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New sample preparation technique for the determination of avoparcin in pressurized hot water extracts from kidney samples

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Abstract

A new approach is presented for the determination of avoparcin in tissue. Complete recovery from spiked swine kidney was achieved with hot water modified with 30% ethanol (v/v). The samples were extracted at 75 °C and 50 atm by accelerated solvent extraction. In situ sample clean-up was achieved by using matrix solid-phase dispersion utilizing the acrylic polymer XAD-7 HP, and by adding triethylammonium phosphate (TEAP) to the extraction solvent. The aqueous extracts were concentrated by solid-phase extraction (SPE) on the hydrophilic interaction chromatography (HILIC) material polyhydroxyethyl aspartamide. Complete analyte retention was possible during SPE when the kidney extracts were modified with 70% ethanol. A 200 Å, 5 μm HILIC column with UV_{225} detection was used for the separation of avoparcin. The retention time was less than 15 min with 47% aqueous component in acetonitrile and 15 m*M* TEAP as eluent. The average recovery of avoparcin from kidney samples was 108%. Published by Elsevier Science B.V.

Keywords: Sample preparation; Pressurized liquid extraction; Hydrophilic interaction chromatography; Avoparcin; Glycopeptides; Subcritical water

1. Introduction

Avoparcin is a mixture of the two polar glycopeptide antibiotics α- and β-avoparcin that occur in the compositional ratio 2:1=β:α. The β form contains an additional chlorine atom that has been substituted in place of a hydrogen atom on the α structure. These antibiotics exhibit activity against gram-positive bacteria, and have been used for the growth promotion of poultry, swine, and beef cattle in agriculture.

The structural similarity of avoparcin to the human glycopeptide antibiotic vancomycin has raised concern regarding cross-species antibiotic resistance. There is copious evidence that the appearance of vancomycin-resistant enterococci (VRE) is associated with the widespread agricultural use of avoparcin in the countries where it has been licensed [1–5]. These antibiotics have never been licensed for use in the USA (or Canada) and, coincidentally, environmental VRE isolates have not been observed in this country [6]. The use of avoparcin as an animal food additive was banned in the European Union in 1997.

The fact that avoparcin is water-soluble (>5000 ppm) facilitates its extraction with pressurized hot water (i.e. subcritical water). Raising the temperature

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also enables fast and efficient extractions, and has permitted the extraction of relatively non-polar analytes from foods of plant origin [7–11]. Hot water extractions have also been performed on animal tissue, for the isolation of the pesticide atrazine from beef and swine kidney [12]. Relatively clean extracts were obtained from kidney, since the tissue was dispersed with the acrylic polymer XAD-7 HP during matrix solid-phase dispersion.

Modifying the hot water with cosolvents [11–13] can improve analyte recovery, while the addition of a salt can further facilitate sample clean-up. For example, the inclusion of 30% ethanol (v/v) at 100 °C resulted in complete recovery of atrazine from beef kidney. It was evident, however, that higher amounts of lipids and proteins were also removed with ethanol [12]. This problem has been overcome by adding the organic buffer triethylammonium phosphate (TEAP) to the extraction solvent.

We also present a hydrophilic interaction chromatography (HILIC) method for the determination of avoparcin in aqueous solution. HILIC is a variant on normal-phase chromatography that utilizes a mobile phase that is usually up to 50% aqueous [14]. When the stationary phase adsorbs or imbibes water, it becomes hydrophilic and polar analytes such as carbohydrates or peptides selectively partition into the stagnant enriched aqueous layer on the surface. Analyte retention therefore increases with analyte polarity. HILIC has already been applied to the separation of vancomycin [15,16]. To our knowledge, only a single analytical-scale liquid chromatographic method has been reported to date for the separation of avoparcin [17], which is similar to a preparative-scale method utilizing a reversed-phased column and ultraviolet detection at 254 nm [18].

