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Analytical procedure for the determination of eprinomectin in soil and cattle faeces

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ABSTRACT

A new analytical HPLC-fluorescence method was developed for the quantitative determination of eprinomectin (EPM) in soil and cattle faeces. EPM was extracted with acetone and acetonitrile from soil and cattle faeces, respectively. Solid phase extraction and derivatization reaction with N-methylimidazole in the presence of trifluoroacetic anhydride and acetic acid were applied. The limit of quantitation was 1 ng g^{-1} air dried soil and 2.5 ng g^{-1} moist cattle faeces. Overall recovery (RSD) was 89% (8) in soil and 85% (10) in cattle faeces and its good reproducibility (RSD < 15%) allows the application of the method in advanced ecotoxicological studies, required for the environmental fate assessment of EPM.

1. Introduction

Eprinomectin (EPM) is a member of the avermectins, belonging to a broader group of chemical compounds named macrocyclic lactones (MLs). MLs are natural fermentation products of soil dwelling *Streptomyces* bacteria and have found widespread application in human and animal health and crop protection [1–3]. Avermectins have a 16-membered macrocyclic ring, containing a spiroketal group, a benzofuran ring and disaccharide functionality. They are highly lipophilic substances and dissolve in most organic solvents. Their solubility in water is low (0.006–0.009 mg kg^{-1} [4]). Avermectins are acid, base and UV light (<280 nm) sensitive. They demonstrate high potency against a broad spectrum of endo- and ectoparasites of farm animals and agricultural mite and insect pests [4]. Moreover, monosaccharides, aglycones and many other reduced derivatives of avermectins demonstrate high potency. It was found that the EC_{90} for spider mites was from <0.005 to 0.1 mg kg^{-1} for ivermectin monosaccharide and aglycone derivatives [4]. EC_{90} for *Tetranychus urticae* was 0.038–4.0 mg kg^{-1} for various avermectin isomers in a foliage assay on bean leaves [4]. It has been proposed that the mode of action of MLs is based on their interaction with the receptor channels for inhibitory neurotransmitters [3]. Synthesis of 4″-epiacetylaminoavermectins from avermectin B1 resulted in an up to 1500-fold increase in potency [2].

EPM [(4″R)-4′-(Acetylamino)-4′-deoxy-avermectin B_1] is a mixture of two closely related homologues, EPM B1a (>90%; M.W.: 914) and EPM B1b (<10%; M.W.: 900) which differ by a methylene group in the C-25 position [2]. It is produced after modification of the naturally composed abamectin (avermectin B1a) and is licensed for the treatment of a broad spectrum of endo- and ectoparasites of cattle (e.g. gastrointestinal roundworms, lungworms, lice, grubs and mange mites). Eprinomectin is the active ingredient of Ivomec Eprinex. It has zero slaughter withdrawal and zero milk discard, due to low residuals in these matrices [5].

After administration (0.5 mg kg^{-1} body weight; applied along the middle line of the animal's back) EPM is excreted in the bile and faeces [5,6]. It is found that about 85.9% of the applied dose is excreted in faeces as unchanged drug [6]. Merck identified only one major, more-polar, 24a hydroxymethyl metabolite in cattle faeces which accounts for 7.4% of the total drug residues [6]. Also, 24a hydroxy, 26a hydroxymethyl and N-deacetylated metabolites were identified in rat faeces [5]. Current literature does not provide information about metabolites fate in soil.

EPM can cause detrimental effects on non-target species and can affect complex processes like dung degradation [7–16]. No observed effect concentration of eprinomectin in cattle faeces and for the dipera species *Neomyia cornicina* (L.) was found to be 7 ± 5 ng g^{-1} wet weight [15]. Faeces voided by cattle after they were treated with eprinomectin were found toxic for the common dung beetle *Onthophagus Taurus* [16]. Sun et al. [11] found that avermectin B1a could be present in the body of adult earthworm (*Eisenia fetida*). These researchers tested only LC_{50} which was found at 17.1 mg kg^{-1} for the exposure of 14 days. Halley et al. [10] also

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tested LC_{50} of eprinomectin in *Lumbricus terrestris* and found that 0.43 mg kg^{-1} faeces wet weight did not affect survival and weight gain of adult worms.

