

# Development and Validation of RP-HPLC Method for the Estimation of Oxyclozanide in Bulk Drug and Pharmaceutical Formulations

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**Abstract:** A new, simple, sensitive, rapid, accurate and precise RP-HPLC method was developed for the estimation of Oxyclozanide in bulk drug and pharmaceutical formulation. Oxyclozanide was chromatographed on a reverse phase C18column (25cm x 4.6 mm i.d; particle size 5  $\mu$ m) in a mobile phase consisting of methanol and water in the ratio of 68:32 % v/v. The mobile phase was pumped at a flow rate of 1.0 ml/min with detection at 302 nm. The detector response was linear in the concentration of 10-30  $\mu$ g/ml. The limit of detection and limit of quantitation was found to be 0.7582 and 2.297  $\mu$ g/ml, respectively. The intra and inter day variation was found to be less than 2%. The mean recovery of the drug from the solution was 100.27%. The proposed method is simple, fast, accurate, precise and reproducible, hence it can be applied for routine quality control analysis of Oxyclozanide in bulk drug and pharmaceutical formulation.

**Key words:** RP-HPLC, Quantitation, Oxyclozanide, chromatographed.

## INTRODUCTION:

Oxyclozanide<sup>1-6</sup> is a anthelmintic drug indicated mainly in treatment of important helminth disease caused by two trematodes Fasciola hepatica and Fasciola gigantica. It is believed to work by increasing capillary endothelial resistance and promoting platelet adhesion. Uncoupling of oxidative phosphorylation processes has been demonstrated for the salicylanilides, which are mainly fasciolicides. These compounds act as protonophores, allowing hydrogen ions to leak through the inner mitochondrial membrane. Although isolated nematode mitochondria are susceptible, many fasciolicides are ineffective against nematodes in vivo, apparently due to a lack of drug uptake. Exceptions are the hematophagous nematodes, eg, Haemonchus and Bunostomum.

Oxyclozanide is chemically 3,3',5,5',6-pentachloro-2'-hydroxysalicylanilide. The molecular formula of Oxyclozanide is : C<sub>13</sub>H<sub>6</sub>Cl<sub>5</sub>NO<sub>3</sub>. The molecular mass of Oxyclozanide is 401.5 g/mol. It is official drug in British Pharmacopoeia. Freely soluble in *acetone*, soluble in *ethanol* (95%) and Very slightly soluble in *water*.

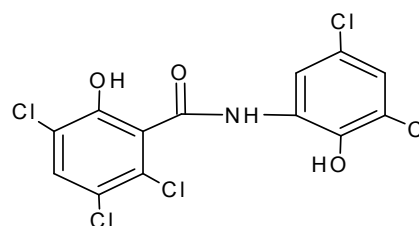


Figure 1. Chemical structure of Oxyclozanide

Literature survey reveals that, only spectrophotometric multicomponent resolution<sup>7</sup>, LC assay of Oxfendazole and Oxyclozanide in pharmaceutical preparation<sup>8</sup> and Determination of Oxyclozanide in beef and milk using High-Performance Liquid Chromatography<sup>9</sup> have been reported.

## **EXPERIMENTAL**

### **MATERIALS AND METHODS:**

The specifications of HPLC system used for the study are given below.

Quantitative HPLC was performed on a gradient high pressure liquid chromatography (Perkin Elmer HPLC 1100) with one LC-10 AT VP pumps, with UV/VIS detector SPD-10A VP, CTO-10 AS VP column oven (Perkin Elmer), SCL-10AVP system controller (Perkin Elmer), a disposable guard column LC-18 (PELLIGUARD)<sup>TM</sup>, LC-18, 2 cm, supelco,inc.,Bellefonte, and a Reverse Phase C-18 Column (25cm x 4.6 mm i.d; particle size 5  $\mu$ m) was used. The HPLC system was equipped with the software TOTAL CHROMATOGRAPHIC NAVIGATOR (Perkin Elmer).

### **REAGENTS AND CHEMICALS:**

Pure drug sample of Oxyclozanide was received as a gift sample from Siflon Drugs, Andhra Pradesh, India and was used as such. The water and methanol used were of HPLC grade from Merck, Mumbai.

### **PREPARATION OF MOBILE PHASE:**

Mobile phase was prepared by mixing 680 ml of methanol and 320 ml of double distilled water.

