielding percentage recoveries of 99.89% for Xanomeline and 99.57% for Trospium. The limits of detection (LOD) and quantification (LOQ) were found to be 0.11 µg/mL and 0.33 µg/mL for Xanomeline, and 0.02 µg/mL and 0.07 µg/mL for Trospium, respectively. Linearity was established with regression equations of $y = 30104x + 1865.4$ for Xanomeline and $y = 23625x + 228.82$ for Trospium. The results demonstrate that the proposed RP-HPLC method is robust, reliable, and suitable for routine quality control analysis of Xanomeline and Trospium in pharmaceutical formulations.

IndexTerms **- Xanomeline, Trospium, RP-HPLC.**

1.INTRODUCTION

Xanomeline was developed in the 1920s as a potential treatment for the Alzhemer's disease due to its muscarinic receptor agonist properties. But later it is used for the treatment of schizophrenia due to its effects on muscarinic receptors [1-4]. Xanomeline is a muscarinic agonist that was approved for the treatment of schizophrenia by the FDA in September 2024,becoming the first approved treatment for schizophrenia to target muscarinic receptors as opposed to dopamine receptors. It is approved as a part of a combination product alongside Trospium, a muscarinic antagonist that acts primarily on peripheral muscarinic receptors in order to mitigate the risk and severity of peripheral cholinergic adverse effects [5-10]. Trospium chloride is for treating overactive bladder in the early 2000s due to its antimuscarinic properties. An overactive bladder causes frequent urination, increased urge to pee, and lack of control [11].

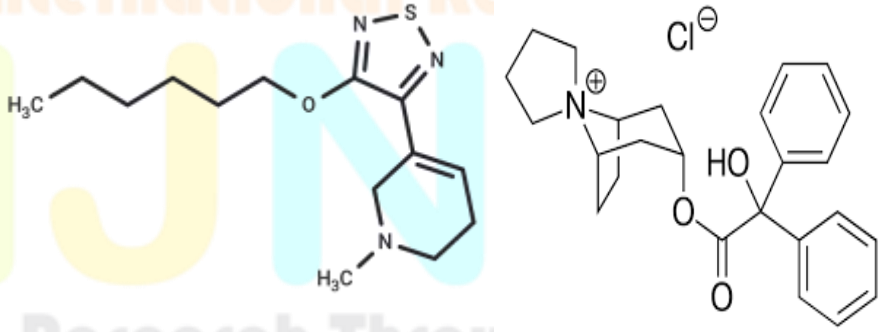

Figure 1: Structures of Xanomeline and Trospium

II. MATERIALS AND METHODS

2.1 Active Pharmaceutical Ingredients (APIs)

Pure drug samples of Xanomeline and Trospium were obtained as gift samples from Spectrum Laboratories. These APIs were used without further purification.

2.2 Marketed Formulation

Commercially available combination tablets of Xanomeline and Trospium (brand name *Cobenfy*) were procured from the local market to evaluate the applicability of the developed method for routine quality control analysis.

2.3 Chemicals and Reagents

All chemicals used in the study were of high purity (Table 1). Acetonitrile, methanol, and water of **HPLC grade** were used for chromatographic analysis and preparation of mobile phases. Analytical reagent (AR) grade potassium dihydrogen orthophosphate and orthophosphoric acid were utilized in buffer preparation. Membrane filters (0.2 µm) were used for filtration of mobile phase and sample solutions prior to injection into the HPLC system.

Table1: Chemical and Reagents

Sr.No.	Chemicals	Quality
1	Acetonitrile	HPLC grade
2	Water	HPLC grade
3	Methanol	HPLC grade
4	Potassium di hydrogen orthophosphate	AR grade
5	Orthophosphoric acid	AR grade
6	Millipore membrane 0.2um	-

2.4 Equipment and Instruments

The experimental work was carried out using calibrated and well-maintained laboratory instruments.

Table2:Equipment and Instruments

Sr. No.	Instrument	Make/Model	Description
1	UV–Visible Spectrophotometer	Microprocessor UV–Visible Single Beam	Used for preliminary wavelength selection and solubility studies
2	HPLC System	Waters Alliance 2695	Equipped with a quaternary pump and autosampler
3	Analytical Balance	Sartorius, Scaletec BSA224S-CW	Used for accurate weighing of samples and standards
4	Ultrasonicator	Lab Man	For dissolving and degassing solutions
5	pH Meter	Lab Man	Used during buffer preparation
6	Hot Air Oven	Sisco	For drying glassware
7	Vortex Mixer	Remi	For mixing solutions uniformly

2.5 Selection of Diluent

A suitable diluent is essential for achieving complete and uniform dissolution of drug molecules. Based on solubility trials conducted using different solvent combinations, a mixture of **Acetonitrile and Water (50:50 v/v)** was selected as the diluent for both drugs due to its ability to dissolve the APIs completely and its compatibility with the mobile phase used in HPLC analysis [12].

2.6 Preparation of Standard Stock Solutions

Accurately weighed quantities of **25 mg Xanomeline** and **5 mg Trospium** were transferred into separate 50 mL volumetric flasks. Each flask was filled to approximately three-fourths with the prepared diluent and sonicated for 10 minutes to ensure complete dissolution of the drug powders. After sonication, the solutions were cooled to room temperature and the volume was made up to the mark with diluent to obtain standard stock solutions of:

Xanomeline: 500 µg/mL
Trospium: 100 µg/mL

These solutions were mixed well and labeled appropriately.

2.7 Preparation of Standard Working Solution (100% Level)

From each standard stock solution, **1 mL** was pipetted out into a 10 mL volumetric flask. The solutions were diluted up to the mark with the diluent, resulting in the following working concentrations

Xanomeline: 50 µg/mL
Trospium: 10 µg/mL

These concentrations were used for system suitability testing, linearity, and validation studies.