EPM could end up in soil directly with or from grazing livestock and indirectly through application of manure in agricultural land [6,12,17]. As a hydrophobic organic compound, it is expected to be strongly bounded in the soil [6]. But, it is rather difficult to assess movement and bioavailability of such compounds through the soil profile [18,19].

A detailed review of the quantification procedure for avermectins in biological matrices is presented by Danaher et al. [3]. Residues of avermectins in sample extracts are typically determined quantitatively directly by liquid chromatography (LC) with UV or mass spectrometric (MS) detection. Alternatively, avermectins can be derivatized pre-column to produce a fluorescent molecule before the determination by LC with fluorescence detection [3]. Fluorescence detection is the most commonly used technique and superior to LC/MS–MS detection with respect to cost and sensitivity [3,20–22].

Analytical methods have been reported for the determination of EPM in sheep faeces [13], in bovine faeces and urine [23] and in rabbit faeces [24]. Krogh et al. [25] had developed a multiresidue method for the determination of avermectins in soil, using LC–MS. It is possible that this method is not applicable to all laboratories due to lack of MS equipment. Also, recovery for eprinomectin is relatively low. The development of a low cost and easily applicable method for EPM in environmental matrices like soil and faeces is useful, due to high toxicity of EPM on non-target species living in soil and faeces.

The objective of our work was to develop a sensitive and selective analytical method for the determination of EPM in soil and cattle faeces, in order to be employed in advanced ecotoxicological studies, as those being established by the EMEA directives [26].

2. Materials and methods

2.1. Reagents

Acetonitrile and methanol of LiChrosolv quality, acetone of analytical grade, N-methylimidazole for synthesis and glacial acetic acid (100% purity) were supplied by Merck (Darmstandt, Germany). Trifluoroacetic anhydride (TFAA; 99% purity) was supplied by Sigma–Aldrich (Steinheim, Germany). Triethylamine (99.5% purity) was supplied by Panreac (Barcelona, Spain). Eprinomectin [(4″R)-4′-(Acetylamino)-4′-deoxy-avermectin B_1] solution in acetonitrile (99 ± 5 ng μL^{-1}; 95.5% purity; Cas no.: 123997-26-2) was supplied by Sigma–Aldrich (Steinheim, Germany). Doramectin [(25-Cyclohexyl-5-O-demethyl-25-de(1-methylpropyl) avermectin; Cas no.: 117704-25-3)], abamectin (Avermectin B1; Cas no.: 71751-41-2), ivermectin (22,23-Dihydroavermectin B1; Cas no.: 70288-86-7), moxidectin (Cas no.: 113507-06-5) and emamectin [(4″R)-4′-Deoxy-4′-(methylamino) avermectin B1 benzoate; Cas no.: 155569-91-8] were also supplied by Sigma–Aldrich (Steinheim, Germany), in order to examine possible interference of them to the determination of EPM and also to explore if a multiresidue determination could be based on the approach we followed.

2.2. Equipment

For the extraction of the EPM from soil and faeces, the mixing device Vortex Genie 2 (Scientific Industries Inc., Bohemia, NY, USA), the Raypa ultrasonic cleaner (Raypa, Barcellona, Spain) and the orbital shaker SSL1 (Stuart, Staffordshire, UK) were used, as well as the centrifuge 5800R (Ependorf, Hamburg, Germany). The J.T. Baker's Bakerbond SPE G12 device (Deventer, Holland) and C18 cartridges (Altech, Germany; C18, 8 mL, 500 mg) were used for the cleanup of the samples. Finally, the evaporator Reacti-therm III (Pierce Chem., Rockford, IL, USA) was used for evaporation under nitrogen.

