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Research Article

Development and Validation of HPTLC Method for Estimation of Cyamemazine Tartrate and its Formulation

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ABSTRACT
A simple, precise, accurate high performance thin layer chromatographic (HPTLC) method has been developed for estimation of cyamemazine tartrate from a bulk drug and combined dosage form. The separation of drugs was carried out on Merck HPTLC aluminium sheets of Silica gel 60 F_{254} TLC plates as stationary phase and the chromatogram was developed using Benzene: Methanol (4.2:0.8v/v) as the mobile phase. Cyamemazine tartrate showed R_f values 0.45, when scanned densitometrically at 260 nm using Camag TLC Scanner. The described method was linear over a concentration range of 200 ng/spot to 1200 ng/spot for cyamemazine tartrate. Results of analysis were validated according to International Conference on Harmonization ICH Q2B guidelines statistically, and by recovery studies. The limit of detection (LOD) and limit of quantification (LOQ) for cyamemazine tartrate was found to be 28.47 ng/spot 86.29 ng/spot respectively. The results of the study showed that the proposed HPTLC method is simple, rapid, precise and accurate, which is useful for the routine determination of cyamemazine tartrate bulk drug and in its pharmaceutical dosage form.

Keywords*:* HPTLC, Cyamemazine tartarate, ICH, Validation, LOD and LOQ.

INTRODUCTION

Cyamemazine Tartrate (CYMT) (Fig. 1) is 10-(3-dimethylamino-2-methyl-propyl)- Phenothiazine-2-carbonitrile tartrate. CYMT is a phenothiazine derivative, used for the treatment of schizophrenia and, especially, for psychosis associated anxiety, due to its unique anxiolytic efficacy[1]. Cyamemazine (CYM), also known as cyamepromazine, is a typical antipsychotic drug of the phenothiazine class used primarily in the treatment of schizophrenia and psychosis-associated anxiety. Cyamemazine actually behaves like an atypical antipsychotic, due to its potent anxiolytic effects (5-HT2C) and lack of extrapyramidal side effects (5-HT2A)[2]. Cyamemazine has been given orally as the base or the tartrate and by injection as the base[1].

Very few methods has been developed for the analysis of CYM by LC-MS/MS tandem technology for the identification of CYM and its metabolites during characterization of human cytochrome P450 enzymes involved in the metabolism of CYM[3], UV-spectrophotometric method[4] for CYMT determination while there was not a single method published in the literature for the analysis of CYMT in bulk as well as in pharmaceutical formulations. Even though there is no simple HPLC, HPTLC methods for the routine determination of CYM.

Considering the need of analytical research appropriate to the CYMT, HPTLC method will help towards determination of drug in bulk and pharmaceutical formulation. Hence present investigation was undertaken to develop, and validate a simple, rapid, accurate, precise and specific method for the determination of CYMT[5]. The developed method was applied effectively for determination of drug in bulk and in-house tablet formulation.

MATERIALS AND METHODS

CYMT was procured as a generous gift sample. As the marketed formulation was unavailable in India, the in-house tablets have been prepared for the analysis of drug from pharmaceutical formulation and to justify the applicability sensitivity of the method for future analysis. The excipients used for preparation of in-house tablets were microcrystalline cellulose (MCC), and magnesium stearate[6]: were purchased from Sigma Aldrich, Mumbai. Silica gel 60 F_{254} TLC plates (10cm x 10cm, layer thickness 0.2 mm, E-Merck, Darmstadt, Germany) were purchased from Merck Ltd India, Mumbai. All the chemicals used were of HPLC grade (Merck laboratories)

Instrumentation

The HPTLC system **(**CAMAG Muttenz, Switzerland), consisted of CAMAG Linomat V applicator connected to nitrogen cylinder, CAMAG Hamilton Micro syringe (100µL), and CAMAG Twin Trough Chambers. Thin layer chromatography was performed on aluminum backed silica gel 60 F_{254} TLC plates, (10 cm × 10 cm, layer thickness 0.2 mm, E-Merck, Darmstadt, Germany). The plates were prewashed by methanol and activated at 110°C for 10 min prior to use for chromatography. The space between two bands was maintained at 6 mm. The slit