2.8 Preparation of Sample Stock Solution

Five tablets of Xanomeline–Trospium combination were weighed, and the average tablet weight was determined. A quantity of powder equivalent to one tablet was accurately transferred to a 100 mL volumetric flask. To this, 5 mL of diluent was added and the mixture was sonicated for 25 minutes to ensure complete extraction of both drugs from the tablet matrix.

After sonication, the solution was diluted to 100 mL with the diluent, mixed thoroughly, and filtered through a 0.2 µm HPLC membrane filter to remove undissolved excipients. The resulting sample stock solution contained:

Xanomeline: 500 µg/mL
Trospium: 200 µg/mL

2.9 Preparation of Sample Working Solution (100% Level)

To obtain the working sample solution, **0.5 mL** of the filtered stock solution was transferred into a 10 mL volumetric flask and diluted to the mark with the selected diluent. The final concentrations obtained were:

Xanomeline: 50 µg/mL
Trospium: 10 µg/mL

This sample solution was used for assay and validation studies.

2.10 Preparation of Buffer (0.1 N Potassium Dihydrogen Orthophosphate)

A buffer solution was prepared by accurately weighing **1.36 g** of potassium dihydrogen orthophosphate and transferring it into a 1000 mL volumetric flask. About 900 mL of Milli-Q water was added, and the mixture was sonicated to remove air bubbles. The volume was then made up to 1000 mL with water. To improve peak shape and reduce tailing, **1 mL of triethylamine** was added to the buffer. The final pH was adjusted to **3.3** using dilute orthophosphoric acid. The prepared buffer was filtered through a 0.2 µm membrane filter before use.

III.RESULTS AND DISCUSSION:

3.1 Optimised Chromatographic Method

A series of preliminary trials were performed using different mobile phase compositions, flow rates, and buffer systems to achieve optimal separation of Xanomeline and Trospium. After several evaluations, the chromatographic conditions described below were found to provide sharp, symmetrical peaks with satisfactory resolution and system suitability parameters.

Chromatographic conditions:

Mobile phase	**:** Formic acid and methanol taken in the ratio 60:40
Flow rate	**:** 1ml/min
Column	**:** STD Phenomenex C18 (4.6x 150mm, 5µm)
Detector wavelength	**:** 230.0nm
Column temperature	**:** 30^0C
Injection volume	**:** 10µL
Run time	**:** 5 min
Diluent	**:** Water and Acetonitrile in the ration 50:50

Under these optimized conditions, both analytes demonstrated good retention behavior and symmetrical peak shapes. The method provided consistent theoretical plate counts, acceptable tailing factors, and good peak resolution, indicating suitability for quantitative estimation.

3.2 Chromaatographic Observations

The optimized chromatogram showed clear and well-resolved peaks for both drugs in figure 2.

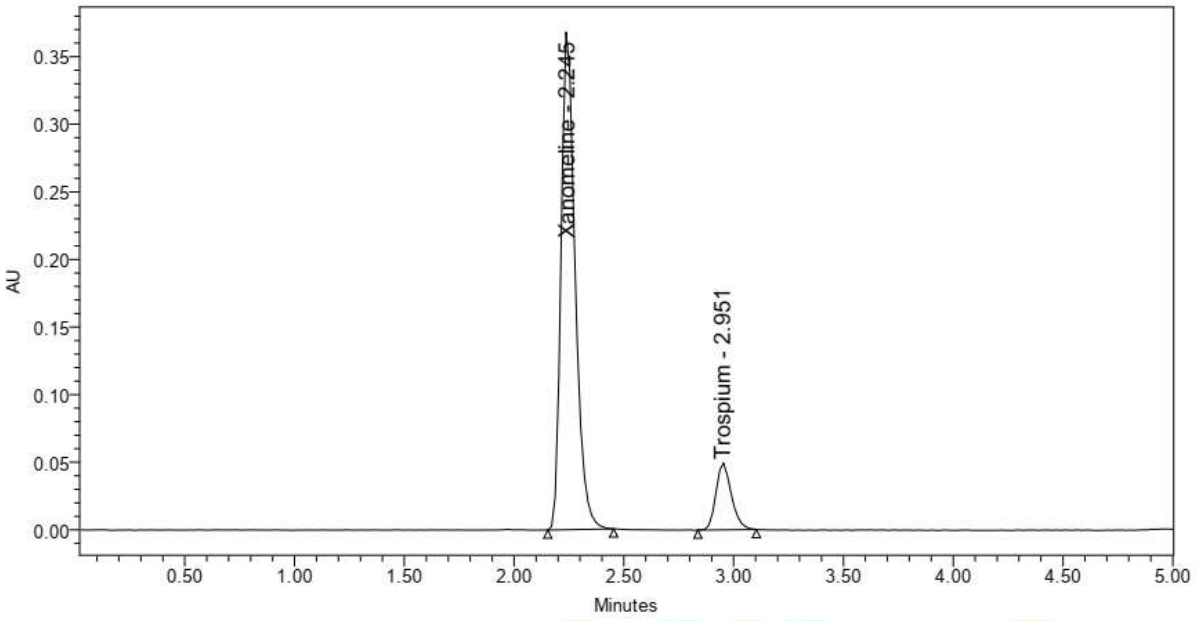

Figure 2: Optimized Chromatogram

Xanomeline eluted at 2.245 min
Trospium eluted at 2.951 min
Both peaks showed adequate retention with no interference from excipients or mobile phase components. The plate count, tailing factor, and overall resolution between the two peaks were found to be highly satisfactory, confirming the reliability of the optimized method. Therefore, the method was finalized and considered appropriate for validation as per regulatory guidelines.

3.2 System Suitability Parameters

System suitability testing was performed by injecting the standard solution six times under optimized chromatographic conditions. The parameters evaluated included retention time (RT), USP plate count, tailing factor, and resolution. All values (Table 3) were found to be within acceptable limits, demonstrating adequate performance of the HPLC system.