2.3. Standard solutions

Working standard solutions of 500 and 100 ng mL^{-1} in acetonitrile were prepared on weekly basis. The first stock solution was made by dissolving 50 μL of the 100 ng μL^{-1} solution in 9.95 mL of acetonitrile. For the 100 ng mL^{-1} solution, 2 mL of the 500 ng mL^{-1} solution was diluted with 8 mL acetonitrile. These two solutions were used for preparing standards for calibration (0.25, 0.5, 1, 2, 4, 8, 10 ng 50 μL^{-1} injected) and for spiking soil (1, 2, 8, 16 ng g^{-1} wet weight) and (2.5, 5, 20, 40 ng g^{-1} wet weight) manure samples.

Moreover, standard solutions (2000 ng mL^{-1}) of doramectin, abamectin, ivermectin, moxidectin and emamectin were prepared in acetonitrile in order to examine possible peak interference of these avermectins with EPM in soil and cattle faeces.

2.4. Soil and cattle faeces sampling

Soil samples were collected from a clay loam Entisol (School of Agriculture research site, Aristoteleian University of Thessaloniki; 40°32′N, 22°59′E) not receiving herbicides for at least one year. The first 10 cm of the soil profile was collected. Soil was homogenized, air dried, sieved (2 mm) and maintained in 4 °C, in a plastic container.

Moist faeces samples were collected from pasture cattle in Galatista, Anthemountas region (Chalkidiki, Greece; 40°28′N, 23°16′E). These animals were not taking any kind of medical treatment for at least six months. Samples were immediately transferred to the laboratory, homogenized, placed in plastic bags and refrigerated at −20 °C.

2.5. Validation and stability

Validation control samples were prepared by spiking blank-drug free soil and faeces samples with EPM.

For the soil, 5 g was spiked with 5 ng (50 μL standard solution 100 ng mL^{-1}), 10 ng (20 μL standard solution 500 ng mL^{-1}), 40 ng (80 μL standard solution 500 ng mL^{-1}) and 80 ng (160 μL standard solution 500 ng mL^{-1}) of EPM. Then, 2 mL of methanol was added and followed by vortex-mixing for 2 min to assist spreading of the EPM homogenously in the soil mass [27]. Methanol was left to evaporate at room temperature and the sample was placed in 4 °C for 24 h.

For the moist faeces 2 g was spiked with 5, 10, 40 and 80 ng (same standard solutions as in soil). After spiking and vortex-mixing for 2 min, the sample was maintained at 4 °C at least for 24 h.

Intraday (four concentrations) and interday (three days) assays were performed in order to evaluate repeatability and reproducibility of the methods in soil and cattle faeces. Also, soil and faeces samples were spiked with doramectin, abamectin, ivermectin, moxidectin and emamectin in order to examine possible peak interference of these avermectins with EPM.

Stability of the fluorescent molecule of EPM was tested in standard samples, in soil and faeces samples extracts left in room temperature (22–24 °C) and in 4 °C for 24 h.

After assessment of EPM stability throughout daily analysis, two more experiments were conducted. In the first experiment the stability of EPM in soil and faeces after storage at −84 °C was determined. In the second experiment blank soil and cattle faeces

samples fortified at 8 ng g^{-1} (n = 8) and 20 ng g^{-1} (n = 8), respectively, underwent a three freeze-thaw cycle.

2.6. HPLC conditions

The method was developed on a Shimadzu LC-10A series chromatographic system (Shimatzu Corporation, Kyoto, Japan). The system consisted of a Model CBM-10A controller unit, a Model DGU-2A degasser, two Model LC-10AD piston pumps, a Model SIL-10A$_{XL}$ autosampler, a Model CTO-10A column oven and a Model RF-551 fluorescence detector, its excitation and emission wavelengths set at 365 and 470 nm, respectively. The sensitivity of the detector was set at "High" and the response time was at 1.5 s. Integration was performed with the use of the Class-LC10 program (version 1.41, Shimadzu). Separation was achieved on an MZ-Analysentechnik (Mainz, Germany) Spherisorb ODS-2 (250 mm × 4 mm i.d., 5 μm particle size), C_{18} reversed-phase analytical column. The LC system operated isocratically. Column temperature was maintained at 31 °C and the mobile phase was 47.5:47.5:6.0 (v/v/v) acetonitrile/methanol/water, at a flow rate of 1.0 mL min^{-1} [13]. Injection volume was 50 μL.