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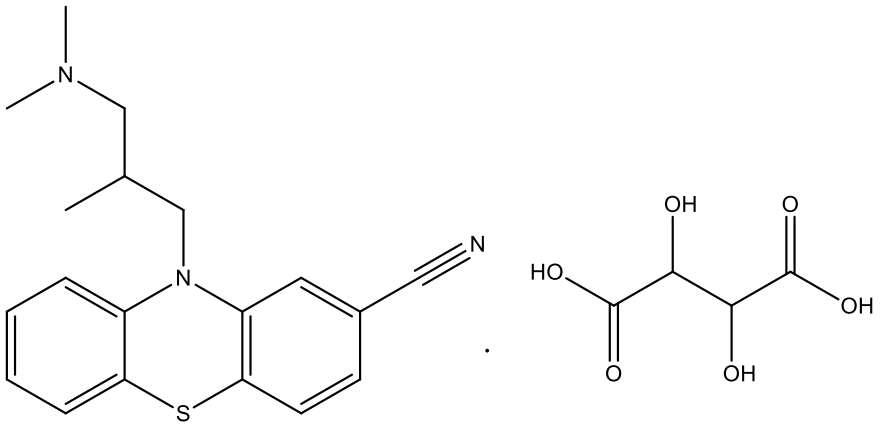

Figure 1: Chemical Structure of Cyamemazine tartarate.

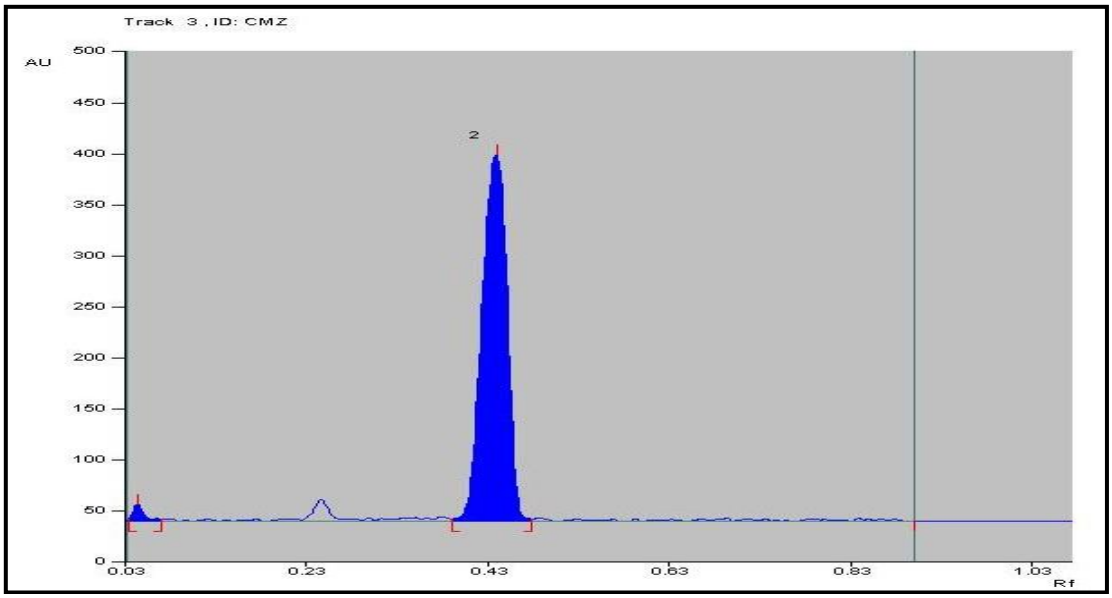

Figure 2: HPTLC chromatogram of standard CYMT.