Xanomeline and trospium were eluted at 2.245 min and 2.951 min respectively with good resolution (Figure 3). All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Table 3: System suitability parameters for Xanomeline and Trospium

S. no	Xanomeline			Trospium			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.219	5852	1.25	2.794	8120	1.19	4.7
2	2.219	5572	1.27	2.796	8166	1.19	4.7
3	2.221	5818	1.24	2.800	8287	1.20	4.7
4	2.222	5504	1.29	2.801	8667	1.20	4.7
5	2.223	5478	1.25	2.801	8211	1.19	4.6
6	2.223	5515	1.24	2.809	8243	1.18	4.8

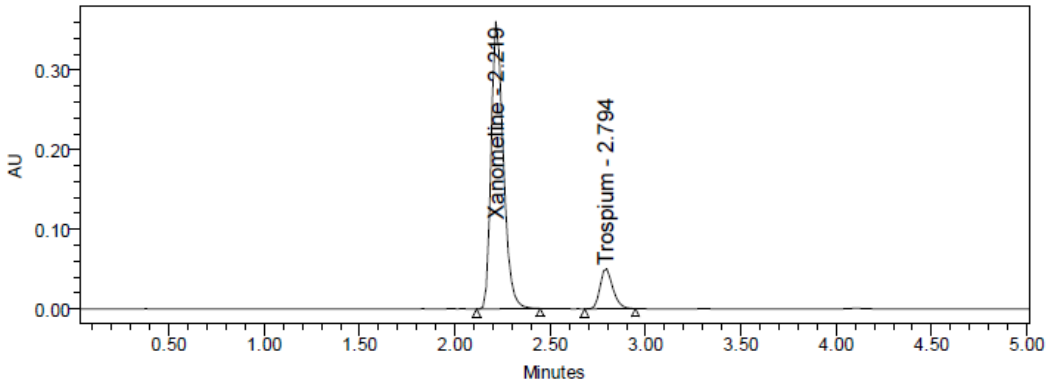

Figure 3: System suitability chromatogram

3.3 Linearity

Linearity studies were performed to assess the relationship between concentration and peak area for Xanomeline and Trospium. Five concentration levels—25%, 50%, 75%, 100%, and 125%—were prepared by pipetting 0.25 mL, 0.50 mL, 0.75 mL, 1.0 mL, and 1.25 mL, respectively, from each of the two standard stock solutions into 10 mL volumetric flasks and making up the volume with the selected diluent. The resulting concentration ranges were 12.5–75 µg/mL for Xanomeline and 2.5–15 µg/mL for Trospium. Each concentration level was injected in duplicate, and the average peak areas were recorded (Table 4).

Table 4: Linearity table for Xanomeline and Trospium

Xanomeline		Trospium	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
12.5	369089	2.5	59408
25	755452	5	119555
37.5	1139933	7.5	178805
50	1507357	10	232569
62.5	1908078	12.5	295201
75	2235549	15	356387

