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Development and Validation of RP-HPLC Method for Ziprasidone Hydrochloride Monohydrate

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ABSTRACT

A new isocratic simple and rapid reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and successively validated for the estimation of ziprasidone hydrochloride monohydrate (ZHM). In this newly developed method chromatographic separation of ZHM was achieved on a Hemochrom-Intertsil C18-5U column (250 × 4.6) mm within a short runtime of 6.5min using mobile phase containing HPLC grade water (pH adjusted to 3.0 with glacial acetic acid AR) and methanol in the ratio of 45:55% v/v. ZHM was estimated with UV detection at 317nm and it was found to be eluted at 4.8min. The above mentioned method was validated as per International Conference on Harmonization (ICH) guidelines with respect to accuracy, precision, linearity, lower limit of detection (LOD) and lower limit of quantitation (LOQ) and robustness. The method was found specific for ZHM and linear (r^2 =0.998) over concentrations ranging from 2 to12µg/ml. The method was found statically accurate (mean recovery = 100.46%), precise with both intra-day and inter-day relative standard deviation (RSD) values < 1.0% and robust. The obtained results concluded that the proposed RP-HPLC method is convenient, reliable and useful in routine analysis for estimation of ZHM in its bulk form and dosage form.

Keywords: Ziprasidone hydrochloride monohydrate (ZHM), RP-HPLC method development and validation, ICH and recovery.

INTRODUCTION

Reversed-phase high performance liquid chromatography (RP-HPLC) is known by various names such as reversed-phase high pressure liquid chromatography and reversed-phase high speed liquid chromatography. RP-HPLC is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in the solvent and includes any chromatographic method that uses a hydrophobic stationary phase. [1] The primary difference between reversed-phase chromatography (RPC) and normal phase chromatography is that the stationary phase in RPC utilizes a non-polar or hydrophobic surface as opposed to a polar (Si-OH) surface used in normal phase chromatography. [2] Reversed-phase chromatography employs a polar (aqueous) mobile phase. As a result, hydrophobic molecules in the polar mobile phase tend to adsorb to the hydrophobic stationary phase, and hydrophilic molecules in the mobile phase will pass through the column and are eluted first. [3]

Ziprasidone HCl Monohydrate (ZHM) is an antipsychotic agent that belongs to a group of benz-isothiazoylpiperazine derivative [4] that is chemically unrelated to phenothiazine or butyrophenone

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antipsychotic agents. [5] ZHM is chemically known as 5-[2-[4-(1, 2-benzothiazol-3-yl) piperazin-1-yl] ethyl]-6-chloro-1, 3-dihydroindol-2-one hydrochloride (shown in Figure 1). ZHM is approved by the Food and Drug Administration as a typical second-generation antipsychotic drug [6] used in schizophrenia, mixed states associated with bipolar disorder and acute agitation. [7] ZHM's antipsychotic activity is likely due to a combined antagonistic function at D_2 receptors in the mesolimbic pathways and at $5HT_2A$ receptors in the frontal cortex. [8] ZHM is available in the market as capsules of different strengths.

Therapeutic category: Schizophrenia and acute mania

The objective of this work was to develop an economic and safe analytical method and validate the same as per the recommendations of ICH guidelines of analytical method validation.

Rationale behind the present investigation is that an economic solvent i.e. water is used for development of the method. Water has following advantages as compared to buffer during Method development and Validation:

- Water is universally available
- Water is economical
- Water prevents precipitation during long term use which is a common disadvantage seen with buffers.
- With water back pressure can be prevented
- Time taken for preparation of buffers will be saved
- Increase in life span of column

The main objective of the work is to introduce with an economical and time saving RP-HPLC method for estimation of ziprasidone from its bulk as well as from its marketed formulation. During literature survey we found the following RP-HPLC methods reported in analytical scientific citations given in Table 1. From Table 1 we can conclude that everywhere buffers are used which can be replaced with HPLC grade water and sharp peak can thus be obtained at flow rate 1ml/min and at short elution time of 4.8min.

