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Review

Analytical Method Development and Validation of Zuclopenthixol by RP-HPLC in compliance with ICH-Q2R1 guidelines

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Abstract

Zuclopenthixol, a thioxanthene-class antipsychotic, is extensively used in the management of schizophrenia, bipolar disorder, and behavioural disturbances in patients with intellectual disabilities. Its therapeutic effect arises from potent antagonism at dopamine D1 and D2 receptors, producing marked sedative and antipsychotic activity. The present research focuses on developing and validating a simple, precise, and stability-indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method for the quantitative estimation of Zuclopenthixol in pharmaceutical formulations, following ICH Q2(R1) guidelines. Chromatographic separation was achieved using a Waters X-Bridge C18 (150 × 4.6 mm, 3.5 µm) column with a mobile phase of 20 mM potassium dihydrogen phosphate containing 0.1% v/v triethylamine and acetonitrile in a 45:55 v/v ratio at a flow rate of 1.0 mL/min. Detection was performed at 257 nm, and the drug showed a retention time of 5.52 minutes. The method exhibited excellent linearity (r^2 = 0.9945) over the concentration range of 25–150%, with mean recovery values between 98–102% and %RSD below 2%. Specificity was confirmed through forced degradation studies, demonstrating no interference from excipients or degradation products. The method was found robust and rugged under varied chromatographic conditions, ensuring reproducibility and reliability. Thus, the validated RP-HPLC method is accurate, precise, and suitable for routine quality control, assay determination, and stability evaluation of Zuclopenthixol in parenteral and other pharmaceutical dosage forms.

Keywords: Zuclopenthixol, Method development, Method validation, ICHQ2R1,RP-HPLC

INTRODUCTION

A common antipsychotic medication in the thioxanthene class, zuclopenthixol is mostly licensed in Europe to treat schizophrenia. It is also used to control hostility and agitation in people with intellectual disabilities. Oral, intramuscular acetate, and long-acting intramuscular decanoate depot preparations are among the various forms of the medication. Due to its dual antagonistic effects on dopamine D1 and D2 receptors, zuclopenthixol is known as the most sedative antipsychotic. Its sedative effects are caused by this antagonistic action, which blocks the cerebral effects of dopaminergic firings in the ventral tegmental area (VTA). Zuclopenthixol's wider neuropsychiatric uses have been highlighted by its effectiveness in reducing aggression in adults with autism, patients with schizophrenia, and kids with bipolar illnesses.[1]

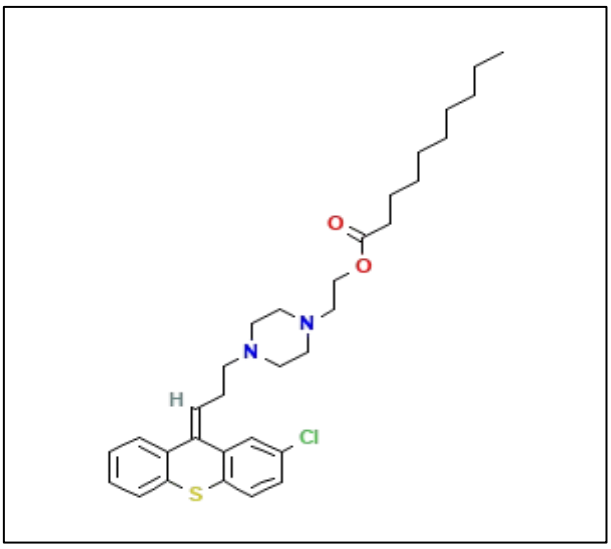

Fig 1: Structure of Zuclopenthixol[2]

A review of the literature reveals that Zuclopenthixol can be estimated using a number of bioanalytical techniques and the HPLC method for quantifying contaminants in Zuclopenthixol followed LCMS/MS[3]. Other methods include UHPLC-MS/MS, HPLC-DAD, HPLC-MS, TLC had been performed[4-9]. The goal of the current study is to use RP-HPLC to establish a precise, accurate, linear, robust, and rugged method for the analysis of zuclopenthixol in tablet dosage forms in accordance with ICH guiding lines Q2 R1[10], based on the literature review.

MATERIALS AND METHODS

Drugs and chemicals

Zuclopenthixol Reference standard procured from USP and TLC pharmaceutical standards. HPLC grade potassium dihydrogen phosphate, triethylamine, acetonitrile and Milli-Q water obtained from Merck.

Instrumentation

Empower-3 software is installed on a waters HPLC (E-2695) device. Waters X Bridge C18, 3.5µm column, 150 x 4.6 mm. Fischer Scientific sonicator used for degassing. The materials were weighed using a Sartorius electronic balance. A thermo Scientific pH meter was used for all pH adjustments.

Mobile Phase preparation

20mM potassium dihydrogen phosphate with 0.10% v/v triethylamine: Acetonitrile (45:55) filtered through the 0.45µm membrane. Mixed and sonicated to degas.