2.7. Extraction procedure for soil samples

Eprinomectin was extracted by adding 10 mL of acetone, vortexing for 2 min, placing the sample in the ultra sonic bath for 15 min [27] and then to the centrifuge (2800 rpm, 20 °C, 10 min). This procedure was repeated twice and 16 mL of 20 mL of acetone was diluted with 50 mL of redistilled water and 50 μL of triethylamine was added. The sample was transferred to the C18 cartridge, which was previously activated with 10 mL acetone and 10 mL acetone/water (3:7, v/v). After application of the sample at a flow rate of 3 mL min^{-1}, the cartridge was immediately washed with acetone/water (1:1, v/v) at a flow rate of 8 mL min^{-1}. The analyte was eluted with 5 mL of methanol, under gravity, collected in a polypropylene test tube and evaporated, under a gentle stream of nitrogen, to dryness at a 60 °C water bath. The test tube was left at room temperature for 5 min.

The sample was then derivatized following the procedure introduced by Danaher et al. [28]. First, 225 μL of N-methylimidazole/acetonitrile (2:7, v/v) was added. The tube was stoppered and vortexed for 3 min. Then, 225 μL of TFAA/acetonitrile (2:7, v/v) was added and the tube was stoppered and vortexed for another 2 min. Finally, 50 μL of glacial acetic acid was added and the tube was stoppered and vortexed for another 1 min.

The sample was then placed for 30 min to 65 °C and after that to 4 °C for approximately 12 h. After this it was transferred in autosampler vials and 50 μL was injected in the HPLC system.

2.8. Extraction procedure for cattle faeces samples

The extraction procedure was based on the research work of Erzen et al. [13]. Change was made in some points, especially in cleanup and derivatization, in order to adjust the method to our laboratory conditions. Eprinomectin was extracted from the sample of 2 g faeces with 25 mL acetonitrile, vortexing for 2 min and then shaking for 40 min at 300 rpm. After centrifugation (2100 × g; 10 min; 20 °C) a 15 mL portion of the extract was diluted with 50 mL ultra pure water and 50 μL triethylamine was added. The sample was transferred to the C18 cartridge, which was previously activated with 10 mL acetonitrile and 10 mL acetonitrile/water (3:7, v/v). After application of the sample at a flow rate of 3 mL min^{-1}, the cartridge was immediately washed with acetonitrile/water (1:1, v/v) at a flow rate of 8 mL min^{-1}. The analyte was eluted with 5 mL of acetonitrile, under gravity, collected in a polypropylene test tube and evaporated, under a gentle stream of nitrogen, to complete dryness at a 60 °C water bath. The test tube was left at room temperature for 5 min. The derivatization procedure was exactly the same as described for soil. The sample was then placed for 30 min to 65 °C and after that to 4 °C for approximately 12 h. The sample was then transferred to a vial and 50 μL was injected to the HPLC system.

2.9. Determination

Calibration curves were obtained by running a total of 7 standard working solutions and plotting the recorded peak heights versus the corresponding mass of EPM injected, using the least square method. The calibration curve covered the entire range of concentrations (0.25–10 ng 50 μL^{-1} injected).

2.10. Statistical analysis

The Statgraphics Centurion-v. XV (StatPoint, Warrenton, VA, USA) software was used for data evaluation and analysis. First, the Grubb's outliers test [29] was performed for each concentration validated for standard solutions of EPM and for EPM in faeces and soil samples. One-way ANOVA test was used for evaluating differences between the same concentrations tested through three different days of validation. A t-test was used to compare means of the same concentration through the 24 h stability experiments. Also, the t-test was used to evaluate the effect of the three freeze-thaw cycle in EPM stability in soil and cattle faeces, respectively.