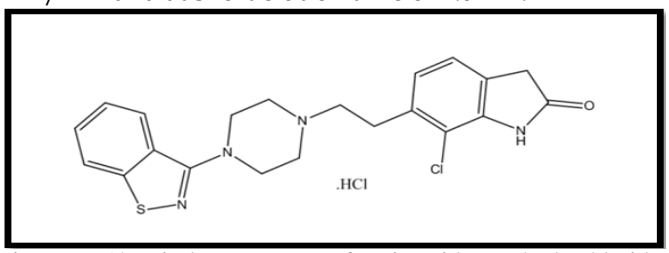

Fig. 1: Chemical structure of Ziprasidone hydrochloride Monohydrate (ZHM); Molecular formula: $C_{21}H_{21}ClN_4OS.HCl.H_2O$.; Molecular weight: 412.936

MATERIALS AND METHODS

Experimental work is generally presented in two sections, namely analytical method development and analytical method validation.

Analytical Method Development

Materials and Reagents

Ziprasidone hydrochloride Monohydrate (ZHM) was obtained as generous gift sample from Wockhardt Limited, Mumbai. Fixed dose tablets (Brand Name: Zipsydon) containing 20 mg ZMH were procured from SUN Pharma. HPLC grade methanol, HPLC grade glacial acetic acid was purchased from SD Fine Chemicals, Mumbai.

Instrument

Quantitative HPLC was performed on Agilent 1200 series with Auto Sampler equipped with variable wavelength detector (UV detector). The chromatograms were recorded using EZChrom software.

Selection of wavelength detection

The multiple spectra scan of 10µg/mL of the ZHM were recorded on UV- visible spectrophotometer in the UV range of 200-700nm. From the UV spectrum, wavelength showing maximum absorbance was selected for detection of HPLC method.

Stationary phase

Hemochrom C18-5U reverse phase column (250 × 4.6) mm dimension was used as stationary phase.

Selection of the mobile phase

Selection of appropriate mobile phase is major step in HPLC method development. Mobile phase selection and optimization was done on "trial and error" basis using literature search. 10µg/mL (10 ppm) solution of ZHM was used to study chromatographic behaviour. Chromatographic behaviour of ZHM was studied under various set of chromatographic conditions. Different mobile phases were made by changing mobile phase composition and flow rate. Run time for each mobile phase composition was set as per retention time of analyte obtained for a specific condition. The best suited mobile phase in terms of resolution, shape of chromatographic peak, run time and cost effectiveness was selected for estimation of ZHM.

Optimisation of Chromatographic Conditions

Selection of appropriate mobile phase is major step in HPLC method development. Mobile phase selection and optimization was done on "trial and error" basis using literature survey. Different mobile phases were made by changing mobile phase composition and flow rate. Run time for each mobile phase composition was set as per retention time of analyte obtained for a specific condition. The best suited mobile phase in terms of resolution, shape of chromatographic peak, run time and cost effectiveness was selected for estimation of ZHM.

Preparation of Standard Stock solution of ZHM

About 25 mg of ZHM was accurately weighed and transferred into 25 ml of volumetric flask then 10 ml of HPLC grade methanol was added and kept for sonication for 5 min to solubilise the drug properly. After 5 min a milky solution was formed and at that time volume was made up to 25 ml with HPLC grade methanol to form final standard concentration of 1000µg/ml of stock solution and again the flask was sonicated for 5 min.

Table 1: Summary including literature review on method development using different mobile phases with its ratio and run time for ZHM

