

## LC assay method for oxfendazole and oxyclozanide in pharmaceutical preparation

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### Abstract

A method has been developed for the simultaneous determination of oxfendazole and oxyclozanide in a pharmaceutical preparation. The method involves reversed phase chromatography with isocratic elution of the mobile phase and detection at 300 nm. The range of quantification for oxfendazole and oxyclozanide was found to be 2–7  $\mu\text{g ml}^{-1}$  and 3–10  $\mu\text{g ml}^{-1}$ , respectively. The validity of the method was evaluated in terms of linear regression analysis, precision, specificity and accuracy. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* RP-LC; Isocratic elution; Oxfendazole; Oxyclozanide

### 1. Introduction

Oxfendazole and Oxyclozanide are an anthelmintic (Flukicide) compound, which are commonly used for the veterinary products of a pharmaceutical preparations. The formulation may contain Oxfendazole alone or in combination with Oxyclozanide. A spectrophotometric method has been described for the assay of suspension containing these two compounds alone and not in combination with each other. An iodometric titra-

tion for the assay of raw material of these compounds have been reported. However, a HPLC method has been reported for the related substance of Oxyclozanide [1,2]. HPLC methods, reported earlier [3–6] describes only the determination of Oxfendazole in pharmaceutical preparations and biological samples. Similarly oxyclozanide has been determined by various HPLC and GC techniques [7,8].

So far no HPLC method has been reported for the simultaneous determination of Oxfendazole and Oxyclozanide, therefore it was considered necessary to develop HPLC method for quantification of these compounds in the pharmaceutical preparation. Details of the method and its validation are now reported.

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## 2. Materials

Sodium dihydrogen phosphate; orthophosphoric acid of extra pure grade and methanol of HPLC grade were obtained from Merck. Freshly prepared standard solutions were used.

## 3. HPLC method

The chromatograph used in this study consisted of a LC-10AS pump (Shimadzu, Japan), a SPD-10AV detector, a C-R6A integrator and SIL-9A injector. Hypersil-5  $\mu\text{m}$  ODS,  $200 \times 4$  mm column was used. A degassed filtered mixture of methanol and 0.1 M sodium dihydrogen phosphate (7:3; v/v) having pH  $4 \pm 0.1$  adjusted with orthophosphoric acid (44%), was used as eluent. The flow rate was maintained at 1.0 ml/min.

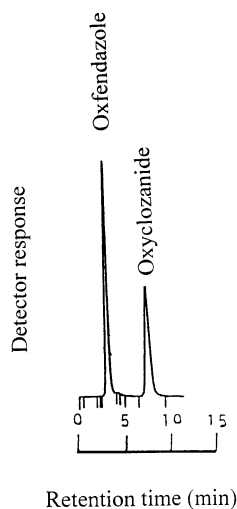


Fig. 1. Chromatogram of authentic mixture containing  $6 \mu\text{g ml}^{-1}$  of Oxfendazole and  $8.5 \mu\text{g ml}^{-1}$  of Oxyclozanide.

Table 1  
Regression analysis of the calibration data<sup>a</sup>

Drug	Slope	Intercept	Correlation coefficient ( $r^2$ )
Oxfendazole	1850	3135	0.9984
Oxyclozanide	1643	-3625	0.9959

<sup>a</sup> An average of three experiments.

Detection was performed at 300 nm. An aliquot of 20  $\mu\text{l}$  was chromatographed. The separations were carried out at ambient temperature.

### 3.1. Preparation of standard solution

An accurately weighed 10 mg Oxfendazole and 14 mg oxyclozanide were transferred into a 100 ml volumetric flask. The contents of the flask were dissolved in a mixture of methanol and water (5:1) with the sonication for 2 min and diluted to volume with methanol. An aliquot of 3 ml of this solution were transferred into 50 ml volumetric flask. The contents of the flask were diluted to volume with mobile phase. This was the reference standard solution. The solution was filtered through a  $0.45 \mu\text{m}$  membrane filter before injection into the liquid chromatograph.

### 3.2. Preparation of test solution

A sample containing oxfendazole and oxyclozanide equivalent to 10.0 mg and 14.0 mg, respectively, were weighed and transferred into 100 ml volumetric flask. The contents of the flask were dispersed in 10 ml of water and dissolved in 50 ml of methanol. The flask was sonicated for 2 min and diluted to volume with methanol. An aliquot of 3 ml of this solution were transferred into 50 ml volumetric flask and diluted to volume with mobile phase. The solution was filtered through a  $0.45 \mu\text{m}$  membrane filter before injection into the liquid chromatograph.

## 4. Results and discussion

Standard solutions of oxfendazole and oxyclozanide containing 40, 60, 80, 100, 120, 140 and 160% of theoretical assay concentration ( $6.0$  and  $8.5 \mu\text{g ml}^{-1}$ ) were examined by the proposed HPLC method and the responses measured. A linearship of peak area and concentration was observed. A typical chromatogram is shown in Fig. 1.