Table 1: Selection of mobile phase.

Sr. no.	Solvents	Proportion (mL)	R_f of CYMT
1	Benzene: Methanol	4:1	0.68
2	Benzene: Methanol	3:2	Does not run
3	Benzene : methanol	4.2:0.8	0.45

Table 2: Linearity study of CYMT.

Sr. No.	Concentration [ng/spot]	Mean Peak Area ± SD [n = 5]	% RSD
1	200	3442.7 ±4.62	0.134087
2	400	5576.6 ± 3.15	0.056618
3	600	7663.2 ± 1.31	0.013895
4	800	9485.4 ± 3.35	0.030119
5	1000	11129.2 ± 2.03	0.015562
6	1200	13073.98 ± 1.81	0.013491

dimension was kept at 6×0.45mm. The mobile phase consisted of Benzene: Methanol (4.2: 0.8 *v/v*). Linear ascending development was carried out in 20×10cm twin trough glass chamber. The optimized chamber saturation time for mobile phase was 15min. Densitometry scanning was performed on Camag TLC Scanner equipped with winCATS software version 1.3.0 at 260 nm. The source of radiation utilized was deuterium lamp. Evaluation was performed using peak area with linear regression. A typical chromatogram of FFD was shown in Fig. 2.

Table 3a: Results of recovery studies.

Drug	Initial amount [ng]	Amount added [ng]	Amount recovered ± SD [ng] [n = 3]	% Recovered	% RSD
CYMT	400	320	385.20± 8.58	96.3	0.15
	400	400	382.04 ± 7.041	95.51	0.13
	400	480	385.45± 4.09	96.11	0.076

Table 3b: Results of precision studies (Intra day and Inter day).

Drug	Conc. [ng/spot]	Intra day Amount found [ng]		Inter day Amount found [ng]	
		Mean ± SD	% RSD [n = 3]	Mean ± SD	% RSD [n = 3]
CYMT	400	5274.99 ± 14.54	0.22	5345.55 ± 43.8	1.03
	600	7497.36 ± 18.15	0.28	7556.1 ± 62.9	0.83
	800	9451.16 ± 27.12	0.26	9492.2 ± 45.6	0.97

Results of repeatability studies.

Sr. No.	Application volume [µL]	Area of CYMT [600 ng/spot]
1	1.0	7371.4
2	1.0	7409.4
3	1.0	7433.17
4	1.0	7458.71
5	1.0	7475.2
6	1.0	7481.8
Mean ± SD		7437.91
% RSD		0.75

Preparation of standard stock solution and samples

A fresh stock solution of CYMT was prepared in methanol (1000 µg/mL). One microlitre from stock solution was spotted on the TLC plate to obtain a final concentration range of 200–1200ng/spot. The standard curve was replicated six times on different plates.

Method Validation

Validation of the developed HPTLC method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1)(5) for specificity, sensitivity, accuracy, precision, repeatability, and robustness.

Specificity

The specificity of the developed method was established analyzing the sample solutions containing CYMT from tablet formulation in relation to interferences from formulation ingredients. To confirm the specificity of the proposed method CYMT tablet solution was spotted on TLC plate, developed and scanned as described earlier. The spectrum of CYMT extracted from tablet was compared with spectrum of standard CYMT, which showed good correlation.

Sensitivity

In order to determine the limit of detection (LOD) and limit of quantification (LOQ), blank methanol was spotted six times following the same method as explained above. Solutions containing 5–100 ng of CYMT were spotted on TLC plate. The signal to noise ratio was determined. The LOD and LOQ for CYMT were found to be 28.47ng and 86.29ng respectively [Where SD = 54.45, S = 6.3108].

Linearity and calibration curve

Linearity study was performed using working standard of CYMT. Calibration was done by applying standard stock solution ranging from 0.4 – 2.4µL by micro liter syringe with the help of automatic sample applicator Linomat V on TLC Plate which gives concentration of 200–1200ng/spot. The plate was developed and scanned. Calibration curve was constructed by plotting the peak area *vs.* corresponding drug concentration.

