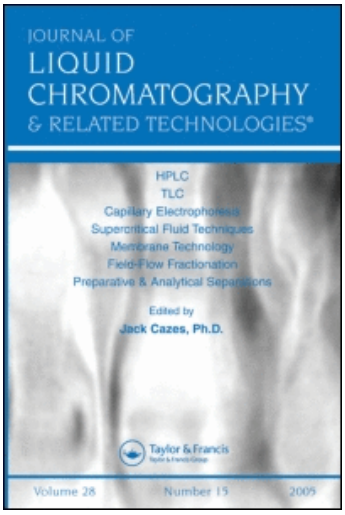

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HPLC Assay of Levamisole and Abamectin in Sheep Plasma for Application to Pharmacokinetic Studies

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Abstract: High performance liquid chromatography (HPLC) techniques were developed to quantify levamisole and abamectin in sheep plasma. UV detection (225 nm) for levamisole and fluorescence detection (excitation at 365 nm and emission at 470 nm) for abamectin were used. Separation was achieved on a C18 Prodigy ODS column with a mobile phase of phosphate (NaH_2PO_4 and Na_2HPO_4)-acetonitrile (60 : 40, v/v) (pH 7.5; 0.01M) for levamisole and methanol-acetonitrile-water (95 : 3 : 2, v/v/v) for abamectin. The retention times were 5.7 minutes for levamisole phosphate, 4.9 minutes for abamectin, 7.4 minutes internal standard (ivermectin). Calibration curves for levamisole phosphate and abamectin were linear over the range 0.05–10 μg/mL for levamisole phosphate and 0.25–20 ng/mL for abamectin, with the correlation coefficients for both drugs exceeding >0.999. The LOQ was achieved as the lowest point on the standard curve, 0.05 μg/mL for levamisole phosphate with the RSD 18.2% and 0.25 ng/mL for abamectin with the RSD 19.6%. The maximum intra-day and inter-day coefficients of variation were 9.1% and 15.0%, respectively, at 0.1 μg/mL (lowest concentration) for levamisole phosphate, and 11.9% and 19.3%, respectively, at 0.5 ng/mL (lowest concentration) for abamectin. Accuracies were 107.5% and 91.9%

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for levamisole and abamectin, respectively, at lowest concentrations of 0.1 μg/mL for levamisole phosphate and 1 ng/mL for abamectin. The recoveries from frozen and thawed plasma samples were 86.3% at 0.1 μg/mL for levamisole phosphate, and 105.8% at 2 ng/mL for abamectin. Both methods were successfully applied for analysis of levamisole and abamectin in plasma after subcutaneous injection to sheep of a formulation of medium chain mono- and diglyceride-propylene glycol-glycerol formal containing both levamisole phosphate and abamectin.

Keywords: High performance liquid chromatography, Levamisole, Abamectin, Validation, Plasma

INTRODUCTION

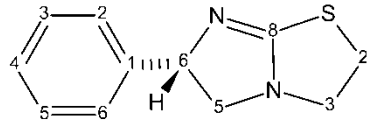
Levamisole (2,3,5,6-tetrahydro-6-phenylimidazole[2,1-b]thiazole) is an imidazothiazole derivative that has been used as an anthelmintic (Figure 1A). It is the levo-rotatory isomer of tetramisole an anthelmintic introduced in 1966.[1] The dextro isomer showed more adverse effects, so it has been removed in marketed preparations.[2]

Abamectin (avermectin B_{1a}) was introduced as an animal endoctocide in 1985 in Australia.[3] It was marketed as an injectable formulation in the same excipient as injectable ivermectin for use in cattle, and was administered at the same dose level of 200 μg/kg. The basic structure of the avermectins is a 16 membered lactone ring, with three main substituent groups: a hexahydrobenzofuran group, a disaccharide group at C-13, and a spiroketal ring (C-17 to C-28) (Figure 1B). Abamectin is at least 80% avermectin B_{1a} and not more than 20% avermectin B_{1b}. The a- and b-compounds have similar biological activities, therefore, they are not separated for commercial use.[4]

Several papers report the determination of levamisole[5–7] and avermectins[8–10] in biological fluids, including plasma, by HPLC. However, no reports have been found for HPLC determination of these two drugs in plasma after parenteral injection of levamisole and abamectin combinations. There was, therefore, a need for a HPLC technique for determination of levamisole and abamectin in plasma.

