METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CLOSANTEL IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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This article is available online at **www.ssjournals.com**

ABSTRACT

A rapid, sensitive and specific RP-HPLC method involving UV detection was developed and validated for determination of closantel in bulk and tablet dosage form. Chromatography was carried out on a phenomenex ODS C-18 column (250 x 4.6 mm), 5 µ particle size using filtered and degassed mixture of water pH adjusted to 3 and acetonitrile in the ratio 10:90 as mobile phase at a flow rate of 1.2 ml/min and effluent was monitored at 333 nm. The method was validated in terms of linearity, precision accuracy and specificity. The assay was linear over the concentration range of 100-500 µg/ml. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the pre analysed test solution and was found to be 99.53% - 101.06% within precision RSD of 0.00502 for closantel. The system suitability parameters such as theoretical plates, retention time and tailing factor were found to be 10832, 6, and 1.27 respectively. The method does require only 10 mins as run time for analysis, which proves the adoptability of the method for the routine quality control of the drug.

KEY WORDS: Closantel, RP-HPLC, Estimation, Validation.

INTRODUCTION

Closantel is an anthelmentic (veterinary) drug[(1)]. Chemically it is N-{5-chloro-4-[(4-chlorphenyl) cyanomethyl] -2-methylphenyl}-2 -hydroxy-3,5-diiodobenzamide. The literature survey showed that an HPLC method with fluorescence detector was developed for the determination of closantel[(2-5)] from the plasma and milk. In this paper we describe a simple, inexpensive, sensitive and validated HPLC[(6-8)] method for the determination of closantel in bulk and pharmaceutical formulation.

MATERIAL AND METHODS

Closantel (working standard) was obtained as a gift sample from VETINDIA Pharmaceuticals Ltd., Hyderabad. HPLC grade acetonitrile and Merck grade orthophosphoric acid were procured from the market. The instrument used was HPLC-LC 2010 with Class VP software.

Chromatographic conditions

The HPLC system consisted of an isocratic pump, an auto sampler and UV detector. The stationary phase used was Phenomenex ODS C_{18} column (250×4.6 mm), 5 µ particle sizes and the mobile

phase was prepared by mixing water pH adjusted to 3 with acetonitrile in the ratio 10:90 (v/v). Chromatography was performed at 25ºC at a flow rate of 1.2 ml/min and the detection was performed by an UV spectrometer at wavelength of 333 nm. The injection volume was 10 µl. The runtime was set to 10 minutes. The column was equilibrated for 30 to 40 minutes with mobile phase.

Mobile phase

Solution –A: 500 ml of HPLC water was adjusted to pH 3 with phosphoric acid.
Solution –B: Acetonitrile Solution A and B were mixed in the ratio of 10:90, filtered and sonicated to degas.

Standard Preparation

Accurately weighed and transferred closantel equivalent to 50 mg of closantel working standard into 50 ml clean dry volumetric flask, 5 ml of tetra hydro furan and 15 ml of methanol were added, sonicated for 5 mins, and diluted to volume with methanol. Further diluted 5 ml to 25 ml with methanol.

Sample Preparation

Weighed accurately a quantity of sample equivalent to 50 mg of closantel into a 50 ml clean dry volumetric flask, 5 ml of tetra hydro furan and 15 ml of methanol were added, sonicated for about 15 mins, further made up the volume with methanol and then filtered through 0.45 micron filter. Further diluted 2.5, 5, 7.5 ml of the filtrate to 25 ml with methanol.

Procedure

Flow rate - 1.2 ml/min; detection wavelength - 333 nm; injection volume - 10 µl; column used - Phenomenex ODS C-18 column (250 x 4.6); column temperature: 25ºc; mobile phase water pH adjusted to 3 and acetonitrile in the ratio 10:90.
Ten µl of each of the working standard and assay preparation was injected and chromatographed. The chromatogram of closantel working standard was given in figure-1 and that of sample in figure-2. The result was given in table – 1.

