Technical paper

Validation of Analytical Procedure for Simultaneous Determination of Two Avermectins in Various Soils

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Abstract

An analytical method for determination of abamectin and/or doramectin in various soils has been developed. Classical extraction procedure for extraction of both avermectins from soils, clean-up of extracts using an appropriate solid-phase extraction (SPE) and a high performance liquid chromatography (HPLC) with fluorimetric detection (FLD) were introduced in the analytical procedure.

The recoveries were in the range from 64 to 81% for abamectin and from 73 to 89% for doramectin in silty clay soils and 55 to 59% for abamectin and 63 to 70% for doramectin in clay soil, respectively. Limits of detection were 0.5 and 0.7 ng g^{-1} of moist soil for abamectin and doramectin, respectively for silty clay soil and 2.0 ng g^{-1} for clay soil.

Keywords: Avermectins, abamectin, doramectin, analytical procedure, soil

1. Introduction

Avermectins (e.g. abamectin, doramectin, or ivermectin), natural fermentation products of a soil-dwelling microorganism *Streptomyces avermitilis,* belong to a group of 16-membered macrocyclic lactons. They are widely used in agriculture as pesticides and in farm animals as veterinary medicinal products indicated for the treatment of a variety of parasitic diseases. Abamectin or avermectin B_{1A} is a mixture of two homologues containing not less than 80% of avermectin B_{1a} and not more than 20% of avermectin B_{1b}. Doramectin is 25-cyclohexylavermectin B_1. Chemical structures of both avermectins are shown in Fig.1.

A majority of the given dose of avermectins is excreted in forms of non-metabolised parent compounds

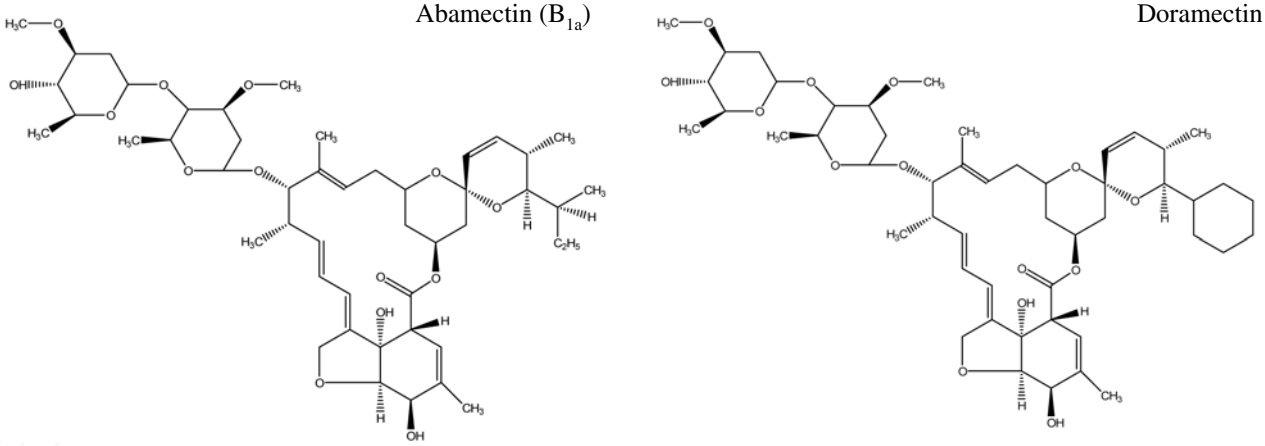

Figure 1. Chemical structure of avermectins.