S. No.	Title	Mobile phase	Column Used	Ratio	Run Time (min)	Ref. Number
1.	Development and validation of a rapid RP-HPLC method for the estimation of Ziprasidone hydrochloride monohydrate in bulk and its capsule dosage forms	Phosphate buffer (pH 3.0 adjusted with orthophosphoric acid) and methanol	C18 column (150 × 4.6 mm, 3µm)	60:40% v/v	2.750	[4]
2.	Development and validation of a rapid RP-HPLC method for the estimation of Ziprasidone hydrochloride monohydrate in drug substance and its dosage forms	Sodium phosphate monohydrate buffer (pH 6.0 adjusted with orthophosphoric acid) and Acetonitrile	Sunsil C18 column (150 × 4.6 mm, 5µm)	40:60% v/v	2.50	[5]
3.	A new simple and rapid validated RP-HPLC method for determination of Ziprasidone in Ziprasidone capsules	Buffer (pH =3.0 adjusted with orthophosphoric acid) and methanol	Zorbax SB C-8 (50 × 4.6 mm, 3.5µm)	45:55% v/v	2	[6]
4.	Method development and validation for the estimation of Ziprasidone hydrochloride in pellets by RP-HPLC	Methanol: 0.05%v/v ortho-phosphoric acid in water	Inertsil ODS C18 column (150 × 4.6 mm, 5µm)	90:10% v/v	3	[7]
5.	RP-HPLC method for the estimation of Ziprasidone	20 mM ammonium acetate buffer (pH adjusted to 3.0 with orthophosphoric acid) and methanol	Lichrospher RP-18 column (250 × 4.0 mm, 5µm)	30:70% v/v	4.76	[8]
6.	Development and Validation of an HPLC Method for Determination of Ziprasidone and Its Impurities in Pharmaceutical Dosage Forms	0.05 M buffer solution, adjusted to pH 2.5 with orthophosphoric acid) and acetonitrile	Waters Spherisorb® ODS (5.0µm particle size, 250 × 4.6 mm)	80:20% v/v	7.9	[9]
7.	Development and Validation of Stability Indicating RP-HPLC method for the estimation of Ziprasidone in Capsule Dosage Form	0.05 M KH_2PO_4 (pH-3) buffer, Methanol and Triethanolamine	Hypercil C_8 column (150 × 4.6mm, 5.0 µm)	70:30:0.1% v/v/v	6.204	[10]

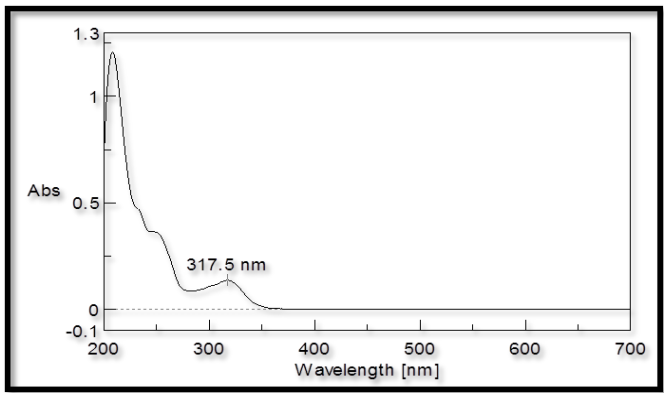

Fig. 2: UV Spectrum of 10µg/mL ZHM in methanol solution

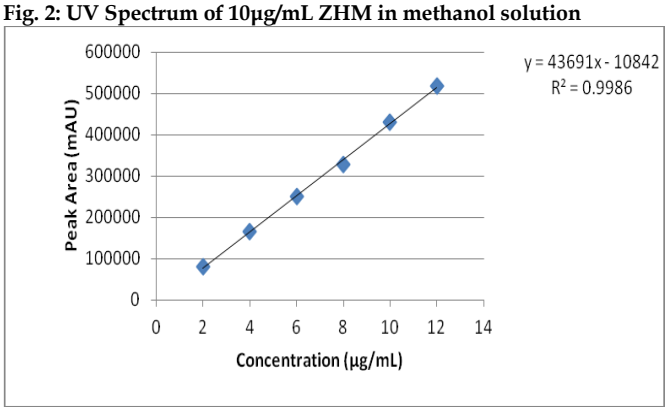

Fig. 3: Calibration Curve Indicating Linearity for ZHM

Preparation of Working Standard solutions of ZHM

For the preparation of working Standard solutions of ZHM suitable aliquots of drug solution from standard stock solution were pipetted and transferred into 10 ml volumetric flask and volume was made up with mobile phase to get concentrations in the range of 2 to 12µg/ml respectively.

Preparation of Sample solutions

For the preparation of sample solutions, ten capsules were weighed, opened and the powder was collected and mixed. A quantity equivalent to 20 mg of ZHM was transferred into 10ml volumetric flask, to this flask 5ml of mobile phase was added and the mixture was subjected to sonication for 20 min for complete extraction of drug. After 20 min. the solution was filtered into 10ml of volume flask through 0.45µ membrane filter before injection. The volume was then made up to the mark with mobile phase. Further, it was diluted with methanol to get the concentration of 10µg/ml concentration of ZHM.

