



## Research Article

### DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF CYAMEMAZINE

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#### ABSTRACT

Selective and precise RP-HPLC method was developed for estimation of Cyamemazine in pure form. Water: Acetonitrile: Methanol (60:10:30 %v/v) was used as optimized mobile phase during the method development at 270 nm. Retention time of Cyamemazine was 4.862 min. The developed method was validated according to ICH guidelines in the range of 5µg to 30µg/mL. The linearity of Cyamemazine shows a co-relation coefficient of 0.999. Precision was found to be 1.53. Percentage mean recovery of Cyamemazine was found to be 100.62%. The developed method can be successfully employed for the quality control analysis of Cyamemazine in its pure form.

**Keywords:** Cyamemazine, RP-HPLC, Method Development, Validation, ICH guidelines

#### INTRODUCTION

Cyamemazine is a typical antipsychotic drug and used to treat schizophrenia and especially, for psychosis-associated anxiety. It is Soluble in ethanol, methanol, chloroform, dimethyl sulfoxide and partially soluble in water<sup>1</sup>. One of the Manufacturer of Cyamemazine pure drugs is Toronto Research Chemicals. Literature survey reveals that few methods were reported for estimation of Cyamemazine<sup>2-4</sup>. The present research work was aimed to develop and validate <sup>5</sup> RP-HPLC method for the quantification of Cyamemazine in pure form.

#### MATERIALS AND METHODS

##### Instruments

RP-HPLC instrument of Shimadzu make and LC-20 AD model equipped with a PDA detector was used for the study.

##### Reagents and Chemicals

Cyamemazine pure drug was procured as gift sample from Varun Herbals, Hyderabad, India. Water, Acetonitrile and Methanol used are of HPLC grade.

##### Liquid chromatographic conditions

Mobile phase containing Water: Acetonitrile: Methanol in the ratio of 60:10:30 %v/v at a flow rate of 1mL/min through C18 Phenomenex Luna (250x4.6 mm; 5µ) at 270 nm was used for quantification. Injection volume was 20µL.

##### Preparation of standard stock solution

Accurately weighed 100 mg of standard Cyamemazine was dissolved in 100 mL of mobile phase with proper sonication which gives strength of 1000 mcg/mL.

##### Calibration curve for Cyamemazine

From the standard stock solution Cyamemazine respected aliquots are pipette out into 10mL volumetric flask and dilutions are made with mobile phase to obtain concentration range from 5-30µg/mL

##### Sample Preparation

Drug equivalent to 10 mg of Cyamemazine was weighed and transferred into 10 mL volumetric flask. The drug was dissolved in 10 mL of mobile phase and sonicated for 5mins.

#### RESULTS AND DISCUSSION

RP-HPLC method was developed for Cyamemazine which can be conveniently employed for routine analysis in pure drug form and will eliminate unnecessary tedious sample preparations. Method validation was done according to ICH guidelines Q2 (R1). Optimized chromatographic condition was developed in order to provide a good performance of the assay. The retention times (Rt) of Cyamemazine was 4.862 min. The chromatograms have been shown in Fig. 2. A six point calibration curve was constructed with working standards in the concentration range of 5-30 µg/mL and was found linear ( $r^2 \geq 0.999$ ) for each of the analytes over their calibration range. The slope was calculated using the plot of drug concentration versus area of the chromatogram. The mean % recovery was found to be 100.62% and %RSD was found to be less than 2% for precision studies. The developed HPLC method was very sensitive, precise, accurate and reproducible. All the method validation parameters are well within the limits as specified in the ICH Q 2 (R1) guidelines. The intra- and inter-day precision (%R.S.D.) at different concentration levels was found to be less than 2% (Table 3). Table 5 lists the percent recovery of Cyamemazine by HPLC. The calculated LOQ and LOD concentrations confirmed that the

methods were sufficiently sensitive. The method was specific as no interferences were found with the analytes of interest (Table 1). The assay of Cyamemazine bulk drug was found to be 100.98 %.

**CONCLUSION**

Selective and precise RP-HPLC method was developed for estimation of Cyamemazine in pure form. Retention time of Cyamemazine was 4.86 min which reduces the analysis time. The method underwent various validation parameters and the results were below the acceptance criteria. So the method can be used for estimation of Cyamemazine pure form in routine analysis.

