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Simple, robust and accurate high-performance liquid chromatography method for the analysis of several anthelmintics in veterinary formulations

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Abstract

A simple, specific and accurate reversed-phase high-performance liquid chromatographic method to assay several anthelmintics in veterinary products is reported. The drugs analysed included albendazole, fenbendazole, niclosamide and oxyclozanide. This method afforded rapid and efficient separation, good resolution and identification of all the examined compounds, alone or combined. Method validation included range, linearity, precision, accuracy, specificity and recovery. The method was used to quantify these drugs, alone or in combination, in tablet, powder and liquid formulations.

Keywords: Pharmaceutical analysis; Anthelmintics; Albendazole; Fenbendazole; niclosamide; Oxyclozanide

1. Introduction

One of the most demanding tasks confronting the pharmaceutical analyst is the development and validation of rugged high-performance liquid chromatographic (HPLC) methods [1]. Owing to the widespread use of HPLC in routine analysis, it is important that good HPLC methods are developed and that these are thoroughly validated. The treatment of worm infestations in man usually implies the use of dosage forms containing single compounds, i.e. niclosamide, mebendazole, etc., and several analytical methods for such products are reported [2,3]. However, the treatment of worm infestations in animals is mostly through multi-component dosage forms containing combinations of well-known anthelmintics. It was found that there are few, if any, analytical methods described for these combinations.

Here, a simple, specific and accurate reversed-phase HPLC method to assay several anthelmintics in veterinary and pharmaceutical products is reported. The drugs analysed were albendazole, fenbendazole, niclosamide and oxyclozanide. The chemical structures of the drugs are shown in Fig. 1. The method was used to quantify these drugs, alone or in combination, in tablet, powder and liquid formulations. Method validation included range, linearity, precision, accuracy, specificity and recovery [4].

2. Experimental procedures

Albendazole [methyl-5-(propylthio)-2-benzimidazole carbamate], fenbendazole [methyl-5-(phenyl-

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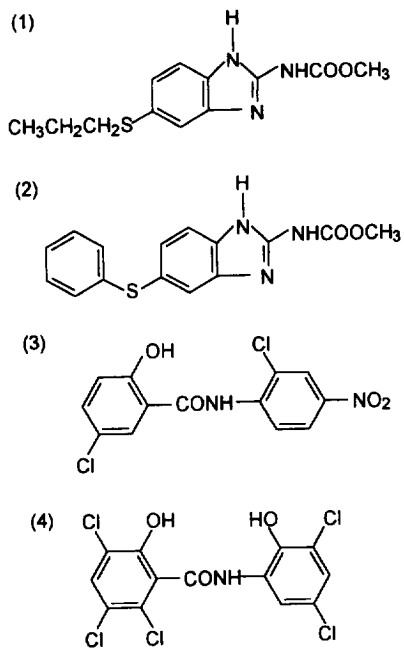

Fig. 1. Chemical structures of anthelmintics albendazole (1), fenbendazole (2), niclosamide (3) and oxyclozanide (4).

thio)-2-benzimidazole carbamate], niclosamide (2′,5-dichloro-4-nitrosalicylanilide) and oxyclozanide [2,3,5-trichloro-N-(3,5-dichloro-2-hydroxyphenyl)-6-hydroxy benzamide] were obtained from Sigma (St. Louis, MO, USA; USP or BP grade; purity above 98%). HPLC methanol from BDH (Poole, UK) and analytical grade formic acid and dibasic ammonium phosphate (Saarchem, South Africa) were used. Dosage forms tested were obtained from companies who are selling it in South Africa, while some were newly formulated products.

The HPLC systems consisted of either a Shimadzu LC-10A system equipped with a Model LC-10AS pump, SPD-10A variable-wavelength detector, SIL-10A autosampler, SCL-10A system controller and C-R6A integrator (Shimadzu, Kyoto, Japan) or a Hewlett-Packard HP1050 system with an HP3395 integrator (Hewlett-Packard, Waldbronn, Germany). The flow-rate employed was 1 ml min^{-1}. Either a Phase Sep, Hypersil C_{18} cartridge (Phase Separation, Norwalk, USA; 250×4.6 mm I.D., 5 μm particle size) or a Nova Pak C_{18} cartridge (Waters, Milford, MA, USA; 250×3.9 mm I.D., 4 μm particle size) was used and UV detection was at 254 nm.

