ELSEVIER

Journal of Chromatography B, 761 (2001) 115–123

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Extraction and isolation of avermectins and milbemycins from liver samples using unmodified supercritical CO_2 with in-line trapping on basic alumina

Martin Danaher[a,b,*], Michael O'Keeffe[a], Jeremy D. Glennon[b]

[a]*Teagasc, The National Food Centre, Dunsinea, Castleknock, Dublin 15, Ireland*
[b]*Department of Chemistry, University College Cork, Cork, Ireland*

Received 13 April 2001; received in revised form 5 July 2001; accepted 6 July 2001

Abstract

A multi-residue supercritical fluid extraction (SFE) method has been developed for the extraction and isolation of eprinomectin, moxidectin, abamectin, doramectin and ivermectin residues from animal liver. Liver samples are mixed with hydromatrix and packed into a vessel containing 2 g of basic alumina. The samples are extracted at 100°C using unmodified supercritical carbon dioxide (SF-CO_2) at a pressure of 300 bar and flow-rate of 5.0 l/min. The analytes are adsorbed in-line on the basic alumina trap, which is later eluted with 4 ml of methanol–ethyl acetate (70:30, v/v). After evaporating to dryness, sample extracts are derivatised using methylimidazole, trifluoroacetic anhydride and acetic acid at 65°C for 30 min. Derivatised sample extracts are analysed by high-performance liquid chromatography (HPLC) with fluorescence detection. The method was validated using bovine liver fortified at levels of 4 and 20 μg/kg with the drugs. The mean recovery ranged between 76 and 97%. The intra- and inter-assay variations showed RSD values <10 and <16%, respectively. The procedure was also applied to ovine and porcine liver, giving similar results. The limit of quantitation of the method is 2 μg/kg.

Keywords: Supercritical fluid extraction; Avermectins; Milbemycins; Alumina

1. Introduction

Supercritical fluids were used initially for industrial extraction processes, such as decaffeination of coffee [1] and extraction of residual solvents and monomer from polymers [2]. In recent years, supercritical fluids have become widely used in the field of residue analysis for the selective extraction of drugs [3], food contaminants [4], banned substances [5], pesticides [6] and environmental pollutants [7] from food and soil. Supercritical fluids have been used also for the extraction of metals [8] and as solvents for synthesis [9]. Supercritical carbon dioxide (SF-CO_2) is the most widely used supercritical fluid because of its low toxicity, low cost, wide availability, ease of disposal, chemical stability and easily attainable critical parameters (critical temperature 31°C and critical pressure 73 atm). In cases where solvation of the target compounds cannot be achieved using SF-CO_2, modifiers may be added to enhance the solvation power of the supercritical fluid.

*Corresponding author. Present address: Teagasc, The National Food Centre, Dunsinea, Castleknock, Dublin 15, Ireland. Tel.: +353-1-805-9500; fax: +353-1-805-9550.

0378-4347/01/$ – see front matter
PII: S0378-4347(01)00315-2

A recent review of applications of supercritical fluid extraction (SFE) has indicated that the majority of applications of SFE in residue analysis have been in the area of pesticide analysis and environmental analysis [10]. Many of these compounds are lipophilic in nature and as a result are readily extracted using SF-CO_2. Many authors have observed that use of SFE can greatly reduce extraction and clean-up time [11–15]. Applications of SFE to analysis of veterinary drugs and related compounds have not been as plentiful [10]. This may be because other techniques, such as solvent extraction and solid-phase extraction (SPE), have been well developed in the area of veterinary drug residue analysis. Traditionally, SFE was regarded as a slow technique because only a limited number of samples (eight to ten) could be processed per day. However, Zoontjes et al. have described an automated SFE method for steroids in animal tissue that can process up to 20 samples per day [16]. SFE has an obvious advantage over other techniques in that extraction and clean-up may be combined into one procedure. Furthermore, SFE may be highly selective through manipulation of a range of variables such as temperature, pressure, flow-rate and trapping sorbents.