Diluent Preparation

Mobile Phase used as diluent

Standard preparation

Weighed accurately and transferred about 50 mg of Zuclopenthixol RS into a 50 ml volumetric flask. Added 30 ml of diluent sonicated to dissolve and diluted to the volume with diluent and mixed well. Further transferred 2.5 ml of above stock solution into a 50 ml volumetric flask. Diluted to the volume with diluent and mixed well

Sample preparation

Transferred 0.5 ml of sample (Zuclopenthixol decanoate 200 mg/ml injection) into a 100 ml volumetric flask. Added 70 ml of diluent and sonicated for 5 minutes with intermittent shaking. Diluted to the volume with diluent and mixed well. Transferred 2.5 ml into a 50 ml volumetric flask. Diluted to volume with diluent and mixed well. Filtered through 0.45µ nylon filter by discarding first 4 ml of filtrate. (50 ppm)

Chromatographic conditions

For chromatographic separation, a Waters X Bridge C18, 150 x 4.6 mm 3.5µm column was used. Acetonitrile and 20mM potassium dihydrogen phosphate (0.10% v/v triethylamine) make up the mobile phase (55:45), which runs for 10 minutes at a flow rate of 1.0 ml/min. 257 nm is the detection wavelength. Zuclopenthixol was reported to have a retention time of 5.52 minutes. The temperature of the sampler is kept at 10°C, the injection volume is 20 µl, and the column oven is thermostatically controlled at 30°C.

METHOD VALIDATION

The developed method was validated by following parameters precision, accuracy, linearity, specificity, robustness and as per the ICH guidelines.

System precision

A standard solution was prepared in accordance with the procedure to determine the system precision. The USP tailing factor, USP plate count for Zuclopenthixol peak, and relative standard deviations for peak area responses from five replicate injections of the standard solution were presented (Table 1). A maximum of 2.0% should be the percentage relative standard deviation from five replicate standard injections. Zuclopenthixol peak obtained from standard solution should have a USP plate count of at least 2000 and a USP tailing factor of not more than 2.0. (Figure 2).

Method precision

Six separate samples were made in order to test the assay method's precision. (Figure 2). The assay findings ought to fall between 90.0% and 110.0%. A maximum of 3.0% should be the percentage relative standard deviation from six distinct sample preparations. (Table 2).

Linearity

The method's linearity was assessed by injecting the HPLC system with concentrations ranging from 25% to 150% of the standard concentrations. A correlation coefficient of at least 0.98 is required. (Table 3).

Accuracy

In the accuracy trial, several concentrations of Zuclopenthixol drug material, ranging from 50% to 150% of the typical concentration of Zuclopenthixol, were processed along with a placebo. The process was followed in preparing the spiked samples. For Zuclopenthixol, both the average and individual recovery percentages at each level must be between 95.0% and 105.0%. According to Table 4, the total percentage RSD should not exceed 3.0%.

Specificity

To show that the procedure is stability indicating, forced degradation research is completed. The final product of Zuclopenthixol was exposed to heat, acid, base, peroxide, and UV radiation. To identify and prevent impurity interference with the Zuclopenthixol peak, blank, standard, control, and stress sample solutions were injected into the HPLC. Peak purity analysis using a PDA detector should show peak homogeneity any secondary peak resulting from forced degradation study should not interfere with the Zuclopenthixol peak, diluent, placebo, and all known impurities should not interfere at the retention time of the Zuclopenthixol peak. Stress samples should have a purity threshold greater than the purity angle. (Table 5).

Robustness

Standard solution was made and injected into HPLC in accordance with the method's requirements for the robustness investigation. By altering the procedure parameters, the identical standard solution was injected again. For standards injected under modified method conditions, a set of system suitability data was computed and compared to the values produced under normal conditions. (Table 6).

Ruggedness

Six separate sample solutions of Zuclopenthixol made by a second analyst using a different HPLC and a different column on a different day were injected to test the intermediate precision. The assay findings ought to fall between 90.0% and 110.0%. The mean percentage assay difference between intermediate precision and

method precision should not exceed 3.0%, and the percentage RSD from six separate sample preparations should not exceed 3.0%.(Table 7).

RESULTS AND DISCUSSION

Table 1: System Precision Results

S.No	Name	Retention time	Area	USP Tailing	USP Plate count
1	Standard-1	5.510	3655765	1.1	5221
2	Standard-2	5.512	3665601	1.2	5219
3	Standard-3	5.514	3667629	1.1	5329
4	Standard-4	5.512	3658434	1.2	5382
5	Standard-5	5.513	3669906	1.1	5253
Mean			3663467		
% RSD			0.17		

Table 2: Method Precision Results

S.No	Name	Area	Assay
1	Sample-1	3685765	100.61
2	Sample-2	3655680	99.79
3	Sample-3	3664585	100.03
4	Sample-4	3654755	99.76
5	Sample-5	3645863	99.52
6	Sample-6	3685765	99.94
Mean			100.61
% RSD			0.48

