RESEARCH ARTICLE

Analytical Method Development and Validation of a Novel RP-HPLC Method for Simultaneous Quantification of Xanomeline and Trospium Chloride in Pharmaceutical Dosage Form

Pasupuleti Laxmiprasanna[1], Chandan R S[1*] Haritha Reddy Nagidi[2] Nithin Vidiyala[3]

[1]Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, Karnataka-570015,
[2]Department of Pharmaceutical Analysis, Care College of Pharmacy, Oglapur, Warangal, Telangana, India
[3]Cerevel Therapeutics, 222 Jacobs St. Suite 200, Boston, Massachusetts 02141, United States

Received: 21st Nov, 2024; Revised: 19th Feb, 2024; Accepted: 5th Mar, 2024; Available Online: 25th Mar, 2025

ABSTRACT
This study aimed to develop and validate a novel RP-HPLC method for the simultaneous estimation of Xanomeline and Trospium Chloride in combination dosage forms, addressing the lack of existing methods for their concurrent analysis. Xanomeline, a muscarinic receptor agonist for schizophrenia, and Trospium Chloride, an anticholinergic for mitigating adverse peripheral effects, are combined for therapeutic synergy. The method utilized a Kromasil C18 column with a mobile phase of acetonitrile and 0.1% formic acid (40:60), a flow rate of 1 mL/min, and a detection wavelength of 231 nm. Validation parameters followed ICH guidelines, demonstrating specificity, linearity (R^2 = 0.99979 for Xanomeline and 0.99988 for Trospium Chloride), precision (%RSD < 0.8), and robustness under varied chromatographic conditions. Forced degradation studies confirmed the method's stability-indicating capability, with Xanomeline showing maximum degradation under peroxide (14.1%) and alkali (12.2%) stress, while Trospium Chloride exhibited significant degradation under peroxide (14%) and acid (11.2%). Accuracy studies revealed mean recoveries of 99.7%–100.0% for Xanomeline and 99.4%–100.0% for Trospium Chloride. The method is suitable for routine analysis, ensuring quality control for combination therapies.

Keywords: Xanomeline, Trospium Chloride, RP-HPLC, Stability Indicating, Forced Degradation, Validation.
How to cite this article: Pasupuleti Laxmiprasanna, Chandan R S Haritha Reddy Nagidi Nithin Vidiyala. Analytical Method Development and Validation of a Novel RP-HPLC Method for Simultaneous Quantification of Xanomeline and Trospium Chloride in Pharmaceutical Dosage Form. International Journal of Drug Delivery Technology. 2025;15(1):119-25. doi: 10.25258/ijddt.15.1.16
Source of support: Nil
Conflict of interest: None

INTRODUCTION

Schizophrenia involves various neurotransmitters, with positive symptoms linked to increased dopaminergic activity in mesolimbic pathways and negative symptoms and cognitive impairment associated with reduced activity in mesocortical pathways.[1] Recent research highlights the potential of muscarinic agonists, particularly targeting M1 and M4 receptors, to enhance cognition in schizophrenia.[2] 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.[3] It is approved as 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.[4] Trospium treats overactive bladder, which causes bladder muscles to contract excessively.An overactive bladder causes frequent urination, increased urge to pee, and occasionally lack of control.[5] Trospium from Indevus Pharmaceutical Inc. was FDA-approved in 2007.[6] According to the Literature Review RP-HPLC[7,8], RP-UPLC[8], two UV spectrophotometric methods[10,11] and LC-MS/MS[12] method.

Materials and methods

Equipment used

The instruments used in this study included a Waters Alliance HPLC system (e2695) with Empower 2.0 software, an Eutech pH meter, a Sartorius weighing balance, and a UV-1700 UV/VIS spectrophotometer. Additionally, Borosil pipettes, beakers, and burettes were utilized, along with a Unichrome UCA 701 ultra sonicator and an isocratic model pump to support sample preparation and analysis.

Chemical used

The chemicals and reagents used in the study included Acetonitrile (HPLC grade, Rankem), Formic Acid (AR grade, Analytical Reagents), HPLC grade Water (Milli-Q or equivalent), and Ortho Phosphoric Acid (AR grade, Analytical Reagent). These high-purity reagents ensured the reliability and accuracy of the analytical method.