The avermectins (eprinomectin, abamectin, doramectin and ivermectin) and the milbemycins (moxidectin) belong to a group of compounds called the macrocyclic lactones, which are used in the treatment of parasitic infections in food producing animals. A number of methods have been developed for the analysis of these compounds. These methods involve extraction using organic solvents or matrix solid-phase dispersion [17], followed by liquid–liquid partitioning [18], SPE [19,20], or immunoaffinity clean-up [21]. Sample extracts may be derivatised and analysed by HPLC with fluorescence detection [18] or analysed directly by LC–MS [22]. Only one application of SFE to the analysis of avermectins has been found in the scientific literature, a method for extraction of abamectin residues from soil and tissue using SF-CO_2 modified with 2-methoxyethanal [23].

The method described here is a multi-residue method and uses unmodified SF-CO_2. Samples are dispersed on hydromatrix and, following extraction, the analytes are trapped in-line using basic alumina. The analytes are eluted from the alumina with organic solvent, evaporated to dryness, derivatised and analysed by HPLC with fluorescence detection.

2. Experimental

2.1. Materials and reagents

Water, methanol, acetonitrile and ethyl acetate (HiPerSolv grade) were obtained from BDH (Merck, Poole, Dorset, UK). Triethylamine, phosphoric acid, glacial acetic acid and hexane (analytical grade) were from BDH. *N*-Methylimidazole and trifluoroacetic anhydride (analytical grade) were obtained from Sigma (St. Louis, MO, USA). Polypropylene SPE reservoirs (6 ml), polyethylene frits (13 mm) and hydromatrix (Celite 566) were obtained from International Sorbent Technology (Mid-Glamorgan, UK). Silanising reagent (Dow-Corning Z-1219) was from BDH. Basic alumina was prepared weekly by heating neutral alumina (Merck, Darmstadt, Germany) at 500°C overnight and cooling in a dessicator. The alumina was stored in a sealed container in a dessicator until use. Polypropylene wool was obtained from Applied Separations (Allentown, PA, USA). Food grade CO_2 (99.95%) was purchased from Air Products (Basingstoke, UK).

2.2. SFE apparatus

A Spe-ed 680 Bar SFE instrument, developed by Applied Separations, Allentown, PA, USA, was used in these studies. This instrument was configured for the simultaneous extraction of two samples. The extraction vessels (24 ml, 14×150 mm) used were constructed of high pressure stainless steel capable of withstanding pressures up to 690 bar. These were connected to the system using hand-tightenable slip free connectors. The restrictors used were micrometering valves encased in an aluminium block fitted with a heater and a thermocouple. Commercial SPE cartridges (6 ml) containing neutral alumina (2 g) packed between polyethylene frits (IST, Mid-Glamorgan, UK) were attached directly to the micrometering valves, and functioned as off-line traps. A Floline SEF-51 Flowmeter totalizer (Horiba, Sunnyvale, CA, USA) was used to measure the flow of expanded CO_2 gas.

2.3. HPLC conditions

The HPLC system consisted of a model 600 HPLC pump with a model 717 autosampler and

model 420-ac fluorescence detector, excitation wavelength 365 nm and emission wavelength 470 nm, all from Waters (Milford, MA, USA). The separation was carried out on a stainless-steel analytical column (150×3.9 mm I.D.) packed with Novapak C_{18} material (Waters) and equipped with a guard column containing μBondapak C_{18} material (Waters). The column temperature was maintained at 30°C. The mobile phase, consisting of methanol–acetonitrile–1% triethylamine/1% phosphoric acid in water (60:30:10, v/v/v), was pumped at 1 ml/min. A Shimadzu (Dusseldorf, Germany) CR-5A integrator (chart speed 5 mm/min, attenuation 7) was used for recording and processing chromatograms.

2.4. Standard solutions

Ivermectin (Sigma), abamectin (Supelco, Bellefonte, PA, USA), eprinomectin (Merck, Rahway, NJ, USA), moxidectin (American Cyanamid, Princeton, NJ, USA), and doramectin (Pfizer, Groton, CT, USA) were used as standard materials. Standard stock solutions of eprinomectin, moxidectin, abamectin, doramectin and ivermectin (all at 1 mg/ml) were prepared in methanol. All standard stock solutions were stored at −20°C. A working standard solution (0.2 μg/ml of eprinomectin, moxidectin, abamectin, doramectin and ivermectin) was prepared from the standard stock solution on the day of use.

2.5. Liver samples

Liver samples (~100 g) were homogenised in a Robot Coupe™ bowl-blender (Montceau-en-Bourgogne, France) and stored at −20°C in 50-ml tubes. Liver samples analysed and found to contain no detectable residues of the analytes were used as negative controls.