In this study, avoparcin was extracted from spiked swine kidney, since it is expected to remain intact in the animal gut following ingestion and any tissue absorption would most likely occur in the kidney [19]. We are not aware of any earlier reports that describe the determination of avoparcin in tissues, nor of any methodologies that are routinely used by regulatory agencies. Although assay methods have been submitted for consideration to the Australian National Registration Authority for Agricultural and Veterinary Chemicals [19], an instrumental method for avoparcin is still lacking.

2. Experimental

2.1. Equipment

Pressurized hot water extractions of kidney samples were performed with an ASE 200 Accelerated Solvent Extractor (Dionex, Sunnyvale, CA, USA). An ASE 200 solvent controller was used to deliver pure and buffered water in addition to the ethanol modifier. The addition of modifier to the water was controlled by the instrument, while buffer was mixed directly into the water prior to being dispensed by the solvent controller. An 11 mL ASE extraction cell was used for all experiments.

During the liquid chromatographic (LC) analysis of avoparcin, the analyte was separated on a polyhydroxyethyl aspartamide column (200×4.6 mm I.D.; 5 μm; 200 Å) (PolyLC, Columbia, MD, USA). Two different LC delivery systems were used. For the first system, the LC mobile phase was delivered with a Beckman 114M solvent delivery pump (Schaumberg, IL, USA). The samples were manually injected with a six-port valve (Valco Instruments, Houston, TX, USA). The second chromatographic system utilized an AS3000 autosampler coupled to an SP8800 ternary pump (Thermo Separation Products, San Jose, CA, USA). The injection volume was 100 μL in each case. Detection was with a Thermo Separation Products SP8490 UV detector for both systems.

Aqueous solutions were concentrated on polyhydroxyethyl aspartamide (12 μm, 100 Å) solid-phase cartridges (PolyLC) that contained 0.6 g of material. The cartridges were mounted on a vacuum manifold (Supelco, Bellefonte, PA, USA) for elution of the samples.

2.2. Chemicals

Avoparcin was supplied as avoparcin sulphate (Roche, Gosport, UK). The ethanol (Aaper Alcohol and Chemical, Shelbyville, KY, USA) was absolute 200 proof. Distilled water was passed through a Milli-Q water system for deionization prior to use. Acetonitrile (HPLC grade), *o*-phosphoric acid (85%), and monobasic sodium phosphate (ACS certified) were all supplied by Fisher Scientific (Pittsburg, PA, USA). Triethylamine (99.5%), am-

monium acetate (99+%), and sodium acetate (99+%) were purchased from Aldrich (Milwaukee, WI, USA). Glacial acetic acid (99.8%) was supplied by J.T. Baker (Phillipsburg, NJ, USA).

Amberlite XAD-7 HP resin (Supelco) was used to disperse the kidney samples. The diatomaceous earth (Hydromatrix) that was used in the experiments was from Varian Corp. (Harbor City, CA, USA). Methanol (HPLC grade) (Fisher Scientific) was used to wet the XAD-7 HP polymer. Swine kidney was obtained from a local retail outlet. Whole kidneys were homogenized in a blender (Waring Products, New Hartford, CT, USA) prior to be being frozen.

2.3. Sample preparation

2.3.1. Standards and solutions

Avoparcin stock solutions (1 mg mL^{-1}) were prepared by dissolving the appropriate amount of avoparcin sulphate in water. Pure standards in the 0.1 to 20 ppm range were prepared by diluting an aliquot of the stock solution in water–acetonitrile (1:1), or by serial dilution. Standards prepared from blank kidney extracts were also spiked with the stock solution.

Triethylammonium phosphate (TEAP) stock solutions (0.5 *M*) were prepared by first dissolving 14.4 g of 85% H_3PO_4 in about 150 mL of water. Triethylamine was added to the stirred solution until the pH reached 3.0. Care was taken to ensure the solution had cooled to room temperature before being brought to the final pH. The solution was then brought to a final volume of 250 mL. An ammonium acetate stock solution (0.5 *M*) was prepared by dissolving 9.6 g of ammonium acetate in 250 mL of water after being brought to pH 5.0 with acetic acid.