Tolan et al.[8] originally studied an analytical method for determination of avermectins in animal plasma, and demonstrated that analytical methods that measure the components involve HPLC separation and fluorescence detection of derivatives formed by converting the cyclohexene ring to an aromatic. The usually low dosages of avermectins require an extremely sensitive and selective analytical method for their determination in the plasma of dosed animals in support of pharmacokinetic studies. It has been demonstrated, that the sensitivity and selectivity of fluorescence detection in liquid chromatography makes this technique the preferred method of detection. The preparation of a fluorescent derivative of the avermectins for determination by HPLC was first described by Tolan et al.[8]

A



B

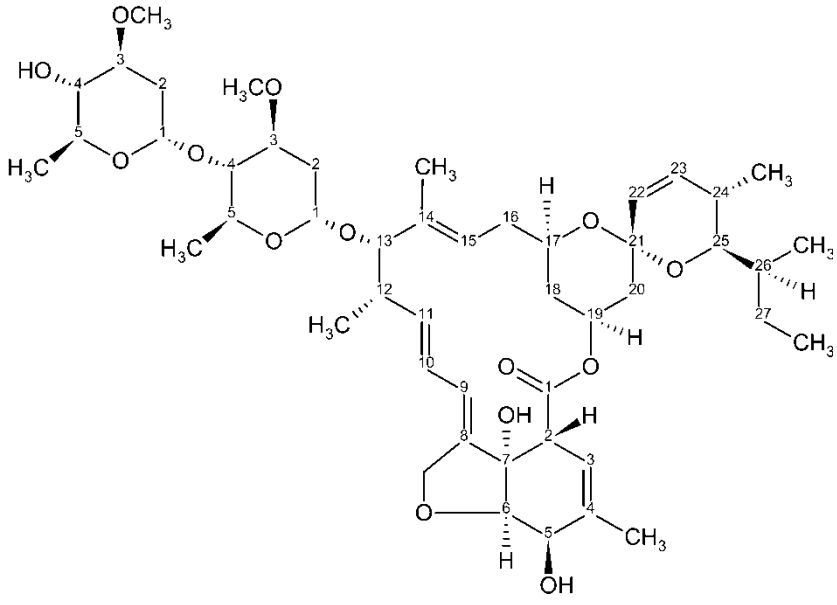


Figure 1. Chemical structure of levamisole (A) and abamectin (avermectin B_{1a}) (B).

The aim of this study was to develop a procedure for the isolation, derivatization, and determination of abamectin using HPLC fluorescence detection, and to isolate and determine levamisole in sheep plasma using HPLC-UV.

EXPERIMENTAL

Chemicals and Reagents

Ivermectin was purchased from Haimen Pharmaceutical Factory (China). Ivermectin was used as an internal standard. The derivatization reagents were trifluoroacetic anhydride (99%) obtained from Avocado Research

Chemicals Ltd, USA, and 1-methylimidazole (99%) obtained from Sigma-Aldrich Corporation, USA. The silylation reagent of dichlorodimethylsilane (99%) was purchased from Aldrich Chemical Co. Inc. (Sigma-Aldrich, Australia). Toluene and acetone were purchased from APS Finechem (Auckland, NZ). Drug free sheep plasma was provided by the Department of Laboratory Animal Science (University of Otago, Dunedin, NZ). HPLC grade acetonitrile and methanol were purchased from BHD Laboratory Supplies (England). Ultrapure analytical grade water for HPLC was produced by a Milli-Q Plus water system (Millipore Corporation, USA). Levamisole was supplied by Ancare, Auckland, New Zealand. Abamectin was purchased from Zhejiang Hisun Pharmaceutical, China.

Stock Solutions

Abamectin, ivermectin, and levamisole phosphate were weighed on an analytical balance (Mettler At201, Mettler-Toledo Ag, Switzerland) and dissolved in methanol to make separate stock solutions of 0.25 mg/mL abamectin, 1 mg/mL ivermectin (internal standard), and 0.2 mg/mL levamisole. Working standards for each concentration of the standard curve were prepared by serial dilutions of the stock solutions. All stock and standard solutions were stored at 4°C.