Table 3: Linearity Results

S.No	Name	Area
1	Linearity - 25%	2398080
2	Linearity - 50%	2830774
3	Linearity - 100%	3663467
4	Linearity - 125%	4396160
5	Linearity - 150%	5861547
Correlation coefficient square = 0.9945		

Table 4: Accuracy data for Zuclopenthixol

S.No	Name	% Recovery	Mean	% RSD
1	Recovery 50% -1	98.52	98.94	0.67
2	Recovery 50% -2	99.70		
3	Recovery 50% -3	98.60		
1	Recovery 100% -1	101.55	100.90	1.15
2	Recovery 100% -2	99.56		
3	Recovery 100% -3	101.58		
1	Recovery 150% -1	101.87	100.97	0.90
2	Recovery 150% -2	100.06		
3	Recovery 150% -3	100.98		

Table 5: Specificity data for Zuclopenthixol

S. No	Name	Purity angle	Purity threshold
1	Control sample	0.145	0.364
2	Acid degradation sample 2N HCl/75°C/4 hrs	0.548	1.454
3	Base degradation sample 0.5N NaOH/75°C/4 hrs	0.211	0.325
4	Peroxide degradation sample 2% H_2O_2/80°C/4 hrs	0.241	0.356
5	Heat degradation /80°C/15 hrs	0.145	0.245
6	Uv light degradation sample Uv light/15 hrs	0.154	0.214
7	Spiked sample	0.125	0.189

Table 6: Results for Robustness

Parameter	% RSD
Column temperature plus	0.15
Column temperature minus	0.25
Flow rate plus	0.45
Flow rate minus	0.98
Mobile phase composition plus	0.84
Mobile phase composition minus	0.28

Table 7: Ruggedness data for Zuclopenthixol

S.No	Name	Area	Assay
1	Sample-1	3695754	99.56
2	Sample-2	3685688	101.25
3	Sample-3	3654587	100.34
4	Sample-4	3664754	99.98
5	Sample-5	3655868	99.25
6	Sample-6	3656559	100.35
Mean			100.12
% RSD			0.71
The % Assay between method precision and ruggedness = 0.48 %			

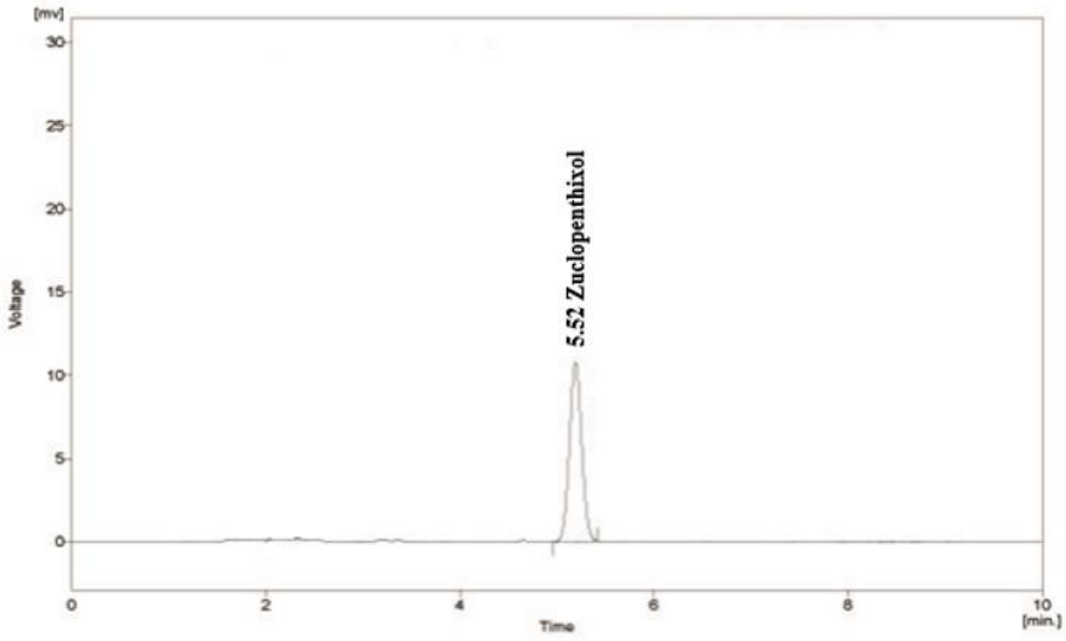

Fig 2: Typical Standard Chromatogram for Zuclopenthixol

CONCLUSION

The proposed RP-HPLC method was found to be precise, linear, accurate, specific, robust and rugged for the estimation of Zuclopenthixol in parenteral dosage form. According to ICH criteria, the suggested method satisfies regulatory requirements and is appropriate for a regulatory approach. This approach is therefore readily used for regular Zuclopenthixol analysis.

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Conflict of Interest
The authors declare that there is no conflict of interest

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