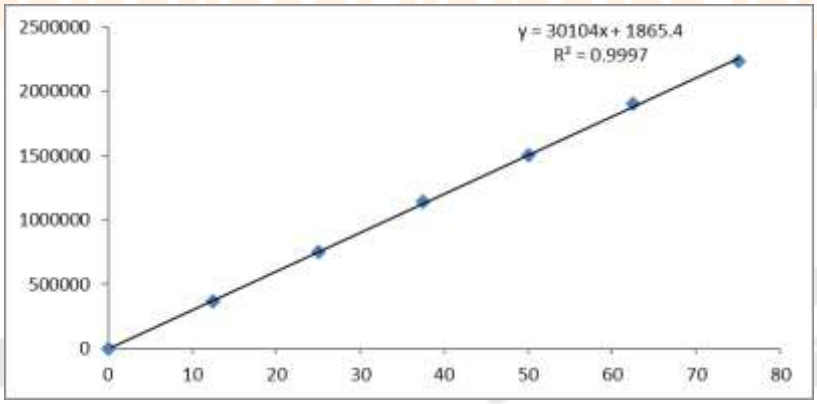

Figure 4: Calibration curve of Xanomeline

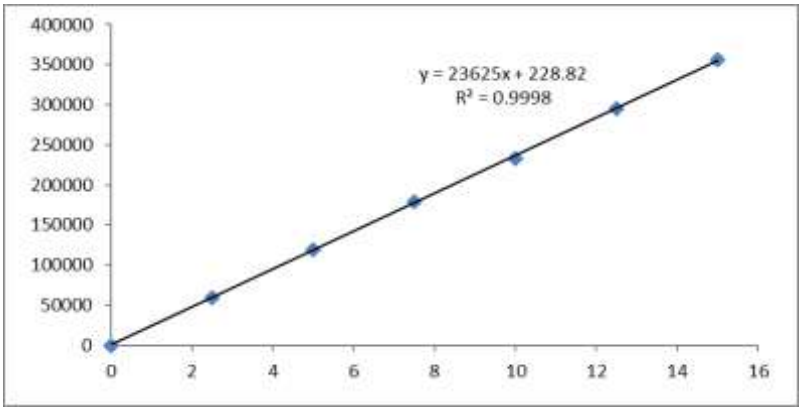

Figure 5: Calibration curve of Trospium

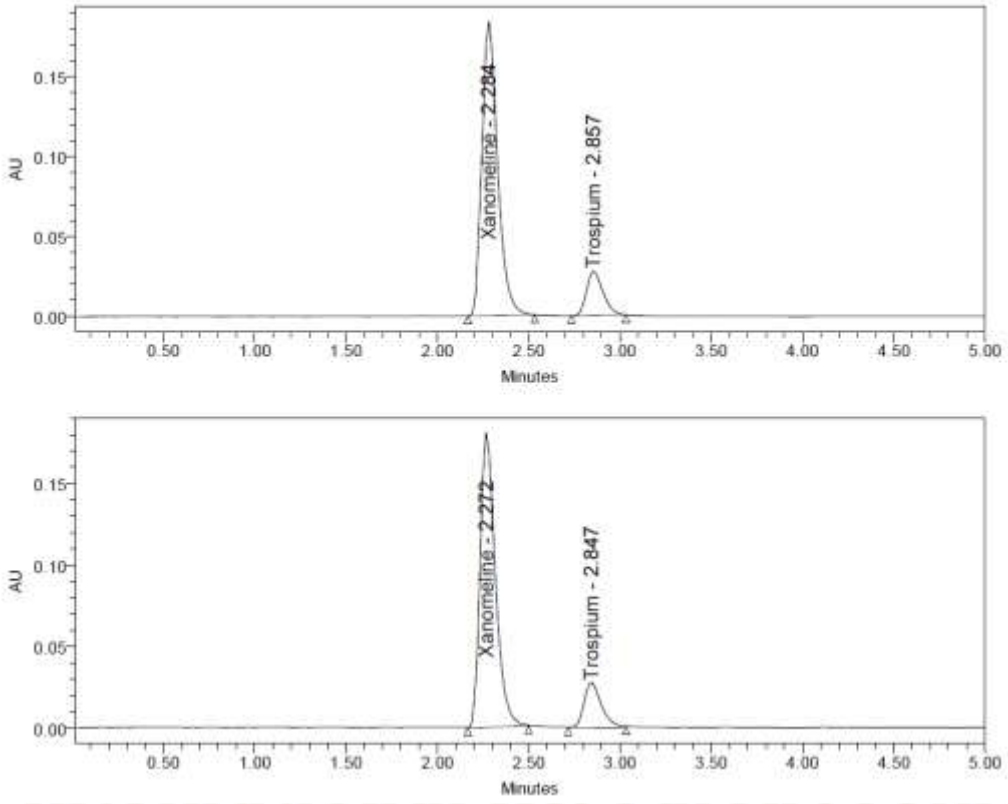

Figure 6: Linearity of 100% Chromatogram of Xanomeline and Trospium

The calibration curves for both analytes demonstrated excellent linearity across the tested concentration ranges. For trospium chloride, the linear regression equation was y = 23,625x + 228.82, with a correlation coefficient (**r**) of 0.999**,** indicating strong linearity. Similarly, xanomeline exhibited a linear regression equation of y = 30,104x + 1865.4**,** with an r-value of 0.999, confirming the method's robust linear response for this compound as well (Figure 4-6)**.**

3.4 Precision

System precision was evaluated by performing six replicate injections of a single working standard solution of xanomeline and trospium. The peak areas obtained for each injection are presented in Table 5. The mean peak areas for xanomeline and trospium were **1,523,050** and **233,969**, respectively. The calculated standard deviations were **7,177.0** for xanomeline and **1,975.0** for trospium. The %RSD values were found to be **0.5%** for xanomeline and **0.8%** for trospium.

As the %RSD values for both analytes were well within the acceptable limit of **≤ 2%**, the system precision results confirm that the analytical system is suitably precise for the determination of both xanomeline and trospium.

Table 5: System precision table of Xanomeline and Trospium

S. No	Area of Xanomeline	Area of Trospium
1.	1532589	236257
2.	1528541	236546
3.	1515876	232512
4.	1515862	233651
5.	1526782	232987
6.	1518652	231862
Mean	1523050	233969
S.D	7177.0	1975.0
%RSD	0.5	0.8

The %RSD values obtained for xanomeline (**0.5%**) and trospium (**0.8%**) were well below the acceptable limit of **≤ 2%**, confirming that the system precision of the method meets the required criteria (Figure 7).

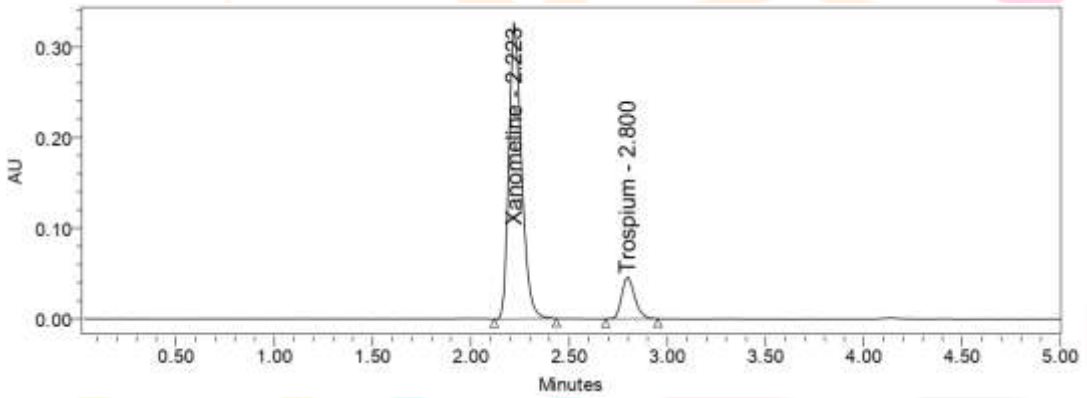

Figure 7: System precision chromatogram

3.5 Repeatability

The repeatability of the method was evaluated by preparing six working sample solutions of identical concentration from a common stock and analysing each solution individually. The mean peak areas obtained were 1,523,622 for xanomeline and 234,196 for trospium. The calculated standard deviations were 5,304.4 and 1,627.4**,** corresponding to %RSD values of 0.3% for xanomeline and **0.7%** for trospium (table 6).

Table 6: Repeatability table of Xanomeline and Trospium

S. No	Area of Xanomeline	Area of Trospium
1.	1525325	233258
2.	1526147	236254
3.	1513579	233254
4.	1522146	236241
5.	1528213	232543
6.	1526322	233626
Mean	1523622	234196

S.D	5304.4	1627.4
%RSD	0.3	0.7

Since the %RSD values for both analytes were well within the acceptable limit of **≤ 2%**, the method demonstrated satisfactory repeatability (Figure 8).