2.6. Fortification of samples

For preparation of fortified liver samples, 2.5-g portions of negative control liver were weighed into 50-ml glass tubes. A 50-μl portion of a 0.2- or 1.0-μg/ml standard solution was added to give residue levels of 4 or 20 μg/kg, respectively. After fortification, samples were allowed to sit for 15 min prior to further preparation.

2.7. Sample preparation

Hydromatrix (4.0 g) was added to the tube containing the liver sample (2.5 g) and the tube contents were mixed using a metal spatula (1 min). The sample–hydromatrix mixture was poured into an SFE vessel sealed with an end cap and containing basic alumina (2.0 g) fitted between two polypropylene wool plugs. A third plug of polypropylene was used to clean the spatula and the tube previously containing the sample. This polypropylene wool plug was added to the extraction vessel above the sample–hydromatrix layer, which was then tightly compressed with a tamping rod. The vessel void space was filled with hydromatrix and a final plug of polypropylene wool was added. The vessel contents were compressed tightly and the vessel was sealed with a second end cap, which was labelled as the inlet side of the vessel. The vessel was stored at 4°C until extraction.

2.8. Extraction and clean-up

Prior to extraction the micrometering valves were heated to 115°C and the inlet, outlet and micrometering valves were closed. Packed SFE vessels ($n=2$) were mounted vertically in the oven with the end of the vessel containing alumina connected as the outlet side. The inlet valves were opened and the vessels were pressurised to 80 bar with CO_2. The vessels were then heated to 100°C for 10 min to equilibrate the vessels. The pressure was not allowed to go over 300 bar during this period, with the outlet and micrometering valves being opened to reduce pressure, if necessary. The outlet and micrometering valves were opened at the end of the equilibration period, and the pressure and flow-rate were set at 300 bar and 5 l/min of expanded gas. These conditions were maintained until a total 50 l of expanded gas had passed through, as measured on the flow-meter. The inlet valves were closed and the system was depressurised. The vessels were disconnected and were cooled to 4°C. The vessel end cap (outlet side) was removed carefully so as not to lose any alumina. The polypropylene plug was removed from the outlet side and the alumina was removed and transferred into an empty SPE cartridge (6 ml) fitted with a polyethylene frit. The outside of the vessel was tapped with a tamping rod to ensure

that all the alumina was transferred from the extraction vessel to the SPE cartridge. The cartridge was tapped on the outside to settle the sorbent bed, a polyethylene frit was added and the alumina was compressed tightly with a glass rod. The analytes were eluted with methanol–ethyl acetate (4 ml; 70:30, v/v), which was collected in a silanised test tube. The solvent was evaporated at 60°C under nitrogen.

2.9. Derivatisation

Portions (225 μl) of methylimidazole–acetonitrile (2:7, v/v) and trifluoroacetic anhydride–acetonitrile (2:7, v/v) were added to the test tube, which was stoppered and vortexed after each addition for 2 and 1 min, respectively. A 50-μl portion of glacial acetic acid was added and the tube was stoppered and vortexed for 1 min. The derivatised sample extract was filtered through a 0.45-μm filter (13 mm, polyvinylidene difluoride) into an HPLC vial, which was incubated in a fan-assisted oven (30 min, 65°C). Samples vials were then cooled (4°C, 3 min) and let sit at room temperature (12 min). An aliquot (100 μl) was injected onto the HPLC column.

2.10. Calibration

Standards were prepared by adding 0, 25, 50, 100, 250 and 500 μl of the working standard solution (0.2 μg/ml) to silanised test tubes, evaporating to dryness under nitrogen at 60°C and derivatising as described above. Calibration curves were prepared by plotting peak area as a function of analyte concentration (0–200 ng/ml). Recovery was measured from the peak areas obtained for fortified sample extracts, as calculated from the standard curve.

