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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CYAMEMAZINE TARTRATE IN FORMULATION BY RP-HPLC WITH STABILITY INDICATING

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ABSTRACT

High-performance liquid chromatography (HPLC) technique for cyamemazine tartrate (CYMT) was developed and validated according to the International Conference on Harmonization (ICH) Q2 (R1) guidelines. The developed and validated method for estimating CYMT from a bulk and its pharmaceutical dosage form was found to be a simple, precise, accurate, fast, and stable reverse phase HPLC (RP-HPLC) approach. For chromatographic separation, a Hypersil BDS C18 (250 mm × 4.6 mm, particle size: 5 µm) column was employed with a mobile phase of methanol and buffer (80:20 v/v) flow rate at 1.0 ml/min at room temperature. For detection, a wavelength of 270 nm was utilized. With a run period of 10 min, the CYMT was eluted at 4.38 min. With a correlation coefficient (r^2) of above 0.9996, and limits of detection and quantitation (LOD and LOQ) of 0.27 and 0.80 µg, respectively, the method exhibited a dynamic linear response across 30–90 µg/ml. The repeatability of batch injections for intra- and inter-assay precision and accuracy testing was likewise satisfactory. The stability of CYMT was studied under thermal, acid, alkali, and oxidation conditions, as well as photodegradation conditions. The stability of the approach is demonstrated by the presence of CYMT and its breakdown products. The recommended technique exhibited great linearity, accuracy, precision, robustness, LOD, LOQ, and system suitability within the acceptance limit. The study's findings indicate that the method is rapid, simple, accurate, exact, and linearly stable, implying that an HPLC method for CYMT has been developed and validated, and that it may be used for routine quality control analysis.

Keywords: Method development, Forced degradation experiments, Method validation, Cyamemazine tartrate, Reverse phase-high-performance liquid chromatography, International Conference on Harmonization guidelines.

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INTRODUCTION

Cyamemazine tartrate (CYMT) is a novel antipsychotic medication for the treatment of schizophrenia. It is 10-(3-dimethylamino-2-methyl-propyl)-phenothiazine-2-carbonitrile (Fig. 1) [1]. Schizophrenia is a psychotic condition that falls within the schizophrenia category. Alcohol and some narcotics, brain tumors, brain infections, and stroke are all factors that can lead to psychosis [2]. CYMT was first developed as an antipsychotic drug because of its dopamine D2 receptor antagonistic activity and phenothiazine derivative. CYMT is a neuroleptic drug with anxiolytic characteristics in humans [3]. Cyamemazine (CYM) binds to 5-HT_3 and 5-HT_2C receptors with a high affinity [4]. CYMT was discovered to be partially soluble in water and soluble in ethanol, methanol, chloroform, and dimethyl sulfoxide. A review of the literature on CYMT indicated that there are limited methods for analysis. During the study of human cytochrome P450 enzymes involved in the metabolism of CYMT, LC–MS/MS tandem technique was used to identify CYM and its metabolites [5]. Bioanalytical methods such as simultaneous determination for seven antipsychotic drugs [6] and quantification in biological fluids and tissues assisted with automated solid-phase extraction [7] were discovered using GC–MS tandem mass spectrometry, but there was no single method for the analysis of CYMT in bulk or pharmaceutical formulations published in the literature. There are also few easy and reliable high-performance liquid chromatography (HPLC), HPTLC, and UV spectrophotometric techniques for determining CYM and, by extension, CYMT on a regular basis.