Analytical Procedures

Levamisole Analysis and Extraction Technique

Chromatographic separation for levamisole phosphate in sheep plasma was performed on a Shimadzu 10ATvp Pump (Japan), equipped with Shimadzu FCV-10Acvp low pressure mixer (Japan), Shimadzu 10ATvp Autosampler (Shimazdu, Japan), a SPD-10Avp UV-Vis Detector (Shimazdu, Japan), and Shimadzu Class-VP Workstation (Version 5.032) (Shimadzu, Japan). HPLC analysis was undertaken using a guard column (ODS 4 mm × 3.0 mm I.D, securityguard, Phenomenex, Torrance, CA, USA) and a C18 column (Prodigy ODS 250 × 4.6 mm, 5 μm, Phenomenex, Torrance, CA, USA) at ambient temperature. The mobile phase consisted of phosphate buffer (NaH_2PO_4 and Na_2HPO_4 pH 7.5, 0.01M)-acetonitrile (60 : 40, v/v). The flow rate of the mobile phase was 1.0 mL/min and the detection wavelength was 225 nm.

The extraction of levamisole phosphate from spiked and experimental sheep plasma was carried out by vortexing sheep plasma (0.4 mL) with 0.8 mL of acetonitrile. After vortexing for 30 seconds, the sample was centrifuged at 12000 rpm (Eppendorf 5810R, Germany), for 15 min. The supernatant was transferred to the auto sampler vial, and 50 μL was injected.

Abamectin Analysis and Extraction Technique

Chromatographic separation for abamectin and ivermectin in sheep plasma was carried out on a Shimadzu LC-6A pump equipped with HP 1046A programmable fluorescence detector (Hewlett Packard, USA), 20 μL injection loop, and HITACHI D-2500 chromato-integrator (Japan). HPLC analysis was undertaken using a guard column (ODS 4 mm × 3.0 mm I.D, security-guard, Phenomenex, Torrance, CA, USA), and a C18 column (Prodigy ODS 150 × 3.0 mm, 5 μm, Phenomenex, Torrance, CA, USA) at ambient temperature, and a methanol-acetonitrile-water (95 : 3 : 2, v/v/v) mobile phase at a flow rate of 1.0 mL/min. Abamectin and ivermectin were detected using an excitation wavelength of 365 nm and an emission wavelength of 470 nm.

The extraction of abamectin was performed by solid phase extraction (SPE). A 2 mL aliquot of sheep plasma, spiked with 200 ng ivermectin (100 μL × 2 μg/mL) as an internal standard, was precipitated by 2 mL of acetonitrile. After vortexing for 30 seconds, the sample was centrifuged at 4000 rpm (Eppendorf 5810R, Germany) for 10 min. The supernatant was loaded onto the Strata C18-E cartridge, which was previously conditioned with 2 mL methanol followed by 4 mL of water (HPLC grade). The cartridge was washed with 6 mL water. The analytes were eluted with 4 mL acetonitrile into silanized glass tubes and dried in a vacuum evaporator. After evaporation, the dried residue was reconstituted with 50 μL of acetonitrile, followed by 50 μL of 1-methylimidazole-acetonitrile solution (1 : 2.5, v/v). The derivatisation was initiated by adding 50 μL trifluoroacetic anhydride-acetonitrile solution (1 : 2.5, v/v), and shaking for 30 seconds at room temperature; 20 μL was injected for analysis.

HPLC Assay Validation Procedures

Calibration Curves and Linearity

To determine linearity, 0.4 mL of sheep plasma was spiked with levamisole phosphate to obtain 0.05, 0.1, 0.2, 0.5, 1, 2, 5, and 10 μg/mL standard concentrations, and 2 mL plasma was spiked with abamectin to obtain 0.25, 0.5, 1, 2, 5, 10, and 20 ng/mL standard concentrations. Ivermectin (200 ng) (100 μL × 2 μg/mL) was added as internal standard. The ratios of abamectin peak height to internal standard peak height and levamisole phosphate peak areas were plotted against standard concentrations to establish calibration curves and analyzed using least squares linear regression analysis in Excel 97. Analysis of variance (ANOVA) with a lack of fit test, which is designed to assess if the selected model (linear) is adequate to describe the observed data, was done using Minitab Release 12.1.

Precision and Accuracy

Intra-day and inter-day precision was evaluated at three different concentration levels: 0.1, 1, and 10 μg/mL for levamisole phosphate, and 0.5, 2, and 20 ng/mL for abamectin. The relative standard deviation (RSD, %) of each concentration was calculated to determine the precision of the method.

Accuracies of levamisole and abamectin from spiked plasma samples were determined at concentrations of 0.1, 1, and 10 μg/mL for levamisole phosphate, and 1, 5, and 20 ng/mL for abamectin. Six replicates for each spiked plasma sample for abamectin and levamisole were analysed during recovery experiments.

