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Extraction and analysis of avermectines in agricultural soils by microwave assisted extraction and ultra high performance liquid chromatography coupled to tandem mass spectrometry

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ABSTRACT

A method for the analysis of avermectines (abamectine, doramectine and ivermectine) in soils has been developed. The analytes are extracted with acetonitrile/water (90:10) by using microwave assisted extraction. The extract is cleaned-up through solid phase extraction with Oasis HLB cartridges and analyzed by ultra high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS). Separation is obtained in 3 min. Extraction of analytes from the soil, that is the most critical point, has been studied in detail, and the effect of soil composition and aging time on the analytes recovery has been investigated.

1. Introduction

Avermectines (AVs) which are chemically related to macrolides, exhibit a high antihelmintic, as well as insecticide and acaricide activity. They were discovered in 1975 and are produced by natural fermentation of the soil bacterium Streptomyces avermitilis. Avermectines are widely used to treat ecto and endo parasites in livestock, and about 80–90% of the administered dose to animals are excreted without transformation. They persist in manure for long term periods and when manure is spread on fields as fertilizer, AVs reach the environment and can be toxic to non-target organisms [1,2]; for instance for springtails an EC_{50} value of 1.7 mg kg^{-1} has been reported for ivermectine [3].

Liquid chromatography (LC), with either UV absorption or fluorescence detection, has been proposed as determination technique for the analysis of AVs. Fluorescence detection proved to be sensitive enough to allow the determination of AVs at low concentrations in environmental samples [4–7]. However, due to its selectivity and identification capabilities, mass spectrometry (MS) is probably the most extensively applied technique [8–10].

Regarding the analysis of AVs in soils, in addition to sensitive and selective detection techniques, previous sample treatment steps (extraction and clean-up) are required. Extraction from the soil matrix, that being the most crucial step in the analytical procedure, still requires further research work. Pure organic solvents, such as dichloromethane or acetonitrile, or hydroorganic mixtures, such as methanol/water or acetone/water, are used as extractants in the few methods reported for the analysis of AVs in solid environmental samples [4,9,11–13]. As extraction techniques, conventional mechanical shaking and exhaustive extraction techniques, such as pressurized liquid extraction have been proposed [9,11]. Recovery rates from 55% to 89% have been reported, depending on the analyte and on the soil [4].

It is well known that the extraction of organic compounds from soils can be highly influenced by the physico-chemical characteristics of the soil. Parameters such as organic matter content or soil pH, can play a significant role in the interaction between the analytes and the soil matrix and thus on their extraction behaviour. Some authors point out that, due to the lipophilic properties of AVs (octanol–water partition coefficient in the range 10^3–10^5) [9], organic matter plays a main role in the interaction of these compounds with the soil matrix [14–16]. However, other authors suggest that soil affinity is mainly related to the inorganic fraction of the soil [17–20]. Moreover the extraction of organic contaminants in soil matrices may be also influenced by aging processes, with extraction decreasing as aging increases [21,22]. Therefore, the residence time of AVs in the soil may affect extraction efficiency [14].

The aim of this work is to develop analytical methodology to analyze AVs such as abamectine (ABA), doramectine (DOR) and

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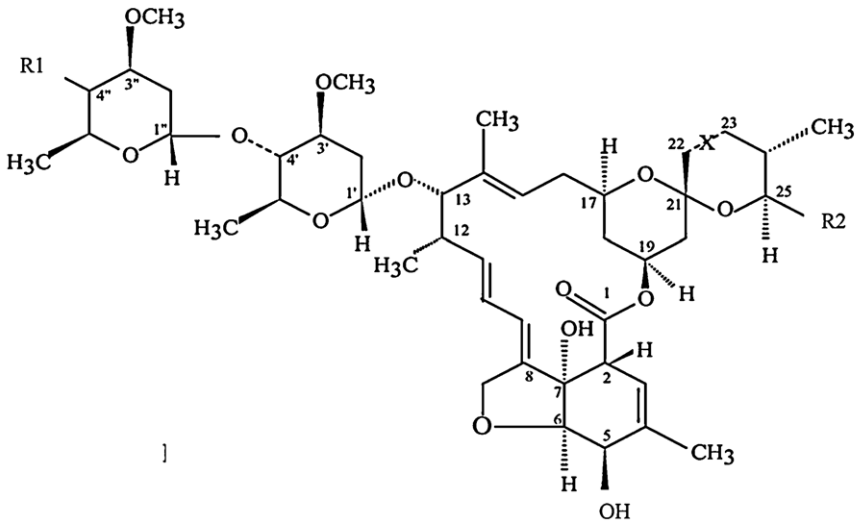

