SIMULTANEOUS DETERMINATION OF ABAMECTIN AND DORAMECTIN IN SOIL FROM A GRAZED PASTURE†

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Abstract

The use of antihelmintics in veterinary medicine, especially avermectins (ivermectin, abamectin, doramectin) has been increasing rapidly in recent years. They cause a serious ecotoxicological problem because of their specific metabolism and their action on non-target organisms.

An analytical procedure for simultaneous determination of abamectin and doramectin in soil from a grazed pasture has been developed. Classical extraction procedure for extraction of both avermectins from soil, a solid phase extraction (SPE) for the clean-up of extracts and a high performance liquid chromatography (HPLC) with fluorescence detection were introduced in the analytical procedure.

Low detection limit (0.5 ng/g of dry soil), good selectivity, recovery of the method in a range of 71-96%, good repeatability (>90%) and limit of quantification of 0.7 and 1.5 ng/g of dry soil for abamectin and doramectin, respectively, enables the determination of abamectin and doramectin in soil.

The developed analytical method can be used for monitoring of soil contamination with abamectin and doramectin and also for eco-toxicological studies of avermectins.

Introduction

Avermectins (ivermectin, abamectin and doramectin), class of 16-membered macrocyclic lactones, are very efficient against a number of nematode and arthropod parasites of cattle, horses, swine and sheep.[1,2] They are fermentation products of the soil-dwelling microorganisms *Streptomyces avermitilis* and are active at very low concentrations. Abamectin consists of a mixture of not less than 80% of avermectin B_{1a} and not more than 20% of avermectin B_{1b}. The major component is the marker substance for abamectin. Abamectin is highly lipophilic and freely soluble in most organic solvents but is poorly soluble in water. It is stable at room temperature in nonacidic solutions but is degradated by UV light. Doramectin differs from abamectin in having a cyclohexyl substituent in the C-25 position. The structure of the major component B_{1a} of abamectin and doramectin is presented in Figure 1.

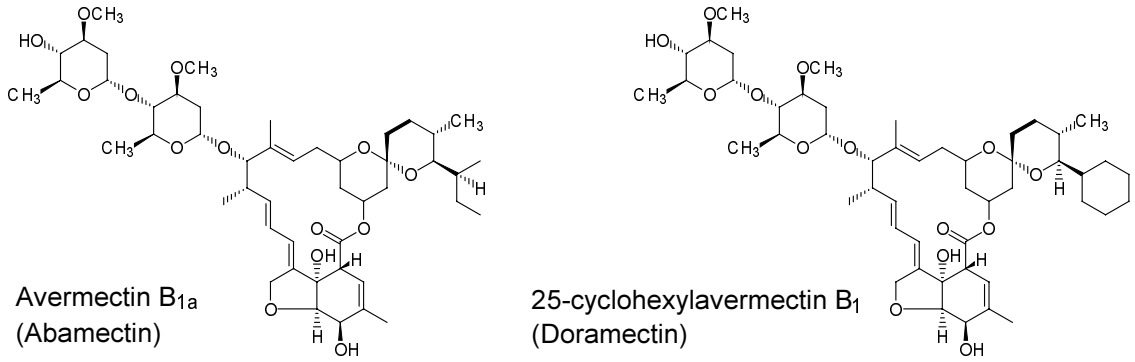

Figure 1. The structure of the major component B_{1a} of abamectin and doramectin.

The most problematic characteristic of the avermectins is that they are eliminated mostly through feces (98%) in non-metabolised, active form. They are very stable in the environment. Time of elimination and toxicity to the non-target species are also very problematic. For that reasons, knowledge of their time of elimination from treated animals, possibility of their bio- and photo-degradation and their distribution in the environment is very important. To study the environmental effects and fate of avermectins, ecological studies have to be performed under laboratory and outdoor conditions. A highly sensitive and specific analytical method for the determination of abamectin and doramectin in soil has to be developed. Several analytical methods for determination of avermectin residues in animal muscle tissue,[3-5] liver,[6-9] plasma,[10,11] milk[12] and feces[13,14] are known. No method has been published for determination of avermectins in soil.

The aim of our work was to develop a sensitive and selective analytical method for simultaneous determination of abamectin and doramectin in soil from the grazed pasture. The method involved extraction using organic solvents, clean-up by solid phase extraction (SPE), derivatization of the sample extracts and determination of abamectin and doramectin using a high-performance liquid chromatography (HPLC) with fluorescence detection.