Buffered LC mobile phases were prepared by mixing the appropriate amounts of buffer stock solutions and water in a 1 L volumetric flask. Acetonitrile was then added to within several milliliters below the volume mark. The flasks were warmed and degassed by placing them in an ultrasonic bath for 5 min. The contents were then brought to the final volume with acetonitrile. Solutions containing TEAP, water, and ethanol that were used during SPE retention experiments were prepared in the same manner using 10 mL volumetric flasks.

2.3.2. Kidney samples

The dispersion of kidney samples has already been described [12]. Briefly, swine kidney samples weighing 0.5 g were spiked to concentrations of 10 or 20 μg avoparcin/g kidney using the appropriate amount of avoparcin stock solution. All of the kidney samples were dispersed with 2 g of Hydromatrix and 2 g of XAD-7 HP (dry weight prior to being wetted) using a mortar and pestle. The XAD-7 HP resin was wetted with methanol and water prior to being dispersed through the kidney samples.

A cellulose fiber was pressed into an 11 mL ASE extraction cell before the cell was filled with the dispersed kidney sample. An additional amount of diatomaceous earth (~0.3 g) was swept through the mortar with the pestle to remove any trace amounts of the kidney sample. This material was also added to the extraction cell, and any remaining void volume in the cell (1–2 mL) was filled with unground Hydromatrix. Unless otherwise specified, the pressurized liquid extractions were performed at 75 °C and 50 atm. The other extraction parameters were the same as those utilized during the pressurized hot water extraction of atrazine from beef kidney [12], as summarized in Table 1.

2.4. Sample analysis

2.4.1. Solid-phase extraction and chromatography

The polyhydroxyethyl aspartamide HILIC column was conditioned according to instructions provided by the manufacturer. The column was flushed with 20 mL of water followed by elution of a solution composed of 0.2 *M* NaH_2PO_4+0.3 *M* sodium acetate (~pH 4) for 1 h. The column was flushed again with 20 mL of water before being equilibrated for 30 min with the mobile phase. The column was equilibrated for at least this length of time for each change in mobile phase composition. All HPLC analyses were performed at a flow-rate of 1.0 mL min^{-1}. The avoparcin complex was detected at 225 nm.

The HILIC–SPE cartridges were conditioned in a similar manner by drawing 5 mL of water through the cartridges with the vacuum. When only about 2 mm of water remained above the solid-phase material, 5 mL of the salt solution were passed through the cartridges, followed by a further 5 mL aliquot of

Table 1
Parameters for the analysis of avoparcin in swine kidney

Sample	
Swine kidney	0.5 g
Diatomaceous earth	2 g
Amberlite XAD-7 HP	2 g[a]
ASE extraction	
Preheat	0 min
Heat	5 min
Static	5 min
Purge	60 s
# cycles	3
Temperature	75 °C
Pressure	50 atm
Solvent	30% ethanol–70% 21.5 m*M* TEAP (v/v)
Flush volume	50%
SPE	
Sample	5 mL extract+7 mL ethanol
Solid phase	Polyhydroxyethyl aspartamide (12 μm, 100 Å)
Wash solution	70% ethanol by volume, 6 m*M* TEAP
Eluent	2–5 mL water
HILIC–HPLC	
Stationary phase	Polyhydroxyethyl aspartamide (5 μm, 200 Å)
Mobile phase	47% aqueous (15 m*M* TEAP) in acetonitrile
Flow-rate	1.0 mL min^{-1}
Wavelength	225 nm

[a] Dry weight prior to wetting, as supplied by manufacturer.

water. The cartridges were then flushed with 5 mL of a wash solution that had a similar organic/aqueous composition to the samples that were being analyzed. (For SPE recovery experiments, the wash solutions had the same composition as the sample solutions, with the exception of the avoparcin component. For the concentration of kidney extracts, the wash solutions were composed of 30% aqueous component and 6 m*M* TEAP in ethanol.) Following the concentration step, aliquots of wash solutions that were used to remove analyte residues from the glassware were transferred to the SPE cartridges. Once no more liquid could be removed from the cartridges under vacuum, avoparcin was eluted with 5 mL of pure water.

