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[Research article]

Development and Validation of RP – HPLC Method for the estimation of Oxyclozanide in Pure and Pharmaceutical formulation

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ABSTRACT

A simple, fast, precise, selective and accurate RP-HPLC method was developed and validated for the simultaneous determination of oxyclozanide from pharmaceutical formulation. Chromatographic separation was achieved gradient on a YMC c18 column (250 x 4.6 mm, 5 µ particle size) using a mobile phase acetonitrile and water in the ratio of 80:20.the flow rate was 1.0ml / min and effluent was detected at 300nm.the retention time of oxyclozanide was found to be 1.89min. Linearity was observed in the concentration range of 10 -100µg / ml .The method was validated according to ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. The method developed can be used for the routine analysis of oxyclozanide.

Keywords: Oxyclozanide, RP-HPLC, Development, Validation.

INTRODUCTION

Oxyclozanide is chemically 2, 3, 5-Trichloro-*N*-(3, 5-dichloro-2-hydroxyphenyl)-6-Hydroxy benzamide used as anti helmenthic.

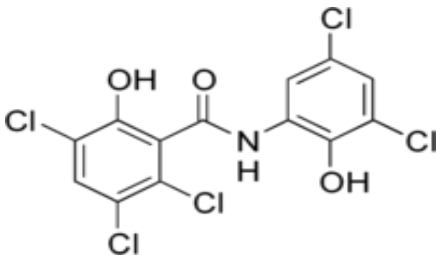

Figure 1: Chemical structure of oxyclozanide

Several HPLC, GC, and LC/MS-MS methods have been reported for the analysis of oxyclozanide in plasma that suffer from either undesirably long chromatographic run times and requirement for gradient analysis or use of an internal standard.

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The objective of this study was to develop reverse phase high performance liquid chromatography method for the estimation of oxyclozanide in pure and pharmaceutical dosage form without any derivatization and having the short retention time. This method was found to be linear, precise, accurate, sensitive, specific, and robust, and therefore suitable for routine analysis.

MATERIALS AND METHOD

HPLC Instrumentation and Chromatographic conditions

The analytical separations were carried out on a waters 2487 HPLC system equipped with Photo Diode Array detector. The output of signal was monitored and integrated using LC – solutions 2000 software. The analytical column was YMC C_{18} (250 × 4.6mm, 5µ). Mobile phase consisted Acetonitrile and water in the ratio of 80:20. Mobile phase was mixed, filtered through 0.45µmembrane filter and degassed under ultrasonication. The mobile phase was used as diluent. The flow rate was 1.0 ml/min and runtime was 5 minute. The column was maintained at ambient temperature. UV detection was measured at 300 nm and the volume of sample injected was 10 µl.

Preparation of standard stock solution

25mg of oxyclozanide was weighed accurately and dissolved in 25ml of mobile phase to get the concentration of 1000 µg/ml. Resultant solution was filtered through Whatman filter paper. The standard chromatogram for oxyclozanide (100 µg/ml) was shown in figure 2.

Preparation of working standard solution

Working standard solutions of oxyclozanide were prepared by accurately transferring the (0.1,0.2,0.4,0.6,0.8 and 1.0 ml) aliquots of the standard stock solution into a series of six 10 ml volumetric flasks. The volume was made up to mark with mobile phase to obtain concentration range of 10 –100 µg/ml.

Preparation of sample solutions

20 tablets were weighed individually and their average weight was calculated. Then the tablets were grounded and weight equivalent to 141.25mg of oxyclozanide was taken into 25mL volumetric flask and then the sample was diluted to 25ml with mobile phase to get concentration of 1000µg/ml and used for analysis.

RESULTS AND DISCUSSION

HPLC method development and optimization

To optimize the chromatographic conditions, different columns, mobile phases, flow rates etc., were tested. Acetonitrile and water in the ratio of 80:20 was preferred as mobile phase because it resulted in a greater response to oxyclozanide after several preliminary investigatory runs compared with the different mobile phase combinations. The effect of the flow rate was studied in the range 0.9 to 1.5ml/min and 1.0ml/min was preferred to be effective. Under these conditions, the analyte peak obtained was well-defined and free from tailing. The retention time (RT) was found to be 1.89 min. The optimized chromatographic parameters were listed in table 1