2.11. Method validation

For the intra-assay study, bovine liver samples ($n=5$, taken from the same animal) fortified at levels of 4 and 20 μg/kg were extracted and analysed on the same day. For the inter-assay study, bovine liver samples ($n=5$, taken from different animals) fortified at levels of 4 and 20 μg/kg were extracted and analysed on 5 different days. To show the ap-

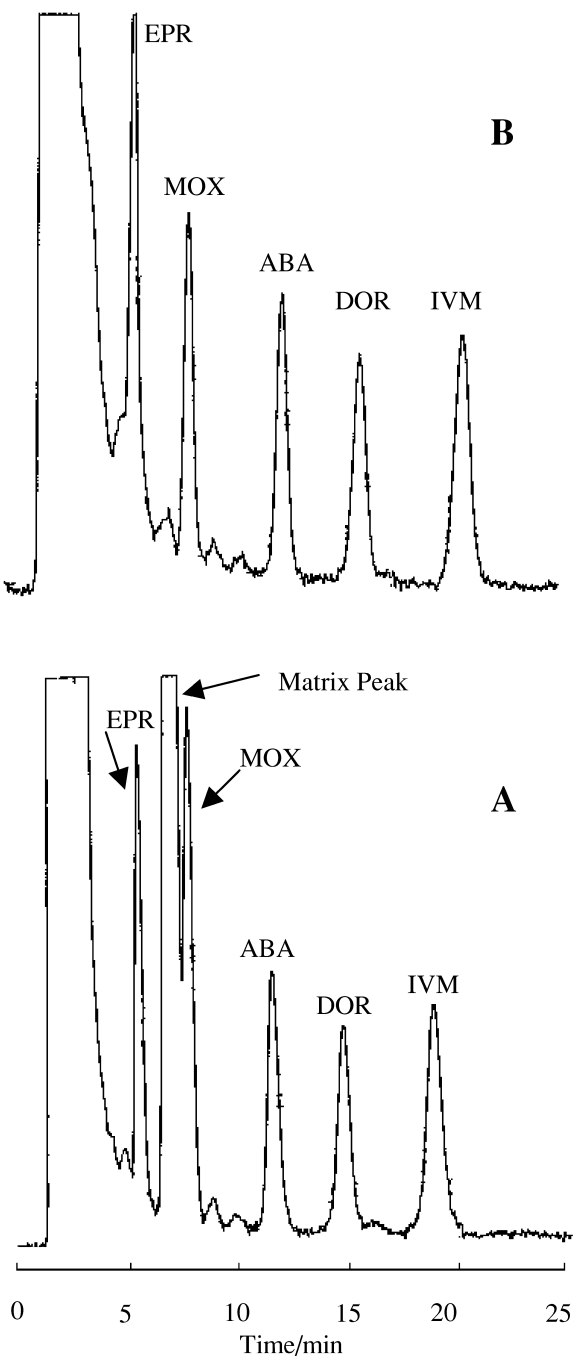

Fig. 1. Chromatograms of porcine liver extracts fortified with 20 μg/kg of eprinomectin (EPR), moxidectin (MOX), abamectin (ABA), doramectin (DOR) and ivermectin (IVM); (A) off-line trap and (B) in-line trap.

Table 1
Effect of weight of basic alumina (g) used for the in-line trap on the recovery of the analytes from liver ($n=2$)

Alumina (g)	Fraction	Recovery (%)				
		Eprinomectin	Moxidectin	Abamectin	Doramectin	Ivermectin
5	In-line eluate	100	87	78	79	84
2	In-line eluate	76	–	89	87	91
	In-line wash	–	98	–	–	–
0.2	In-line eluate	68	–	43	48	46
	In-line wash	6	–	36	33	34
	Off-line eluate	–	79	–	–	–

plicability of the method to other species, an inter-assay study was also completed for porcine and ovine species: five samples (all from different animals) of each species were fortified at a level of 10 μg/kg and extracted and analysed on 5 different days.

3. Results and discussion

3.1. Selection of trapping conditions

Initially, off-line trapping on neutral alumina (2 g) was used, based on a published SPE procedure for these drugs [19]. The neutral alumina trapped the analytes as the expanded CO_2 gas passed through the SPE cartridges fitted to the outlet of the micrometering valves. Following removal of the SPE cartridge from the SFE equipment, the neutral alumina was washed with hexane–ethyl acetate (10 ml; 70:30, v/v) and then eluted with methanol–ethyl acetate (8 ml; 70:30, v/v). The eluates were evaporated to dryness at 60°C under nitrogen and the sample extracts were derivatised for HPLC determination. The sample chromatograms contained a matrix interfering peak eluting close to the moxidectin peak (Fig. 1A).