Analytical Method Validation

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose as stated in ICH guidelines Q2 (R1) on validation of analytical procedures: text and methodology. Parameters to be considered during validation of the developed method as per ICH guidelines are as given in Table 2.

RESULTS AND DISCUSSION

Analytical Method Development

Selection of wavelength detection

UV spectra of ZHM showed maximum absorbance at a particular wavelength of 317.5nm at concentration of 10µg/mL as shown in Figure 2.

Optimization of Chromatographic Conditions

Mobile phase trials for optimisation of chromatographic conditions for ZHM are given in Table 3.

Table 2: Summary of analytical method validation parameters with its method to be followed according to ICH guideline Q2 (R1) [11, 12, 13, 14]

S. No.	Parameter	Definition	Method / Procedure to be followed
1.	Specificity	Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.	To determine specificity chromatograms were obtained for blank and ZHM
2.	Linearity	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.	A linear relationship was evaluated across the range of 2 to 12µg/mL for ZHM. It was obtained by plotting peak area against concentration of standard and finding regression coefficient (r^2).
3.	Accuracy	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.	Accuracy was assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g. 3 replicates each of 3 concentrations each of the total analytical procedure). In the present work percent recovery was calculated by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% by adding known amount of standard solution of DMP. These samples were then analysed and the results obtained were compared with expected results.
4.	Precision	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. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.	Precision is reported as standard deviation and relative standard deviation (Coefficient of variation) for each type of precision investigated. (Acceptance Criteria -% RSD of low, mid and high should be less than 2%)
5.	Repeatability	Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.	Repeatability was assessed by using minimum of 9 determinations covering the specified range for the procedure (e.g. 3 replicates each of 3 different concentrations - low, mid and high i.e. 4, 8 and 12µg/mL)
6.	Intermediate Precision	Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.	Intermediate Precision was established to study the effects of random events i.e. days, on the precision of the analytical procedure. Intra-day and inter-day precision studies were performed by taking 9 determinations of 3 concentrations low, mid and high i.e. 4, 8 and 12µg/mL 3 replicates each, at 3 times in a same day and on 3 different days, respectively.
7.	Limit of Detection (LOD) Limit of Quantification (LOQ)	The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.	The values of Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined based on the standard deviation of the response and the slope of calibration graph. The quantitation was done with the help of following expression, **LOD**=3.3×σ/S **LOQ**=10×σ/S σ = Standard deviation of response estimated based on the calibration curve. S = Slope of the calibration curve.
8.	Robustness	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.	Robustness was evaluated for proving the reliability of an analytical method with respect to deliberate variations in method parameters. To establish robustness of analytical method following factors were studied □ Influence of variations of pH in a mobile phase □ Influence of variations in mobile phase composition □ Temperature □ Flow rate

