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Development and Validation of Novel UV-Spectrophotometric method for estimation of Cyamemazine Tartrate for simple, rapid and cost effective analysis

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Abstract

Simple, novel, rapid and cost effective UV-spectrophotometric method has been developed and validated for estimation of CYMT in bulk and tablet formulation. CYMT is a Phenothiazine derivative from the class of typical antipsychotic and a new drug for the treatment of Schizophrenia. As no analytical method was seen in literature for determination of CYMT; we report here, development and validation of simple, novel UV-spectrophotometric method for analysis of CYMT. Drug was found to be soluble in water and was stable for more than 72 h in same solvent when tested for bench top stability; being ideal solvent, spectrophotometric studies were carried out using distilled water and detection as well as quantitation was performed at wavelength maximum of 267.21 nm. Linearity curve for developed method was generated by measuring absorbance at specified wavelength maximum and plotting it against concentration. The method followed linearity in range of 2 - 12 µg/mL with a correlation coefficient of 0.999. The mean recovery of 98.97 reflects accuracy for developed method and the method precision was found to be well within acceptable limits. The developed method was tested and validated for various parameters as per USP requirements and recent ICH guidelines (addendum 2005). The present UV-spectrophotometric estimation for CYMT was proved to be statistically accurate, precise, and sensitive. The applicability of method can be extended towards rapid routine determination of drug in bulk and pharmaceutical formulations and it has the unique importance of being first and simple UV-Spectrophotometric analytical method for the determination of CYMT in bulk and in pharmaceutical formulation

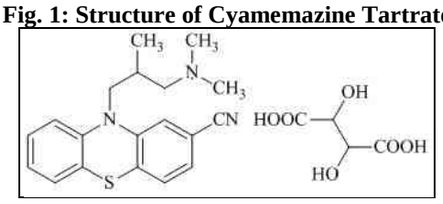
Keywords: Cyamemazine Tartrate, UV-Spectrophotometry, Method Development, Method Validation

1.Introduction

Cyamemazine Tartrate (CYMT) (**Fig. 1**) is 10-(3-dimethylamino-2-methyl-propyl)-Phenothiazine-2-carbonitrile (Cyamemazine)[1] and its tartrate (CYMT) belonging to class of typical antipsychotics and a new drug for the treatment of Schizophrenia[2]. CYM being a Phenothiazine derivative was introduced initially in clinical practice as an antipsychotic agent due to its Dopamine D_2 receptor antagonistic activity, however ample clinical experience indicated that drug was also usefull for the treatment of anxiety; Cyamemazine is a neuroleptic compound which possesses anxiolytic properties in humans[3, 4]. A previous binding study has shown that cyamemazine possesses high affinity for 5-HT_3- and 5-HT_2C-receptor types[5]. The scifinder search was performed for the literature survey of Cyamemazine (CYM) as well as CYMT. Literature survey of CYM revealed very few methods for the analysis; viz…an LC-MS/MS tandem methodology was developed specifically for the identification of CYM and its metabolites during characterization of human cytochrome P450 enzymes involved in the metabolism of CYM[6]. Bioanalytical methods were found with application GC-MS tandem mass spectrometry; as simultaneous determination for seven antipsychotic drugs[7] and quantification in 11 biological fluids and tissues assisted with automated solid phase extraxtion[8], while there was not a single method published in literature for the analysis of CYMT in bulk as well as in pharmaceutical formulations. Even there is scarcity of simple and stability indicating HPLC, HPTLC and UV-spectrophotometric methods for the routine determination of CYM and hence for CYMT too.

Considering the need of analytical investigations pertaining to the CYM and CYMT, simple methods such as estimation by UV-Spectrophotometry will assist towards rapid determination of drug in bulk and pharmaceutical formulation. The objective of the present investigation was to establish simple, Zero order UV-spectrophotometric method for estimation of CYMT. The developed method was applied effectively for determination of drug in bulk and in-house tablet formulation. Method was validated for accuracy, precision, sensitivity and ruggedness as per ICH guidelines[9].