**Author for Correspondence: rschandan@jssuni.edu.in*

Preparation Sample and Standard Solutions

Weigh 100 mg of Xanomeline and 20 mg of trospium chloride accurately, then add diluent, and sonicate to dissolve, then top off the volume with more diluent in a 100 mL volumetric flask. 5 mL of the stock solution should be divided into a 50 mL volumetric flask and diluted with 20 ppm Trospium chloride and 100 ppm Xanomeline. Fill a 10 mL volumetric flask with 17.5 mg of Xanomeline and trospium chloride, weigh them, add diluent, sonicate for 30 minutes, centrifuge, and then cover with stock solution. After passing through a 0.45-micron filter, dilute 1 mL of the stock solution with 100 ppm Xanomeline and 20 ppm Trospium chloride in a 10 mL volumetric flask.

Method Development

Multiple trials were conducted using different columns and mobile phase ratios. Waters X-Bridge Phenyl and Kromasil C18 columns were tested with varying ratios of Acetonitrile and aqueous modifiers (0.1% TFA or Formic Acid). Observations included system suitability and plate count issues, broad and unknown peaks, and suboptimal peak height, indicating the need for further optimization. (Table 1).

Method Validation

The method validation process entails performing a sequence of tests grounded in the analytical method. The purpose of these tests is to assess and record the method's performance characteristics and evaluate its suitability for a particular analytical objective. The performance characteristics of LC methods encompass specificity, selectivity, precision, linearity, robustness, recovery, Minimum detectable concentration, Minimum quantifiable concentration, and ruggedness.[14-17].

Specificity

An analytical method must be capable of identifying a target analyte exclusively among controls and known impurities to assess specificity. Chromatograms were