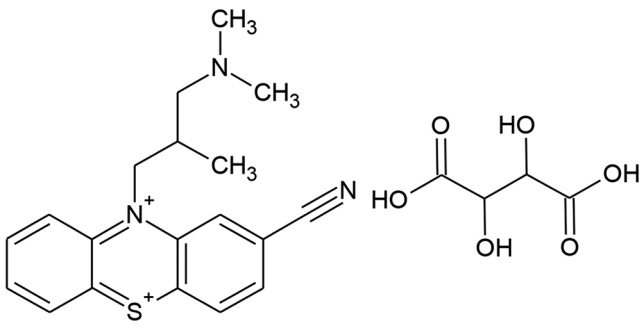

Fig. 1: Chemical structure of cyamemazine tartrate

The product's degradation may be determined by exposing it to severe pH conditions (acidic or basic), oxidative processes, extreme temperature, UV, and dry heat for 5–20% of the period [8]. Only a few methods for estimating CYM have been published, according to a review of the literature. The purpose of this work was to develop and evaluate a stability-indicating reverse-phase HPLC (RP-HPLC) approach for measuring CYM in pure form, as per International Conference on Harmonization (ICH) criteria.

METHODS

Instrumentation

The method was developed using RP-HPLC instrument of Shimadzu LC-20 AD RP-HPLC instrument with UV detector SPD-20A column Hypersil BDS C18 (250 mm × 4.6 mm, particle size: 5 µm) and LC Solution software, including pump and manual injector. Shimadzu (AUW220D) digital weighing balance, a BT ultrasonicator, an ELICO L 1127pH meter, and a Millipore vacuum filter pump (XI 5522050). Merck Millipore's 0.45 µm Nylon filter was employed in the experiment.

Reagents and materials

Varun Herbals, Hyderabad, India, provided a complimentary sample of CYMT pure drug. The HPLC grade water, acetonitrile, and methanol were used. The AR grade triethyl amine, orthophosphoric acid, trifluoroacetic acid, and sodium dihydrogen orthophosphate were utilized.

Conditions for liquid chromatography
Mobile phase methanol: buffer (80:20), column Hypersil BDS C18 (250 mm × 4.6 mm, particle size: 5 µm), 1.0 mL/min flow rate, 20 µL injection volume, 60 ppm sample concentration, ambient temperature, 270 nm wavelength, and 10 min run duration.

Preparation of buffer
With HPLC grade water and 0.5 ml triethyl amine, 0.01M NaH_2PO_4 (sodium dihydrogen ortho phosphate) was produced, and the pH was adjusted to 4.5 with orthophosphoric acid (OPA).

Mobile phase preparation
Prepared an 80:20 v/v ratio of methanol and buffer solution of pH 4.5, mixed, and sonicated. As a mobile phase, they were used.

Preparation of standard stock solutions
By dissolving 30 mg of standard in a 50 ml volumetric flask, adding 30 ml of diluent, and sonicating for 10 min, a working standard stock solution of CYM was created. Allow the solution to cool to room temperature before diluting to volume with diluent to achieve a concentration of 600 ppm.

Standard diluted preparation
With diluent, 2 mL of standard stock solution was diluted to 20 mL and properly mixed. The concentration of the solution was 60 ppm.

Sample solution preparation
Weighed 10 tablets and estimated their average weight then use a mortar and pestle to break them into a fine powder. In a 100 ml volumetric flask, sonicate crushed powder equivalent to 25 mg of CYM for 10 min with intermediate shaking at a temperature not exceeding 20°C in an ultrasonic bath. Before diluting with diluent to the required volume, let the flask to cool to room temperature. Filter the fluid through a 0.45 m diameter nylon membrane filter.

Validation of the method
The method was evaluated according to the ICH requirements, with system suitability, linearity, accuracy, precision, sensitivity limits of detection and limits of quantification (LOQ and LOD), robustness, and a forced degradation study among the validation parameters [9-11].

System suitability

Three injections of 25 ppm CYM solution were used to determine it. Pipetting 1 ml of the stock solution into a 10 ml volumetric flask and diluting to the mark with mobile phase provided a 250 ppm CYM working standard solution. The study takes into consideration elements such as theoretical plates, peak area, retention duration, and asymmetry factor while determining system applicability [12].