Fig 1. optimized chromatogram

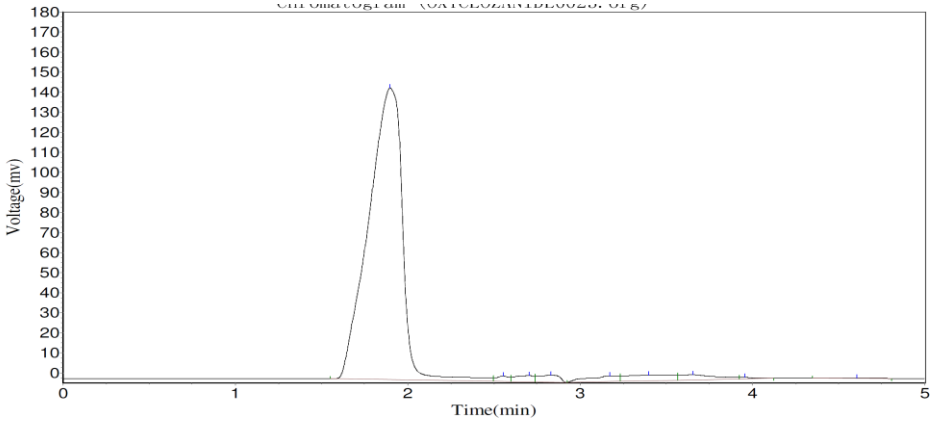


Table 1: Optimized chromatographic parameters

Optimized	Chromatographic parameters
Elution	gradient
Mobile phase acetonitrile	water (80:20)
Column	YMCc18 column
Flow rate	1.0ml/min
Detection	300nm
Injection volume	10µl
Temperature	Ambient
Retention time	1.89min
Run time	5.0 min
Concentration	10 - 100µg/ml

Validation of the method

When method development and optimization are complete, it is necessary to accomplish method validation. The validation studies include linear range (correlation coefficient), method precision (RSD, %), method accuracy (% recovery and RSD, %), sensitivity studies (LOD & LOQ), and robustness.

System suitability studies

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (RT), tailing factor (T), and peak asymmetry (AS), resolution (RS) were evaluated. The system suitability test was performed using five replicate injections of standards before analysis of samples. The system suitability method acceptance criteria set in each validation run were: capacity factor > 2.0, tailing factor ≤ 2.0 and theoretical plates > 2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. System suitability parameters were shown in table 2.

Table 2: System suitability parameters

Parameters	Values
Retention time	1.89min

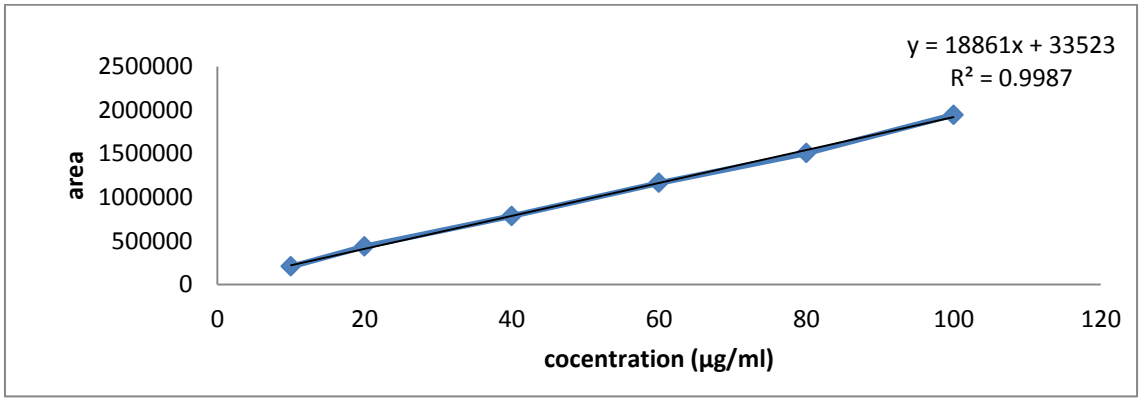
Linearity

The linearity of the method was evaluated by preparing six series of standard solutions of oxyclozanide in the range of 10 – 100 µg/ml in mobile phase and injecting the solutions into the HPLC system. Excellent correlation between oxyclozanide peak area and concentration was observed with R^2 = 0.998 (Figure.3). The regression equation was found to be Y =18861 x +33523. Statistical data are presented in table 3 and the calibration curve was shown in figure 3.

Table 3: Linearity results for oxyclozanide

s.no	concentration	Area
1	10	207406
2	20	437484
3	40	786045
4	60	1506426
5	80	1945004
6	100	2065987

Fig 2. Calibration curve for oxyclozanide



PRECISION

System precision: (Repeatability)

To study precision, five replicate standard solutions of oxyclozanide (40 µg/ml) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated. Results of system precision studies were shown in table 4.

Table 4: Results of system precision for oxyclozanide

s.no	Retention time(min)	Area(Mv. sec)
1	1.193	1023654
2	1.923	1.45897
3	1.857	1056488
4	1.857	1023654
5	1.913	1023564
6	1.848	1023654
Mean	1.8854	1032833.5
Standard deviation	0.047545968	14609.88216
% RSD	1.645189498	1.414543

Method precision: (Reproducibility)

The intraday and inter-day precision of the proposed method was determined by analyzing the corresponding responses 6 times on the same day and on different days for concentration of sample solutions of 100µg/ml. The result was reported in terms of relative standard deviation (% RSD). Results of method precision studies were shown in table 5.

Table 5: Results of Method precision for oxyclozanide

s.no	Peak area	%labelled claim
1	803093	102
2	832580	99.3
3	830258	99.6
4	833425	98
5	803092	102
6	823695	99

Intermediate precision

The intermediate precision of the proposed method was determined by performing the method by two analysts (Analyst 1 and Analyst 2) for concentration of sample solutions 40 µg/ml. The percent relative standard deviation (% RSD) for peak responses was calculated. The results for intermediate precision were shown in table 6.