The stability of levamisole phosphate and abamectin in frozen and thawed plasma samples was studied. Levamisole phosphate and abamectin spiked samples at concentrations of 0.1, 1, and 10 μg/mL, and 2, 20, and 200 ng/mL, respectively, were stored in the freezer at −18°C for one week. The samples were thawed at 37°C, and analyzed.

Applicability of the Method in Pharmacokinetic Studies

A pharmacokinetic study (No: 001-4-MED-1101) was carried out in the female adult sheep (E. Frazian Texcel Cross). The study was approved on 3 December 2001 by Ancare Ethics Committee, Auckland, New Zealand. The animals received a subcutaneous injection of a formulation of a mixture of medium chain mono- and diglyceride (MCMDG), propylene glycol (PG), and glycerol formal (GF) containing 136.5 mg/mL levamisole phosphate and 5 mg/mL abamectin. Blood samples (20 mL) were taken from the jugular vein of each sheep into vacutainers containing heparin at 0, 0.25, 0.5, 1, 2, 3, and 6 hours post-injection and then daily for 1, 2, 3, 6, 9, 15, 21, and 27 days post-injection.

RESULTS

Chromatographic Procedure and Linearity

Representative chromatograms of levamisole and abamectin in plasma are shown in Figures 2 and 3. There were no interfering peaks of endogenous compounds in the chromatographic determinations. The HPLC method achieved good baseline separation of levamisole phosphate and abamectin at retention times less than 6 minutes for levamisole phosphate and 8 minutes for abamectin and internal standard (ivermectin).

Calibration curves for levamisole phosphate and abamectin were linear over the range 0.05–10 μg/mL for levamisole phosphate and 0.25–20 ng/mL

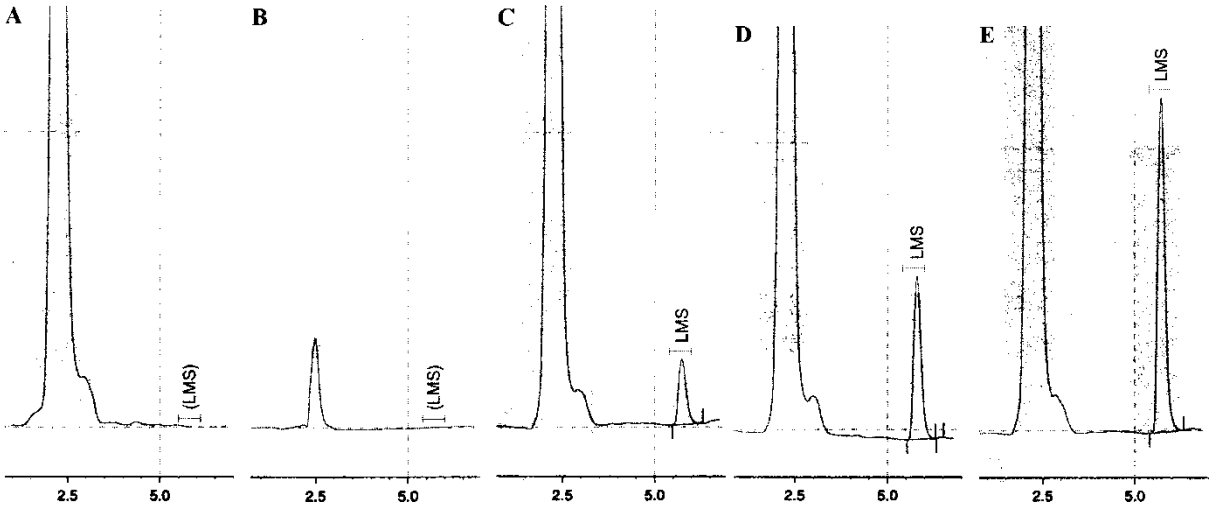


Figure 2. HPLC-UV (λ 225 nm) chromatograms. A) blank sheep plasma; B) blank plasma containing abamectin and ivermectin (abamectin and ivermectin 200 ng/mL); C) 2.0 μg/mL levamisole phosphate; D) 5.0 μg/mL levamisole phosphate; E) 10.0 μg/mL levamisole phosphate in sheep plasma. Retention time of levamisole phosphate varied from 5.71 to 5.79 min. LMS: levamisole phosphate.