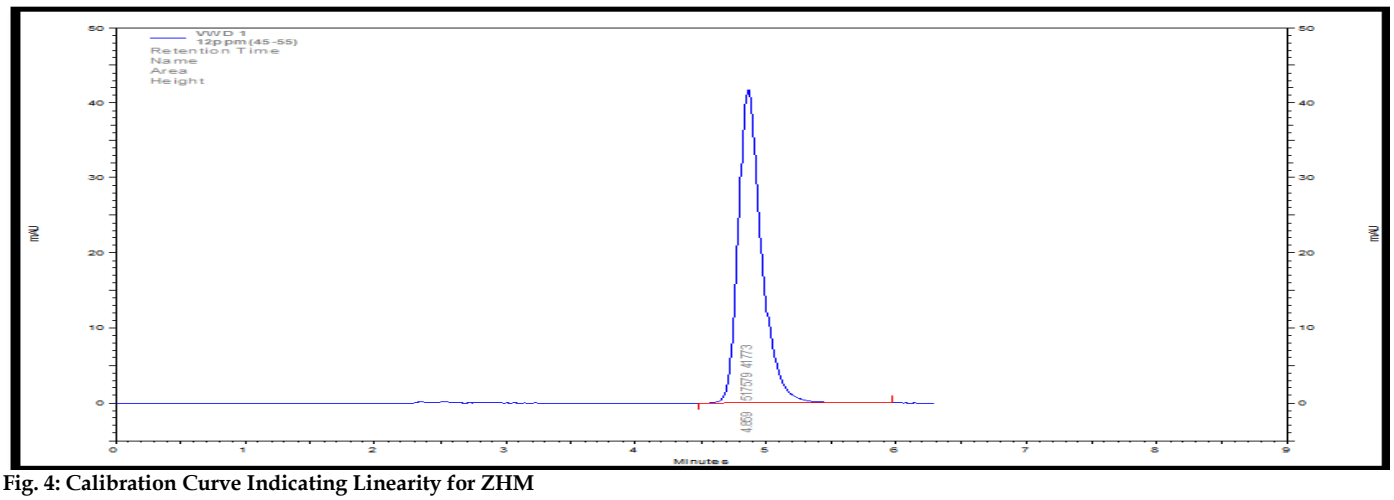

Fig. 4: Calibration Curve Indicating Linearity for ZHM

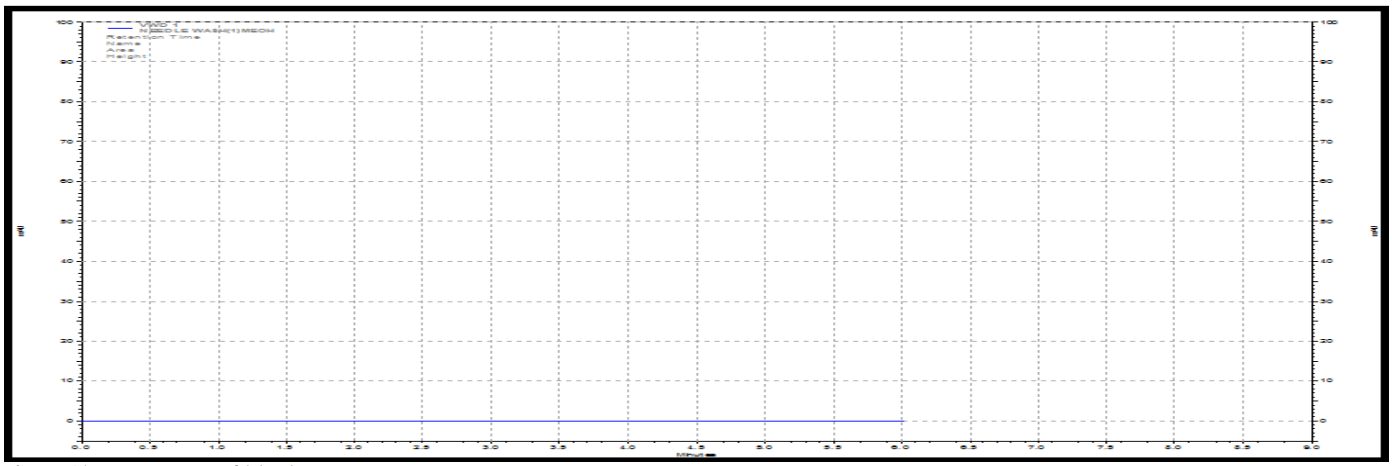

Fig. 5: Chromatogram of blank run

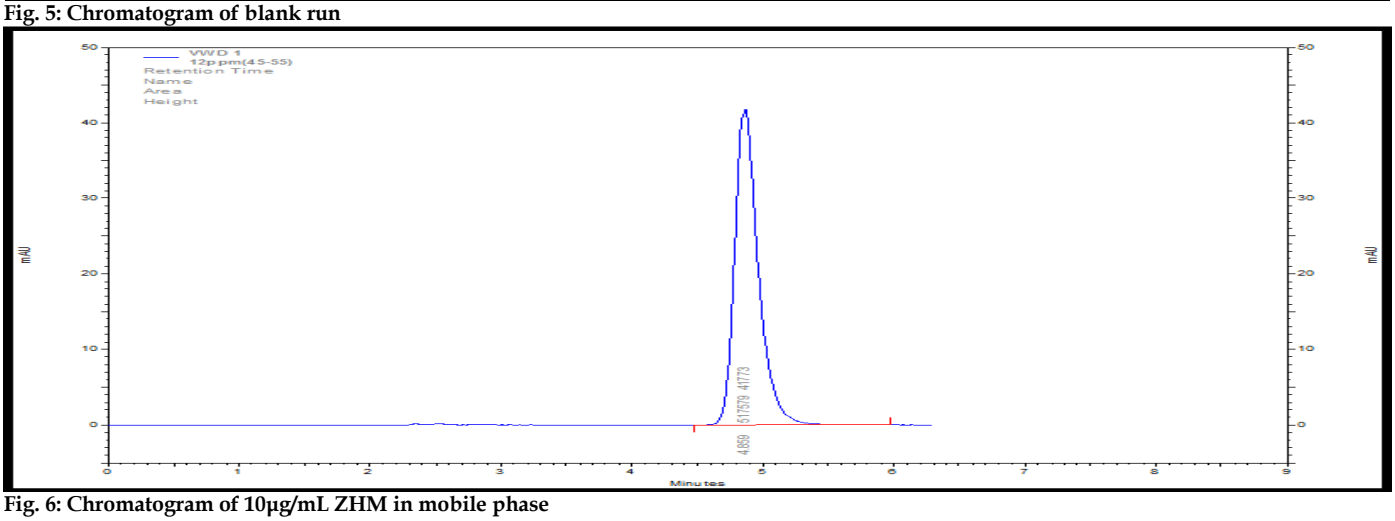

Fig. 6: Chromatogram of 10µg/mL ZHM in mobile phase

Table 3: Mobile phase trials for optimisation at 1 ml/min flow rate

Mobile phase used	Composition (% v/v)	Inference
ACN : Water (pH-3 made up with glacial acetic acid)	50:50	Peak tailing was observed
ACN : Water (pH-3 made up with glacial acetic acid)	30:70	Broad peak with tailing was observed
Methanol : Water (pH-3 made up with glacial acetic acid)	60:40	Peak was eluted very fast i.e. at 2 min
Methanol : Water (pH-3 made up with glacial acetic acid)	50:50	Peak was not sharp
Methanol : Water (pH-3 made up with glacial acetic acid)	45:55	Desired peak with satisfying RT, sharpness and area was obtained

Table 4: Chromatographic method development parameters

Parameters	Specifications
Column Type	Hemochrom-Intertsil C18-5U reverse phase column (250 × 4.6) mm dimension
Temperature	25 ± 2°C
Mobile Phase	Water (pH 3) & Methanol in 45:55% v/v ratio respectively
Flow Rate	1 ml/min.
Injection volume	50µl
Detection wavelength	317 nm
Retention time (RT)	4.8 min.
Run time	6.5 min.
Column Pressure	130 bar