Figure 1: Structure of Xanomeline and Trospium chloride

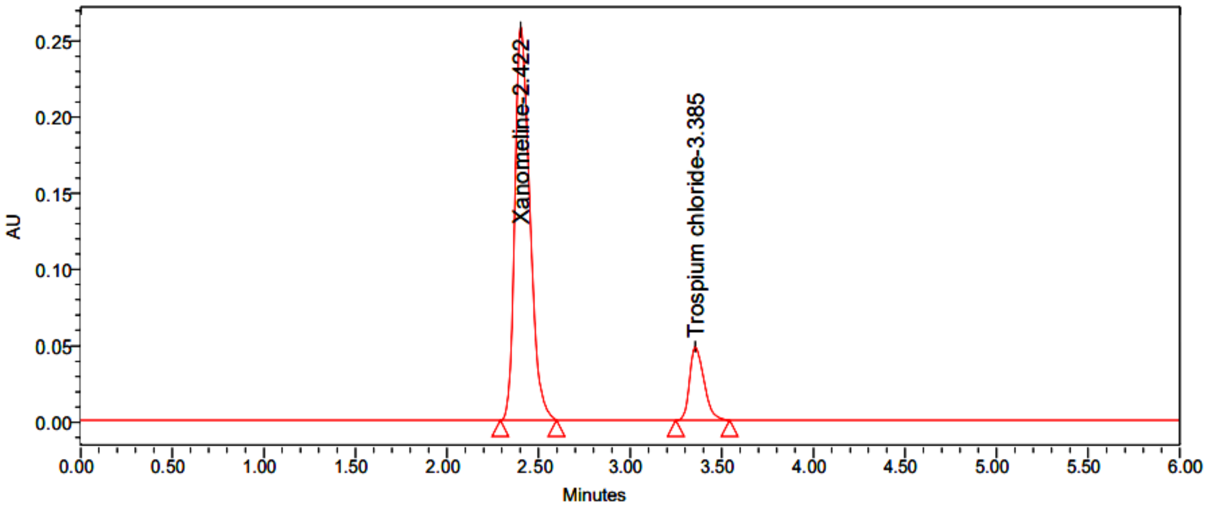


Figure 2: Optimized chromatogram of Xanomeline and Trospium chloride

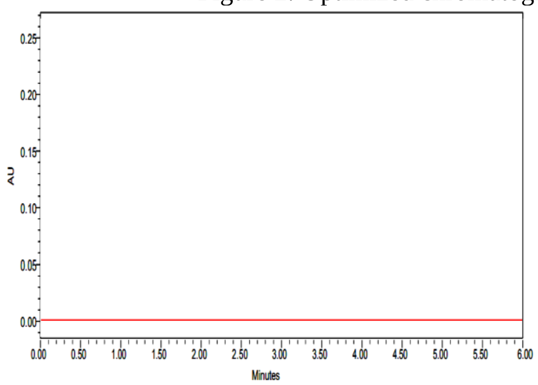


Figure 3: Blank Injection Chromatogram

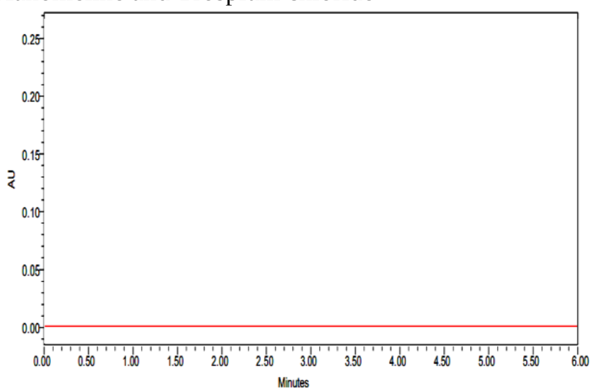


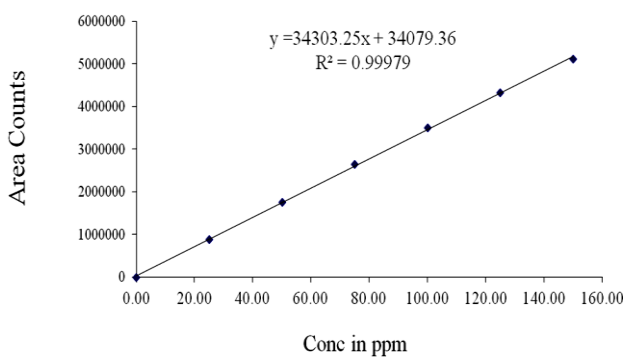
Figure 4: Placebo Injection Chromatogram

Table 1: Chromatographic Trials for Method Optimization

Trial No.	Column	Mobile Phase Ratio	Detection Wavelength (nm)	Run Time (min)	Observation
1	Waters X-Bridge Phenyl	Acetonitrile : 0.1% TFA (20:80)	200–400	10	System suitability conditions are not within the limit
2		Acetonitrile : 0.1% TFA (70:30)	231	10	Plate count is not within the limit
3	Kromasil C18	Acetonitrile : 0.1% TFA (60:40)	231	10	Broad peaks are observed
4		ACN : 0.1% Formic Acid (50:50)	231	10	Unknown peaks are observed
5		ACN : 0.1% Formic Acid (45:55)	231	8	Peak height is not within the limit

Table 2: Results of linearity for Xanomeline & Trospium chloride

S.No	Xanomeline		Trospium chloride	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	25.00	880946	5.00	186943
2	50.00	1755893	10.00	352942
3	75.00	2652366	15.00	547139
4	100.00	3509712	20.00	726318
5	125.00	4326185	25.00	906431
6	150.00	5122659	30.00	1075746
Regression equation	y = 34303.25x+34079.36		y =35997.07x + 3063.68	
Slope	34303.25		35997.07	
Intercept	34079.36		3063.68	
R^2	0.99979		0.99988	



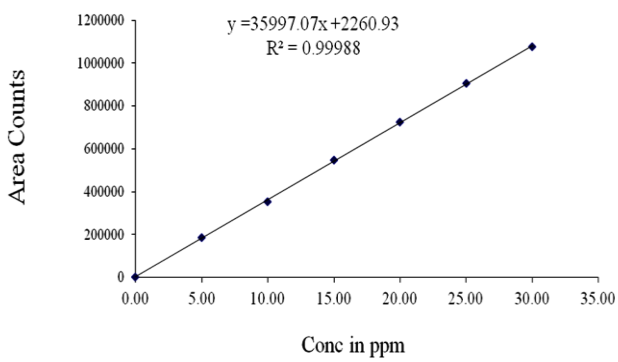


Figure 5: Concentration-Response Curve for Xanomeline and Trospium chloride

acquired for standards, blanks, and samples to accomplish this.