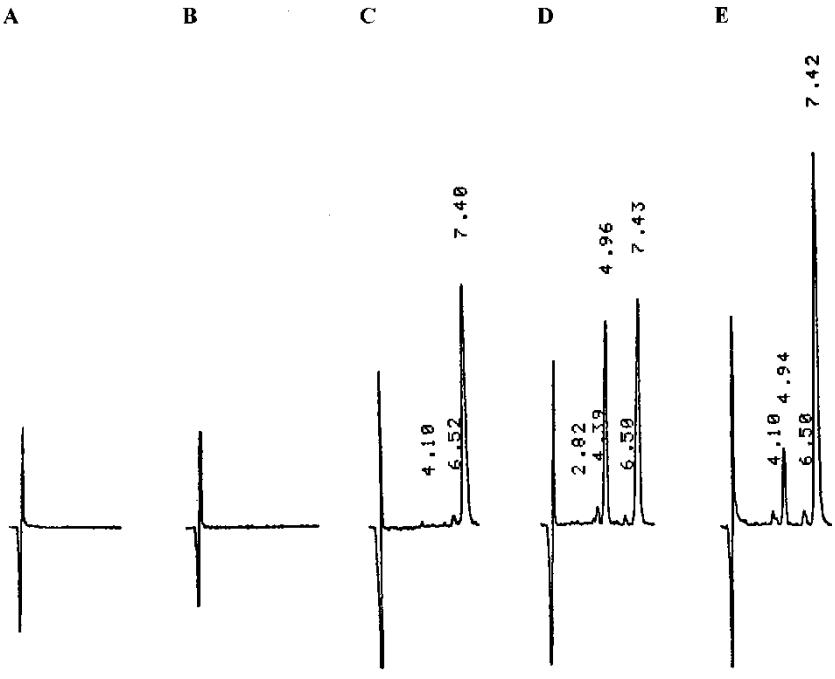


Figure 3. HPLC (fluorescence detection, E_x 365 nm, E_m 470 nm) chromatograms. A) blank sheep plasma; B) blank sheep plasma containing 10 μg/mL levamisole phosphate; C) internal standard (ivermectin, 50 ng/mL), D) 50 ng/mL abamectin and internal standard (ivermectin, 50 ng/mL); E) 20 ng/mL abamectin and internal standard (ivermectin, 100 ng/mL) in plasma. Retention times of abamectin and ivermectin varied from 4.94 to 4.96 min and 7.40 to 7.43 min, respectively.

for abamectin, with the correlation coefficients for both drugs exceeding >0.999 with no significant curvature (ANOVA, lack of fit $P > 0.05$).

The limit of quantitation (LOQ) was achieved as the lowest point on the standard curve, 0.05 μg/mL for levamisole with the RSD 18.2% ($n = 6$), and 0.25 ng/mL for abamectin with the RSD 19.6% ($n = 5$).

Precision and Accuracy

The maximum intra-day and inter-day precision was 15.0% for levamisole phosphate and 19.3% for abamectin (Table 1).

Data on accuracies showed that recoveries of levamisole phosphate and abamectin were 108 ± 12.7% ($n = 6$) and 91.9 ± 18.1% ($n = 6$), respectively, at the lowest concentrations (0.1 μg/mL for levamisole phosphate, and 1 ng/mL for abamectin) in spiked plasma samples (Table 2).

Table 1. Intraday and interday RSD of levamisole phosphate and abamectin in sheep plasma

	Levamisole phosphate			Abamectin	
Conc. added (μg/mL)	Conc. assayed (Mean ± SD) (μg/mL)	RSD (%)	Conc. added (ng/mL)	Conc. assayed (Mean ± SD) (ng/mL)	RSD (%)
Intraday					
0.1 (n = 6)	0.11 ± 0.01	9.1	0.5 (n = 5)	0.59 ± 0.07	11.9
1 (n = 6)	0.99 ± 0.02	2.0	2 (n = 5)	1.90 ± 0.28	14.7
10 (n = 6)	10.0 ± 0.10	1.0	20 (n = 5)	19.9 ± 0.68	3.4
Interday					
0.1 (n = 5)	0.10 ± 0.01	15.0	0.5 (n = 5)	0.57 ± 0.11	19.3
1 (n = 5)	1.01 ± 0.08	7.4	2 (n = 5)	1.98 ± 0.13	6.6
10 (n = 5)	10.0 ± 0.35	3.5	20 (n = 5)	18.9 ± 1.16	6.1

RSD: Relative standard deviation; Conc: Concentration.