and active metabolites either via urine or faeces.[1,2] They enter the environment due to the spreading of manure and slurry onto agricultural land or via direct deposition by grazing livestock. Environmental fate of environment veterinary medicines may include degradation, transport and distribution to different compartments. Distribution of avermectins in soil is limited due to their lipophylic nature and insolubility in water. Sorption coefficient (K_d) varied from 17.4 to 147 for abamectin in sand and clay loam soil[3] and from 70.8 to 562 for doramectin.[5] Due to their low mobility in the environment, avermectins bind strongly to soil particles and show prolonged persistence with a long half-life. The organic carbon normalized sorption coefficients (K_{oc}) varied from 5300 to 7520 for abamectin and doramectin, respectively in the sandy soil and from 15700 to 86900 in the clay soil.[3–5] However, for the binding of avermectins to soil particles the organic carbon content appears to be very important. Residues of avermectins and/or their metabolites adversely affect several species of dung-dwelling organisms and non-target soil invertebrates. Consequently, avermectins may present a serious ecotoxicological risk. Therefore, concentrations of avermectins in faeces as well as in soil are important indicators of the ecological impact. Various analytical methods have been developed for determination of avermectins in biological samples[6–15] but only a few for environmental samples.[16–18] Chromatographic methods are the most widely used. Determination of abamectin and/or doramectin in animal tissue,[6–9] liver,[10,11] animal plasma[12,13] and milk[14,15] is described by several authors. Some methods have been published for sheep sera[9] and faeces,[19] water,[16] sediments[17] and soils[17,18] using analytical techniques such as liquid chromatography (LC) with ultraviolet (UV) or fluorimetric (FL) detection, liquid chromatography mass spectrometry (LC-MS) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The use of LC-MS/MS methods allows simultaneous, sensitive and selective determination of a large number of complex samples (mixture of avermectins, various matrices). However, the analysis of less complex and/or less numerous samples (one or two types of avermectins present in similar matrices) may require less complicated and more readily available chromatographic equipment.

The aim of the presented work was to develop a reliable and sensitive analytical method for the determination of abamectin and/or doramectin in various soils using HPLC with fluorimetric detection. As soil is a complex matrix it is important to introduce the analytical procedure that enable high recovery and low quantification limits. Therefore, an appropriate clean-up procedure has to be developed to remove interfering matrix components for efficient extraction of avermectins from soils. In addition, method could be used in ecotoxicological studies of both avermectins in *in-vitro* as well as in field conditions.

2. Experimental

2. 1. Reagents

Methanol, acetonitrile, acetone, ethyl acetate and n-hexane (all obtained from J.T.B., The Netherlands) were of HPLC grade. Isooctan (analytical grade), *N*-methylimidazole and trifluoroacetic anhydride (TFAA) (for synthesis) were supplied by Merck. Supelco (Bellefonte, PA, USA) Sylon CT was used for deactivating the surface of the glassware.

Reference standards of abamectin and doramectin were purchased from Sigma Aldrich (Steinheim, Germany). Standard solutions of abamectin and doramectin in a concentration of 1 mg mL^{-1} and working standard solutions were prepared in acetonitrile. These solutions were stored at 4 °C and were stable for at least 1 month.

2. 2. Instrumentation

A Vibromix 313 EVT mechanical shaker and Vibromix 204 EV vortex mixer, both from Tehtnica (Železniki, Slovenia) and an IKA®-WERKE ultrasonic shaker (ULTRA-TURRAX T 25 basic) were used for 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 Bakerbond (J.T. Baker, Philipsburg, NJ, USA; 500 mg, 3 mL) were used for the clean-up of extracts and for pre concentration of abamectin and doramectin from soil.

2. 3. HPLC Conditions

A Varian (Palo Alto, CA, USA) ProStar HPLC system consisting of a solvent delivery module, a model 410 autosampler, and fluorescence (excitation wavelength 365 nm; emission wavelength 470 nm) detector was used for determination of abamectin and doramectin. An aliquot of 10 µL of derivatised extract was injected on a Phenomenex Luna 3 µ C18 (2) (150 × 4.6 mm ID; 3 µm particle size) analytical column with Phenomenex pre-column C18 (ODS, Octadecyl) (4.0 × 3.0 mm ID; 5 µm particle size) at 28 ± 1 °C. The mobile phase consisted of methanol, acetonitrile and bi-distilled filtered water (47.5 + 47.5 + 6.0, v/v/v), with a flow rate of 1.1 mL min^{-1}.

2. 4. Test Soils

Two types of soil (silty clay and clay) were collected in three different regions [Vremščica (silty clay), Podpeč (silty clay) and Grosuplje (clay)] of Slovenia from the top 10 – 20 cm of soil and classified according to pedological parameters, i.e. texture, organic matter content, and soil particle size. Properties of the soils are summarized in Table 1. Before the analysis all soils were gently crushed, sieved through a 2 mm sieve, and stored at approximately

–20 °C. The pH was measured in 0.01 M $CaCl_2$ solution.[20] The content of organic matter was determined by Walkley-Black method[21] and texture of soil according the procedure Janytzki[22]. Moisture content was determined at the time of the analysis. It was calculated from the weight loss.