To prepare the mobile phase, phosphate buffer was prepared by dissolving dibasic ammonium phosphate (6.6 g, 0.05 *M*) in deionised water (1000 ml) filtered with a Milli-Q50 system (Millipore, Bedford, MA, USA) and adjusted to pH 3.6 with concentrated phosphoric acid. The optimum mobile phase consisted of a mixture of phosphate buffer and methanol (75:25, v/v) with a pH of 3.6. The isocratic system was operated at ambient temperature and the method did not require any complex sample extraction procedure. For the analysis of combination preparations, the mobile phase was optimised by varying buffer pH and concentration, and organic solvent proportion and type.

To prepare standard solutions, accurately measured amounts of drugs were dissolved in methanol containing 10% formic acid. For each drug, alone or in combination, two samples were weighed and then used to prepare two stock solutions. Dilutions made from both these solutions were injected into the chromatograph and the linearity of peak area versus concentration was calculated. Solutions were diluted with the mobile phase. Samples taken from dosage forms were similarly prepared such that the drug concentration in the samples injected into the chromatograph fell within the range of standards assayed. To check for interference by degradation products, strong ammonia solutions were added to sample solutions, the solutions were then placed at 40°C for 12 h and again injected into the chromatograph.

3. Results and discussion

The described mobile phase and chromatographic conditions were sufficient for selective elution of the anthelmintics. Three chromatograms of artificial combinations of compounds are shown in Fig. 2. Separation and analysis required less than 20 min. The reproducibility of the chromatographic system was high because the resolution of the compounds of interest did not change over time, after repeated injections and throughout the period of testing the retention times of the compounds changed by no more than approximately 1% during a 12 h period.

For the analysis of single drugs, a combination of albendazole, niclosamide and oxyclozanide, and a