Linearity, Precision, Accuracy and Robustness

From the stock, a series of 25-150 µg/mL for Xanomeline and 5.00-30.00. µg/mL for Trospium chloride solutions were made, diluted, and injected three times into the HPLC system; the chromatograms were obtained from each of them. Every chromatogram had a recorded peak area; the mean peak area was computed. The study of precision under varied experimental conditions like different equipment, labs, and days was termed intermediate precision. The method accuracy and recovery rate were assessed at 50%, 100%, and 150% concentrations. Flow rate, organic phase ratio, and temperature were modified to test robustness, and the mean %RSD was analyzed.

Forced degradation studies

These conditions included acidic and alkaline hydrolysis (using 2N HCl and 2N NaOH, respectively), Peroxide (with 10% hydrogen peroxide), thermal stress (in a hot air oven at 105°C), photolytic exposure (under UV light), and simple hydrolysis. Each treatment was applied to Xanomeline and Trospium chloride and maintained for specified durations, such as 30 minutes for chemical exposures and up to 6 hours for thermal stress

RESULTS AND DISCUSSION

Method Optimization

HPLC with a PDA detector was used to optimize the chromatographic technique for estimating Xanomeline and Trospium Chloride together. The optimized settings used a Kromasil C18 column (150 × 4.6 mm, 3.5 µm) for effective separation. The 40:60 mobile phase of acetonitrile and 0.1% formic acid provided optimum resolution and peak symmetry. The detection wavelength was 231 nm for maximum analyte sensitivity. Set the flow rate to 1 mL/min, injection volume to 10 µL, and run time to 6 minutes.

Table 3: System precision table of Xanomeline & Trospium chloride

S.no	Concentration Xanomeline (µg/ml)	Area of Xanomeline	Concentration of Trospium chloride (µg/ml)	Area of Trospium chloride
1.	100	3536891	20	728271
2.	100	3526897	20	726943
3.	100	3551786	20	725849
4.	100	3556841	20	726894
5.	100	3527892	20	729813
6.	100	3547836	20	725840
Mean	3541357		727268	
S.D	12655.74		1535.01	
%RSD	0.36		0.21	

Table 4: Method Precision for Xanomeline & Trospium chloride

S. No.	Area for Xanomeline	Area for Trospium chloride
1	3568490	720954
2	3547810	721842
3	3521698	723146
4	3589742	728469
5	3594281	725843
6	3587468	728215
Average	3568248	724744
Standard Deviation	28613.95	3239.462
%RSD	0.80	0.45

Specificity

While the peaks of the analytes were apparent, and there were no interferences in the chromatograms, the findings of the chromatograms also demonstrated that the approach was selective. The new RP-HPLC method can accurately measure the concentration of Xanomeline and Trospium chloride in both bulk and Pharmaceutical dosage forms, as shown by these chromatograms. Chromatograms of specificity are shown in Figure 3 and 4.

Linearity

The linearity results for Xanomeline (25–150 µg/mL) and Trospium Chloride (5–30 µg/mL) demonstrated strong correlation coefficients (R^2 = 0.99979 and 0.99988, respectively), confirming excellent linear relationships. The regression equations were $y = 34303.25x + 34079.36$ for Xanomeline and $y = 35997.07x + 3063.68$ for Trospium Chloride. The slope and intercept values indicate the method's accuracy and reliability for quantification (Table 2, Figures 5).

System Precision and Method Precision

The system precision results for Xanomeline (100 µg/mL) and Trospium Chloride (20 µg/mL) showed mean peak areas of 3,541,357 and 727,268, respectively, with %RSD values of 0.36% for Xanomeline and 0.21% for Trospium Chloride. (Table 3).

The method precision results for Xanomeline and Trospium Chloride by RP-HPLC showed average peak areas of 3,568,248 and 724,744, respectively, with %RSD values of 0.80% for Xanomeline and 0.45% for Trospium Chloride. These low %RSD values confirm the method's reliability and reproducibility for quantitative analysis (Table 4).

Accuracy

For Xanomeline, the mean recovery values were 99.7%, 100.0%, and 99.4% at the respective levels, indicating accurate quantification. Similarly, for Trospium Chloride, the mean recovery values were 100.0%, 100.0%, and 99.4%, respectively, demonstrating consistent results across all levels (Table 5).

Robustness

The robustness study for Xanomeline and Trospium Chloride under varied flow rates (0.9–1.1 mL/min) and organic phase ratios (34:66 to 44:56) showed retention times and %RSD values within acceptable limits, confirming the method's reliability. For Xanomeline, %RSD ranged from 0.36% to 0.78%, and for Trospium Chloride, it ranged from 0.15% to 0.61% (Table 6).