3. Results and discussion

3.1. Extraction, cleanup and derivatization

The goal when developing a cleanup procedure in matrices like soil and faeces is to achieve maximum possible recovery, combined with minimum interferences from endogenous compounds or reagents used to extract the analyte(s). Sufficient extraction in soil samples was achieved with acetone by twice repeating the extraction procedure. The method described by Erzen and Flajs [30] for the extraction of abamectin and doramectin from soil was not reproducible for EPM in our laboratory. Also, the method described for the extraction of EPM in cattle faeces is not applicable to soil samples (recovery <50%). Acetonitrile was used for the extraction of EPM in cattle faeces [13]. Methanol, n-hexane and methanol/acetonitrile 1:1 (v/v) were also tested in preliminary trials but insufficient extraction was observed (for the first two recovery <60%).

Cleanup was observed to be better after using C18 SPE cartridges than using C8 cartridges both in soil and in faeces samples (P = 0.07, n = 4; 8 ng g^{-1} soil and P = 0.09, n = 6; 20 ng g^{-1} faeces). Recovery using C18 SPE cartridges was always between 75 and 100% (Table 1) while using C8 columns was 40–60%. Problems with EPM recovery (<60%) were observed when sample flow in C18 cartridges, both for soil and for faeces, was above 6 mL min^{-1} and especially for concentrations bellow 20 ng g^{-1}. Finally, preliminary trials were conducted to select the appropriate organic solvent for eluting EPM from SPE cartridges. The best possible results were achieved after using methanol (among acetonitrile, ethyl acetate, chloroform, methanol and methanol/acetonitrile 1:1 (v/v) tested) in soil samples and acetonitrile in faeces (among n-hexane, ethyl acetate, chloroform, methanol and methanol/acetonitrile 1:1 (v/v) tested; <70% recovery). Selecting as elution solvent methanol for soil samples and acetonitrile for faeces samples, lead to enhanced reproducibility of the procedure in our laboratory.

In preliminary trials, we followed the derivatization procedures described by Danaher et al. [28], Erzen et al. [13] and Berendsen et al. [21]. The procedure described by Danaher et al. was selected,

Table 1
Results from intraday and interday assays of recovery of eprinomectin from soil (1, 2, 8, 16 ng g^{-1}) and cattle faeces (2.5, 5, 20, 40 ng g^{-1}).

		Fortification level, ng g^{-1}							
		Soil				Faeces			
		1	2	8	16	2.5	5	20	40
Over all (n = 18)	Recovery %	91	87	89	88	84	83	92	85
	SD	10	8	6	5	8	6	5	6
	cv %	11	9	6	6	9	7	5	7
Reproducibility (n = 6)									
Day 1	Recovery %	89	89	91	92	83	86	93	87
	SD	11	4	4	5	9	6	6	8
	cv %	13	5	4	5	11	7	6	10
Day 2	Recovery %	92	82	89	89	84	80	90	84
	SD	14	9	4	4	6	6	3	6
	cv %	15	11	5	5	7	7	4	7
Day 3	Recovery %	91	93	86	84	87	84	93	85
	SD	6	7	7	5	10	7	4	5
	cv %	6	8	8	6	11	8	5	6
Repeatability (n = 6)	Recovery %	91	88	88	88	85	82	92	85
	SD	1	5	2	3	7	8	4	8
	cv %	1	5	2	4	3	1	1	1

offering less relative standard deviation (<15%) intra and between days, especially in small concentrations of eprinomectin. Relative standard deviation for the derivatization procedure described by Erzen et al. and Berendsen et al. was >35 and >25%, respectively. Moreover, the creation of the derivative was not always successful in our laboratory after applying the derivatization procedures of Erzen et al. (10% failure) and Berendsen et al. (5% failure).

Also, using polypropylene test tubes rather than glass tubes gave better results for the derivatization procedure. Especially for concentrations close to limit of detection the polypropylene tubes gave significantly higher (t-test for comparing average heights) and sharper peaks. Moreover, with polypropylene tubes failure of derivative creation was less than using glass tubes.

3.2. Validation

The linearity of the detector response for eprinomectin was evaluated by injecting seven standards (0.25–10 ng 50 μL^{-1} injected), thus covering the entire working range of the assay. This procedure was performed eight times for soil and seven times for cattle faeces. Least squares linear regression and best fit analysis of the data obtained showed the detector response for eprinomectin to be linear. The calibration curves for standards in pure solvent provided correlation coefficients which always exceeded 0.99. Limit of detection for standard solutions was 0.25 ng 50 μL^{-1} injected, the lower concentration tested.