The amount of closantel present in the sample was calculated using the formula:

$$\text{Amount present} = \frac{\text{Test Peak Are}}{\text{Standard Peak Area}} \times \frac{\text{Standard Dilution}}{\text{Test Dilution}}$$

Calibration procedure

Appropriate aliquots of the drug were pippetted out from the standard stock solution into a series of 25 ml volumetric flasks. The volume was made upto the mark with methanol to obtain a set of solutions for closantel of concentrations 100, 200, 300, 400, and 500 µg/ml. Triplicate dilutions of each concentration of the drug were prepared separately. From these triplicate solutions 10 µl of each concentration of the drug were injected into the HPLC system three times separately and chromatographed under the optimized conditions. Evaluation of the drug was performed with the UV detector set at 333 nm and the peak areas were recorded. The correlation curve was constructed by plotting the peak area ratios obtained versus the injected amounts of the respective concentrations. The Beer - Lamberts law was obeyed in the concentration range of 100 -500 µg/ml for closantel as shown in Figure - 3.

RESULTS

Linearity

The linearity of calibration graph and adherence of the system to Beer's law was validated by high value of correlation co-efficient (r^2 = 0.999 and regression equation is Y= 5021x – 24330. The linearity table of closantel was shown in Table -2.

Precision

Intraday and inter-day precision of the assay samples containing closantel at three different concentrations were analyzed three times in the same day (intraday) and for three consecutive days (interday). The results were shown in Table - 3.

Accuracy

The accuracy of the HPLC assay method was assessed by adding known amount of the drug to a drug solution of known concentration and subjecting the samples to the proposed HPLC method. The drug was estimated as the procedure described above for the estimation of closantel in the tablet formulations. The recovery studies were replicated five times. The accuracy was expressed in terms of percentage recovery. Results of recovery studies were shown in Table - 4.

Repeatability

Repeatability studies were carried out by estimating response of 3 different concentrations of closantel for 3 replicate determinations and results were reported in terms of relative standard deviation (Table - 5).

Robustness

As defined by the ICH, the robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed by small variation in the chromatographic conditions and found to be unaffected by small variations like ±2% variation in volume of mobile phase composition, ±0.1 ml/min in flow rate of mobile phase, ±0.1 variation in pH.

Stability

Results obtained in the study of the solution (both sample & standard solution) where it can be noticed that the solutions were stable for 72 h, as during this time the results does not decrease below the minimum percentage (98%).

System suitability

To know reproducibility of the method, system suitability test was employed to establish the parameters such as retention time, peak area response, theoretical plates, asymmetry factor and repeatability and the values were shown in table-5.

DISCUSSION

In this present study an attempt has been made to develop RP-HPLC method for the determination of closantel in bulk and pharmaceutical dosage form. The results obtained were reproducible and reliable. The validity and precision of the methods were evident from the statistical and analytical parameters obtained. Therefore, it is concluded that the method developed is simple, rapid, selective, economical, accurate and precise. Hence, this method is suitable for application in routine quality control analysis of pharmaceutical preparations.

ACKNOWLEDGEMENT

The authors are thankful to the management of Faculty of Pharmacy, Sri

Ramachandra University for providing the necessary facilities.

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Table – 1: Assay results for Closantel solution

Label claim (mg/ml)	Amount found (mg/ml)	% Label claim	SD	RSD	SE	CV
150	148.90	99.26	0.2002	0.0020	0.1156	0.2000
150	149.60	99.75				
150	149.90	99.93				

SD: Standard deviation RSD: Relative standard deviation
SE: Standard error CV: Coefficient of variation

Table - 2: Data for calibration curve

Concentration of closantel (in µg/ml)	Peak area
100	471362
200	981000
300	1493087
400	1984965
500	2480102

Table – 3: Intra and Inter day precision for Closantel assay

Concentration (µg/ml)	Observed concentration			
	Intraday	%RSD	Interday	%RSD
100	100.65	0.52	100.36	0.32
200	200.10	0.33	200.51	0.55
300	300.23	0.08	299.84	0.03