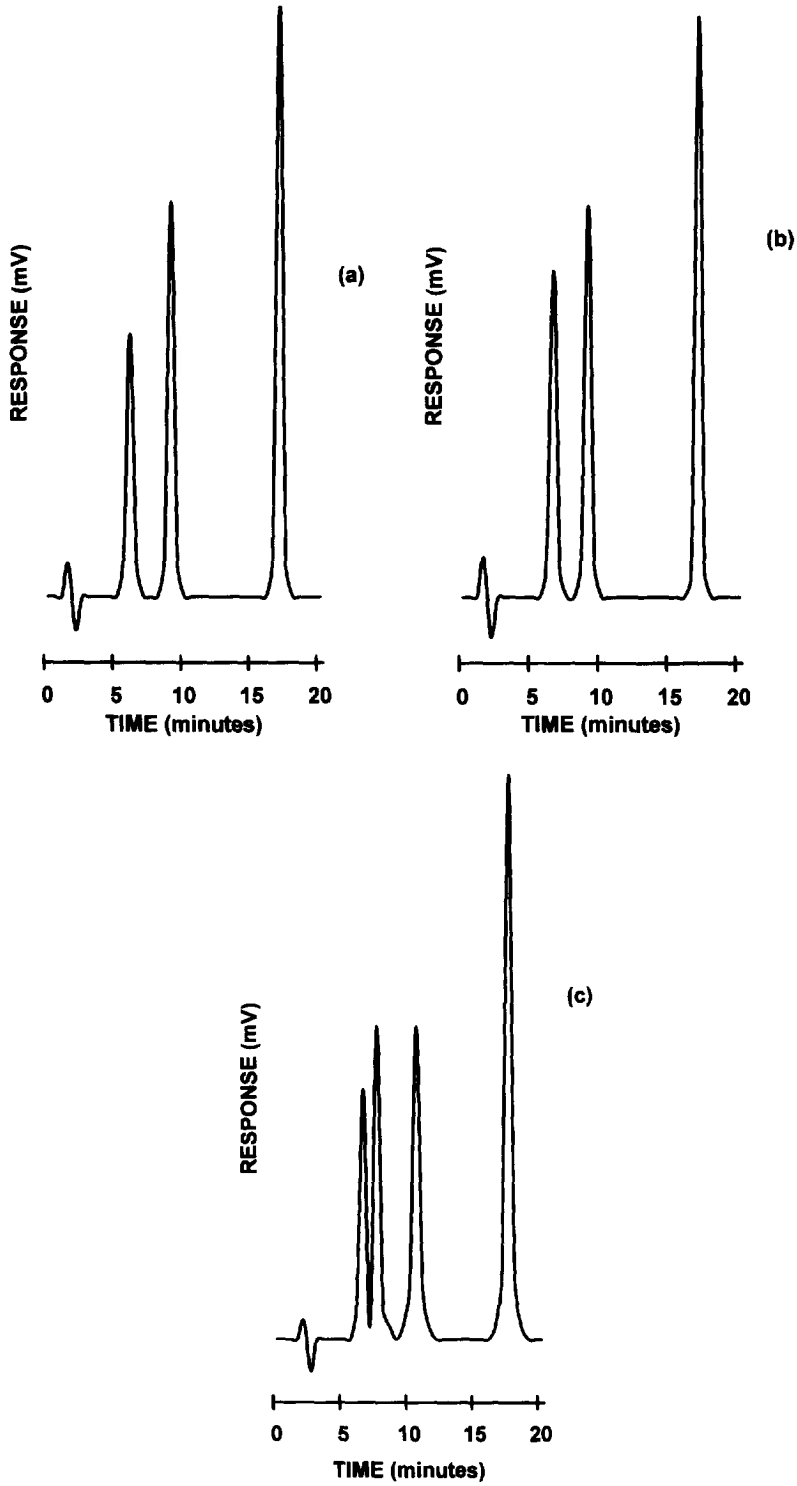

Fig. 2. Chromatograms of (a) mixture of albendazole, niclosamide and oxyclozanide, (b) fenbendazole, niclosamide and oxyclozanide, and (c) albendazole, fenbendazole, niclosamide and oxyclozanide.

Table 1
Selectivity and specificity as shown by retention times, plate numbers, tailing factors and resolution [4]

Compound	Retention time (min)	Plate number	Tailing factor	Resolution (drug:drug)
Albendazole (1)	6.0	12000	1.8	–
Fenbendazole (2)	6.5	12700	1.5	–
Niclosamide (3)	17.0	31551	1.1	–
Oxyclozanide (4)	9.1	24516	1.2	–
Mixture 1:				
Albendazole	5.5	11920	1.8	8 (1;4)
Niclosamide	17.3	34947	1.1	14 (3;4)
Oxyclozanide	9.0	26886	1.2	8;14 (1;4 and 3;4)
Mixture 2:				
Fenbendazole	6.5	12608	1.5	5 (2;4)
Niclosamide	17.3	28155	1.2	13 (3;4)
Oxyclozanide	9.2	22146	1.2	5;13 (2;4 and 3;4)
Mixture 3:				
Albendazole	6.5	8562	1.7	2 (1;2)
Fenbendazole	7.8	9331	1.6	7 (2;3)
Niclosamide	18.1	24335	1.0	9 (3;4)
Oxyclozanide	11.0	19887	1.2	5;9 (2;4 and 3;4)

combination of fenbendazole, niclosamide and oxyclozanide the optimum mobile phase was a mixture of ammonium phosphate buffer and methanol (75:25, v/v) with a pH of 3.6. See results listed in Table 1 and Table 2 and chromatograms shown in Fig. 2. Changes in the methanol concentration from 18 to 30% and the pH from 3.2 to 4 did not change the calibration data or the resolution of the compounds. For the analysis of a combination of albendazole and fenbendazole with niclosamide and oxyclozanide, Fig. 2c, the optimum mobile phase was found to be was a mixture of ammonium phosphate buffer and methanol (81:19, v/v) with a pH of 3.9.

Satisfactory results for the compounds and mix-

Table 2
Calibration data for standard drug solutions, alone and in combination

Compound	Concentration ($\mu g\ ml^{-1}$)	Correlation coefficient	Slope	Intercept
Albendazole (1)	12–60	0.9995	494 117	−40
Fenbendazole (2)	8–41	0.9999	560 225	110
Niclosamide (3)	12–60	0.9999	301 758	−73
Oxyclozanide (4)	16–82	0.9999	390 720	106
Mixture 1:				
Albendazole	12–60	0.9997 [0.9996]	485 755 [1 554 416]	35 [1120]
Niclosamide	12–60	0.9999 [0.9995]	326 715 [1 045 488]	−14 [489]
Oxyclozanide	16–82	1.0000 [0.9998]	406 973 [1 302 314]	192 [652]
Mixture 2:				
Fenbendazole	8–41	0.9999	540 635	134
Niclosamide	12–60	0.9998	276 800	−117
Oxyclozanide	12–60	0.9997	374 467	−56
Mixture 3:				
Albendazole	12–60	0.9996	488 852	67
Fenbendazole	8–41	0.9997	558 671	134
Niclosamide	12–60	0.9999	293 361	−117
Oxyclozanide	12–60	0.9995	384 426	−56

Results in square brackets were obtained on the HP 1050 system with NovaPak column.