3.3. Soil

The results from the interday and intraday assays of recovery of EPM from soil are presented in Table 1. Fig. 1 presents a blank chromatograph and one spiking with 8 ng g^{-1} soil, wet weight. Recovery ranged from 82 to 92. Average recovery of all avermectins in the work of Krogh et al. [25] in soil was 64%.

There were not significant differences between each of the four concentrations tested in three different days [1 ng g^{-1} (n = 24; P = 0.89); 2 ng g^{-1} (n = 24; P = 0.16); 8 ng g^{-1} (n = 18; P = 0.18); 16 ng g^{-1} (n = 18; P = 0.18)]. Limit of detection and limit of quantification were both 1 ng g^{-1} in soil, wet weight, both determined in real samples not theoretically. Krogh et al. [25] theoretically estimated LOQ 0.5–2.5 ng g^{-1} for seven avermectins. Stability of eprinomectin is presented in Table 2. EPM and EPM derivative product proved to be stable. We found that there are not peak interferences between EPM and five other avermectins (Fig. 2). Finally, the method could be further developed to multiresidue determination of avermectins in soil. There is a good separation of all substances and recoveries were found to be above 70%.

3.4. Cattle faeces

In Table 1, the results from intraday and interday assays of recovery of eprinomectin from moist cattle faeces are presented. Fig. 3

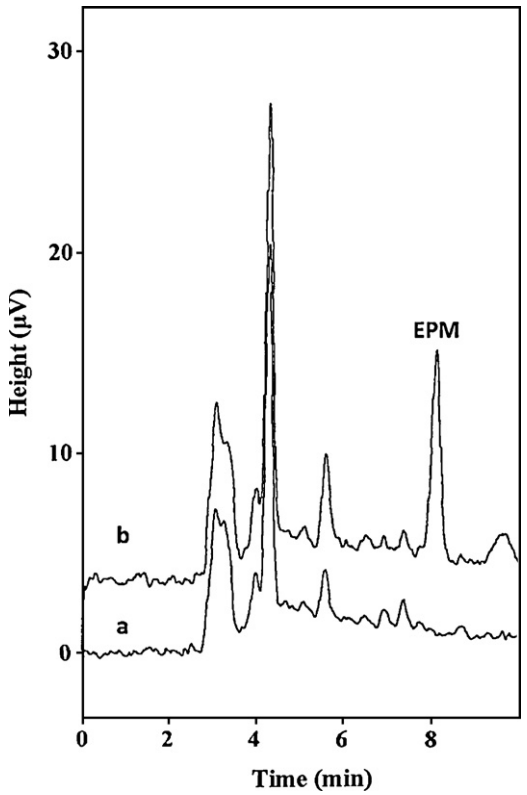

Fig. 1. Chromatogram obtained from soil samples: (a) blank and (b) 8 ng g^{-1}.

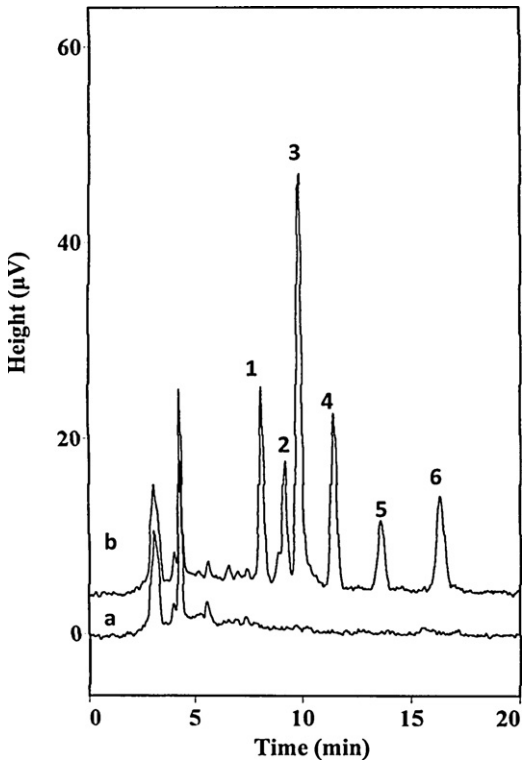

Fig. 2. Chromatogram obtained from soil samples: (a) blank and (b) containing the avermectins: eprinomectin (1), moxidectin (2), emamectin (3), doramectin (4), abamectin (5) and ivermectin (6).