Different flow rate in the range of 0.5 to 1.5 ml/min and different injection volumes in the range of 20µl to 100µl were tried. Mobile phase selected was optimised at the composition of Methanol: Water (pH-3 made up with glacial acetic acid) in the ratio of 45:55%v/v. Optimized chromatographic conditions are tabulated in Table 4. Representative chromatogram using these optimised chromatographic conditions mentioned above retained at 4.8 min is shown in Figure 3.

Analytical Method Validation

Validation of the developed method was carried out as per ICH guidelines with respect to parameters such as specificity, linearity, precision, accuracy, robustness, limit of quantification (LOQ) and limit of detection (LOD).

Linearity

The standard curve was observed by preparing six serial dilutions of ZHM using a standard stock solution and dilution were made with mobile phase Methanol: Water (pH 3 made up with glacial acetic acid) in the ratio of 45:55% v/v. Responses were recorded as peak area. The peak areas were plotted against concentrations to obtain the calibration curve. Linear relationship was observed across the range of 2-12µg/mL. Linearity range and linear regression data of calibration plot for ZHM is given in table no.5 and calibration curve is represented in Figure 4.

Specificity

Specificity was tested by evaluating chromatogram of blank run and standard ZHM. The HPLC

chromatograms recorded for the blank showed almost no peaks, interfering peak or baseline noise within a retention time range of 4.8min. Chromatogram of blank run and of 10 ppm of ZHM in mobile phase is given Figure 5 and 6.