Table 3
Determination of drugs in samples of known concentration

Compound	Concentration (μg ml^{-1})		Recovery (%)
	Added	Found±S.D.[a]	
Albendazole (1)	12.0	12.2±0.13	101.3
Fenbendazole (2)	16.3	16.4±0.05	100.5
Niclosamide (3)	60.0	60.9±0.10	101.4
Oxyclozanide (4)	81.6	83.3±0.87	101.1
Mixture 1:			
Albendazole	60.0	61.1±0.46	101.9
Niclosamide	60.0	61.0±0.33	101.6
Oxyclozanide	81.2	83.2±0.28	101.9
Mixture 2:			
Fenbendazole	8.2	8.3±0.41	101.7
Niclosamide	12.0	12.0±0.07	100.2
Oxyclozanide	16.3	17.0±0.13	104.2

[a]Based on ten replicate analyses of known samples.

tures in terms of both linearity and sensitivity (Table 1 and Table 2) were obtained. From calibration plots, the relative correlation coefficients were above r= 0.999 for both the analysis of single compounds and that of combinations. Linear relationships between peak areas and amounts of products were observed in the range 12–60 μg ml^{-1} for albendazole, 8–41 μg ml^{-1} for fenbendazole, 12–60 μg ml^{-1} for niclosamide and 16–82 μg ml^{-1} for oxyclozanide.

The chromatographic method also provided satisfactory specificity, precision and accuracy for the analysis of compounds 1–4. The lowest recovery after ten replicate injections of known samples (Table 3) was 100.2% and the highest 104.2%, with an overall mean deviation from 100% of 1.1%. Results obtained after replicate injection of the same solutions gave a precision of 0.9% (Table 4). Inter-day repeatability (Table 4) was also excellent. These results illustrated that the method was both accurate, precise and could be repeated.

No interference from the sample solvent, impurities and dosage form excipients could be observed at the detection wavelength (254 nm), as shown in Fig. 2 and Table 5. Degradation products and excipients were found not to interfere with quantification. The ruggedness of the method was demonstrated by the fact that when using the same conditions, but a different HPLC system (HP 1050) and column (NovaPak, Waters), there were no significant differences in the applicability of the method, as illustrated by the calibration data listed in Table 2.

Table 4
Accuracy and precision of method determined over two days

Compound	Concentration (μg ml^{-1})		Accuracy (%)	Precision (C.V. %)
	Added	Found±S.D.[a]		
Mixture 1:				
Albendazole	12.0	12.2±0.04	101.4	0.3
Niclosamide	12.0	12.1±0.04	100.7	0.3
Oxyclosanide	16.3	16.4±0.03	100.5	0.2
Mixture 2:				
Fenbendazole	8.2	8.0±0.17	98.2	2.2
Niclosamide	12.0	12.0±0.18	99.7	1.5
Oxyclosanide	16.3	16.4±0.16	100.3	1.0

[a]Based on five samples measured again after 20 h.

Table 5
Analysis of dosage forms

Product	Label claim (mg[b])	Assay result (mg[b])	Recovery[a] (%)	C.V.[a] (%)
Worm granules				
Fenbendazole	50	49.8	99.6	0.89
Niclosamide	125	126.6	101.3	1.23
Worm suspension				
Albendazole	19	18.6	98.2	0.64
Oxyclozanide	102	100.4	98.4	0.55

[a] Mean of six determinations.
[b] Units: mg per gram for granules; mg per ml for suspension.

4. Conclusions

The use of the described HPLC method allows a selective and quantitatively accurate analysis of several anthelmintics in veterinary dosage forms. The chromatographic method is sufficiently specific, precise and sensitive for the purpose of analytical characterisation of niclosamide, fenbendazole, albendazole and oxyclozanide. Preliminary results not given here also showed that the method was applicable for the analysis of other anthelmintics, including mebendazole, rafoxinide and levamisole.

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