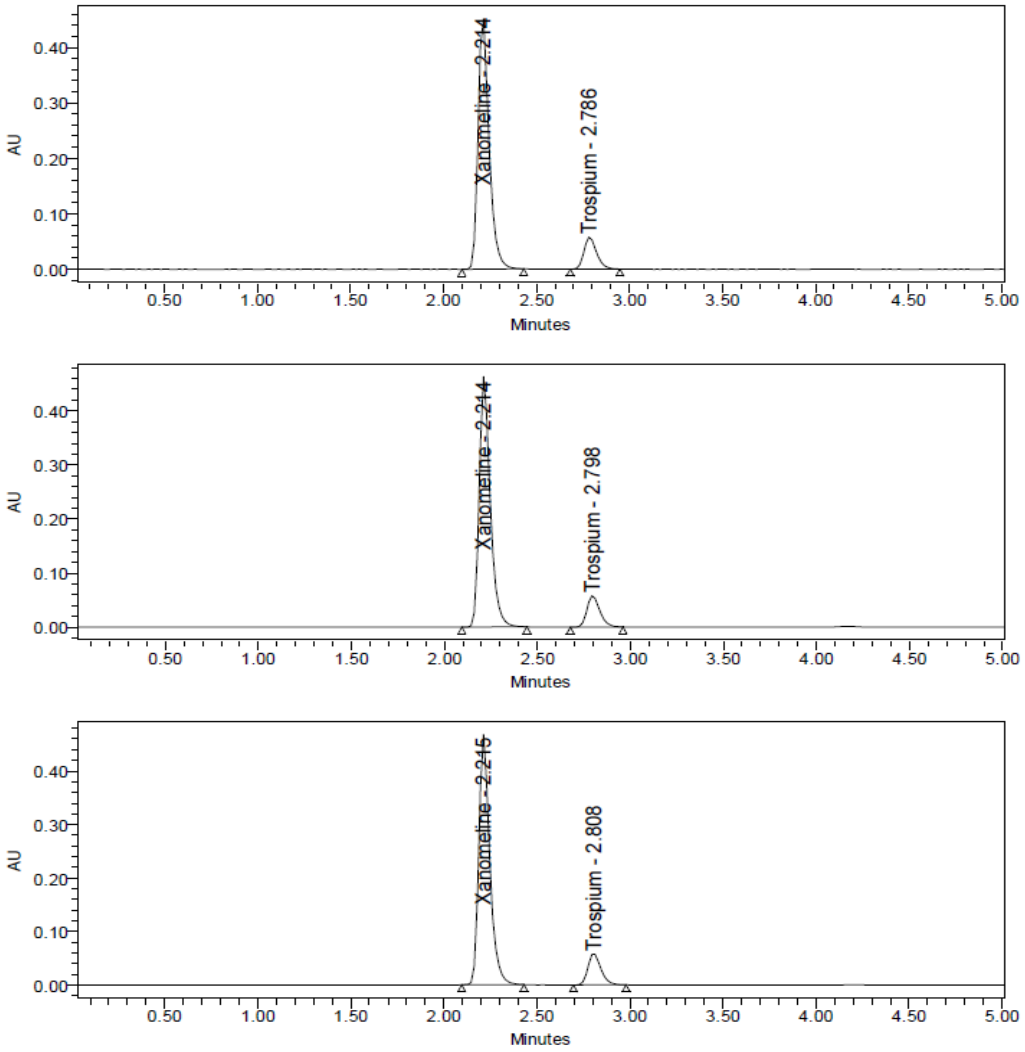

Figure 8: Reapeatability chromatogram

3.6 Intermediate precision

Multiple sampling from a sample stock solution was done and six working sample solution of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned below in table 7, figure 9.

Table 7: Intermediate precision table of Xanomeline and Trospium

S. No	Area of Xanomeline	Area of Trospium
1.	1522156	232657
2.	1515254	230214
3.	1516875	229876
4.	1526785	225743
5.	1520236	228352
6.	1521233	229325
Mean	1520423	229361

%Level	Amount spiked (µg/ml)	Amount recovered(µg/ml)	%Recovery	Mean %Recovery
50%	5	4.97	99.38	99.57%
	5	4.92	98.30	
	5	4.98	99.70	
100%	10	9.99	99.94	

S.D	4086.0	2278.8
%RSD	0.3	1.0

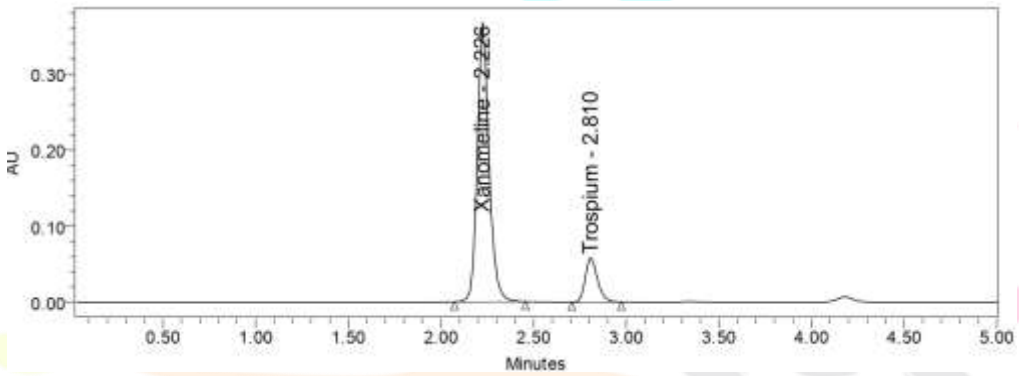

Figure 9: Inter day precision chromatogram

3.7 Accuracy

Accuracy was assessed by the standard addition method at three concentration levels (50%, 100%, and 150%), with each level analysed in triplicate for both analytes. The mean % recovery values obtained for xanomeline ranged from **99.58% to 100.88%**, resulting in an overall mean recovery of **99.89%**. For trospium, recoveries ranged from **98.30% to 101.49%**, with an overall mean recovery of **99.57% (Table 8)**.

As all recovery values fell within the acceptable limits of **98–102%**, the method was confirmed to be accurate for the quantification of both xanomeline and trospium Table 9.