Levamisole phosphate and abamectin stabilities in spiked plasma samples were studied. The recoveries from frozen and thawed plasma samples were 86.3 ± 5.8% at 0.1 μg/mL for levamisole phosphate and 105.8 ± 3.4% at 2 ng/mL for abamectin (Table 3).

Application of the Method for Pharmacokinetic Studies

The validated HPLC methods were used to analyse the plasma samples from the pharmacokinetic study in sheep. The plasma concentration time profiles of levamisole phosphate and abamectin for an individual sheep are shown in Figure 4. The rate constants of levamisole phosphate were calculated as

Table 2. Accuracies for levamisole phosphate and abamectin in spiked plasma samples

Levamisole phosphate			Abamectin		
Conc. added (μg/mL)	Conc. assayed (Mean ± SD) (μg/mL)	Recovery (%) (Mean ± SD)	Conc. added (ng/mL)	Conc. assayed (Mean ± SD) (ng/mL)	Recovery (%) (Mean ± SD)
0.1 (n = 6)	0.11 ± 0.01	108 ± 12.7	1 (n = 6)	0.92 ± 0.18	91.9 ± 18.1
1 (n = 6)	1.06 ± 0.09	106 ± 8.3	5 (n = 6)	5.4 ± 0.65	107 ± 13.0
10 (n = 6)	10.6 ± 0.67	106 ± 6.1	20 (n = 6)	18.7 ± 1.46	93.3 ± 7.3

Conc: Concentration.

Table 3. Recoveries of levamisole phosphate and abamectin from freeze-thawed plasma samples

Levamisole phosphate			Abamectin		
Conc. added (μg/mL)	Conc. assayed (Mean ± SD) (μg/mL)	Recovery (%) (Mean ± SD)	Conc. added (ng/mL)	Conc. assayed (Mean ± SD) (ng/mL)	Recovery (%) (Mean ± SD)
0.1 (n = 6)	0.09 ± 0.01	86.3 ± 5.8	2 (n = 4)	2.1 ± 0.07	105.8 ± 3.4
1 (n = 6)	1.06 ± 0.01	106 ± 1.3	20 (n = 5)	18.0 ± 1.50	90.2 ± 7.5
10 (n = 6)	10.5 ± 0.14	105 ± 1.4	200 (n = 5)	199.5 ± 3	99.7 ± 1.5

Conc: Concentration.

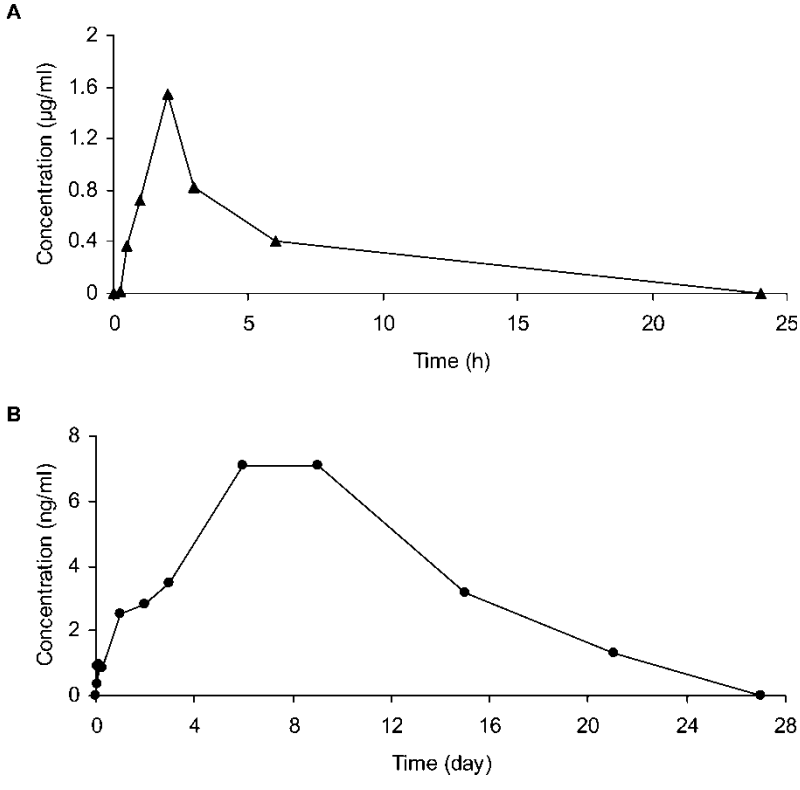


Figure 4. Plasma concentration time curves A) levamisole and B) abamectin for a sheep treated with a formulation containing 5.5 mg/kg of levamisole phosphate and 0.2 mg/kg of abamectin.