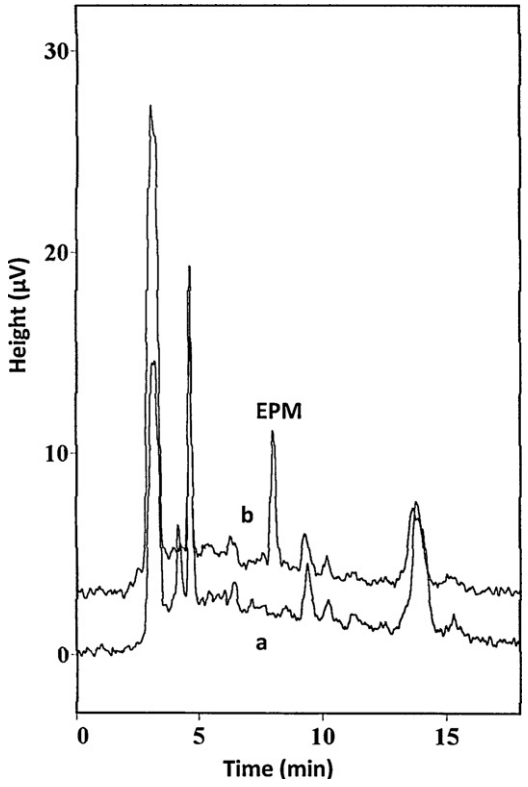

Fig. 3. Chromatogram obtained from cattle faeces samples: (a) blank and (b) 20 ng g^{-1}.

Table 2
Stability of EPM derivative in soil samples after 24 h waiting time in the autosampler (room temperature, approximately 22 °C), after 24 h waiting in 4 °C and stability of EPM after 2 months storage at −84 °C and after a three freeze-thaw cycle.

EPM added, ng g^{-1}	Mean concentration found, ng g^{-1} (SD)		*P*-value
	0 h	24 h	
Autosampler			
1	0.89 (0.12)	0.92 (0.14)	0.70
2	1.65 (0.19)	1.73 (0.09)	0.33
8	7.33 (0.31)	7.02 (0.34)	0.13
16	14.64 (0.77)	14.20 (0.70)	0.37
4 °C			
1	0.91 (0.12)	0.92 (0.06)	0.90
2	1.74 (0.18)	1.86 (0.14)	0.25
8	6.50 (0.39)	6.84 (0.56)	0.26
16	13.17 (0.64)	13.78 (0.33)	0.65

EPM added, ng g^{-1}	Mean concentration found, ng g^{-1} (SD)		*P*-value
	0 h	2 months	
−84 °C			
16	14.67 (2.38)	14.32 (1.68)	0.34

EPM added, ng g^{-1}	Mean concentration found, ng g^{-1} (SD)		*P*-value
	Before	After	
Three freeze-thaw cycle			
8	7.33 (0.31)	7.34 (0.52)	0.97

presents a blank chromatograph and one for 20 ng g^{-1} moist cattle faeces. Recoveries were between 84 and 92%, greater than those of Erzen et al. [13] which were between 78.8 and 87.1% and Jiang et al. [23] which were 79–86%. Limit of detection (LOD) and quantification (LOQ) for cattle faeces were 2.5 ng g^{-1} wet weight, the

Table 3
Stability of EPM derivative in spiked faeces samples after 24 h waiting time in the autosampler (room temperature, approximately 22 °C), after 24 h waiting in 4 °C and stability of EPM after 2 months storage at −84 °C and after a three freeze-thaw cycle.