Accuracy

Accuracy of the method was evaluated by carrying the recovery study at three levels. It was done by adding three different amount of standard drug CYMT that is 80, 100 and 120 % level to pre analyzed sample (400ng of CYMT) and subjected them to the proposed HPTLC method.

Precision (Intra day and inter day precision)

Intra day precision was determined by analyzing, the three different concentrations 400ng, 600ng and 800ng of CYMT, for three times within the day. Day to day variability was assessed using above mentioned three concentrations and analyzing it for three different days, over a period of one week which shows reproducibility of the method.

Repeatability

Repeatability of measurement of peak height and area were performed by spotting 1.0 µl (600ng of CYMT) of standard drug solution on TLC plate and developing the plate. The separated spot was scanned 6 times without changing the position of the plate.

Robustness

Robustness was studied in six replicate at the concentration level of 1000 ng/spot. In this study, seven parameters (mobile phase composition, mobile phase volume, development distance, relative humidity, duration of saturation, time from spotting to chromatography and chromatography to spotting) were varied and the effects on the results were examined.

Application of the proposed method to Tablets formulation

To determine the content of CYMT in tablets (label claim: 25mg per tablet); twenty tablets were powdered and powder equivalent to 25 mg was weighed. The drug was extracted with methanol. To ensure complete extraction, it was sonicated for 20 min and the volume was made up to 100mL to get a solution of 200 µg/mL. The resulting

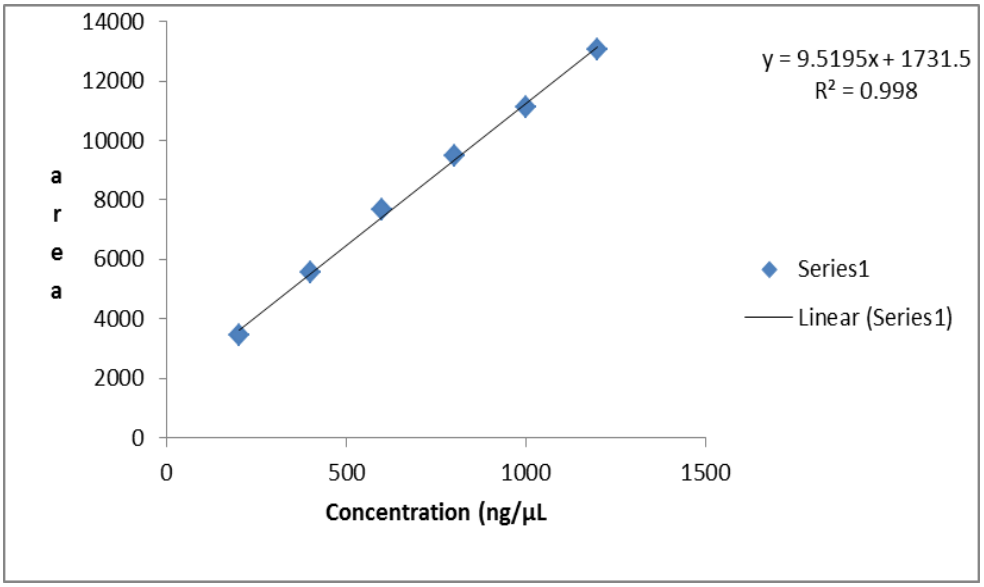

Figure 3: Calibration curve of CYMT.

Table 5: Results of robustness studies.