Fig. 1: Structure of Cyamemazine Tartrate



2. Experimental

2.1 Materials and Equipments

Cyamemazine Tartrate was supplied as generous gift sample. Water used for spectrophotometry was distilled water and used throughout the experimental work described. 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: were purchased from Sigma Aldrich, Mumbai.

Spectrophotometric studies were performed using UV-visible spectrophotometer (2450, Shimadzu), UV Probe 2.21, a pair of 10 mm matched quartz cells and spectral bandwidth 1 nm were employed for all samples. Solvents were investigated to develop UV-spectrophotometric method for the estimation of CYMT in in-house tablet formulations. Criteria taken into consideration while selecting solvent for present spectrophotometric analysis were solubility of Cyamemazine Tartrate in solvent systems, stability in different solvents or solvent compositions, steps involved in sample preparation, sensitivity, simplicity, cost effective analysis and applicability of method towards rapid estimation of drug in bulk and pharmaceutical formulations. Absorbance of CYMT in the selected solvent at wavelength maximum was determined. All the criteria listed above are fulfilled by water being universal solvent. As the drug was found to be soluble in water, method development

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and validation of present UV-spectrophotometric method was assisted with distilled water as solvent.

2.2 Physical Properties and Characteristics ad uses of Cyamemazine tartrate

-Yellowish Colour
-Soluble in Water and Methanol
-Melting Point- 180-184^{0}C
-Bioavailability- 10-70%
-Metabolism-Hepatic
-Half-Life- 10 hrs.
-Excreted in urine.
-Routes - Oral, IM, IV.
-Molecular formula: $C_{23}H_{27}N_3O_6S$
-Used in Schizophrenia and especially for psychosis, anxiety, anxiolytic efficacy.

2.3 Preparation of standard solution and study of linearity curves

Standard solution was prepared by dissolving 10 mg of CYMT in 100 mL of distilled water to obtain concentration of 100 µg/mL. It was further diluted with the same solvent to obtain concentration of 10 µg/mL and scanned in the UV-region of 400 – 200 nm. Drug showed maximum absorption at a λmax of 267.21 nm **Fig 2**. From the standard solution, aliquots in the range of 0.2 to 1.2 mL were transferred into a series of six 10 mL volumetric flasks and volume was made up to the mark to obtain concentration in the range of 2 - 12 µg/ mL. The absorbance obtained was plotted against the concentration to generate linearity curve for the method as depicted in **Fig 3**. The results for the linearity study of CYMT are as represented in **Table 1.**

Table 1: Results of linearity studies for estimation of Cyamemazine Tartrate

Sr. No	Concentration of Cyamemazine Tartrate (µg/ml)	Absorbance Mean ± SD (n = 6)	% RSD
1	2	0.142 ± 0.0008	1.47
2	4	0.245 ± 0.0008	1.71
3	6	0.393 ± 0.0012	1.42
4	8	0.519 ± 0.0014	1.21
5	10	0.642 ± 0.0023	1.74
6	12	0.765 ± 0.0022	1.19

Fig. 2: UV-Spectrum for Estimation of Cyamemazine Tartrate depicting λmax at 267.21 nm