Regression analysis of the calibration data ( $n = 3$ ) for each compound gave the value for slope, intercept and correlation co-efficient for each calibration curve (Summarized in Table 1).

Table 2  
Simultaneous analysis of Oxfendazole and Oxyclozanide by the proposed HPLC method<sup>a</sup>

Oxfendazole			Oxyclozanide		
Amount added (% w/w)	Amount found (% w/w)	Recovery (%)	Amount added (% w/w)	Amount found (% w/w)	Recovery (%)
1.904	1.825	95.851	2.701	2.793	103.406
1.981	1.964	99.142	3.000	2.916	97.200
2.335	2.313	99.058	3.408	3.406	99.941
2.428	2.394	98.599	3.332	3.365	100.990
2.527	2.478	98.061	3.689	3.712	100.623
2.427	2.345	96.621	3.494	3.532	101.088
2.234	2.274	101.791	3.217	3.207	99.689
2.216	2.168	97.834	3.230	3.244	100.433
2.556	2.562	100.235	3.986	4.123	103.437
2.612	2.631	100.727	3.752	3.675	97.948
Mean		98.792	Mean		100.476
S.D.		1.728	S.D.		1.899
% RSD		1.749	% RSD		1.889

<sup>a</sup> An average of six experiments.

The validity of the listed regression data was tested by the assay of an authentic mixture containing known quantities of oxfendazole and oxyclozanide in pharmaceutical formulation at 80, 100 and 120% level theoretical assay concentration [9–11]. The result showed good accuracy (as revealed by the percentage recovery, see Table 2) which clearly demonstrates that other constituents of the formulation are not interfering with the assay.

Precision was assessed by the assay of a multiple sample (six) of homogenous test solution, using the proposed HPLC method. The results obtained were statistically valid (Table 3). The assay results (Table 4) by two operators each carried out three determinations on the same batch of a formulation indicates the robustness of the method. The analytes remain stable in the solution 24 h after its preparation. The stability indicating results are summarized in Table 5. The LOD for Oxfendazole and Oxyclozanide was  $1.0 \mu\text{g ml}^{-1}$  whereas LOQ lies within the range of  $2\text{--}7 \mu\text{g ml}^{-1}$  and  $3\text{--}10 \mu\text{g ml}^{-1}$ , respectively.

The dependence of retention time on pH of mobile phase has also been observed and found that an increase in pH decreases the retention time of oxyclozanide with no marked influence on

oxfendazole peak. This behaviour clearly demonstrates that at higher pH level the oxyclozanide molecule becomes ionized and hence, do not retain on the non-polar surface of the reversed phase analytical column [12]. The solubility of oxyclozanide in alkali hydroxides and carbonates also indicate the change in the ionic state of the molecule in alkaline region [13].

The proposed HPLC method has also been applied on pharmaceutical preparation containing oxfendazole alone and observed that the assay results are comparable with the spectrophotomet-

Table 3  
Precision of sample measurement

S. No.	Peak area for Oxfendazole (a.u)	Peak area for Oxyclo- zanide (a.u)
01	198 405	162 572
02	197 020	162 273
03	198 207	167 936
04	198 005	162 967
05	199 673	165 369
06	201 899	164 819
Mean	198 868	164 322
S.D.	1562	1979
% RSD	0.785	1.204

Table 4  
Robustness of the proposed HPLC method

Operator	Determination	Oxfendazole (% L.C)	Oxyclozanide (% L.C)
A	01	97.83	98.57
	02	100.25	101.65
	03	100.21	101.44
B	01	98.53	98.25
	02	97.74	97.27
	03	97.83	98.97
Mean		98.73	99.35
S.D.		1.09	1.63
% RSD		1.10	1.64

Table 5  
Stability indicating results of Oxfendazole and Oxyclozanide

Time period (h)	Peak area for Oxfendazole (a.u)	Peak area for Oxyclozanide (a.u)
0	190 632	161 614
16	190 890	158 066
24	189 816	163 158

Table 6

A comparison of assay results obtained by the Spectrophotometric method and proposed HPLC method

Batch number	Spectrophotometric method <sup>a</sup> (% L.C)	Proposed HPLC method <sup>a</sup> (% L.C)	% RSD
01	99.55	99.67	0.06
02	101.76	98.28	1.73
03	102.10	104.64	1.23

<sup>a</sup> L.C, Label claim.

ric method as shown in Table 6. However, the HPLC method has been found time saving as it provides the analysis of two compounds simultaneously with the high degree of precision and accuracy. The HPLC method presented here, has been considered to be well appropriate for the use in Quality Assurance and R&D Laboratories for similar type and composition of products.

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