In-line trapping on basic alumina, which had been used in other SFE applications [24], was evaluated as an alternative to off-line trapping. Three weights of basic alumina (5, 2 and 0.2 g) were evaluated for in-line trapping, using the same wash and elution conditions as were used for the off-line trap except that the volumes of wash (1 ml) and elution (0.8 ml) solvent mixtures for the 0.2-g trap were one-tenth those used for the 2- and 5-g traps. The matrix interfering peak could be largely eliminated using in-line trapping on basic alumina (Fig. 1B). Recovery of the analytes was affected by the weight of basic alumina used for trapping (Table 1). With the exception of the 5-g trap, losses of analytes occurred either through early elution from the basic alumina in the wash step (2-g trap) or through non-retention and passage through the in-line trap, to the off-line trap (0.2-g trap). These losses may be due to deactivation of a portion of the basic alumina by water extracted from the liver sample in the SFE procedure, which would be more significant where lower quantities of basic alumina were used in the in-line trap. These results indicate that weights of alumina between 2 and 5 g successfully trap the analytes. Because of the loss of moxidectin in the wash step of the 2-g traps, this step was omitted, and the analytes were eluted directly from the basic alumina. Similar, high re-

Table 2
Recovery of analytes from basic alumina in the in-line trap using direct elution with methanol–ethyl acetate ($n=2$)

Alumina (g)	Recovery (%)				
	Eprinomectin	Moxidectin	Abamectin	Doramectin	Ivermectin
2	106	83	92	91	92
3	119	89	95	95	93
4	112	84	93	92	90

covery of all analytes was obtained under these conditions using amounts of basic alumina between 2 and 4 g (Table 2) and the resulting chromatograms were free of interferences. The conditions selected for trapping of the analytes were 2 g basic alumina in-line. The effect of volume of eluent was tested by eluting the alumina with 2×4-ml volumes of methanol–ethyl acetate (70:30, v/v). All analytes were eluted in the first 4-ml volume with none being detected in the second. Consequently, an eluent volume of 4 ml methanol–ethyl acetate (70:30, v/v) was selected.

The effect of water, in the sample, on the extraction of the analytes from liver was evaluated (Table 3). Fortified samples were dispersed on hydromatrix and dried in an oven (100°C, 30 min) prior to extraction and trapping in-line on basic alumina. However, the recovery was much lower compared with the conventional non-dried sample–hydromatrix mixture. Oven dried sample–hydromatrix mixture was rehydrated by adding water (1.6 ml) directly to it and then packing it into the vessel. Results indicated that recovery of the analytes was higher from hydrated as opposed to dehydrated tissue. Oven dried sample–hydromatrix mixture was packed into the vessel and water was then added to the rear of the vessel. Both rehydration experiments gave equivalent results, with recovery similar to that obtained for non-dried samples, indicating that sample moisture assists the extraction of the analytes.

3.2. Development of SFE variables

A step-by-step approach for SFE method development, as described by McNally [25], was used as an alternative to a factorial design approach [14,26]. In practical terms, this approach can give similar results but requires less data processing. The order chosen