Table 8: Accuracy table of Xanomeline

%Level	Amount spiked(µg/ml)	Amount recovered(µg/ml)	%Recovery	Mean %Recovery
50%	25	25.21	100.82	99.89%
	25	24.90	99.58	
	25	24.96	99.84	
100%	50	50.01	100.02	
	50	49.67	99.34	
	50	50.44	100.88	
150%	75	74.38	99.18	
	75	74.59	99.45	
	75	74.95	99.93	

Table 9: Accuracy table of Trospium

	10	9.99	99.89	
	10	10.15	101.49	
150%	15	14.91	99.37	
	15	14.85	98.99	
	15	14.87	99.11	

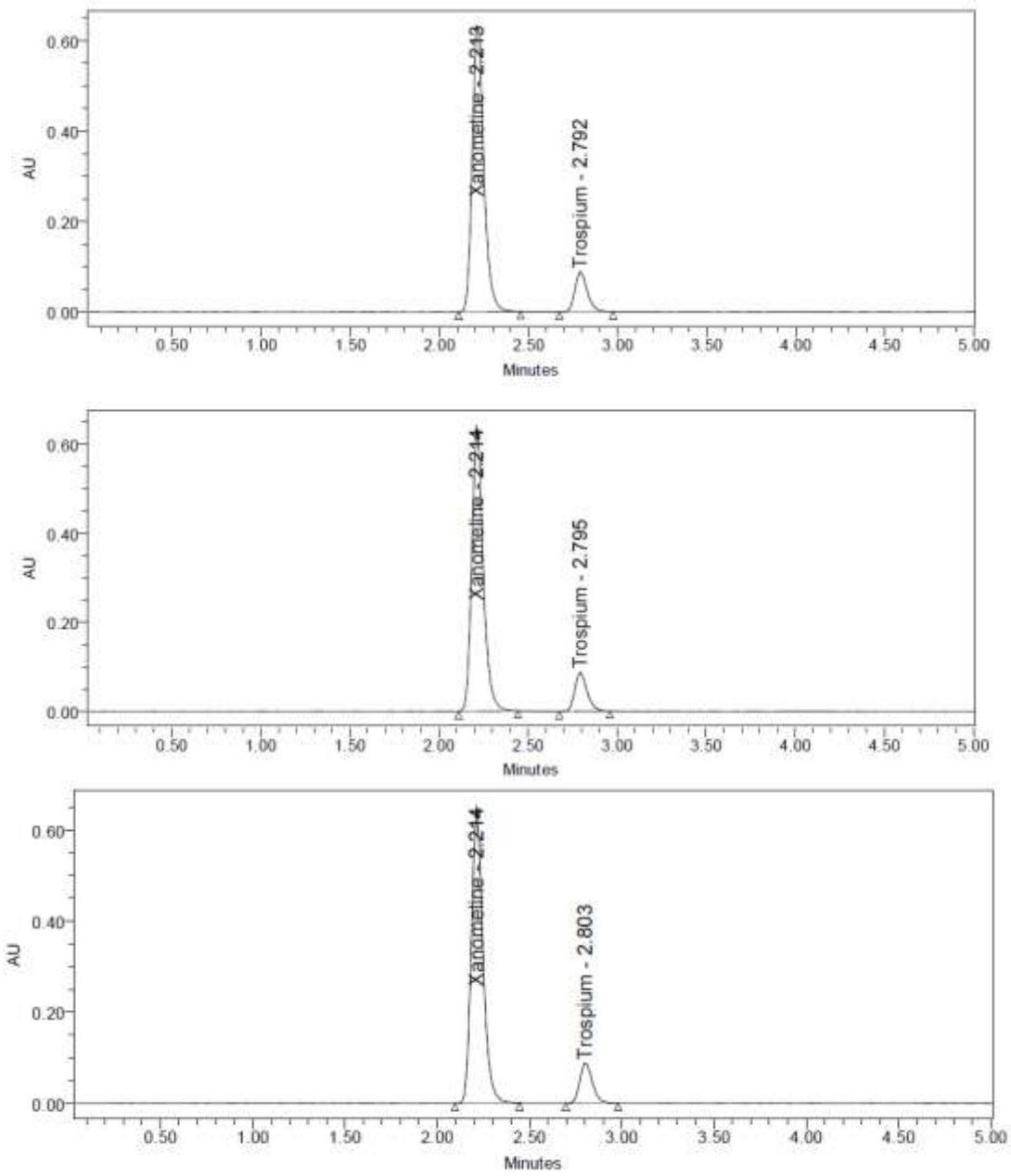

Figure 10: Accuracy of 50%