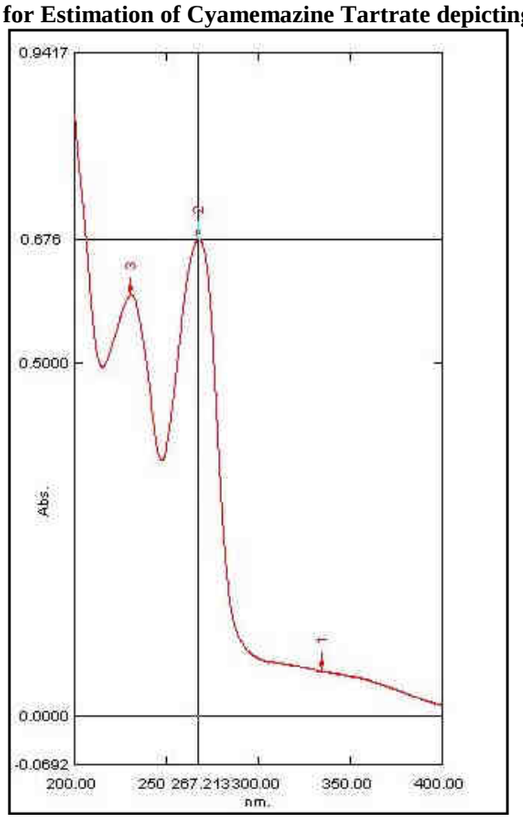
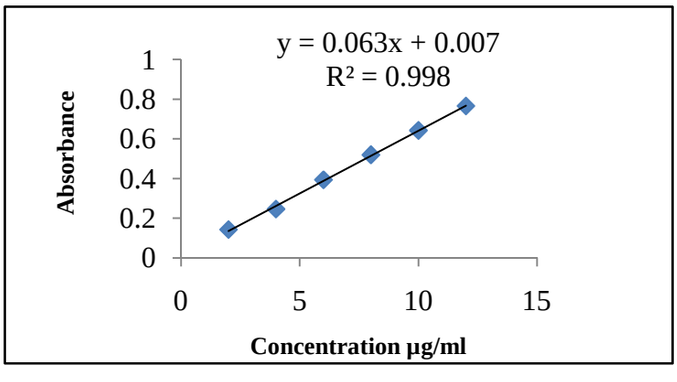


Fig. 3: Calibration Curve for UV-Spectrophotometric estimation of Cyamemazine Tartrate



2.4 Preparation of in-house tablet formulation

In-house tablets, containing 25 mg of CYMT per tablet, were prepared using simple direct compression technique. The excipients used were microcrystalline cellulose (MCC), and magnesium stearate.

2.5 Validation of methods

The developed UV-spectrophotometric method was validated for Precision, Accuracy, Repeatability, Ruggedness, Sensitivity and the validated method was thus applied for the determination of CYMT in bulk and in in-house tablet formulation.

2.6 Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the 6, 8 and 10 µg/mL of Cyamemazine Tartrate solutions for three times in the same day. Inter-day precision was determined by analyzing the 6, 8 and 10 µg/mL of Cyamemazine Tartrate solutions daily for three days, results are reported in **Table 2**.

Table 2: Results from precision studies for estimation of Cyamemazine Tartrate

Drug	Cyamemazine Tartrate	
Concentration [µg mL^{-1}]	**Amount Found [µg mL^{-1}] [n= 9] ± SD**	**RSD [%]**
Intra-day Precision		
6	5.89 ± 0.0064	1.65
8	8.06 ± 0.0060	1.13
10	9.84 ± 0.0061	1.74
Inter-day Precision		
6	5.79 ± 0.0062	1.58
8	8.03 ± 0.0076	1.18
10	9.74 ± 0.0060	0.67

2.7 Recovery experiments

Accuracy of the methods was studied at three different levels i.e. 80, 100 and 120 % levels. To the pre-analyzed sample solution (6 µg/mL of CYMT) a known amount of standard drug was added and it was then reanalyzed by the proposed methods. The results for the recovery studies are as depicted in **Table 3.**

Table 3: Results from Recovery Studies for estimation of Cyamemazine Tartrate

Pre-analysed sample solution [µg/mL]	Excess drug added [µg/mL] (%)	Amount recovered [µg/mL]	% Recovery (n = 3)	% RSD
4	3.2 (80)	3.92	99.21	1.54
	4.0 (100)	3.89	99.47	1.67
	4.8 (120)	3.84	98.25	1.72

2.8 Repeatability

Repeatability was determined by analyzing 6 µg/mL concentration of Cyamemazine Tartrate solution for six times.

2.9 Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous solution by two analysts using same operational and environmental conditions and the results are reported in **Table 6**. Proposed method was evaluated for ruggedness by analyzing fixed concentration of 6 µg/mL of sample solution; by two different analysts keeping operational and environmental conditions identical and the results are reported in terms of % RSD.

2.10 Sensitivity

The sensitivity of the method was determined as Limit of Detection (LOD) and Limit of Quantification (LOQ). To determine the limits of detection and quantification, concentrations at the lower end of the linear range of the calibration plot were analyzed. The LOD and LOQ were calculated with application of equations; LOD = 3.3 × *N/B* and LOQ = 10 × *N/B;* where, 'N' is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and '*B*' is the slope of the corresponding calibration plot.