Avermectin	R1	R2	C22–x–C23
Abamectine B_{1a}	–OH	$-CH(CH_3)CH_2CH_3$	–CH=CH–
Doramectine A_{1a}	–OH	$-C_6H_{11}$	–CH=CH–
Ivermectine B_{1a}	–OH	$-CH(CH_3)CH_2CH_3$	$-CH_2-CH_2-$
Eprinomectine B_{1a}	$-NH-COCH_3$	$-CH(CH_3)CH_2CH_3$	$-CH_2-CH_2-$

Fig. 1. Structure of the studied avermectines.

ivermectine (IVER) in soils (Fig. 1). The study is mainly focused in the extraction of AVs from soil, accounting for the effect of soil physicochemical properties and aging on the extraction. New information about the extraction behaviour of these anti-parasitic agents in several soils, as well as the effect of soil aging, is provided.

2. Experimental

2.1. Chemicals and solutions

ABA, DOR and eprinomectin (EP) were purchased from Riedel-de-Haën (Seelze, Germany), while IVER was purchased from Sigma–Aldrich (Steinheim, Germany). The commercial products are mixtures but the study was focused on the major component of each AV (i.e. ABA B_{1a} 98%, DOR A_{1a} 92%, EP B_{1a} 97% and IVER B_{1a} 94%. Individual primary stock standard solutions of the compounds (100 mg L^{-1}) in acetonitrile were prepared and stored at −18 °C. They were stable for several months. Secondary stock standard solutions, containing 5 mg L^{-1} of each AV, were prepared weekly in acetonitrile and stored at 4 °C. Working solutions were prepared freshly everyday by further dilutions of the stock solutions in acetonitrile.

Acetonitrile (ACN) and methanol (MeOH) were both HPLC grade supplied by Merck (Darmstadt, Germany). Other chemicals were analytical grade. All aqueous solutions were prepared using doubly de-ionized water (Milli-Q, Millipore, Molheim, France) with a resistivity of 18.2 MΩ cm^{-1}.

Mobile phase consisted of ACN and 10 mM acetic acid–ammonium acetate buffer pH 4.

0.1 M buffer solutions at pH 3.5, 7.0 and 10 were prepared from formic acid/sodium formate, sodium dihydrogenphophate/sodium hydrogenphosphate and sodium hydrogencarbonate/sodium carbonate, respectively.

The SPE cartridges used in this study were Oasis HLB 30 mg from Waters (Milford, MA, USA).

Glassware used for experiments was previously soaked in 10% nitric acid for 24 h and rinsed with ultrapure water.

Table 1
Physico-chemical characteristics of the soil samples.

Soil	S-A	S-B	S-C	S-D
pH	8.2	7.0	5.4	4.9
CEC (meq/100 g)	10.6	–	30.6	24.5
Organic carbon (%)	1.5	9.2	1.1	8.2
Sand (%) 2 mm > d > 0.05 mm	38.6	36.7	36.7	59.9
Silt (%) 0.05 mm > d > 0.002 mm	37.7	52.0	42.8	31.0
Clay (%) d < 0.002 mm	23.7	11.3	20.5	9.1

d: particle diameter (mm).

2.2. Samples

Soil samples were collected from four agricultural fields in Spain. Samples were air-dried, sieved (2 mm), irradiated with γCo^{60} to avoid microbial activity and stored in the dark at −20 °C. Before extraction, samples were thawed and then splittered. Table 1 shows parameters referring to texture and other properties of the soil samples.

2.3. Apparatus

Chromatographic analysis was performed in an Acquity Ultra Performance LC Waters consisting of a binary pump, an automatic injector and a UV detector with an Acquity UHPLC® BEH C_{18} column (50 × 2.1 mm I.D., 1,7 μm particle size). The LC system was coupled to a PE-Sciex API3000 triple quadropole mass spectrometer (Foster City, CA, USA) equipped with an Atmospheric Pressure Chemical Ionization (APCI) source working in positive mode. Data were collected using Analyst® 1.4.2 software (MDS Sciex, Concord, ON, Canada).

Microwave assisted extraction was carried out using an *ETHOS E* closed-vessel system (1000 W) supplied by Milestone (Sorisole, Italy). The system is designed for extraction using organic solvents and is able to hold twelve extraction vessels.

For SPE preconcentration a Rapid Trace Workstation (Caliper LifeSciences, Inc., Massachusetts, USA) was used.