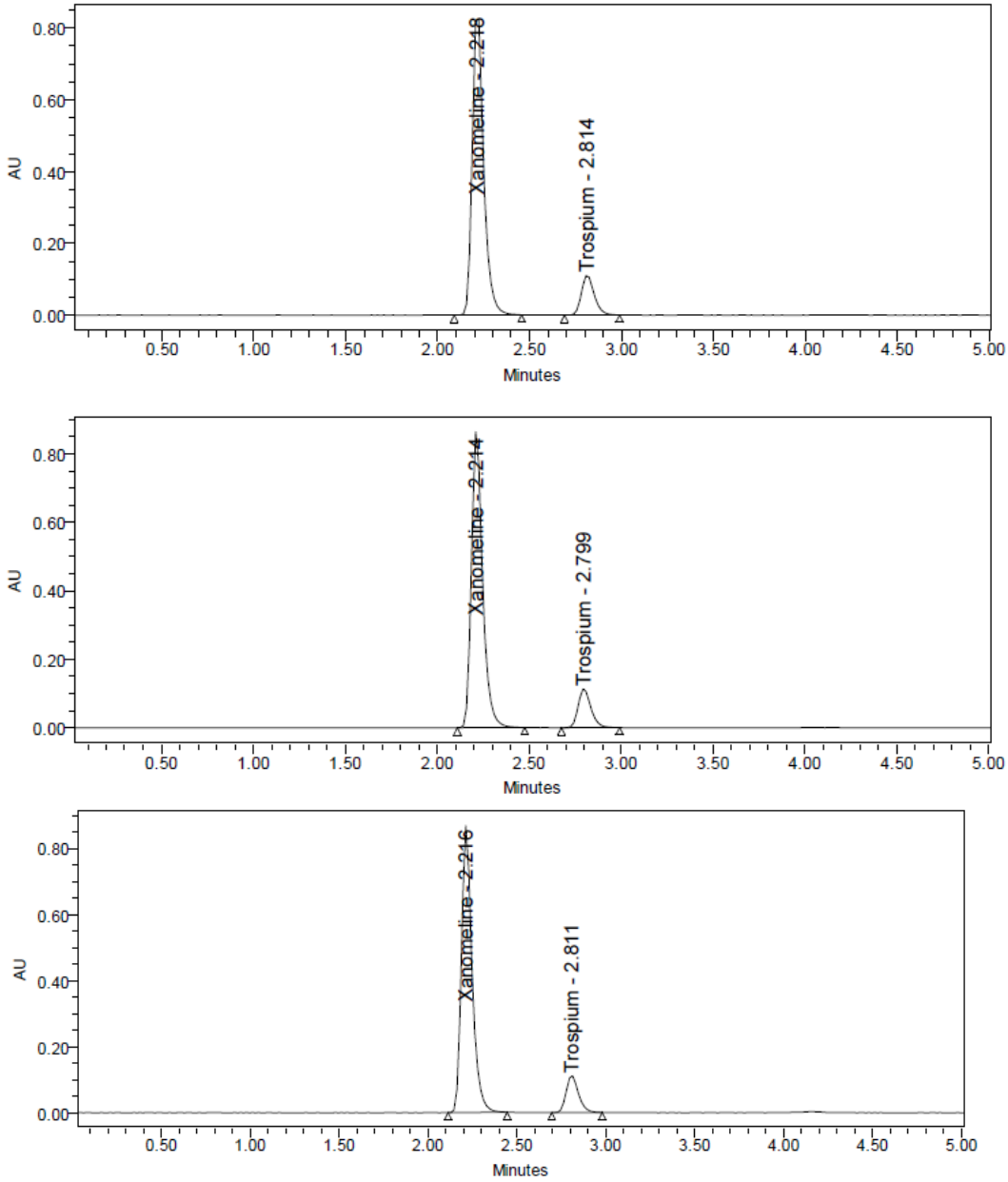

Figure 11: Accuracy of 100%

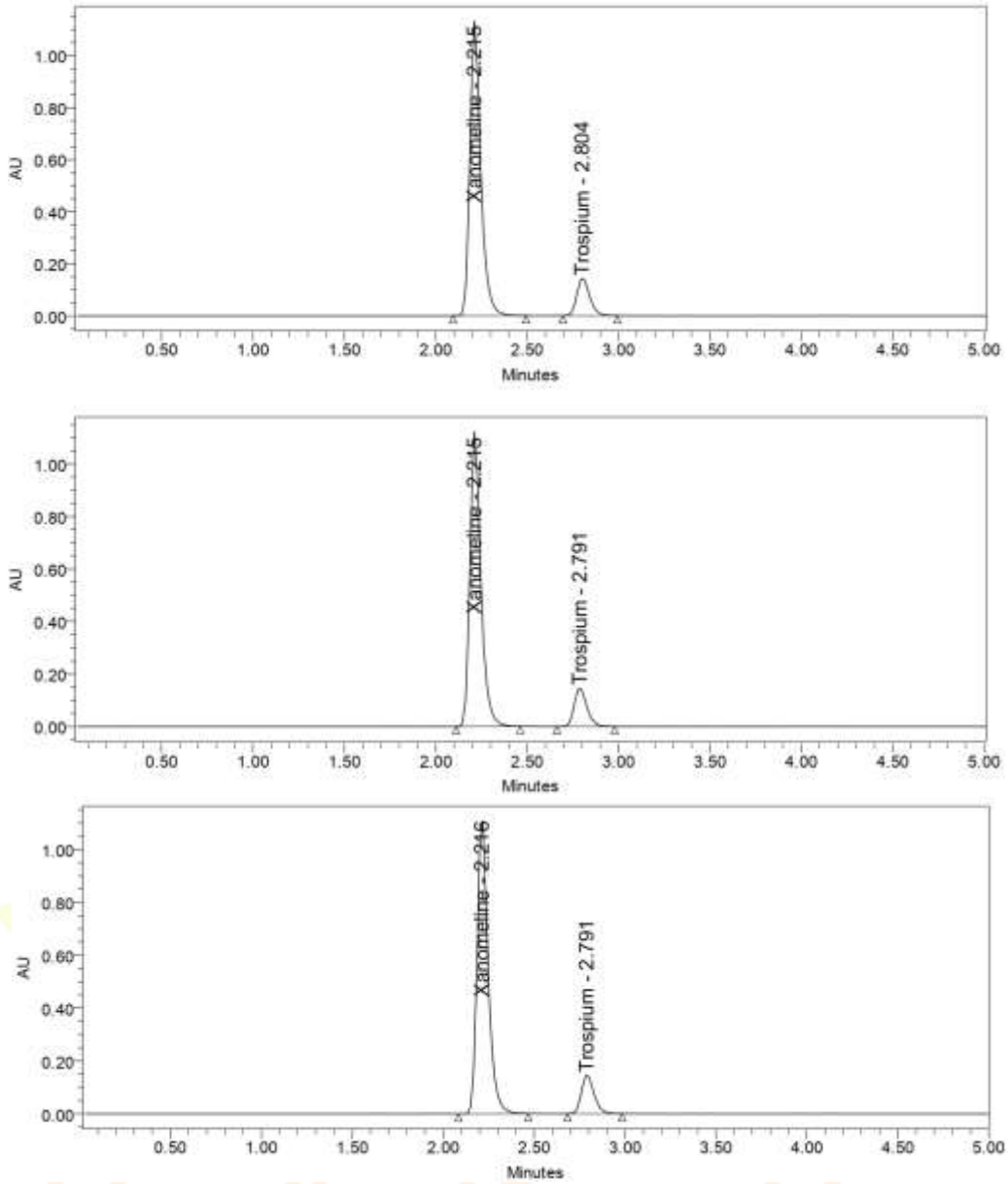

Figure 12: Accuracy of 150%

Sensitivity

The sensitivity of the method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ) for both xanomeline and trospium (table 10). The results are presented in Table 10. The LOD values were found to be 0.11 µg/mL for xanomeline and 0.02 µg/mL for trospium, while the LOQ values were 0.08 µg/mL and 0.07 µg/mL, respectively.