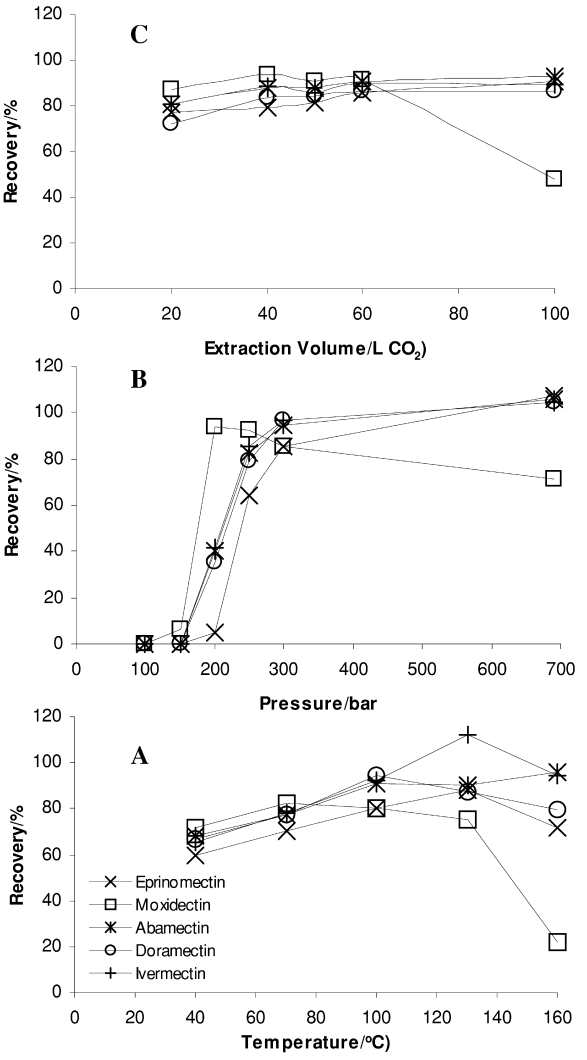

Fig. 2. Plots of analyte recovery (%) from liver samples as a function of extraction temperature (°C), extraction pressure (bar) and extraction volume of (CO_2 l).

Table 3
Effect of water in the sample on extraction of analytes

Treatment	Recovery (%)				
	Eprinomectin	Moxidectin	Abamectin	Doramectin	Ivermectin
Fresh sample	100	96	87	85	83
Oven-dried sample	37	66	56	55	51
Oven-dried sample, rehydrated directly	107	97	91	105	90
Oven-dried sample, rehydrated in the vessel	114	98	91	95	89

Table 4
Intra- and inter-assay variations for the recovery of the analytes from bovine liver

Analyte	Fortification level (μg/kg)	Recovery (%)			
		Intra-assay		Inter-assay	
		Mean±SD (n=5)	RSD	Mean±SD (n=5)	RSD
Eprinomectin	4	90±8.4	9.4	76±11.9	15.7
	20	82±3.8	4.6	80±7.0	8.8
Moxidectin	4	89±7.1	8.0	85±10.1	11.9
	20	87±2.6	3.0	83±12.0	14.4
Abamectin	4	93±3.9	4.2	85±10.9	12.9
	20	88±5.3	6.0	85±8.6	10.1
Doramectin	4	97±4.9	5.1	76±12.9	16.9
	20	85±3.9	4.7	81±7.7	9.5
Ivermectin	4	83±3.9	4.7	80±8.8	10.9
	20	87±4.9	5.6	82±8.0	9.8

for evaluating the variables was temperature, pressure and extraction time (or volume of CO_2); the order in which the variables are evaluated does not matter provided that two of the three variables are fixed during each evaluation.

The analytes were extracted using a range of temperatures (40–160°C), with the pressure at 300 bar and volume of CO_2 at 60 l. Results show high recovery of the analytes using an extraction temperature of 100°C and, at temperatures greater than 130°C, there was a notable decrease in recovery (Fig. 2A). The presence of a portion of the analytes on the off-line trap indicated that this reduced recovery was due to poorer trapping efficiency by the in-line trap at higher temperatures (160°C).

Pressure was evaluated next (oven temperature set at 100°C) using a range of pressures (100–690 bar). Results show an increase in recovery of the analytes between 200 and 300 bar (Fig. 2B). At pressures greater than 250 bar lower recovery of moxidectin was observed. However, a pressure of 300 bar was needed to extract eprinomectin, which appeared to be the most strongly adsorbed analyte. The off-line traps indicated that at more extreme conditions (>300 bar) poor trapping efficiency of moxidectin was observed.

The extraction time was evaluated by varying the volume of CO_2 used (20–100 l CO_2) with temperature and pressure at their newly optimised values (100°C and 300 bar). Results indicate high recovery of all analytes between 40 and 60 l CO_2; when greater than 60 l of CO_2 was used, poor trapping efficiency was observed for moxidectin (Fig. 2C).