In addition, for the method development each soil was spiked with standard mixture solutions of abamectin and doramectin at the given fortification levels of 2.5, 5.0, 10 and 50 ng g^{-1} (as described in Section 2.6), therefore both tested compounds were present in the soil. Fortification levels were chosen according to predicated environmental concentration. Blank soil was analyzed as well.

The recovery of the method was tested daily within the set of sample determinations by addition of abamectin and doramectin to blank moist soil samples in three concentrations near that expected in the samples. All soil samples were analyzed in four parallel determinations. The blank soil sample without abamectin and doramectin was served as a control.

2. 6. Method Validation

The method was *in-house* validated using the following performance criteria: linearity and a range of linearity, selectivity, intra-day and inter-day precision, detection and quanfication limits, and stability of *N*-methylimidazole derivatives of both avermectins in soil extracts. Solutions for calibration and fortification were prepared in acetonitrile and stored at 4 °C. Linearity was investigated over a six-point calibration ranging from 0.5 to 500 ng mL^{-1}. Selectivity was evaluated by comparing chromatograms of blank samples with those of spiked samples. Limits of detection (LOD) for standards were tested under optimum chromatographic conditions, by injection of abamectin and doramectin standard solutions at concentrations below 50 ng mL^{-1}, and calculated as signal to noise ratio (S/N = 3).

The intra-day precision of the method, expressed as the relative standard deviation of peak area measurements (n = 6), was evaluated through the results obtained with the method operating over one day under the same conditions, using three different fortification levels: 5.0, 10 and 50 ng g^{-1}. The inter-day precision was determined using four different concentration levels (2.5, 10, 20 and 50 ng g^{-1}) on three successive days.

The stability of abamectin and doramectin was determined in derivatized samples over a period up to 48 h after derivatization.

Table 1. Selected soil properties for the silty clay (Vremščica and Podpeč) and the clay soil (Grosuplje)

Soil	pH[a] ($CaCl_2$)	RSD (%)	Organic matter[b] (%)	RSD (%)	Texture[c] (%)		
					Clay (< 2 μm)	Sand (2–50 μm)	Silt (50–2000 μm)
Vremnščica	6.2	0.5	8.8	0.2	45.3	2.9	51.8
Podpeč	5.5	0.4	14.5	0.5	42.1	4.0	53.9
Grosuplje	4.6	0.4	0.5	0.1	83.3	3.0	13.7

[a] ISO 10390.[20] [b] Walkley-Black method.[21] [c] ISO 11277.[22]

2. 5. Sample Preparation Procedure

Sample preparation was performed according to previously published analytical procedure for determination of abamectin and doramectin in soil from a grazed pasture.[19] Several modifications of the procedure were applied. Approximately 5.0 g (wet weight) of the soil sample was weighted into a 50 mL extraction tube and spiked with the standard mixture solution of abamectin and doramectin at different fortification levels. The sample was extracted with 15 mL acetone-water (1 + 1, v/v) by shaking on a mechanical shaker at room temperature for 30 min at 350 rpm. A 15 mL of isooctane was added and shake for an additional 5 min at 350 rpm. After centrifugation (10 min at 3000 rpm) samples were re-extracted twice more with isooctane. Isooctane layers were collected and combined. The SPE cartridges were activated with 6.0 mL methanol and conditioned with 6.0 mL isooctane. Sample extracts were loaded using polypropylene reservoirs above the cartridge. The cartridge was rinsed with 10 mL *n*-hexane-ethyl acetate (70:30 (*v/v*)), while 9.0 mL of methanol-ethyl acetate (70:30 (*v/v*)) was used for elution into polypropylene test-tube. The eluate was evaporated to dryness under nitrogen at 60 °C. Samples and standards were derivatized with 100 μL *N*-methylimidazole-acetonitrile, (1:1 (*v/v*)) and 150 μL trifluoroacetic anhydride-acetonitrile, (1:2 (*v/v*)) by vortex for 10 s. After 20 s sample was diluted with 750 μL acetonitrile and transferred to an HPLC vial. An aliquot of 10 μL was injected into the HPLC system.

3. Results and Discussion

Optimization of the clean-up procedure for all soil extracts and enrichment of abamectin and doramectin were based on analytical method for determination of both

avermectins in soil from a grazed pasture published by Kožuh Eržen et al.[18] The analytical procedure is described in detail in the Experimental section.