EPM added, ng g^{-1}	Mean concentration found, ng g^{-1} (SD)		*P*-value
	0 h	24 h	
Autosampler			
2.5	2.07 (0.22)	2.08 (0.33)	0.73
5	4.52 (0.39)	4.31 (0.53)	0.31
20	18.61 (1.20)	17.98 (0.63)	0.21
40	34.96 (3.33)	34.24 (2.32)	0.67
4 °C			
2.5	2.09 (0.14)	2.17 (0.24)	0.46
5	3.51 (0.96)	3.54 (0.27)	0.14
20	17.48 (1.67)	17.98 (0.43)	0.21
40	33.53 (1.78)	34.06 (2.89)	0.78

EPM added, ng g^{-1}	Mean concentration found, ng g^{-1} (SD)		*P*-value
	0 h	2 months	
−84 °C			
40	34.29 (2.49)	36.95 (2.66)	0.09

EPM added, ng g^{-1}	Mean concentration found, ng g^{-1} (SD)		*P*-value
	Before	After	
Three freeze-thaw cycle			
20	17.98 (0.63)	17.77 (0.77)	0.08

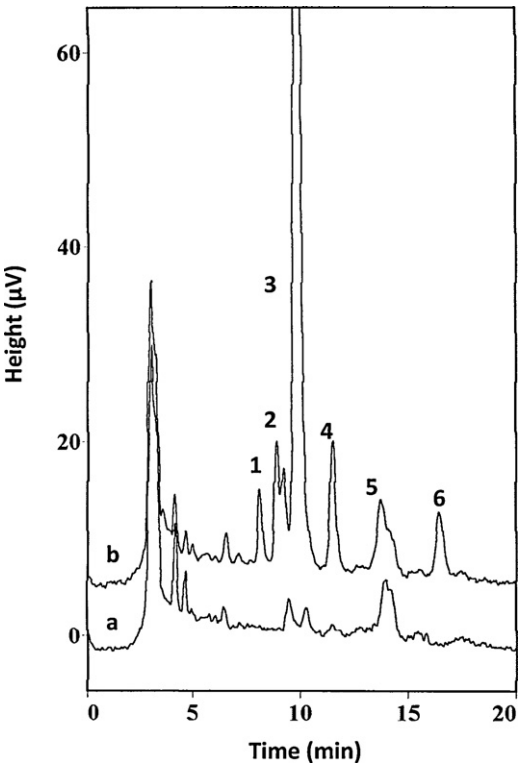

Fig. 4. Chromatogram obtained from cattle faeces samples: (a) blank and (b) containing the avermectins: eprinomectin (1), moxidectin (2), emamectin (3), doramectin (4), abamectin (5) and ivermectin (6).

lower concentration tested. There were not significant differences between each of the four concentrations tested in three different days [2.5 ng g^{-1} (n = 20; P = 0.36); 5 ng g^{-1} (n = 21; P = 0.19); 20 ng g^{-1} (n = 22; P = 0.31); 80 ng g^{-1} (n = 15; P = 0.69)]. Results dealing with stability of EPM derivative are presented in Table 3. The EPM derivative proved to be very stable. Finally, we found that there are not peak interferences between EPM and five other avermectins (Fig. 4).

Erzen et al. [13] use an extra cleanup step with n-hexane when eprinomectin is less than 10 ppb in order to achieve recoveries higher than 80%. We found that this step is not necessary when the sample flow in the SPE C18 cartridges is 2–4 mL min^{-1}, and the elution solvent is completely and very carefully dried with N_2. Eliminating this step from the cleanup procedure reduces time and organic solvent consumption per sample.

3.5. Selectivity

The method was checked to ensure that there was no interference with EPM from matrix co-extractives. Chromatograph obtained from blank soil and faeces samples showed that the peak attributable to EPM was resolved sufficiently from other peaks (Figs. 1 and 3). Additionally, five structurally related compounds were tested for non-interference in the method. Neither compound interfered in the chromatographic analysis of EPM (Figs. 2 and 4).

4. Conclusions

A new analytical method for the determination of EPM in soil and cattle faeces was developed. The low detection and quantification limits, the repeatability and reproducibility allow the application of the methods in studies concerned with the environmental fate of EPM.

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