Linearity

In terms of test concentration, linearity of the detector response was demonstrated for all known impurities, including CYM, with concentrations ranging from LOQ to 150% of the specification threshold (0.15%). The samples were assessed using the test method specified. Plotting the responses of impurity (Y-axis) versus actual concentration in ppm (X-axis) on a linearity graph yielded the correlation coefficient and Y-intercept at 100% response [13].

Precision

Precision of an analytical method, as defined by ICH Q2 (R1), is the degree of agreement for a measurement process performed on repeated samples. According to the recommendations, method precision and intermediate precision were determined on a homogenous sample, and the percent relative standard deviation (RSD) of individual impurity for precision and intermediate precision was computed and reported [14,15].

The sample solutions were made using 10 mg of CYM. The percent RSD. of CYM was computed after it was tested 3 times on the same day at three different concentrations (10 µg/ml, 30 µg/ml, and 60 µg/ml). Standard deviation or RSD are typically used to describe it.

Preparation of standard stock solution
Thirty milligrams working standards were weighed and transferred to a 50 ml volumetric flask, where the volume of the aforementioned concentration was made up with diluent. With diluent, this solution was diluted up to 20 ml.

Accuracy

Recovery experiments were used to assess the accuracy of the latest investigation, which comprised mixing standard drug solution with reanalyzed sample solution at three significant concentrations: 80%, 100, and 1200% spiked levels. The degree to which the true value and the experimental value agree is the accuracy of an analytical technique. The accuracy of the three contaminants was evaluated at five levels, ranging from LOQ to 150% of the impurity's specification level in respect to the test concentration level. The % recovery was calculated by comparing the impurity level at each level of the spiked sample to that of the control sample.

Standard drug solution preparation
To obtain 600 µg/ml stock solutions, a precisely weighed quantity of 30 mg CYMT was dissolved in diluent and the volume was increased to 50 ml by the same.

Standard solution in work
Pipette out 1.6 ml for 80%, 2 ml for 100%, and 2.4 ml for 120% of the standard stock solution and dilute it with diluents in a 20 ml volumetric flask.

LOD and LOQ

For all three contaminants, the linearity technique was utilized to estimate the LOD and LOQ. Impurity solutions ranging from 0.1 to 15 parts per million (ppm) were created and injected at five different levels. Based on the impurity reaction and STEYX value, the lowest concentration of each impurity that can be detected and measured was calculated and validated.

Robustness

The method's robustness was assessed to determine its capabilities by varying the experimental settings and analyzing the impact on system suitability. Changes in process parameters such as mobile phase, flow rate, and column temperature were used to test robustness. The method's robustness was tested by making small and deliberate modifications to the experimental studies, such as: (i) Temperature of the column: ±5°C, (ii) ±1 ml/min flow rate, (iii) approximately ±2 nm in length, and (iv) content of the mobile phase, organic composition 5%. The change was done to see how it affected the procedure. The obtained data were analyzed by computing percent RSD and percent recovery for each case [16].

Force deterioration study

Forced degradation, also known as accelerated disintegration, is a process in which a product's or material's natural degradation rate is accelerated by applying additional stress. Forced degradation experiments are used to detect reactions that could cause a treated product to degrade. Forced degradation is usually carried out before final formulation and involves applying external stress conditions and checking for material stabilities quickly [17,18].

The following formula was used to calculate the % degradation:

(Area of Unstressed-Area of Stressed)/(Area of Unstressed)*100=% Degradation

- Acid degradation
- Basic degradation

- Peroxide degradation
- Thermal degradation
- Photolytic degradation.

Analytical solution stability

The stability of analytical solutions was determined by analyzing the standard and sample preparations at 0 h, 1 day in the refrigerator, and at ambient room temperature 30°C. From three injections of each solution, the average peak and RSD were computed [19,20].