Results and discussion

A sensitive and selective analytical method for determination of abamectin (B_{1a} component) and doramectin in soil from the grazed pasture has been developed. An extraction of abamectin and doramectin from soil samples, clean-up of extracts using

solid phase extraction and a determination by high-performance liquid chromatography with fluorescence detection were employed in the analytical procedure. The optimal clean-up procedure and the enrichment of abamectin and doramectin in soil samples were slightly modified, on the basis of the HPLC method for the determination of avermectins and moxidectin in animal liver published by Danaher et al.,[8] to achieve good recovery, selectivity and sensitivity of the method. The analytical procedure is presented in detail in a section Experimental. The clean-up of extracts is the most critical and difficult step of the residual analysis. Due to the possible matrix interferences, extracts have to be appropriately cleaned-up. Various solid phase extraction cartridges i.e. Merck LiChrolut RP-18 endcaped (500 mg; 3 mL), Merck LiChrolut SI 60 (500 mg, 3 mL), Merck LiChrolut RP-select B (500 mg, 3 mL), Varian Bond Elut Al-N (500 mg, 3 mL) and J.T. Baker Bakerbond spe Octyl (C_8) (500 mg, 6 mL) and various types of eluent were employed in the clean-up procedure. The best recovery was achieved using Varian Bond Elut Al-N (500 mg, 3 mL) extraction cartridges and elution with methanol and ethyl acetate in a volume ratio of 70 : 30.

The optimal chromatographic separation, selectivity and sensitivity were tested with various analytical columns i.e. LiChrospher 60 RP-Select B (250 x 4.6 mm ID; 5 μm particle size); Hypersil ODS (120 x 4.0 mm ID; 5 μm particle size); Chromolith Performance RP-18e (100 x 4.0 mm ID); BetaBasic-4 (150 x 4.6 mm ID; 5 μm particle size) and Supelcosil LC-8-DB (250 x 4.6 mm ID; 5 μm particle size) and with different combinations of solvents composing a mobile phase (methanol, acetonitrile, water). The optimal chromatographic separation, selectivity and the highest sensitivity were achieved using the Supelcosil LC-8-DB column (250 x 4.6 mm ID; 5 μm particle size) with the guard column Supelguard LC-8-DB (20 x 4.6 mm ID; 5 μm particle size) and a mobile phase composing of methanol, acetonitrile and water in a volume ratio of 47.5 : 47.5 : 6.0 and at a flow rate of 1.1 mL/min.

The detection limit (LOD) of standards was tested at the optimal chromatographic conditions, by injection of abamectin and doramectin standard solutions in concentrations below 50 ng/mL prepared in acetonitrile. LOD was 0.25 ng/mL for both, abamectin and doramectin. The linearity of the method was in the range from 0.5 to 500 ng/mL.

To determine the recovery of the method, blank samples of moist soil were spiked with abamectin and doramectin in a concentration of 2.5, 5.0, 10, and 20 ng/g (intra day analysis). Table 1 and Table 2 represent recovery and repeatability of determination of abamectin and doramectin in soil, respectively.

The recoveries, tested with addition of abamectin and doramectin in a concentration of 2.5, 5.0, 10, and 20 ng/g of moist soil, were between 71.9-91.0% for abamectin (Table 1) and between 70.6-96.3% for doramectin (Table 2).

Table 1. Recovery and repeatability of determination of abamectin in moist soil from the grazed pasture (addition of abamectin in a concentration of 2.5, 5.0, 10, and 20 ng/g).

abamectin in soil from the grazed pasture							
addition of 2.5 ng/g		addition of 5.0 ng/g		addition of 10 ng/g		addition of 20 ng/g	
found (ng/g)	recovery (%)	found (ng/g)	recovery (%)	found (ng/g)	recovery (%)	found (ng/g)	recovery (%)
2.36	94.5	4.10	80.2	7.43	74.3	16.1	80.7
2.53	101	3.93	78.7	7.43	74.3	14.7	73.7
2.06	82.4	3.86	77.1	7.43	74.3	15.0	74.8
2.14	85.9	3.80	75.9	6.57	65.7	12.6	63.1
2.86	91.4	3.72	74.4	6.42	64.2	14.0	70.1
mean±s (%)	91.0 ± 7.3	mean±s (%)	77.3 ± 2.3	mean±s (%)	71.9 ± 5.3	mean±s (%)	72.5 ± 6.5
RSD (%)	8.0	RSD (%)	3.0	RSD (%)	7.3	RSD (%)	9.0