3.3. Results of method validation

The method was validated using the developed conditions (100°C, 300 bar, 50 l CO_2). Calibration

Table 5
Inter-assay variation for the recovery of the analytes from ovine and porcine liver samples, fortified at 10 μg/kg

Analyte	Recovery (%)			
	Porcine liver		Ovine liver	
	Mean±SD (n=5)	RSD	Mean±SD (n=5)	RSD
Eprinomectin	72±2.9	4.0	75±6.4	8.6
Moxidectin	77±6.0	7.8	72±11.3	15.6
Abamectin	77±3.4	4.4	79±2.6	3.3
Doramectin	71±5.6	7.9	73±5.2	7.1
Ivermectin	76±3.6	4.8	79±3.1	3.9

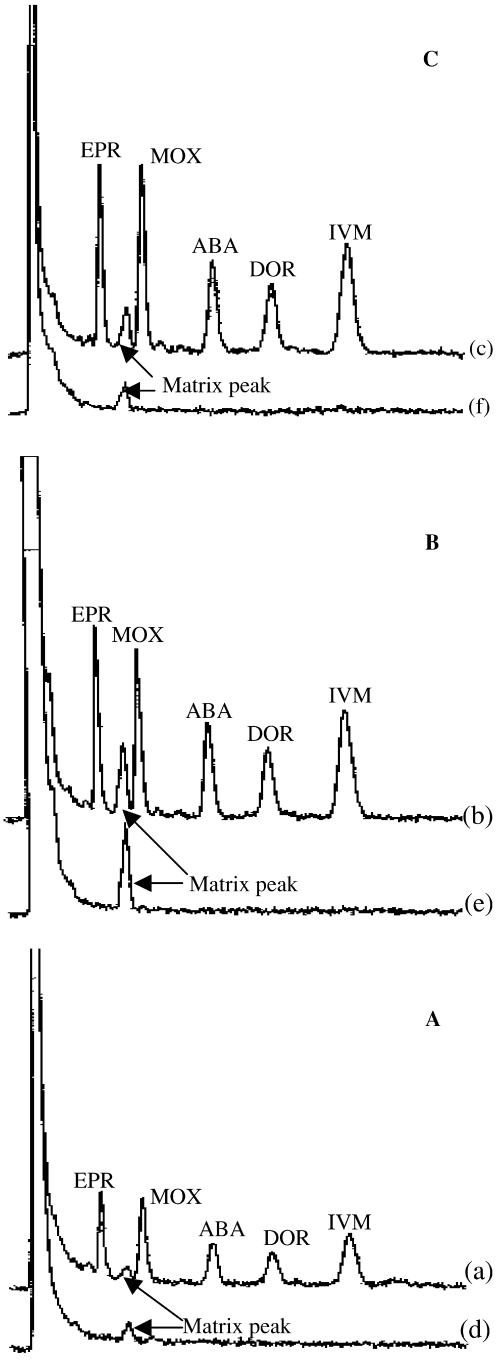

Fig. 3. Chromatograms of (A) bovine, (B) ovine and (C) porcine liver extracts fortified at levels of (a) 4 μg/kg, (b) 10 μg/kg and (c) 10 μg/kg with avermectin and milbemycin standards. Chromatograms of negative control bovine, ovine and porcine livers are also shown.

curves were linear (r^2=0.999) over the range 0–200 ng/ml. The limit of quantitation for the method, as determined from the lowest standard on the calibration curve (10 ng/ml), was 2 μg/kg. The accuracy and precision of the method were determined using bovine liver samples fortified at levels of 4 and 20 μg/kg. Mean recovery of the analytes was between 76 and 97%. Intra-assay variation was determined by analysing five samples within a single run; RSD values were at less than 10 and 6% at the 4- and 20-μg/kg levels, respectively (Table 4). Inter-assay variation was determined by analysing samples on five different occasions, to evaluate the run to run variation in the method; RSD values were less than 17 and 15% at the 4- and 20-μg/kg levels, respectively (Table 4).

The method was also applied to ovine and porcine livers fortified with the analytes at 10 μg/kg. Mean recovery of the analytes from ovine and porcine livers was between 71 and 79%, with RSD values being less than 16 and 8%, respectively (Table 5). Fig. 3 shows chromatograms for fortified bovine, ovine and porcine samples extracted using SFE. A matrix peak was present in these chromatograms, eluting between eprinomectin and moxidectin. This peak was found to be more abundant in ovine liver than in porcine or bovine livers, but did not prevent quantitative analysis of the analytes.

4. Conclusions

An SFE method has been developed for the analysis of avermectin and milbemycin compounds in bovine, ovine and porcine liver samples, giving acceptable recovery and repeatability. The analytes could be eluted from an in-line alumina trap using 4 ml of solvent, producing sufficiently clean extracts for analysis by HPLC, without further clean-up. SFE offers an alternative to published methods for avermectin and milbemycin compounds based on solvent extraction and multiple SPE clean-up [18,19,26]; apart from low solvent usage, the SFE method requires only an elution from alumina to give a clean sample extract for chromatography. A total of ten samples can be analysed routinely per day on the instrumentation available, but with automated SFE

(or multiple SFE units), sample throughput could be increased.