RESULTS

Method development and experimental parameter optimization

During technique development and optimization, certain physical and chemical properties of CYMT were obtained from the literature. The analytical technique was used to choose preliminary reversed phase HPLC chromatographic parameters, such as detection wavelength, mobile phase, stationary phase, and sample preparation procedure. A number of tests were carried out on the Hypersil BDS C18 (250 cm 4.6 mm) 5 µm column to improve the chromatographic conditions by altering the methanol and buffer ratios and optimizing the chromatographic conditions. The outcomes of method optimization are summarized in Table 1. The mobile phase of methanol and buffer in the ratio of 80:20 v/v, flow rate of 1 mL/min, injection volume 20 l, run time 10 min, and column temperature 30°C at wavelength (λ) 270 were optimized as the best chromatographic conditions for the entire study, where CYMT was eluted forming symmetrical peak shape, resolution, and suitable analysis time with retention time of 4.38 min (Fig. 2). Finally, the buffer (0.01M Na_H2P_O4 [sodium dihydrogen orthophosphate] in 100 ml HPLC grade water, 0.5 ml triethyl amine, and OPA to adjust the pH to 4.5). At a flow rate of 1 ml/min and a column temperature of 30°C, methanol in the ratio (20:80) of v/v was chosen as the best option because it produced a well-defined and well-resolved peak of CYM. The wavelength of 270 nm was chosen as the best for detecting and quantifying CYM. The retention period for CYM was reported to be 4.38 minutes under optimal chromatographic conditions. Fig. 2 shows a typical chromatogram.

Validation of the method

System suitability

The peak area, retention period, tailing factor, and theoretical plate were compared to determine system suitability parameters. Table 2 shows the result. The % RSD of peak area and retention duration were found to be within acceptable limits. Both the theoretical plate and tailing factor are within acceptable bounds. This means that the parameters of system suitability meet acceptable standards.

Linearity

The proposed approach was tested for linearity by graphing peak area versus concentration of CYM working standard solutions. The plot of peak area versus corresponding concentrations of CYM was proven to be linear in the concentration range of 30–90 µg/ml. The findings are shown in Fig. 3 and Table 3. With a goodness of fit (r2) of 0.9996, the regression analysis revealed the following linear equation: y=13.338 × + 6.2707, indicating a linear connection between analyte concentration and area under the peak.

Precision

The precision findings for both the system and the technique revealed that the approach is accurate within acceptable limits. The RSD, tailing factor, and number of theoretical plats were determined for both solutions, and all of the numbers were within acceptable limits. Tables 4 and 5 show that acceptable accuracy for the RSD and tailing factor was <2.0%, and the number of plates was fewer than 1000.

Accuracy

The correctness of an analytical approach indicates how close the findings obtained by that method are to the real value. At all three levels, accuracy data revealed percentage recovery in the range of 99.33–101.10%, and percent RDS values in the range of 1.35–1.41%, as shown in (Table 6). The percentage recovery and percent RSD values were both within acceptable ranges of 98.0–102.0% and not more than 2.0%, indicating that the technique may be used for routine drug analysis, as shown in Table 7.

Robustness

Six sample solutions were prepared and analyzed under controlled conditions, with flow rate, mobile phase ratio, buffer pH, and detection wavelength varied at three levels. The mobile phase flow rate was increased to 1.1 ml/min from 0.9 ml/min before. From 268 nm to 272 nm, the wavelength has been altered. Sample was injected to assess their retention duration and tailing factor. The mobile phase composition also changed from methanol to buffer. Small adjustments in different factors such as wavelength and flow rate were used to test

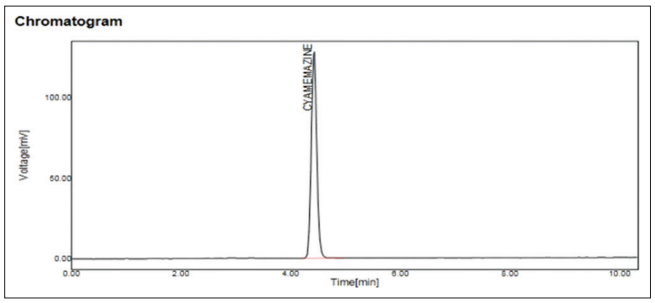

Fig. 2: Chromatogram of cyamemazine obtained using methanol:buffer (80:20)