Assay

The assay results for Xanomeline and Trospium Chloride showed % assay values of 100.3% and 99.7%, respectively, indicating compliance with the labeled amounts. (Table 7).

Forced Degradation studies

Forced degradation studies for Xanomeline and Trospium chloride showed varied % assay and % degradation under different stress conditions (acid, alkali, peroxide, reduction, thermal, photolytic, and hydrolysis). Purity angles remained below their thresholds, indicating no significant co-eluting impurities, ensuring method specificity. The highest

Table 5: Accuracy results of Xanomeline and Trospium Chloride

Concentration Level	Xanomeline			Trospium Chloride		
	Peak Area	% RSD	Mean Recovery	Peak Area	% RSD	Mean Recovery
50%	1765428, 1745896, 1785463	0.6	99.7	365982, 364213, 360542	0.6	100.0
100%	3564821, 3547920, 3512069	0.4	100.0	724569, 726341, 729620	0.4	100.0
150%	5248931, 5296067, 5294782	0.5	99.4	1082732, 1092543, 1081370	0.5	99.4

Table 6: Robustness Results of Xanomeline and Trospium Chloride

Parameter	Condition	Xanomeline Retention Time (min)	Xanomeline %RSD	Trospium Chloride Retention Time (min)	Trospium Chloride %RSD
Flow Rate Change	Less Flow (0.9 mL)	2.659	0.53	3.488	0.61
	Actual (1.0 mL)	2.422	0.36	3.385	0.21
	More Flow (1.1 mL)	2.350	0.78	3.229	0.31
Organic Phase Change	Less Org (34:66)	2.765	0.51	3.620	0.55
	Actual (40:60)	2.425	0.36	3.383	0.21
	More Org (44:56)	2.131	0.64	3.101	0.15