Several solid phase extraction cartridges i.e. Varian Bond Elut Al–N (500 mg, 3 mL), J. T. Baker alumina (1000 mg, 6 mL) and J. T. Baker alumina (500 mg, 3 mL) were initially tested in the clean-up procedure. Kožuh Eržen et al.[18] reported good recovery and LOQ using Varian Bond Elut AL–N cartridge in clean-up procedure, while in our study a very low recovery (22%) and poor repeatability (RSD = 36%) were observed in clay soil Grosuplje. The best recovery of the method for all tested soils was achieved with J. T. Baker alumina (500 mg, 3 mL) extraction cartridges employing elution with methanol and ethyl acetate in a volume ratio of 70 : 30. In addition, good separation and good sensitivity were achieved using analytical LC column Phenomenex Luna 3 μ C_{18} (2) column (150 × 4.6 mm ID; 3 μm particle size) with the pre-column C_{18} (ODS, Octadecyl) (4.0 × 3.0 mm ID; 5 μm particle size), and employing a mobile phase of methanol, acetonitrile and water in a volume ratio of 47.5 : 47.5 : 6.0, at a flow rate of 1.1 mL min^{-1}.

Calibration curves for abamectin and doramectin standards prepared in acetonitrile in the concentration range from 0.5 to 500 ng mL^{-1} provided correlation coefficients (R^2) which always exceeded 0.999. LODs for standard solutions were estimated to be 0.5 ng mL^{-1} for abamectin and 0.5 ng mL^{-1} for doramectin, respectively. Selectivity was evaluated by comparing chromatograms of blank samples with spiked samples (with standards) in all three soils. The results are presented in Fig. 2.

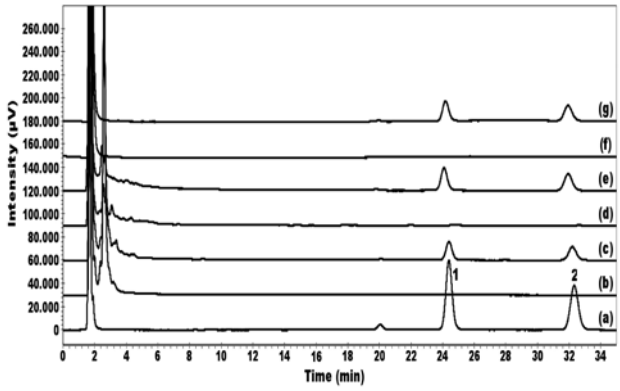

Figure 2. LC-FL chromatograms of standard solutions of abamectin and doramectin in a concentration of 100 ng mL^{-1} (a), blank soil extracts Vremšica (b), Podpeč (d) and Grosuplje (f) and spiked soil extracts Vremšica (c), Podpeč (e) and Grosuplje (g) with abamectin (1) and doramectin (2) in a concentration of 10 ng g^{-1}.

In addition, the stability of abamectin and doramectin *N*-methylimidazole derivatives were previously investigated at room temperature for respective standard solutions (they were very stable up to 48 h).[16] The same stability was found for abamectin and doramectin in extracts of all investigated soils as well (fortification levels of abamectin and doramectin in concentrations of 5.0 ng g^{-1} and 50 ng g^{-1}).

To determine recovery of the method, blank samples of moist soils were spiked with abamectin and doramectin in concentrations of 5.0, 10 and 50 ng g^{-1} (intra day analysis – repeatable conditions). The results are presented in Tables 2, 3 and 4.

Mean recovery of samples spiked with abamectin in concentrations 5.0, 10, and 50 ng g^{-1} were between 68 and 81% (RSD = 5%) for soil Vremščica, 64 and 79% (RSD = 3%) for soil Podpeč and 55 and 59% (RSD = 5%) for soil Grosuplje, respectively.

On the other hand, mean recoveries of samples spiked with doramectin in concentrations 5.0, 10, and 50 ng g^{-1} were between 75 and 85% (RSD = 2%) for soil Vremščica, 73 and 89% (RSD = 4%) for soil Podpeč, and 63 and 70% for soil Grosuplje (RSD = 4%) (Tables 2, 3 and 4). Recoveries for soil Vremščica and Podpeč were comparable, but with tendency towards higher recoveries in soil Vremščica. Moreover, recoveries for both avermectins in soil Vremščica and soil Podpeč were higher in comparison to soil Grosuplje. In these soils the content of clay particles is lower (42.1%) than in soil Grosuplje (83.3%) (Table 1). The content of clay particles in soil Vremščica and in soil Podpeč is similar (Table 1), but the organic carbon content is lower in soil Vremščica. Slightly better recovery for both compounds was achieved in Vremščica soil samples. That may further support the conclusion that avermectins are strongly adsorbed to clay particles and/or organic matter.[3–5]