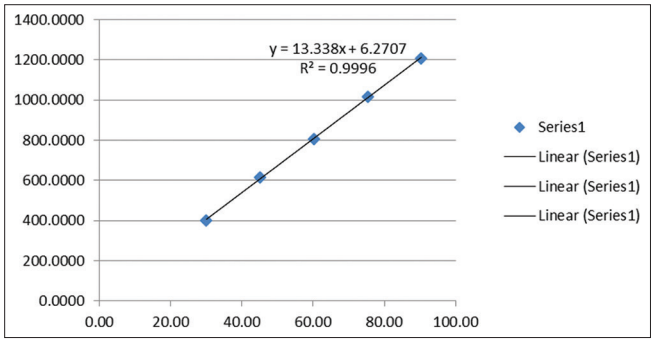

Fig. 3: Standard calibration curve for cyamemazine

Table 1: Method development and optimization results

Column used	Mobile phase	Flow rate	Wavelength	Observation	Result
Hypersil BDS C18 (250 mm × 4.6 mm, Particle size: 5 µm)	Methanol: water (80:20)	1.0 ml/min	270 nm	Poor resolution	Method rejected
Hypersil BDS C18 (250 mm × 4.6 mm, Particle size: 5 µm)	Methanol: water (75:25)	1.0 ml/min	270 nm	Poor resolution	Method rejected
Hypersil BDS C18 (250 mm × 4.6 mm, Particle size: 5 µm)	Methanol: buffer (80:20)	1.0 ml/min	270 nm	Good resolution	Method accepted

Table 2: Result of system suitability for cyamemazine

S. No.	Peak Area	Retention time	Tailing factor	Theoretical plate
1	805.3428	4.43	1.32	4252
2	798.9524	4.42	1.31	5021
3	810.8641	4.40	1.34	5921
Mean	805.0531	4.42	-------	-------
SD	5.9611	0.0153	-------	-------
RSD	0.74	0.35	-------	-------

Table 3: Standard linearity calibration curves of cyamemazine

Conc. (ppm or μg/ml)	Area
30.10	401.9248
45.15	615.4218
60.20	807.0649
75.25	1016.8143
90.3	1204.9289
Correlation coefficient	0.9996
Intercept	6.2707
Slope	13.338

Table 4: System precision data from the standard solution of the proposed HPLC method

S. No.	RT	Peak area	Theoretical plate	Tailing factor
1	4.42	798.95	7027	1.33
2	4.43	806.82	7059	1.31
3	4.39	811.90	7019	1.34
4	4.40	810.86	7048	1.30
5	4.38	812.38	7129	1.32
6	4.40	821.52	7112	1.29

Table 5: Method precision data from the sample solution of the proposed HPLC method

S. No.	RT	Peak area	Tailing factor	Theoretical plate	% assay
1	4.43	792.64	1.29	7055	98.51
2	4.42	802.41	1.33	7027	99.59
3	4.41	799.46	1.34	7105	99.57
4	4.39	794.94	1.30	7049	99.93

Table 6: Recovery study of cyamemazine

Recovery level	Area	Amount added, μg/ml	Amount found, μg/ml	Amount recovered, μg/ml	% recovery
Accuracy. 80%	1454.5264	48.0000	107.6037	47.6037	99.17
Accuracy. 80%	1442.5754	48.0000	106.7196	46.7196	99.33
Accuracy. 80%	1459.4289	48.0000	107.9664	47.9664	99.93
Accuracy. 100%	1620.5671	60.0000	119.8871	59.8871	99.81
Accuracy. 100%	1607.2187	60.0000	118.8996	58.8996	98.17
Accuracy. 100%	1631.0067	60.0000	120.6594	60.6594	101.10
Accuracy. 120%	1787.2657	72.0000	132.2193	72.2193	100.30
Accuracy. 120%	1791.9515	72.0000	132.5659	72.5659	100.79
Accuracy. 120%	1766.0893	72.0000	130.6527	70.6527	98.13

the method's robustness. The flow rate was 2 nm, 1 ml/min, and the wavelength changes were 2 nm, 1 ml/min, respectively. The method's robustness was evaluated by calculating % RSD values, as shown in Table 8.