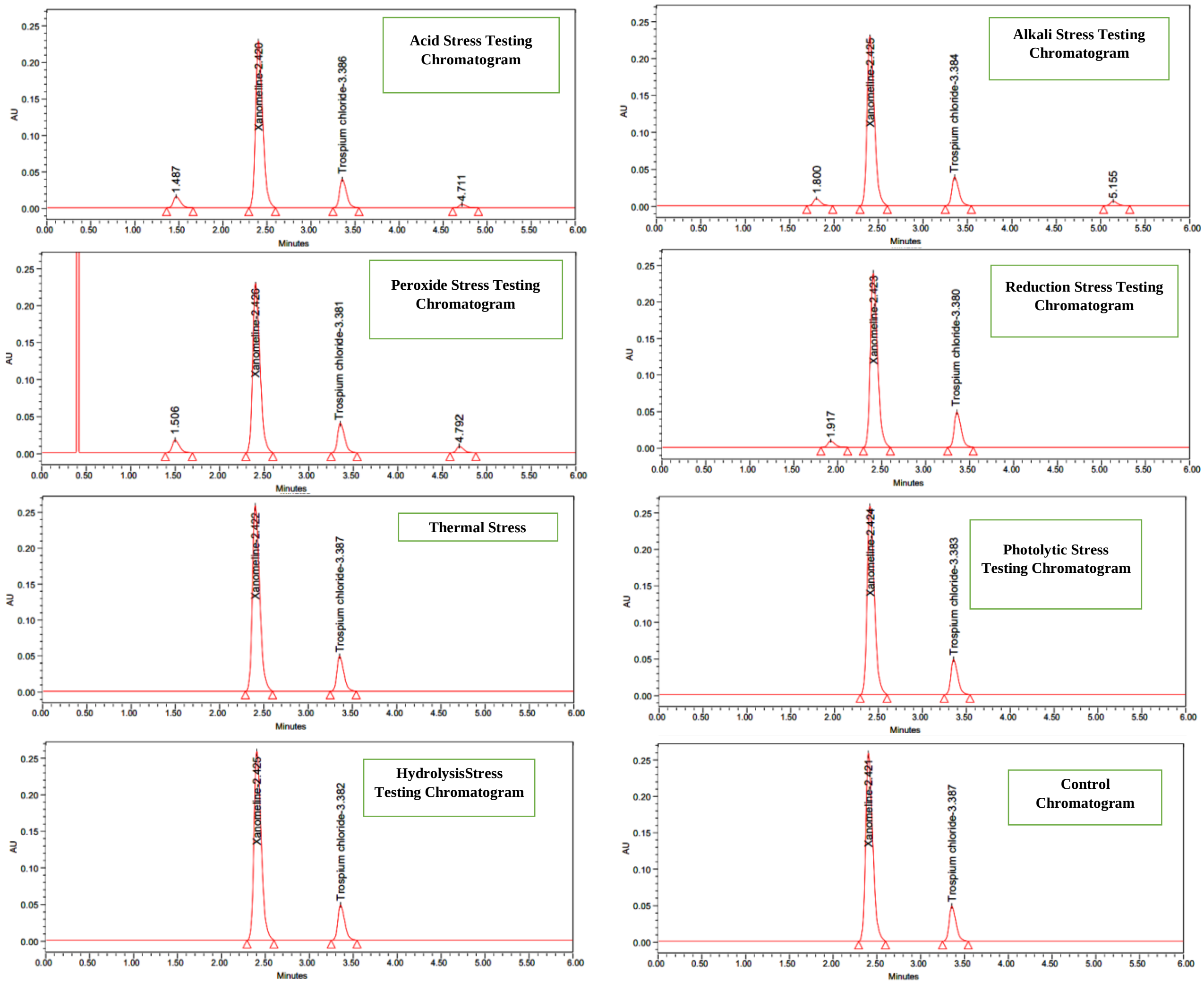


Figure 6: Chromatograms observed during Stress study

degradation was observed under peroxide stress for both compounds. (Table 8, Figure 6).

CONCLUSION

The developed RP-HPLC method provides a robust, validated approach for the simultaneous quantification of Xanomeline and Trospium Chloride, ensuring precise and reliable analysis in combination dosage forms. The method achieved excellent separation with retention times of 2.242 minutes for Xanomeline and 3.385 minutes for Trospium Chloride, with %RSD values within acceptable limits, confirming precision and repeatability. Forced degradation studies validated the method's stability-indicating capability, effectively differentiating degradation products under varied stress conditions. Accuracy was demonstrated through recovery rates exceeding 99%, while linearity across specified concentration ranges confirmed the method's reliability. The method's robustness under minor chromatographic variations further underscores its suitability for routine pharmaceutical analysis, contributing significantly to the quality control of combination therapies targeting schizophrenia. This novel method bridges a critical analytical gap, providing an efficient tool for regulatory compliance and therapeutic efficacy monitoring.

Table 7: Assay of Xanomeline & Trospium chloride

Brand	Drug	Avg sample area (n=2)	Std. Conc. (μg/ml)	Sample Conc. (μg/ml)	Label amount (mg)	% assay
COBNEFY	Xanomeline	3549411	100	100	100	100.3
	Trospium chloride	725352	20	20	20	99.7

Table 8: Forced Degradation results for Xanomeline and Trospium chloride

Deg .Parameters	Xanomeline				Trospium Chloride			
	% Assay	% Deg	Purity Angle	Purity Threshold	% Assay	% Deg	Purity Angle	Purity Threshold
Control	100	0	7.125	12.355	100	0	2.536	6.893
Acid	88.9	11.1	7.136	12.347	88.8	11.2	2.544	6.852
Alkali	87.8	12.2	7.124	12.324	88.2	11.8	2.570	6.847
Peroxide	85.9	14.1	7.106	12.363	86	14	2.525	6.841
Reduction	90.1	9.9	7.165	12.394	96.5	3.5	2.591	6.82
Thermal	96.9	3.1	7.138	12.335	98.7	1.3	2.547	6.855
Photolytic	98.6	1.4	7.145	12.374	96.9	3.1	2.533	6.892
Hydrolysis	99.3	0.7	7.198	12.392	97.8	2.2	2.582	6.838

Acknowledgements
The authors extend gratitude and thanks to the Principal, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Mysuru for support and completion of this research work.