### **CHROMATOGRAPHIC CONDITIONS:**

The contents of the mobile phase were methanol and water in the ratio of 68:32 v/v. The mobile phase was filtered through 0.45  $\mu$ m-membrane filter and sonicated for 8 min. The flow rate of the mobile phase was maintained at 1.0 ml/min. The column temperature was set at  $20 \pm 1^{\circ}$  C and the detection was carried out by UV-Detector wavelength was set at 302 nm. The run time was set at 15 min and the volume of the injection loop was 20  $\mu$ l. Prior to injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The data were acquired, stored and analyzed with the software TOTAL CHROMATOGRAPHIC NAVIGATOR (Perkin Elmer).

### **PROCEDURE:**

About 100 mg of Oxyclozanide was accurately weighed and transferred to a 100 ml volumetric flask. It was dissolved in 65 ml of methanol and made up to the volume with methanol and sonicated for 8 min. From this a working standard solution of 100  $\mu$ g/ml of strength was prepared. From this dilution 10, 15, 20, 25 & 30  $\mu$ g/ml were prepared in 100 ml volumetric flasks with methanol. 20  $\mu$ l of each dilution was injected each time into the column at a flow rate of 1.0 ml/min. each dilution was injected 3 times into the column and the corresponding chromatograms were obtained.

The retention time of Oxyclozanide was found to be 4.569 min. The calibration curve was constructed by plotting concentration versus peak area ratio. The amount of Oxyclozanide present in sample was calculated through the standard calibration curve. The linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method. The peak area ratios of the drug versus concentration were found to be linear and the results are furnished in Table 1.

### **ASSAY:**

Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of Oxyclozanide was transferred to 100 ml volumetric flask containing 65 ml of ethanol and the contents of the flask were sonicated for 8 min, to ensure the complete solubility of the drug, The mixture was then made up to 100 ml with ethanol. The resulting solution was thoroughly mixed and filtered through a 0.45  $\mu$ m membrane filter. Using this a working standard solution of 100  $\mu$ g/ml of strength was prepared. From this dilution 10, 15, 20, 25 & 30  $\mu$ g/ml were prepared in 100 ml volumetric flasks with methanol. This solution (20  $\mu$ l) was injected three times into the column. The mean values of peak areas of five such determinations were calculated and the drug content in the tablet was quantified using the regression equation. The results were furnished in Table 2.

### **VALIDATION OF PROPOSED METHOD:**

Selectivity of the method was assessed on the basis of elution of Oxyclozanide using the above mentioned chromatographic conditions. The linearity, precision, accuracy, limit of detection, limit of quantitation and robustness has been validated for the determination of Oxyclozanide. The results are furnished in Table 3.