LOD and LOQ

The slope and standard deviation were used to calculate LOD and LOQ. The LOD and LOQ, respectively, are the lowest amounts of analyte in a sample that can be detected but not necessarily quantified, and the LOQ is the lowest amount of analyte in a sample that can be quantitatively determined with sufficient precision. For CYMT, the LOD and LOQ were determined to be 0.27 and 0.80 g, respectively.

Solution stability

The percent of recovery ranged from 98.0 and 102.0%, with an RSD of <2.0%, indicating that the sample and standard solutions were stable for 24 h under both conditions. The percent RSD was <2.0%, and the percentage of recovery was within acceptable norms. The theoretical plate number and tailing parameters were also found to be within acceptable limits.

Force degradation study

Table 9 shows the % degradation and % area after degradation results.

DISCUSSION

CYM was eluted at 4.38 min in this method. Under ambient temperature, the current technique was developed employing methanol and buffer (80:20) v/v at a flow rate of 1.0 ml/min. The pH was adjusted with OPA to 4.5 using a 0.01M NaH_2PO_4 buffer. The new approach was more sensitive and cost effective. The idiosyncrasy between active pharmaceutical components and any degradation products formed under given circumstances is due to the stability indicating RP-HPLC. The proposed approach proved to be effective in separating the chemical from its degradation.

Table 7: Statistical validation of recovery study

Recovery at	Mean	SD	%RSD
80%	98.81	1.34	1.35
100%	99.69	1.47	1.47
120%	99.74	1.42	1.42

Table 8: Robustness data of the proposed HPLC method

Parameters	Chromatographic conditions	% RSD of STD drug area	% RSD assay
Wavelength	268 nm	1.36	0.79
	272 nm	1.60	0.68
Mobile phase composition	Methanol: buffer (85:15)	0.97	0.49
	Methanol: buffer (75:25)	0.83	0.70
Flow rate	0.9 ml/min	0.93	0.29
	1.1 ml/min	0.45	0.51

Table 9: % degradation and % area after degradation results

Stress condition	Min	% area cyamemazine tartrate observed after degradation	% of degradation
Acidic	30 min	96.65	3.35
Basic	30 min	84.06	15.94
Peroxide	12 h	84.89	15.11
Thermal	1 h	99.30	0.7
Photolytic	1 h	99.76	0.24

Table 10: The proposed method's assay results

Parameters	Results
Standard area	805.3428 798.9524 810.8641
Mean area	805.0531
Amount found	24.87 mg
% Assay	99.45%

Analysis of a commercial sample

The proposed approach may be used to analyze CYM in a 25 mg marketed tablet dosage form. The amount detected was up to 24.87 mg, and the percent assay findings were confirmed to be 99.45% accurate. Table 10 is a summary of the work.

CONCLUSION

This study developed and validated a speedy, simple, precise, exact, and linear stability-indicating HPLC approach for CYMT, which can now be utilized for routine quality control analysis. The analytical method parameters and mobile phase solvents for CYMT have good resolution. Furthermore, the suggested method has a short run time and a retention time of around 4.38 min. The process was verified in accordance with ICH guidelines. The method is robust enough to duplicate precise and exact results under a variety of chromatographic conditions.

AUTHORS' CONTRIBUTIONS

All the authors have contributed to the study design. SJ has performed the HPLC method development and validated study under the guidance of PK. SJ and PK have drafted the manuscript as per the journal submission format. All the read and approved the final manuscript. The authors declare that they have no competing interests.

CONFLICTS OF INTEREST

The author declared no conflicts of interest.

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