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Solid-phase extraction and HPLC determination of levamisole hydrochloride in sheep plasma

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ABSTRACT

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The anthelmintic, levamisole, was determined in sheep plasma by means of ion-pair solid-phase extraction (SPE) and reverse-phase liquid chromatography. The SPE columns were conditioned with 2 mℓ of methanol followed by 1 mℓ of octane sulphonic-acid buffer. After sample application, the columns were washed with 2 mℓ of the same buffer, followed by elution with 90/10 acetonitrile: buffer. A phenyl reverse-phase column (Spherisorb S5 Phenyl, 250 x 4,6 mm) was used with a mobile phase of acetonitrile: 0,005 M of octane sulphonic-acid sodium salt and 0,2% triethylamine in water, pH 3,5, 38/62. Extraction recoveries of 89–94% were achieved over the range from 100–3 750 ng/mℓ. Accuracy and precision were better than 96 % and 2,6%, respectively, over said range, with a limit of quantitation of 50 ng/mℓ.

Keywords: HPLC determination, levamisole hydrochloride, sheep plasma, solid-phase extraction

INTRODUCTION

Levamisole is a well-known anthelmintic which has been in use since the late sixties (Rayemackers, Allewijn, Vanderberck, Demoen, Van Offenwert & Jansen 1966). Several methods, including polarography, have been described for the determination of levamisole in body fluids (Holbrook & Scales 1967), thin-layer chromatography (Otteneder & Hezel 1975), gas chromatography (Nose, Kobayashi, Tanaka, Horose & Watanabe 1977; Woestenborghs, Michielsen & Heykants 1981), capillary gas-chromatography electron-impact mass spectrometry (Stout, Dacunha, Tondreau & Boyd 1988) and high-performance liquid chromatography (HPLC) (Garcia, Diez, Sierra & Teran 1990; Alvinerie, Galtier & Escoula 1981; Marriner, Galbraith & Bogan 1980). The preferred method of analysis at present is HPLC, and the existing methods were reviewed. Most methods consisted of laborious extraction procedures with ether (Marriner *et al.* 1980), ether-hexane (Garcia *et al.* 1990) or chloroform (Alvinerie *et al.* 1981). Apart from being tedious, these extractions resulted in poor recoveries (83, 74 and 70%, respectively, for the above methods). Österdahl, Johnsson & Nordlander (1985) used Extrelut columns to extract levamisole from milk, but even this gave poor recovery with high variation (78 ± 9,1%). The limit of determination was claimed to be "between 0,02 and 0,05 µg/mℓ", when 50 mℓ of milk were used.

The aim of the present investigation was to develop a sensitive and reliable method by which to conduct a comparative bioavailability study of different levamisole oral suspensions.

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METHOD

The method involves the paired-ion extraction of the analyte and the internal standard, followed by paired-ion reverse-phase chromatography. Thiabendazole (Fig. 1) was found to be a suitable internal standard as it is structurally similar to levamisole.

Reagents and materials

- Supelclean LC-18 100-mg solid-phase extraction tubes (Supelco, Inc. Bellefonte, PA)
- Spherisorb S5 Phenyl HPLC cartridge column, 5 µm, 250 X 4,6 mm (Phase Separations, Deeside, UK)
- Acetonitrile for HPLC (HiPerSolv, BDH, Poole, UK)
- Water for HPLC was prepared in-house with a reverse-osmosis system (Milli-RO and Milli-Q, Millipore, Milford, MA).
- n-Octane sulphonic-acid sodium salt for HPLC (Romil chemicals, Leicestershire, UK)
- Ortho-phosphoric acid and triethylamine of analytical reagent grade (E Merck, Darmstadt, FRG)
- Levamisole, QC no. 94/3436 (Twins Animal Health, Wadeville, RSA)
- Thiabendazole BP, Batch no 56645 (Dolder Ltd, Basle, Switzerland)
- Paired-ion chromatography (PIC) buffer: dissolve 1 g of n-octane sulphonic-acid sodium salt in about 950 mℓ of water. Add 2 mℓ of triethylamine, stir and adjust the pH to 3,5 with 25% phosphoric acid. Make up to 1 ℓ with water.

Chromatographic conditions and equipment

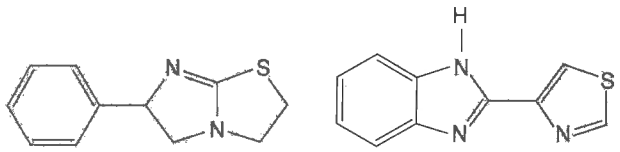
Mobile phase: acetonitrile/PIC buffer 38/62, detection UV at 215 nm, flow rate 1,0 mℓ/min, injection volume 100 µℓ. A Hewlett Packard 1050 HPLC system equipped with an isocratic pump, autosampler, variable wavelength detector and HP Chemstation computer-based control- and data-handling system (Hewlett Packard, Palo Alto, CA) was used.