Table 2. Recovery and repeatability of determination of doramectin in moist soil from the grazed pasture (addition of doramectin in a concentration of 2.5, 5.0, 10, and 20 g/g).

doramectin in soil from the grazed pasture							
addition of 2.5 ng/g		addition of 5.0 ng/g		addition of 10 ng/g		addition of 20 ng/g	
found (ng/g)	recovery (%)	found (ng/g)	recovery (%)	found (ng/g)	recovery (%)	found (ng/g)	recovery (%)
2.43	97.5	4.40	88.1	8.07	80.7	17.1	85.5
2.65	106	4.17	83.5	7.37	73.7	15.6	78.2
2.27	91.1	4.08	81.6	7.48	74.8	16.0	80.0
2.28	91.2	4.00	80.0	6.31	63.1	13.6	67.7
2.39	95.8	3.91	78.1	7.01	70.1	14.9	74.5
mean±s (%)	96.3 ± 6.1	mean±s (%)	82.3 ± 3.8	mean±s (%)	70.6 ± 5.1	mean±s (%)	77.2 ± 6.6
RSD (%)	6.3	RSD (%)	4.6	RSD (%)	7.2	RSD (%)	8.5

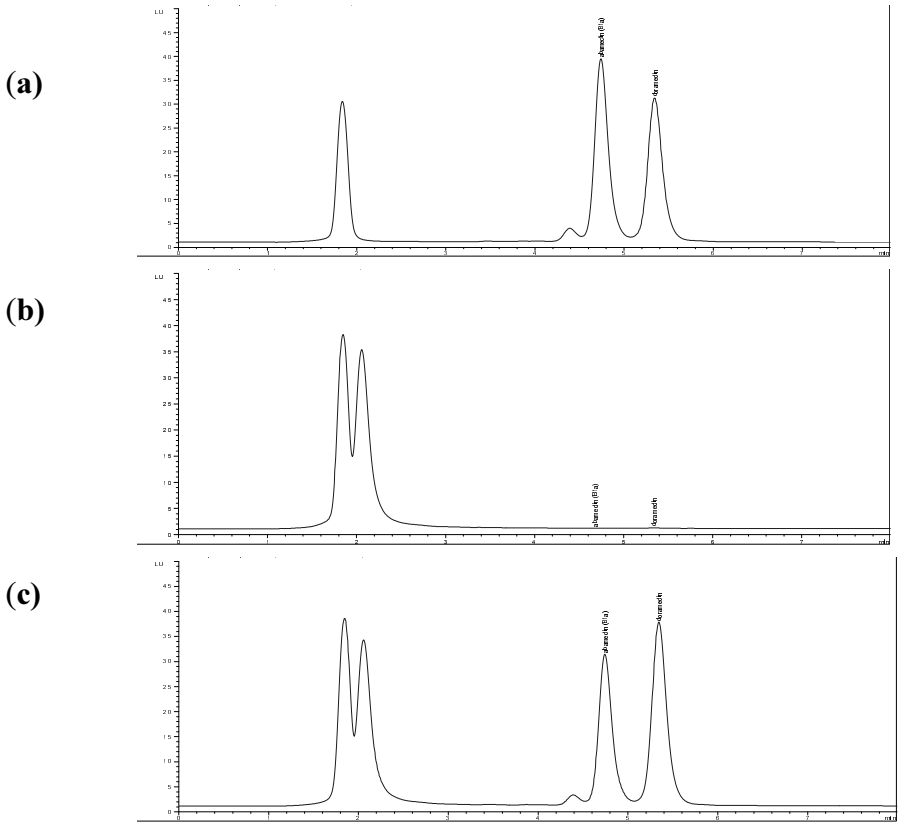

Figure 2. The chromatogram of abamectin and doramectin standard solution in a concentration of 250 ng/mL (a) and of a blank soil sample (b) and a soil sample spiked with abamectin and doramectin in a concentration of 50 ng/g (c).

It is also evident from Table 1 and Table 2 that the repeatability of spiked soil samples with abamectin and doramectin is better than 90%.