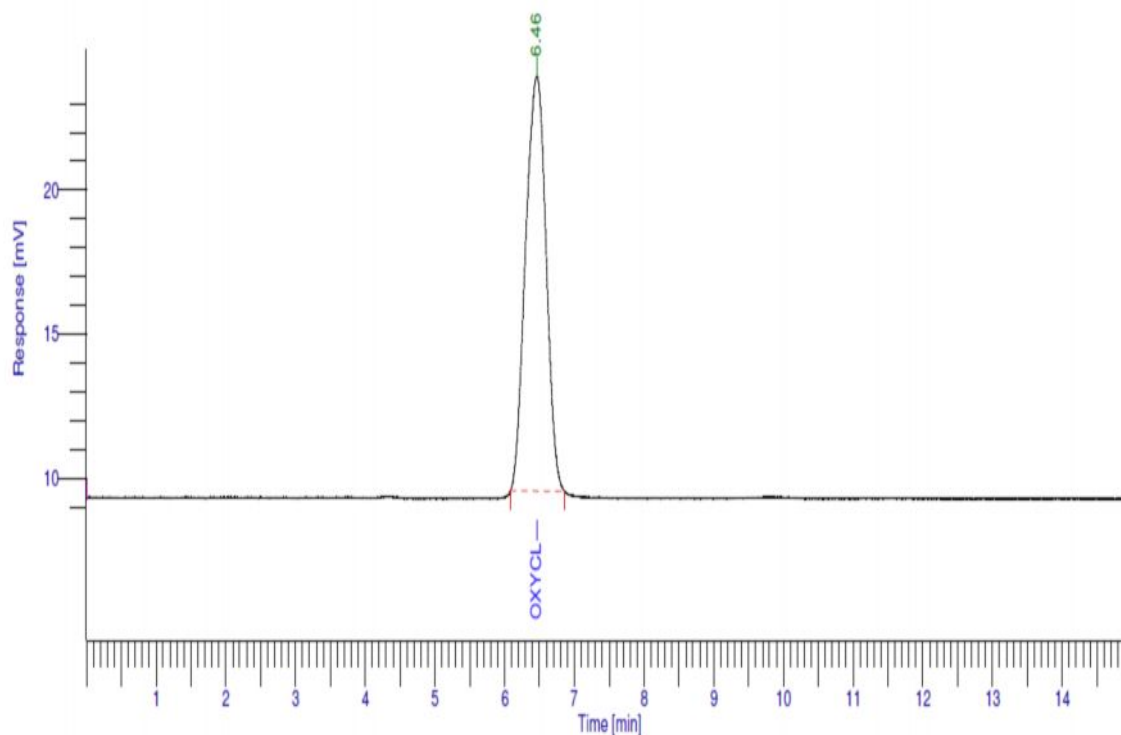


Figure 2. Typical chromatogram of Oxyclozanide

Table 1. Calibration data of the method

Sl. No	Concentration ( $\mu\text{g/ml}$ )	Area
1	0	0
2	10	196246.85
3	15	289285.01
4	20	375051.3
5	25	459985.1
6	30	553410.42

Table 2. Assay of Oxyclozanide

Components	Label Claim (mg)	Amount found(mg)	% Found
Brand 1	1000	1000.19	100.01
Brand 2	1000	1007.8	100.78

\*n = 3

Table 3. Optical Characteristics of Oxyclozanide by RP-HPLC method.

Parameters	RP-HPLC
Linear Range ( $\mu\text{g/ml}$ ) (C)	10-30
Slope(m)	18294
Intercept(c)	6694
Correlation co-efficient ( $r^2$ )	0.999
Range of % RSD	0.5179-1.2158
LOD ( $\mu\text{g/ml}$ )	0.7582
LOQ ( $\mu\text{g/ml}$ )	2.2978
Tailing factor	0.942
Theoretical plates	2492.2

\*n = 6

**Table 4. Precision of the proposed HPLC method**

Components	Precision	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
OXY	Intra-day	100.17%	0.4072	0.4065	0.2347
	Inter-day	99.82%	0.4072	0.4080	0.2355

\*n = 3

**Table 5. Recovery studies of the proposed HPLC method.**

Level of % recovery	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
80%	100.38%	0.5352	0.5331	0.3078
100%	100.05%	0.6118	0.6115	0.3531
120%	100.12%	0.5792	0.5785	0.3340

\*n = 3

**LINEARITY:**

The standard curve was obtained in the concentration range of 10-30 µg/ml. The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits.

**PRECISION:**

The precision of the assay was determined in terms of intra-day and inter-day precision. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V.) obtained by multiplying the ratio of standard deviation to mean with 100. The results are furnished in Table 4.

**LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)**

The LOD and LOQ for Oxyclozanide were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of Oxyclozanide.

**ROBUSTNESS:**

The robustness was checked by changing the flow rate to 0.9 and 1.1 ml/min and the wavelength at 300 and 304 nm, the method suits best.

**ACCURACY:**

The accuracy of the HPLC method was assessed by adding known amount of drug solution to a solution of known concentration and subjecting the samples to the proposed HPLC method. The recovery studies were replicated 3 times. The accuracy was expressed in terms of recovery and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration so as to give the percentage recovery. The results are furnished in Table 5.

**RESULTS AND DISCUSSION:**

By applying the proposed method, the run time of the method was set at 15 min and Oxyclozanide appeared on the typical chromatogram at 4.569 min, which indicates a good base line. When the same drug solution was injected 3 times, the retention time of the drug was same. Linearity range was observed in the concentration range of 10-30 µg/ml. The regression equation of Oxyclozanide concentration over its peak area ratio was found to be  $Y=18600+4273x$  ( $r = 0.999$ ) where Y is the peak area ratio and X is the concentration of Oxyclozanide (µg/ml). The proposed HPLC method was also validated for intra-day and inter-day variation. The coefficient of variation in the peak area of the drug for 3 replicate injections was found to be less than 1%. The tailing factor was found to be 0.942, which indicates good shape of peak. The number of theoretical plates were found to be 2492.2, which indicates efficient performance of the column. The limit of detection and limit of quantitation was found to be 0.7582 µg/ml and 2.297 µg/ml, indicates the sensitivity of the method. To optimize the chromatographic conditions, various combinations of water and methanol were tested. The use of methanol and water in the ratio of 68:32 % v/v resulted in peak with good shape and resolution. The high percentage of recovery of Oxyclozanide ranging from 99.78 to 100.78 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by proposed HPLC method.

**CONCLUSION:**

The proposed HPLC method was found to be simple, rapid, sensitive, precise and accurate for the estimation of Oxyclozanide in pharmaceutical formulations. Hence, this method can be easily and conveniently

adopted for routine quality control analysis of Oxyclozanide in bulk drug and its pharmaceutical formulations.

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