Parameters	SD of peak area	%RSD
Mobile phase composition		
Benzene:methanol(4.2:0.8)	2.71	0.029
Mobile phase volume		
5.1 mL	9.21	0.17
10.2 mL	6.28	0.09
Development distance		
7.0 cm	13.83	0.29
7.5 cm	18.98	0.23
8.0 cm	8.87	0.15
Relative humidity		
55	19.84	0.56
65	26.7	0.76
Duration of saturation		
10 min	13.6	0.17
15 min	21.9	0.23
20 min	19.4	0.19
Activation of prewashed TLC plates		
8 min	28.14	0.50
10 min	29.64	0.53
12 min	26.83	0.43
Time from spotting to chromatography	35.57	0.54
Time from chromatography to scanning	25.11	0.38

solution was filtered and 5µL (400 ng per spot) was applied on TLC plate followed by development and scanning. The possibility of excipients interference in the analysis was studied.

RESULT AND DISCUSSION

To develop HPTLC method of analysis for CYMT for routine analysis, selection of mobile phase was carried out by using different proportions of mixture of Benzene: Methanol (Table 1). A solvent system that would give dense and compact spots with appropriate and significantly

Table 6: Assay of CYMT tablet.

Drug	Amount of CYMT in formulated tablet	Amount found [mg]	Amount found [%]
CYMT	25	19.82	99.08
	25	19.84	99.19
	25	19.89	99.46
	25	19.97	99.86
	25	19.77	99.83
	Mean ± SD	19.86 ± 0.08	99.48 ± 0.36
	% RSD	0.40	0.36

different *Rf* value for CYMT was desired. Among these proportions, the solvent system comprising of Benzene: Methanol (4.2:0.8v/v) gave peak shape and not tailing of CYMT from its matrix with an *Rf* value of 0.45. It was also observed that chamber saturation time and solvent migration distance are crucial in chromatographic separation as chamber saturation time of less than 15min and solvent migration distances greater than 70mm resulted diffusion of analyte spot. Therefore, Benzene: Methanol solvent system in (4.2:0.8v/v) proportion with chamber saturation time of 15min at 25◦C and solvent migration distance of 80mm was used as mobile phase. These chromatographic conditions produced a well defined compact spot of CYMT with optimum migration at *Rf* = 0.45 (Fig 1). It also gave a good resolution of analyte from excipients used in tablet formulation.

Specificity is the ability of an analytical method to assess unequivocally the analyte in the presence of sample matrix. CYMT was separated from excipients with an *Rf* = 0.45. It was observed that excipients present in formulation did not interfere with peak of CYMT.

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample[5]. Method was found to be linear in a

concentration range of 200–1200 ng/spot (n = 6), with respect to peak area (Table 2). Calibration curve was constructed by plotting the peak area *vs.* corresponding drug concentration. (Fig. 3)

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found[5]. It was determined by the application of analytical procedure to recovery studies, where known amount of standard is spiked in pre-analyzed samples solutions. Results of accuracy studies from excipients were shown in Table 3; recovery values demonstrated the accuracy of the method in the desired range.

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Intraday precision refers to the use of analytical procedure within a laboratory over a short period of time using the same operator with the same equipment whereas Interday precision involves estimation of variations in analysis when a method is used within a laboratory on different days, by different analysts. The results obtained are shown in Table 4. In all instances, %RSD values were less than 5% confirming the precision of the method.

Repeatability of measurement of peak height and area were performed by spotting 1.0 µl (600ng of CYMT) of standard drug solution on TLC plate and developing the plate. The separated spot was scanned 6 times without changing the position of the plate. The results are as shown in Table 4.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The low values of % RSD after introducing seven parameters in the developed HPTLC method confirms robustness of the method (Table 5). The developed assay method was applied for the estimation of CYMT in-house tablet. The drug content shown in Table 6 confirms that method was successfully applied to the formulation.

CONCLUSION

A new HPTLC method has been developed for the identification and quantification of CYMT. Low cost, faster speed, and satisfactory precision and accuracy are the main features of this method. Method was successfully validated as per ICH guidelines and statistical analysis proves that method is sensitive, specific, and repeatable. It can be conveniently employed for routine quality control analysis of CYMT as bulk drug in in-house tablets, without any interference from excipients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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