The limit of detection (LOD) and the limit of quantification (LOQ) were also determined. LOD was 0.7 ng/g of dry soil for abamectin and 1.5 ng/g of dry soil for doramectin. It was calculated as the mean value for twenty blank determinations plus three times the standard deviation.[15] The mean value for twenty blank soil determinations and the standard deviation for abamectin were 0.29 and 0.14 ng/g of dry soil, respectively and for doramectin 0.71 and 0.27 ng/g of dry soil, respectively. LOQ was 1.0 ng/g of dry soil for abamectin and 2.5 ng/g of dry soil for doramectin which was the lowest analyte content for which the method has been validated with specified degrees of accuracy and repeatability[15] (recovery at standard addition of 1.0 ng/g of dry soil for abamectin: mean ± s = 92.6 ± 10.2%, RSD = 11.0%; recovery at standard addition of 2.5 ng/g of dray soil for doramectin: mean ± s = 90.8 ± 8.7%, RSD = 9.6% (under the reproducibility conditions)).

The chromatograms of abamectin and doramectin standard solution in a concentration of 250 ng/mL, of blank soil sample and of soil sample spiked with abamectin and doramectin at a concentration of 50 ng/g are presented in Figure 2.

Conclusions

An analytical method for simultaneous determination of abamectin and doramectin in soil from the grazed pasture has been developed. Good selectivity, recovery of the method and precision as well as low detection and quantification limit enable simultaneous determination of abamectin and doramectin in soil. This method can therefore be used for eco-toxicological studies of avermectins in various soils.

Experimental

Reagents and equipment. Methanol, acetonitrile, isooctane, acetone, ethyl acetate and n-hexane (LiChrosolv quality) were obtained from Merck. *N*-methylimidazole and trifluoroacetic anhydride (analytical grade) were also supplied by Merck. Supelco Sylon CT was used for deactivating the surface of the glassware.

A Tehtnica Železniki mechanical shaker (VIBROMIX 313 EVT) and a vortex (VIBROMIX 204 EV) from the same producer were employed for the extraction of samples. A Hettich centrifuge (ROTIXA/RP) was used for the centrifugation of samples. Extracts were evaporated using an Organomation N-EVAP No. 111 evaporator. A Supelco Vacuum Manifold and alumina SPE cartridges Varian Bond Elut Al-N (500 mg, 3 mL) were introduced into the clean-up procedure and to enrich abamectin and doramectin in soil extracts.

The HPLC system (Thermo Separation Products) that consisted of a Spectra Systems P2000 pump, an AS300 auto injector and a Shimadzu RF-535 fluorescence (excitation wavelength 365 mm; emission wavelength 470 mm) detector was used for the simultaneous determination of abamectin and doramectin. The separation was carried out on a Supelco Supelcosil LC-8-DB column (250 x 4.6 mm ID; 5 μm particle size) with a Supelco guard column Supelguard LC-8-DB (20 x 4.6 mm ID; 5 μm particle size). The column temperature was maintained at 28 °C. The mobile phase consisting of methanol-acetonitrile-water (47.5 + 47.5 + 6.0, v/v/v) was pumped at 1.1 mL min^{-1}.

Standard solutions. Abamectin (obtained as a gift from Krka d.d., Novo mesto, Slovenia) and doramectin (Pfizer Inc., Groton, USA) were used as standard reference materials. The stock solution of abamectin and doramectin in a concentration of 100 μg/mL and working standard solutions were prepared in acetonitrile.

Soil samples. Samples (rendzik leptosol; A_h horizon (0-10 cm)) were collected from the grazed pasture in Vremščica, Slovenia. They were homogenized and stored at -20 °C. Soil characteristics are presented in Table 3. The moist content (at the time of the analysis) was calculated from the weight loss.

Table 3. Soil characteristics.

soil type	Moisture (%)	pH	organic matter (%)	soil particle diameter (μm)	cationic capacity (mmol/100 g of soil)
rendzik leptosol	28.0	6.3	18.7	20-50; 9.8 % 20-2; 68.9 % < 2; 21.3 %	30-45

Extraction and clean-up procedure. Homogenized, moist soil sample (5.0 g) was weighted into a 50 mL extraction tube. A 15 mL portion of acetone-water (1 + 1, v/v) was added and sample was shaken for 30 min at 350 rpm. After that, 15 mL of isooctane was added and shaken for additional 5 min at 350 rpm. After centrifugation (10 min, 3000 rpm) the sample was re-extracted twice more with isooctane and isooctane layers were collected together. They were transferred to a reservoir connected to an alumina SPE cartridge. The SPE cartridge was previously activated with 6.0 mL of methanol and conditioned with 6.0 mL of isooctane. After applying the sample extracts, they were washed with 10 mL of n-hexane-ethyl acetate (70 + 30, v/v). The analyte was eluted with 9.0 mL of methanol-ethyl acetate (70 + 30, v/v), collected in a polypropylene test-tube and evaporated to dryness under nitrogen at 60 °C. Sample was then derivatized with 100 μL of *N*-methylimidazole-acetonitrile (1 + 1, v/v) and 150 μL of trifluoroacetic anhydride-acetonitrile (1 + 1, v/v) on a vortex for 10 seconds and after 20 seconds sample was diluted with 750 μL of acetonitrile. 150 μL of sample was injected into the HPLC system.