Reproducibility of the method for determination of abamectin and doramectin in all investigated soils was tested employing inter-day analyses (three successive days; two analysts) by determination of recoveries of spiked soil samples with 2.5, 5.0, 10 and 20 ng g^{-1} of both avermectins. The results are presented in Tables 2, 3 and 4. Due to the low standard deviations (4%) obtained for six replicates, recoveries are comparable for two investigated soils. The overall average recovery for abamectin was 68% and 75% for doramectin in soils Vremčica and Podpeč, respectively (RSDs for both avermectins < 10%). On the other hand, in soil Grosuplje the recovery was only 57% for abamectin and 65% for doramectin, respectively (RSDs for both avermectins < 8%).

LOD and LOQ for abamectin and doramectin in soils were determined as well. LOD was calculated as apparent content corresponding to the three times the peak-to-peak noise ratio.[23] It was determined at levels of 0.5 ng g^{-1} (abamectin) and 0.7 ng g^{-1} (doramectin) of moist soil Vremšica and Podpeč, respectively, while in soil Grosuplje it was 2.0 ng g^{-1} for both avermectins.

LOQ was determined as the lowest amount of the analyte for which the method could be validated with specified accuracy and repeatability. LOQs were determined at levels of 1.0 ng g^{-1} (abamectin, doramectin) for silty clay soil and 3.0 ng g^{-1} (abamectin, doramectin) for clay soil.

Table 2. Results of intra-day (5.0, 10 and 50 ng g^{-1}) and inter-day (2.5, 10, 20 and 50 ng g^{-1}; three days; two analysts) assay of abamectin and doramectin in soil Vremščica.

Compound	Fortification level (ng g^{-1})	Repeatability (n = 6)		Reproducibility (n = 4)							
				Day 1		*Day 2*		*Day 3*		*Overall*	
		Recovery (mean ± S.D.) (%)	RSD (%)	Recovery (mean ± S.D.) (%)	RSD (%)	Recovery (mean ± S.D.) (%)	RSD (%)	Recovery (mean ± S.D.) (%)	RSD (%)	Recovery (mean ± S.D.)	RSD (%)
Abamectin	2.5	–	–	75 ± 5	6	62 ± 2	3	61 ± 2	3	66 ± 3	4
	5.0	68 ± 3	5	71 ± 7	10	59 ± 6	10	61 ± 6	10	66 ± 6	10
	10	73 ± 1	3	70 ± 6	9	70 ± 6	8	65 ± 6	10	69 ± 6	9
	20	–	–	76 ± 5	6	61 ± 3	5	72 ± 2	3	74 ± 3	5
	50	81 ± 5	6	–	–	–	–	–	–	–	–
Doramectin	2.5	–	–	86 ± 3	3	74 ± 2	2	68 ± 2	3	76 ± 2	3
	5.0	75 ± 3	2	71 ± 7	10	81 ± 9	11	66 ± 6	7	73 ± 7	11
	10	83 ± 2	2	80 ± 2	3	84 ± 2	3	83 ± 8	4	83 ± 4	5
	20	–	–	79 ± 6	7	68 ± 3	4	76 ± 2	3	75 ± 3	5
	50	85 ± 2	2	–	–	–	–	–	–	–	–

S.D.: standard deviation

Table 3. Results of intra-day (5.0, 10 and 50 ng g^{-1}) and inter-day (2.5, 10, 20 and 50 ng g^{-1}; three days; two analysts) assay of abamectin and doramectin in soil Podpeč.