**Table 1: Specificity Data**

S. No	Peak Name	Observation
1	Blank	Nil
2	Placebo	Nil
3	Standard	R <sub>t</sub> :4.862 min   Peak area: 195421

**Table 2: Results of System Suitability**

Parameter	Result	Acceptance Limit
Retention time (R <sub>t</sub> )	4.862 min	--
Resolution factor	NA	--
Number of theoretical plates (N)	3127	More than 2000
Tailing factor (T)	1.52	Less than 2
Number of injections: 6 replicates		

**Table 3: Precision results of Cyamemazine**

S.No.	Intraday precision Area	Inter day precision Area
1	194854	196452
2	195741	196241
3	189124	196141
4	195812	189451
5	195666	196421
6	199214	196241
<b>Mean</b>	195068.5	195157.8
<b>Std Dev</b>	2998.064	2554.458
<b>%RSD</b>	1.5	1.3

**Table 4: Results of calibration curve at 270 nm for Cyamemazine**

S. No	Concentration (µg/mL)	Peak Area
1	5	50125
2	10	102152
3	15	151242
4	20	194785
5	25	241521
6	30	291452

**Table 5: Determination of Accuracy results for Cyamemazine**

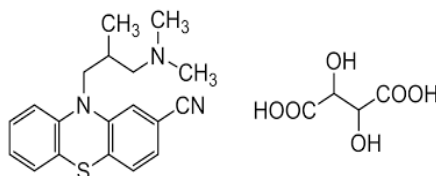
Spiked Concentration (µg/mL)	Peak area	Amount added (µg/mL)	Amount Found (µg/mL)	Recovery	% Mean Recovery
10	102415	10.01	10.14	101.29	100.83
	102411		10.13	101.19	
	102418		10.01	100	
20	194615	20.01	20.02	99.60	100.86
	194785		20.65	102.73	
	194521		20.15	100.24	
30	291459	30.02	30.12	99.73	100.19
	291452		30.51	101.02	
	291411		30.15	99.83	

**Table 6: Results for Detection and Quantitation limits**

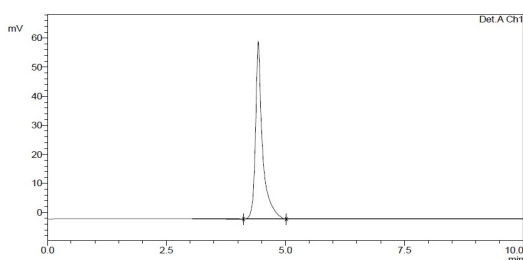
S.NO	Parameter	Slope	Standard Deviation	Value (µg/mL)
1	Limit of Detection	9533	2998	<b>1.03</b>
2	Limit of Quantification			<b>3.14</b>

**Table 7: Change of Flow rate ( $\pm 10\%$ ) and Temperature ( $\pm 5^\circ\text{C}$ )**

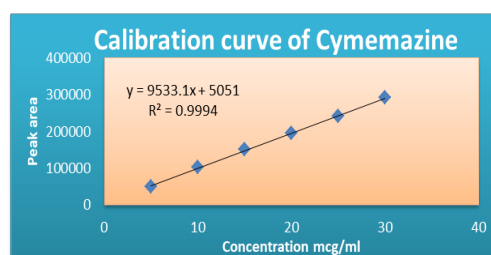
S.No		Flow Rate			Temperature		
		1mL/min	1.1mL/min		30 °C	35 °C	40 °C
2		194758	194521	194100	2310456	471849	463065
3		194521	194587	189412	2264765	463065	471849
4		189541	187451	194124	2354946	458652	478412
5	Mean	192940	192186.3	192545.3	2310055.667	464522	471108.7
6	Std dev	2405.402669	3348.495	2215.623	36817.32735	5485.274	6287.218
7	% RSD	1.24	1.74	1.15	1.59	1.18	1.33



**Fig. 1: Cyamemazine chemical structure**



**Fig.2: Optimized Chromatogram of Cyamemazine**



**Fig. 3: Calibration Curve of Cyamemazine**

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