The recovery of the method must be tested daily within the set of sample determinations by addition of abamectin and doramectin to blank moist soil sample in two concentrations expected in the measured samples. The blank soil sample must also be tested as a control.

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References and Notes

1. W. C.Campbell, M. H. Fisher, E. O. Stapley, G. Albers-Schonberg, T. A. Jacobs, *Science* **1983**, *221*, 823–828.
2. G. W. Benz, R. A. Roncalli, S. J. Gross, *Ivermectin and abamectin*, Springer-Verlag, New York 1989, pp 215–229.
3. H. S. Rupp, S. B. Turnipsed, C. C. Walker, J. E. Roybal, A. R. Long, *J. AOAC Int*. **1998**, *81*, 549–553.
4. S. V. Prabhu, T. A. Wehner, P. C. Tway, *J. Agric. Food Chem*. **1991**, *39*, 1468–1471.
5. D. G. Kennedy, A. Cannavan, S. A. Hewitt, D. A. Rice, W. J. Blanchflower, *Food Adit. Contam*. **1993**, *10(5)*, 579–584.
6. B. Roudaut, *Analyst* **1998**, *123*, 2541–2544.
7. G. Scarano, M. Esposito, L. Grasso, V. Soprano, G. Oliviero, *Analyst* **1998**, *123*, 2551–2553.
8. M. Danaher, M. O'Keeffe, J. D. Glennon, *Analyst* **2000**, *125*, 1741–1744.
9. M. Sher Ali, Tuhg Sun, G. E. McLeroy, E. T. Phillippo, *J. AOAC Int.* **2000**, *83(1)*, 31–38.
10. J. V. Pivnichny, J.-S. K. Shim, L. A. Zimmerman, *J. Pharm. Sci*. **1983**, *72(12)*, 1447–1450.
11. J. F. Sutra, C. Chartier, P. Galtier, M. Alvinerie, *Analyst* **1998**, *123*, 1525–1527.
12. F. J. Schenck, L. H. Lagman, *J. AOAC Int*. **1999**, *82(6)*, 1340–1344.
13. J. L. Bernal, M. J. Del Nozal, M. Salas, E. Galante, J. P. Lumaret, *J. Liq. Chromatogr.* **1994**, *17(11)*, 2429–2444.
14. L. D. Payne, M. B. Hicks, T. A. Wehner, *J. Agric. Food Chem*. **1995**, *43*, 1233–1237.
15. R. J. Heitzman *AGRIGULTURE - Veterinary Drug Residues; Residues in Food-Producing Animals and Their Products: Reference Materials and Methods,* Commission of the European Communities, Brussels 1992, pp 30–31.

Povzetek

Uporaba antihelmintikov, posebno avermektinov (ivermektin, abamektin, doramektin), se je v veterinarski medicini, v zadnjem času, močno povečala. Zaradi njihovega specifičnega metabolizma in njihovega vpliva na neciljne organizme pa te spojine predstavljajo resen ekotoksikološki problem.

Razvili smo analitsko metodo za simultano določanje vsebnosti abamektina in doramektina v zemlji s pašnih površin. Metoda vključuje klasično ekstrakcijo obeh avermektinov iz zemlje, ekstrakcijo trdo-tekoče (SPE) za čiščenje ekstraktov tal in tekočinsko kromatografijo visoke ločljivosti (HPLC) s fluorescenčno detekcijo.

Nizka meja določljivosti (0,5 ng/g suhih tal), dobra selektivnost in izkoristek metode v območju 71–96%, ponovljivost večja od 90% in meja vrednotenja 0,7 ng/g suhih tal za abamektin in 1,5 ng/g suhih tal za doramektin omogočajo določanje vsebnosti abamektina in doramektina v tleh.

Razvito analitsko metodo se lahko uporablja za spremljanje onesnaženosti tal z abamektinom in doramektinom in pri ekotoksikoloških raziskavah avermektinov.