2.11 Application of Developed UV-spectrophotometric Method for Estimation of Cyamemazine Tartrate in Bulk and in in-house tablet formulation

2.11.1 Determination of CYMT in Bulk: Accurately weighed 10 mg of CYMT was transferred to 100 ml volumetric flasks containing about 25 mL of distilled water, sonicated for 5 min and volume was made upto the mark using same solvent to obtain desired concentration 100 µg/ml. Aliquots of 6 µg/mL were prepared and scanned on spectrophotometer in the UV range and absorbance was measured at 267.21 nm. The concentrations of the drug were calculated from linear regression equations; results are shown in **Table 4.**

Table 4: Analysis of Cyamemazine Tartrate in Bulk

Concentration [µg/mL]	Amount Found (mg)	Amount found (%)
6	5.93	98.83333
	5.88	98.00001
	5.89	98.16667
	6.01	100.1667
	6.09	101.5
	5.96	99.33333
Mean ± SD	5.96 ± 0.0079	99.3333 ± 1.3249
% R.S.D.	1.33	1.33

2.11.2 Assay of in-house tablet formulation

Twenty in-house tablets were selected randomly, weighed accurately and ground into fine power. An amount of powdered drug equivalent to 10 mg was accurately weighed and transferred into a 100 mL volumetric flask containing 25 mL of Distilled water, sonicated for 15 min and volume was made up to the mark followed by filtration through Whatmann filter paper number 41. From this solution; appropriate volumes of 0.6 mL were diluted to 10 mL using distilled water. The resulting solutions were scanned by using UV-spectrophotometer in the range of 400 - 200 nm. The same spectrum was recorded and absorbance obtained was measured. The amount of drug was estimated by using established linearity curve for the

method; results for the formulation assay are as represented in **Table 5**.

Table 5: Analysis of Cyamemazine Tartrate in Tablet formulation

Concentration [µg/mL]	Amount Found (mg)	Amount found (%)
6	5.88	98.0
	5.91	98.05
	6.10	101.66
	6.11	101.83
	5.89	98.16
	6.03	100.5
Mean ± SD	5.98 ± 0.010	99.777 ± 1.772
% R.S.D.	1.771	1.776

3. Results and Discussion

CYMT showed absorbance maximum at 267.21 nm distilled water and followed linearity in the concentration range of 2 - 12 µg/mL. The developed zero order UV-spectrophotometric method was found to have the linear regression equation as $y = 0.063\ x + 0.0078$ with regression coefficient (r^2) of about 0.999. The intra-day and inter-day precision values (% RSD) were calculated and proved to be in acceptable limit (≤ 2%) for CYMT. Accuracy of was evaluated by percent recovery studies; performed at concentration levels of 80, 100 and 120 % and found to be within acceptable limits (≤ 2%) with mean percent recovery of 98.97 %. The percent amount of CYMT in in-house tablets as estimated by present UV-spectrophotometric method was found to be 99.77 %. The results are as shown in **Table-2**.The results represented that there was no interference from the excipients that generally occurs in tablet formulation. The summary of validation parameters for the UV-spectrophotometric estimation areas depicted in **Table 6.**

Table 6: Summary of validation parameters for UV-Spectrophotometric determination of Cyamemazine Tartrate

Parameter	UV-Spectrophotometry
% Recovery (n= 9)	98.97
% RSD	1.64
Sensitivity	
LOD (µg/mL)	0.2
LOQ (µg/mL)	0.6
Precision [% RSD]	
Intra-day (n=3)	1.13 - 1.74
Inter-day (n=3)	0.67 - 1.58
Repeatability [%RSD] (n=6)	1.50
Ruggedness [± SD]	
Analyst – I (n= 6)	98.05 ± 0.0067
Analyst – II (n= 6)	99.14 ± 0.031

4. Conclusion

The developed zero order UV-spectrophotometric method was found to be rapid, economical and simple for determination of CYMT. Accuracy and precision are found within acceptable range (ICH Guideline Q2 (R1), 2005). Thus developed method can be used for routine analysis of CYMT in bulk and in its tablet formulation effectively.

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