$0.53\ h^{-1}$ for absorption and $0.48\ h^{-1}$ for elimination. The absorption and the elimination rates of abamectin were calculated as $0.16\ day^{-1}$ and $0.15\ day^{-1}$, respectively. The maximum plasma concentration was 1.6 μg/mL at 2 hours post-dose for levamisole phosphate and 7.1 ng/mL at 6 hours post-dose for abamectin. $AUC_{(0\text{-}24)}$ was $8.3\ \mu g \cdot h \cdot mL^{-1}$ for levamisole phosphate, while $AUC_{(0-27)}$ was $92.6\ ng \cdot day \cdot mL^{-1}$. Detailed results of the full pharmacokinetic study will be published elsewhere.

DISCUSSION

Chromatographic Procedure

In this study, the retention times of abamectin and levamisole phosphate varied from 4.94 to 4.97 and 5.66 to 5.98 minutes, respectively, across all samples. The retention time of ivermectin (internal standard) was within 7.40–7.46 minutes.

The extraction procedure for levamisole phosphate was carried out by treatment of plasma with acetonitrile. This procedure produced a clear extract that could be injected directly in the HPLC without further purification. Therefore, the procedure permitted the rapid and selective determination of levamisole in sheep plasma.

In the present study, derivatization for abamectin and ivermectin was carried out at room temperature for 30 seconds. Tolan et al.[8] reported that the fluorescent derivatives were yielded at 105°C for 20 hours. It has been reported, that the derivatization reaction occurred at 95°C for 1 hour[9] and at 25°C for 30 seconds.[11]

The formulation administered into the sheep contained both levamisole phosphate and abamectin. Therefore, it was necessary to determine whether there were any interfering compounds in the chromatographic determinations. No interference from endogenous substances was observed in the chromatographic determinations (Figures 2 and 3).

Precision and Accuracy

The assay showed that the intra-day and inter-day precisions for levamisole phosphate and abamectin with a RSD of less than 20% were obtained at the lowest concentrations. Typically, the LOQ is defined as the concentration where the RSD is 20%.[12] The assay showed that the intra-day and inter-day precisions for levamisole phosphate and abamectin with a RSD of less than 20% were obtained, which comply with the requirements of CDER FDA.[12]

The recovery of levamisole phosphate was estimated as 108% with the RSD 11.8% at the lowest concentration (0.1 μg/mL). Abamectin recovery was determined as 91.9% with the RSD 19.7% at the lowest concentration (1 ng/mL).

The freeze/thaw consisted of storing at −18°C for one week and then thawing at 37°C. The data for accuracies for both drugs from freeze thawed plasma samples (Table 3) are comparable with those from spiked plasma samples (Table 2), suggesting that both drugs were stable during the freeze/thaw process investigated.

Garcia et al.[5] developed a method for levamisole in plasma using an ion-pair HPLC method in a reversed and isocratic phase at room temperature, using UV detection at 225 nm. A mobile phase of 0.2% (v/v) acetic acid in water-methanol (35 : 65, v/v) with Pic B7-low UV 0.005 M and adjusted to pH 4 with acetic acid, and C18 10 μm reversed-phase column (Bondapack C18 300 × 3.9 mm) were used. The LOQ for levamisole was 0.08 μg/mL and the average recovery was 73.6%.[5]

Du Preez and Lotter[7] determined levamisole in sheep plasma using ion-pair solid phase extraction (SPE) and reverse-phase HPLC. 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 acetonitrile and buffer (90 : 10, v/v). A phenyl reversed-phase column (Spherisorb S5 Phenyl, 250 × 4.6 mm) was used with a mobile phase of acetonitrile, 0.005 M of octane sulphonic-acid sodium salt, and 0.2% (v/v) triethylamine in water, pH 3.5, 38 : 62 (v/v). Extraction recoveries of 89–94% were achieved over the range 100–3750 ng/mL. Accuracy and precision were 96% and 2.6%, respectively, over this range, with a limit of quantitation of 50 ng/mL.[7]

Sahagun et al.[13] found the lowest quantifiable concentration of levamisole in goat plasma was 16 ng/mL, and calculated a RSD and extraction recovery of 4.52 ± 1.21% and 82.8 ± 6.12 %, respectively, using the assay developed by Garcia et al.[5] In this study, the LOQ for levamisole phosphate was 50 ng/mL with the RSD 18.2%. Precision was less than 15.0% at lowest concentration. The recovery achieved was 108 ± 13% at the lowest concentration (0.1 μg/mL) and 106 ± 6 at the highest concentration (10 μg/mL).