Compound	Fortification level (ng g^{-1})	Repeatability (n = 6)		Reproducibility (n = 4)							
				Day 1		*Day 2*		*Day 3*		*Overall*	
		Recovery (mean ± S.D.) (%)	RSD (%)	Recovery (mean ± S.D.) (%)	RSD (%)	Recovery (mean ± S.D.) (%)	RSD (%)	Recovery (mean ± S.D.) (%)	RSD (%)	Recovery (mean ± S.D.)	RSD (%)
Abamectin	2.5	–	–	74 ± 5	6	61 ± 3	4	61 ± 2	4	65 ± 3	5
	5.0	65 ± 3	3	62 ± 4	7	67 ± 4	6	65 ± 3	5	66 ± 4	6
	10	64 ± 1	2	60 ± 2	3	66 ± 8	12	62 ± 1	2	63 ± 3	5
	20	–	–	70 ± 2	3	75 ± 3	4	77 ± 1	2	74 ± 1	2
	50	79 ± 4	4	–	–	–	–	–	–	–	–
Doramectin	2.5	–	–	75 ± 9	12	63 ± 1	1	6 ± 4	6	67 ± 4	6
	5.0	73 ± 2	4	69 ± 2	4	74 ± 5	6	71 ± 4	5	72 ± 4	6
	10	74 ± 2	3	70 ± 2	3	74 ± 9	13	71 ± 1	1	72 ± 4	6
	20	–	–	76 ± 2	2	79 ± 2	3	83 ± 1	2	79 ± 2	2
	50	89 ± 3	3	–	–	–	–	–	–	–	–

S.D.: standard deviation

Table 4. Results of intra-day (5.0, 10 and 50 ng g^{-1}) and inter-day (2.5, 10, 20 and 50 ng g^{-1}; three days; two analysts) assay of abamectin and doramectin in soil Grosuplje.

Compound	Fortification level (ng g^{-1})	Repeatability (n = 6)		Reproducibility (n = 4)							
				Day 1		*Day 2*		*Day 3*		*Overall*	
		Recovery (mean ± S.D.) (%)	RSD (%)	Recovery (mean ± S.D.) (%)	RSD (%)	Recovery (mean ± S.D.) (%)	RSD (%)	Recovery (mean ± S.D.) (%)	RSD (%)	Recovery (mean ± S.D.)	RSD (%)
Abamectin	2.5	–	–	–	–	–	–	–	–	–	–
	5.0	56 ± 3	4	54 ± 6	11	58 ± 3	6	56 ± 4	4	56 ± 4	7
	10	55 ± 1	3	51 ± 6	12	55 ± 4	7	55 ± 1	3	55 ± 3	5
	20	–	–	65 ± 3	5	61 ± 4	7	63 ± 5	7	63 ± 4	6
	50	59 ± 4	6	69 ± 5	7	54 ± 4	8	65 ± 4	5	57 ± 4	8
Doramectin	2.5	–	–	–	–	–	–	–	–	–	–
	5.0	67 ± 3	5	–	–	67 ± 4	6	65 ± 4	5	66 ± 4	6
	10	63 ± 4	6	63 ± 4	6.5	68 ± 5	7	58 ± 3	5	63 ± 4	6
	20	–	–	69 ± 3	4.6	63 ± 4	7	69 ± 4	6	67 ± 4	6
	50	70 ± 5	2	78 ± 6	7.5	62 ± 5	7	59 ± 5	8	66 ± 5	8

S.D.: standard deviation

Typical chromatograms of simultaneous determination of standard solutions of abamectin and doramectin in a concentration of 100 ng mL^{-1}, blank silty clay and clay soil, spiked with abamectin and doramectin in a concentration of 10 ng g^{-1} are presented in Figure 2. Good selectivity of the method allows simultaneous determination of abamectin and doramectin at low LOQs in all three soils.

4. Conclusions

A reliable and sensitive analytical method for simultaneous determination of abamectin and doramectin in three soils [Vremščica (silty clay), Podpeč (silty clay) and Grosuplje (clay)] has been developed. Good recovery of the method, reproducibility, repeatability and selectivity as well as low LOD and LOQ enable determination of both avermectins in all three investigated soils.

Therefore, this method can be used in environmental monitoring of abamectin and doramectine contamination in soils as well as in ecotoxicological studies of these avermectins.

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Povzetek

Razvili smo analitsko metodo za določanje abamektina in/ali doramektina v različnih vrstah tal. Za ekstrakcijo obeh avermektinov iz tal smo uporabili postopek ekstrakcije z ustreznim čiščenjem ekstraktov na trdni fazi (SPE) in uporabo tekočinske kromatografije visoke ločljivosti (HPLC) s fluorescenčno detekcijo (FLD).
Izkoristki so bili med 64 in 81 % za abamektin in med 73 in 89 % za doramektin v meljasto glinastih tleh, v glinastih tleh pa med 55 in 59 % za abamektin in med 63 in 70 % za doramektin. Meja zaznavnosti (LOD) je bila 0,5 ng g^{-1} za abamektin in 0,7 ng g^{-1} za doramektin v meljasto glinastih tleh ter 2,0 ng g^{-1} za obe spojini v glinastih tleh.