Table 10: Sensitivity table of Xanomeline and Trospium

Molecule	LOD	LOQ
Xanomeline	0.11	0.08
Trospium	0.02	0.07

The low LOD and LOQ values indicate that the developed method is highly sensitive and capable of detecting and quantifying very small concentrations of both analytes. The corresponding chromatograms for LOD and LOQ are shown in Figures 13 and 14.

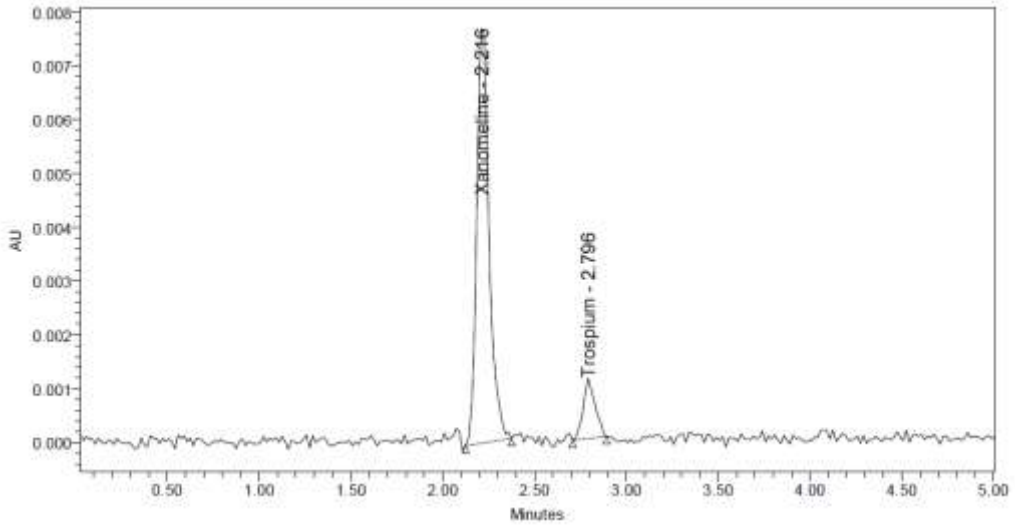

Figure 13: LOD Chromatogram of Standard

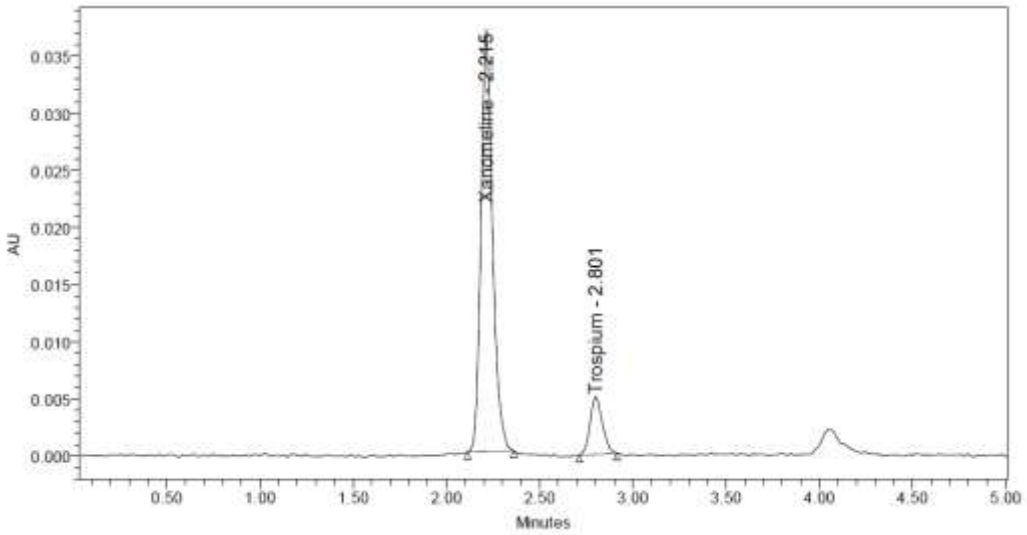

Figure 14: LOQ Chromatogram of Standard

IV.CONCLUSION

The analytical method developed for the simultaneous estimation of xanomeline and trospium was successfully validated as per ICH guidelines. Retention time of Xanomeline and Trospium were found to be 2.245 min and 2.951 min. %RSD of the Xanomeline and Trospium were found to be 0.5 and 0.8 respectively The method exhibited excellent linearity for both drugs with correlation coefficients of **0.999**, confirming a strong proportional relationship between concentration and response. System precision and repeatability studies demonstrated high method reproducibility, with %RSD values well below the acceptable limit of **≤ 2%**.

Accuracy results obtained through standard addition showed mean recoveries of 99.89% for xanomeline and 99.57% for trospium, indicating that the method is highly accurate. Sensitivity evaluation further revealed low LOD and LOQ values for both analytes, confirming the method's ability to detect and quantify trace-level concentrations effectively.

Overall, all validation parameters met the required criteria, establishing that the developed method is precise, accurate, linear, and sensitive, making it suitable for routine analysis of xanomeline and trospium in pharmaceutical formulations.

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