Tolan et al.[8] obtained a limit of quantitation of approximately 0.2 ng/mL with a RSD of 8% for ivermectin in bovine serum. The fluorescent derivatives of the avermectins were separated by HPLC on a 4.6 mm × 250 mm octadecylsilyl column (Zorbax ODS) using a mobile phase of acetonitrile-methanol-water (100 : 15 : 1, v/v), at a flow rate of 2.4 mL/min. Recovery of avermectin from plasma was 81%.[8] Tolan et al.[8] used ethyl acetate-chloroform as a solvent for the isolation of drugs from plasma using Florisil (magnesia-silica gel), and demonstrated that treatment of avermectins with acetic anhydride-pyridine (1 : 3, v/v) resulted in the fluorescent derivative at 105°C for 20 hours. In the present study, accuracy of abamectin was 91.9% with RSD 19.7% at concentration of 0.1 ng/mL, and LOQ achieved was 0.25 ng/mL with the RSD 19.6% using a SPE technique. The LOQ obtained in the present study was similar to that in the study conducted by Tolan et al.,[8] but the RSD reported by Tolan et al.[8] was lower than that in the present study.

A detection limit of less than 0.2 ng/mL ivermectin was achieved by a considerably simpler analytical procedure.[9] The RSD has been calculated by other workers at 6%,[14] 1.84%,[15] and 8%.[16] Lifschitz et al.[15] determined that the mean ivermectin recovery from plasma was 89.5% using the technique described by De Montigny et al.[11] The recovery of abamectin from horse plasma was 80%,[16] using the SPE technique with C_{18} cartridges.[10] De Montigny et al.[11] used acetonitrile and a Superclean LC18 cartridge for the extraction of ivermectin from spiked plasma, and demonstrated that the fluorescent derivatives were formed by reaction with a mixture of trifluoroacetic anhydride, and 1-methylimidazole in acetonitrile at 25°C for 30 seconds. In the present study, recovery for abamectin was higher than that in the studies conducted by De Montigny et al.[11] and Echeverria et al.[16] However, the RSD indicated in the studies conducted by Toutain et al.,[14] Lifschitz et al.,[15] and Echeverria et al.[16] was lower than that in the present study.

Application of the Methods

The validated methods have been successfully applied for pharmacokinetic studies analysing levamisole and abamectin in sheep plasma after subcutaneous administration of a MCMDG-PG-GF formulation[17] containing levamisole and abamectin. The contents of levamisole phosphate and abamectin in formulations were 136.5 and 5 mg/mL (27.3 : 1), respectively. The animals received a dose of 5.5 mg/kg body weight for levamisole phosphate and a dose of 0.2 mg/kg body weight for abamectin. Therefore, 2 mL/50 kg of the MCMDG-PG-GF formulation was injected into the sheep subcutaneously.

Figure 4 shows the plasma concentration time curves for levamisole and abamectin obtained in an individual sheep. Based on the pharmacokinetic analysis, a subcutaneous injection of the MCMDG-PG-GF formulation resulted in a longer t_{max} and slower absorption process for levamisole compared with those reported in previous studies[18,19] in sheep, and similar elimination profiles for abamectin compared to the commercial ivermectin formulation. These methods were suitable for the analysis of levamisole and abamectin in sheep plasma.

CONCLUSION

A HPLC assay method has been developed for determination of levamisole phosphate and abamectin in sheep plasma. The assay uses ivermectin as an internal standard for abamectin determination. Treatment with acetonitrile for levamisole, and the use of a SPE procedure for abamectin results in chromatograms free of interference. The procedures of both levamisole phosphate and abamectin have been validated, and the results demonstrate that the standard curve is linear over the concentration of 0.05–10 μg/mL levamisole phosphate and 0.25–20 ng/mL abamectin. The analysis requires 0.4 mL and

2 mL plasma for levamisole phosphate and abamectin, respectively. The LOQs are 0.05 μg/mL for levamisole phosphate and 0.25 ng/mL for abamectin, and the RSDs for both drugs are less than 20%. The method is suitable for pharmacokinetic studies of levamisole phosphate and abamectin in sheep after subcutaneous administration.

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