Acknowledgements

The authors wish to thank the following for supply of analytical standards: Michel Alvinerie (INRA, Toulouse), Bernd Julicher (BgVV, Berlin), Paul Cooper (Merial, UK), Ado van Langerak (Merial, Holland), Colm Menton (T.P. Whelehan and Sons, Dublin) and Larry Parker (Fort Dodge, UK). This research has been part-funded by grant aid under the Food Sub-Programme of the Operational Programme for Industrial Development, which is administered by the Department of Agriculture, Food and Rural Development, Republic of Ireland, and supported by national and EU funds.

References

[1] K. Zosel, French Patent, 2,079,261, 1971.
[2] V.J. Krukonis, Polym. News 11 (1985) 7.
[3] D.K. Matabudul, N.T. Crosby, S. Sumar, Analyst 124 (1999) 499.
[4] S.L. Taylor, J.W. King, J.L. Richard, J.I. Greer, J. Food Prot. 60 (1997) 698.
[5] M.J. O'Keeffe, M. O'Keeffe, J.D. Glennon, Analyst 124 (1999) 1355.
[6] S.H. Salleh, Y. Saito, K. Jinno, Anal. Chim. Acta 481 (2000) 69.
[7] E. Björklund, S. Bøwadt, T. Nilsson, L. Mathiasson, J. Chromatogr. A 836 (1999) 285.
[8] J.D. Glennon, S.J. Harris, A.J. Walker, C.C. McSweeney, M. O'Connell, Gold Bull. 32 (1999) 52.
[9] J.D. Holmes, P.A. Bhargava, B.A. Korgel, K.P. Johnston, Langmuir 15 (1999) 6613.
[10] R.M. Smith, J. Chromatogr. A 856 (1999) 83.
[11] A. Valvede-García, A.R. Fernandez-Alba, M. Contreras, A. Agüera, J. Agric. Food Chem. 44 (1996) 1780.
[12] K.L. Pearce, V.C. Trenerry, S. Were, J. Agric. Food Chem. 45 (1997) 153.
[13] M. Anastassiades, W. Schwack, J. Chromatogr. A 825 (1998) 45.
[14] R.K. Juhler, Analyst 123 (1998) 1551.
[15] D.H. Kim, G.S. Heo, D.W. Lee, J. Chromatogr. A 824 (1998) 63.
[16] P.W. Zoontjes, A.A.M. Stolker, L.A. van Ginkel, in: L.A. van Ginkel, A. Ruiter (Eds.), Proceedings of EuroResidue IV Conference on Residues of Veterinary Drugs in Food, Veldhoven, The Netherlands, May 2000. National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands, 2000, p. 1152.
[17] E. Iosifidou, P. Shearan, M. O'Keeffe, Analyst 119 (1994) 2227.
[18] P.C. Tway, J.S. Wood Jr., G.V. Downing, J. Agric. Food Chem. 29 (1981) 1059.
[19] M. Danaher, M. O'Keeffe, J.D. Glennon, Analyst 125 (2000) 1741.
[20] M.S. Ali, T. Sun, G.E. McLeroy, E.T. Phillippo, J. AOAC Int. 83 (2000) 31.
[21] J. Li, C. Qian, J. AOAC Int. 79 (1996) 1062.
[22] S.B. Turnipseed, J.E. Roybal, H.S. Rupp, S.A. Gonzales, A.P. Pfenning, J.A. Hurlbut, Rapid Commun. Mass Spectrom. 13 (1999) 493.
[23] M.W. Brooks, P.C. Uden, Pestic. Sci. 43 (1995) 141.
[24] A.A.M. Stolker, L.A. van Ginkel, R.W. Stephany, R.J. Maxwell, O.W. Parks, A.R. Lightfield, J. Chromatogr. B 726 (1999) 121.
[25] M.E.P. McNally, J. AOAC Int. 79 (1996) 2.
[26] M. Danaher, M. O'Keeffe, J.D. Glennon, Analyst 126 (2001) 576.