Journal of Chromatography B, 878 (2010) 1537–1542

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

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

V.D. Litskas[a,*], G.C. Batzias[b], X.N. Karamanlis[a], A.P. Kamarianos[a]

[a] Laboratory of Ecology and Environmental Protection, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece
[b] Laboratory of Veterinary Pharmacology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 1 February 2010
Accepted 8 April 2010
Available online 1 May 2010

Keywords:
HPLC
Eprinomectin
Avermectins
Soil
Cattle faeces

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

* Corresponding author. Tel.: +30 2310 999855; fax: +30 2310 999855.
E-mail address: vlitskas@vet.auth.gr (V.D. Litskas).

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doi:10.1016/j.jchromb.2010.04.007

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