Precision

Intra-day precision: It was performed at three different concentration levels low (4µg/mL), mid (8µg/mL) and high (12µg/mL) within the same day at three different times (session 1, 2, 3).

Inter-day precision: It was carried out at same concentration levels on three consecutive days, using same homogeneous sample. The % RSD values for both intra-day and inter-day precision were found within acceptable limit. Results are presented in Tables 6 and 7 respectively.

LOD and LOQ

Values of LOD and LOQ were calculated using slope of calibration curve. LOD and LOQ values of ZHM for HPLC method are tabulated in Table 8. Determined based on the standard deviation of the response and slope of the calibration curve.

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. Accuracy of the method is reported as present recovery of known added amount of analyte in the sample. The accuracy of the method was established by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% by adding known amount of ZHM. Results obtained were found to be within acceptable limits as shown in Table 9.

Robustness

Robustness of method was studied by making slight but deliberate changes in chromatographic conditions such as proportion of organic phase in mobile phase composition and flow rate. Effects of these changes on both the retention time (RT) and peak area were evaluated by calculating the relative standard deviations (%RSD). The results obtained are tabulated in Table 10.

Table 5: Values for linearity

Parameters	Result Obtained
Linearity range (µg/mL)	2-12
Correlation coefficient (r^2)	0.9986
Slope	43691
Intercept	10842

Table 6: Intra-day precision results

		ZHM			
Level		Low	Mid	High	Inference
Concentration (µg/mL)		4	8	12	
Peak Area (mAU)	Session 1	164950	326213	517748	Acceptable %RSD, hence precise.
	Session 2	165106	327270	521914	
	Session 3	165234	327398	523345	
Average Peak Area		165097	326960	521002	
Standard Deviation		142.229	650.336	2907.74	
%RSD		0.086	0.198	0.0558	

Table 7: Inter-day precision results

		ZHM			
Level		Low	Mid	High	Inference
Concentration (µg/mL)		4	8	12	
Peak Area (mAU)	Session 1	164950	326213	517748	Acceptable %RSD, hence precise.
	Session 2	164026	325310	522152	
	Session 3	162364	320588	515771	
Average Peak Area		163781	324037	503044	
Standard Deviation		1310.716	3020.853	3266.52	
%RSD		0.800	0.932	0.629	

Table 8: Results of LOD and LOQ

Parameters	Result
LOD	0.254 µg/mL
LOQ	0.770 µg/mL

Table 9: Recovery studies indicating accuracy on capsule formulation

Drug	Level of Percentage recovery (%)	Amount present in extract (µg/mL)	Amount added (µg/mL)	Total amount (µg/mL)	Amount recovered (µg/mL)	% recovery	Average % recovery	% RSD	Inference
ZHM	80	10	8	18	18.44	102.49	100.50	0.0031	Acceptable recovery Hence accurate.
	100	10	10	20	19.90	99.44		0.0032	
	120	10	12	22	23.83	99.59		0.0032	

Table 10: Effect on retention time and response by variation in mobile phase composition and its pH, column temperature and flow rate

Method Parameters and variations	Level of variations	Modified Parameters	ZHM	
			%RSD	Retention Time(Min)
Proportion of organic phase in mobile phase 45:55 (±2)	-2	Methanol : Water (pH 3) (53:47)	0.35	0.75
	+2	Methanol : Water (pH 3) (57:43)	0.58	1.02
Flow Rate (1.0± 0.2)	-0.2	0.8	0.160	1.40
	+0.2	1.2	0.156	1.10
pH	-2	2.8	0.23	1.42
	+2	3.2	0.83	0.11

The proposed RP HPLC method developed for ZHM with UV detection has been statistically validated following the ICH guidelines recommendations and it is successfully found to be specific, precise, accurate and robust. This method is economic as water constitute one among the two mobile phase used. So the mobile phase is simple to prepare and economical. Also as elution time of ZHM was fast i.e. 4.8min; it saves time for analysis and reduces mobile phase consumption. Hence, the developed method can be conveniently used for determining the quality control of ZHM in bulk pharmaceuticals.

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