REFERENCES

1. Singh A. Xanomeline and Trospium: A Potential Fixed Drug Combination (FDC) for Schizophrenia-A Brief Review of Current Data. Innovations in Clinical Neuroscience. 2022;19(10-12):43–7.
2. Yohn SE, Harvey PD, Brannan SK, Horan WP. The potential of muscarinic M1 and M4 receptor activators for the treatment of cognitive impairment associated with schizophrenia. Frontiers in Psychiatry. 2024 Oct 4;15.
3. FDA News Release: FDA Approves Drug with New Mechanism of Action for Treatment of Schizophrenia
4. FDA-Approved Drug Products: Cogency (xanomeline tartrate/trospium chloride) capsules for oral use (September 2024).
5. Biastre K, Burnakis TG. Trospium Chloride Treatment of Overactive Bladder. 2009 Feb 1;43(2):283–95.
6. Pak RW, Petrou SP, Staskin DR. Trospium chloride: A quaternary amine with unique pharmacologic properties. Current Urology Reports. 2003 Nov;4(6):436–40.
7. D. Kedar V, N. Dube P, N. Kapse S, B. Thombare Y. Development and Validation of Stability indicating RP-HPLC Assay Method for Trospium Chloride in Tablet Dosage Form. Research Journal of Pharmacy and Technology. 2024 Apr 22;1595–8.
8. Panda SS. New Stability-Indicating RP-UFLC Method for Determination of Trospium Chloride in Tablet Dosage Form. Scientia Pharmaceutica. 2012;80(4):955–64.
9. R Bendale A, Prajapati S V, P Narkhede S, B Narkhede S, G Jadhav A, G Vidyasagar. Analytical Method Development & Validation Protocol For Trospium Chloride In Tablet Dosage Form. Indo Global Journal of Pharmaceutical Sciences. 2011;01(02):166–72.
10. Mishra K, Snigdha Rani Behera, Mandala PR, Sankar DU, Sankar CG. Analytical techniques for the estimation of Trospium chloride in capsule dosage form by spectrophotometric method. International Journal of Pharmacy and Pharmaceutical Science. 2021 Jan 1;3(1):01–4.
11. Jadhav, M. L., S. R. Tambe, and M. V. Girase. “Analytical Quality by Design Approach For Development Of Uv Spectrophotometric Methods In The Estimation Of Trospium Chloride From Capsule Dosage Form”. International Journal of Pharmacy and Pharmaceutical Sciences, vol. 6, no. 10, Oct. 2014, pp. 530-3,
12. Gundu R, Pekamwar S, Shelke S, Kulkarni D, Shep S. Development, optimization and pharmacokinetic evaluation of biphasic extended-release osmotic drug delivery system of trospium chloride for promising application in treatment of overactive bladder. Future Journal of Pharmaceutical Sciences. 2021 Aug 12;7(1).
13. Murphy AT, Kasper SC, Gillespie TA, Delong AF. Determination of xanomeline and active metabolite, N-desmethylxanomeline, in human plasma by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Journal of Chromatography B Biomedical Sciences and Applications. 1995 Jun 1;668(2):273–80.
14. Reddy KT, Gandla K, Suthakaran R, Parija S, Surekha ML, Gandu S. High-Performance Liquid Chromatography for The Simultaneous Estimation of Anti-Ulcer Drugs in Pharmaceutical Dosage Form. Journal of Positive School Psychology. 2022 Oct 20;6(9):4524-8.
15. N. V. V. D. Praveen Boppy, Sharath Babu Haridasyam, Niroja Vadagam, Muvvala Venkatanarayana, Chinnakadoori SR, Lakka NS. Separation and quantification of organic-related impurities of anti-

histamine drug hydroxyzine in pharmaceutical dosage forms using stability-indicating high-performance liquid chromatography, liquid chromatography-mass spectrometry, and high-resolution mass spectrometry techniques. Separation science plus. 2024 Feb 13;7(4).

16. Mohan Pasham, Sharath Babu Haridasyam, Niroja Vadagam, N. V. V. D. Praveen Boppy, Chinnakadoori SR, Lakka NS. Separation and quantification of organic-related impurities of beta-adrenergic receptor blocking agent propranolol in pharmaceutical solid dosage forms: Impurity profiling using stability-indicating HPLC method. Separation science plus. 2023 Nov 13;7(1).
17. Niroja Vadagam, Sharath Babu Haridasyam, Muvvala Venkatanarayana, Lakka NS, Chinnakadoori SR. Separation and simultaneous estimation of enantiomers and Diastereomers of muscarinic receptor antagonist Solifenacin using stability-indicating Normal-phase HPLC technique with chiral stationary phase amylose tris-(3,5-dimethylphenylcarbamate). Chirality. 2023 Nov 23;36(2).