Table – 4: Results of recovery studies

Amount of drug in sample (mg/ml)	Amount of standard added (mg/ml)	Amount recovered (mg/ml)	% recovery	SD	RSD	SE	CV
150	120	158.71	99.58	0.5019	0.00502	0.2898	0.5020
150	150	199.15	99.53				
150	180	237.54	101.06				

Table – 5: System suitability study

S.No	Parameters	Values
1.	Retention Time Peak	6 mins
2.	Area Response	475166
3.	Theoretical Plates	10832
4.	Asymmetry Factor	1.27
5.	Repeatability	0.87

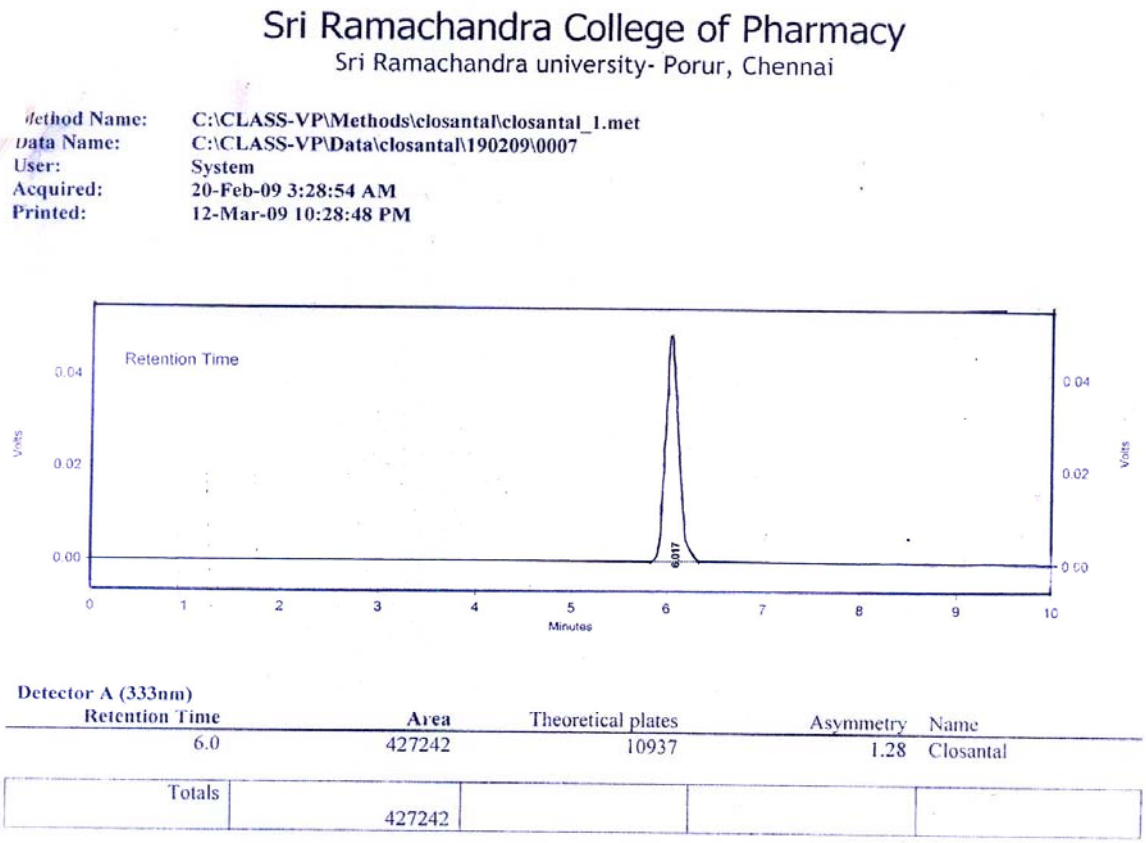

Figure 1: Chromatogram of Closantel sample