Standard solutions

Five milligrams of levamisole were dissolved in 100 mℓ of 0,01 M hydrochloric acid. Two sequential dilutions in water were made to obtain three solutions of 50, 5 and 0,5 µg/mℓ of levamisole. One microlitre of blank plasma was spiked with these solutions to obtain standards of 20, 50, 100, 200, 500, 1 250, 2 500, 3 750 and 5000 ng/mℓ.

Internal-standard solution

Five milligrams of thiabendazole was dissolved in 200 mℓ of 0,01 M hydrochloric acid.



a b

FIG.1 The chemical structures of Levamisole (a) and Thiabendazole (b)

Solid-phase extraction

Fifty microlitres of internal-standard solution and 1 mℓ of PIC buffer solution (and the appropriate amount of levamisole in the case of standards) were added to 1 mℓ of plasma and mixed for 15 s with a vortex mixer.

The solid-phase extraction (SPE) column was activated by passing 2 mℓ of methanol, followed by 1 mℓ of PIC buffer solution through the column.

The prepared samples were loaded onto the columns and drawn through under slight vacuum. Two millilitres of the PIC buffer was used to wash the column, after which full vacuum was applied for 5 min to remove all fluid from the columns.

Five millilitres of siliconized glass tubes were placed in the vacuum manifold, and the needles were wiped clean before the samples were eluted with 2 mℓ of acetonitrile/PIC buffer 90/10. The samples were evaporated to dryness under nitrogen, redissolved in 300 µℓ of mobile-phase vortexes, filled into 300-µℓ sample vials, and 100 µℓ were injected onto the HPLC column.

Method validation

Extraction recovery was determined by adding levamisole and thiabendazole to blank plasma and extracting as described above. Standard solutions corresponding to the final concentration expected, were prepared in mobile phase. The percentage recovery was determined by comparing the peak areas of the extracted levamisole and thiabendazole with the peak areas obtained for the solvent-based standards.

Accuracy and precision were determined by analysing six sets of blank plasma samples spiked with known amounts of levamisole (low, medium and high concentration), and determining the amount present by means of a separate calibration curve. Accuracy was expressed as the percentage of levamisole recovered, and precision, as the relative standard deviation.

RESULTS AND DISCUSSION

The extraction was rapid and precise. One analyst was able to prepare up to 80 samples in 8 h. The

TABLE 1 The extraction recovery of levamisole and thiabendazole from plasma

Amount added	Extraction (ng/mℓ)	Accuracy recovery %	Precision (RSD %)
Levamisole			
100	89,12 ± 3,32	96,01	1,57
1 250	94,93 ± 2,82	98,04	2,67
3 750	94,61 ± 3,12	99,74	2,28
Thiabendazole			
1 250	93,67 ± 2,58		

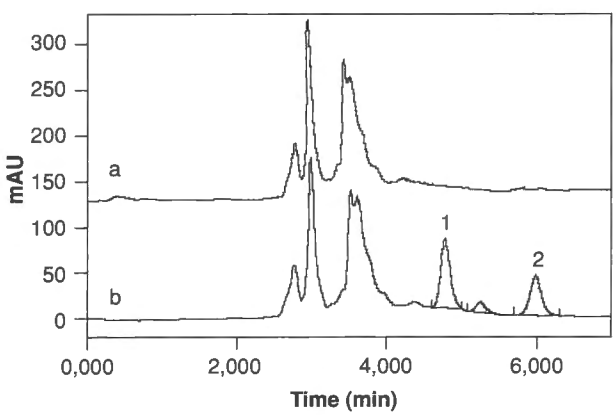


FIG. 2 Chromatograms of extracted blank plasma (a) and a sample (b) containing 486 ng/mℓ of levamisole (2) and the internal standard (1)

recovery (extraction yield), accuracy and precision are listed in Table 1. Calibration curves of peak-area ratio vs concentration were constructed by means of linear regression. Regression values of R^2 [0,9961; 0,9994], slope [0,00056; 0,00126] and y-intercept [–0,056; 0,047] were obtained during the study (n = 6,95% confidence intervals).

The limit of quantitation (lowest concentration where coefficient of variation is ≤ 15%) was 50 ng/mℓ, and the limit of detection (peak height four times baseline noise) was 1 ng/mℓ.

A phenyl reversed-phase column was found to give better peak shapes with much less tailing than was obtained on the more conventional C18 stationary phases. Representative chromatograms of extracted blank plasma collected prior to dosing, and a sample collected from a sheep 2 h after oral administration of a dose of 7,5 mg/kg of levamisole, are shown in Fig. 2. The retention times were 4,80 and 5,95 min for thiabendazole and levamisole, respectively.

No interference with the two analyte peaks was detected. The peak eluting at 5,3 min might have been a metabolite of levamisole, but this was not investigated.

CONCLUSION

The method described was suitable for the rapid, accurate and precise determination of levamisole in sheep plasma.

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