Table 6: Results of Intermediate precision for oxyclozanide

		ANALYST – 1		ANALYST – 2	
	S.NO	**AREA (Mv.sec)**	**Retention time**	**AREA (Mv.sec)**	**Retention time**
OXYCLOZANIDE	1	1088512	1.857	786045	1.857
	2	1088836	1.857	774744	1.865
	3	1085985	1.857	785396	1.857
	4	1045311	1.848	774744	1.865
	5	1055614	1.873	803093	1.865
	MEAN	1072852	1.8584	785011	1.601
	S.D	20789.75	0.009044	10393	0.00438178
	% RSD	1.937803	0.486673	1.323	0.235453007

ACURACY

Accuracy of the method was confirmed by the standard addition method, which was carried out by performing recovery studies at 2 different concentrations 40 and 60 µg/ml of these expected, in accordance with ICH guidelines, by replicate analysis (n=3). Known amount of standard drug solution (40µg/ml) was added to a pre analyzed sample solution (40 and 60 µg/ml) and percentage drug content was measured. The closeness of obtained value to the true value indicates that the proposed method is accurate. Recovery studies were shown in table 7.

$$\% \text{ Recovery} = [(Ct - Cpa)/ Cs] \times 100.$$

Where,

Ct = Total concentration of analyte

Cpa = Concentration of pre-analysed sample

Cs = Concentration of standard added to pre-analysed sample.

Table 7: Results of recovery studies for oxyclozanide by using RP –HPLC method

s.no	std	level	Amount added	Total recovery	recovered	%recovery
1	40	40	40	81.3	41.3	103.25
2	40	40	40	83.93	43.93	109.825
3	40	40	40	81.39	41.39	103.475
4	40	60	60	98.3	58.3	97.1
5	40	60	60	102.46	62.46	104.1
6	40	60	60	100.8	60.8	101.3

ROBUSTNESS

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing parameters like flow rate of mobile phase and detection wavelength

- Change in the detection wavelength by ± 2nm (298 nm and 300 nm)

- Change in flow rate by ± 0.1 ml/minute (1.2 ml/min and 0.9 ml/minute)

After each change, sample solution was injected and % assay with system suitability parameters were checked.

Robustness values were given in table 8

Table 8: Results of Robustness for oxyclozanide

parameter	Rt(min)	Area(mvsec)
Flow rate(ml/min)1.2	1.90	1485965
0.9	1.892	1300145
Wavelength(nm)302	2.08	1328815
298	1.986	1215141

Limit of Detection and Quantitation

Detection and Quantitation limit were calculated by the method based on the standard deviation (σ) and slope of the calibration plot, using the formula

$$\text{Limit of Detection} = \sigma \times 3.3/S$$

$$\text{Limit of Quantitation} = \sigma \times 10/S$$

Where σ = the standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

Table 9: Results of LOD, LOQ for oxyclozanide

S.No	LOD	LOQ
1	0.081	0.075702

Specificity

Specificity of an analytical method is its ability to measure the analyte accurately and specifically in the presence of component that may be expected to be present in the sample matrix. Chromatograms of standard and sample solutions were compared in order to provide an indication of specificity of the method.

Assay of pharmaceutical formulation

The proposed validated method was successfully applied to determine oxyclozanide in their pharmaceutical dosage form and the % Assay results were shown in table 10.

Table 10: Results of % assay by using RP – HPLC method

Drug	Sl.No	Amount found(mg)	Test area	Standard area	% Assay (A_T /A_S*100)
Oxyclozanide	1	999.99	774744	785396.563	98.64%
	2	999.99	803093		102.25%
	3	999.99	786045		100.08%

CONCLUSION

A simple, rapid, accurate, and precise RP-HPLC method for the analysis of oxyclozanide in pure and in pharmaceutical dosage forms had been developed and validated in accordance with ICH guidelines. The RP-HPLC method developed is cost-effective due to short retention time which enabled analysis of oxyclozanide samples with a small amount of mobile phase. From the % RSD values of precision and recovery studies the method was found to be precise and accurate. The low detection and quantification limits achieved

indicate the method is very sensitive. The robustness data gathered during method validation showed that the method is not susceptible to small changes in chromatographic conditions. The proposed RP-HPLC method developed by the author is suitable for routine analysis and quality assessment of oxyclozanide in pharmaceutical products.

Table 12: Summary of validated parameters for proposed method

Parameter	**Result**
Linearity range	10 – 100 µg/ml
Regression equation	Y = 18861 x +33523
Slope	18861
Intercept	33523
Correlation coefficient	0.998
System precision	(% RSD,n=5)1.4145
Intermediate precision	(% RSD, n=5) 0.23
LOD (µg/ml)	0.081
LOQ (µg/ml)	0.075
% Recovery (Accuracy, n =3)	100%
% Assay (% Assay, n=3)	100%

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