All kidney extracts were diluted 1:1 with acetonitrile prior to injection to the LC. This was done by dissolving 0.5 mL of extract in 0.5 mL of acetonitrile. Calibration curves were constructed from pure standards or standards prepared from blank kidney extracts, as appropriate.

2.4.2. Thermal stability studies

Thermal stability studies were performed by placing 1 mg mL^{-1} avoparcin stock solutions into autosampler vials, which were then immersed in a hot water bath for 1 h. The temperature of the water bath was monitored with a thermometer that was accurate to 0.5 °C. Prior to the heating step, small aliquots of the standards were diluted in the mobile phase and injected to the LC so that a reference signal could be obtained for comparison at each temperature. The vials were then heated to 55, 60, 80, or 90 °C, and the resultant solutions were injected to the LC in the same fashion. Degradation of the avoparcin complex was confirmed at a specified temperature if one or more of the following was observed during chromatography: change in

analyte peak area, change in analyte retention time, appearance of a new peak, disappearance of the analyte peak, or a change in the analyte peak shape.

3. Results and discussion

3.1. Effect of water and buffer on avoparcin retention

The retention of avoparcin on a poly(2-hydroxyethylaspartamide) bonded silica column was initially adjusted by altering the amount of aqueous component in a mobile phase composed of acetonitrile and 15 m*M* TEAP (this buffer concentration was a judicious suggestion from the manufacturer). The retention data in Fig. 1 indicate that a mobile phase containing 45% or more of aqueous component is required to achieve a retention time of less than 20 min. As expected, the HILIC hydrophilic retention mechanism caused the retention time for avoparcin to increase by about 10% for every 1% change in the organic solvent content in the mobile phase [14].

TEAP was added to the mobile phase to control the influence of secondary mechanisms, thereby facilitating reproducible analyte retention. Although the HILIC retention mechanism tends to dominate in the presence of 70% acetonitrile for the separation of small peptides [14], ion-exchange interactions and/or ion-exclusion effects can occur in more water-rich eluents [14–16,20]. The presence of a buffer salt has also been shown to improve the peak shape of vancomycin [15].

The data in Fig. 2 indicate that avoparcin becomes more retained by the HILIC column when the amount of TEAP is increased to 40 m*M* in the presence of 53% aqueous component, even though an increase in buffer concentration usually results in a decrease in analyte retention during HILIC [14,15]. Conversely, avoparcin retention decreased with an increasing salt gradient of ammonium acetate (also shown in Fig. 2). This sort of disparity is the subject of continued research in the relatively new field of HILIC. We did not perform further experiments with

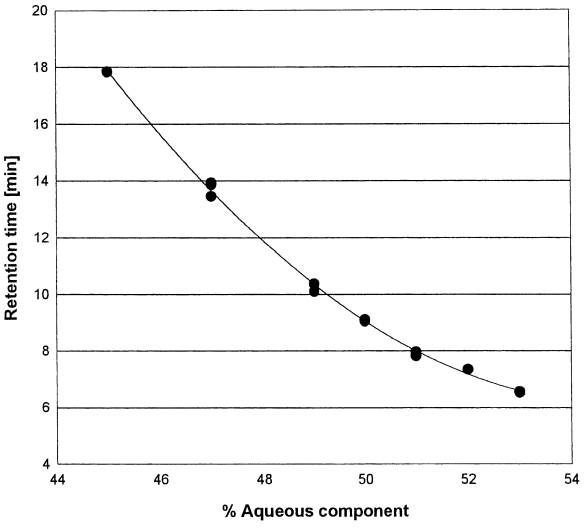

Fig. 1. Effect of the amount of aqueous component in the mobile phase on the retention time of 10 μg mL^{-1} avoparcin during HILIC. Total concentration of TEAP buffer was 15 m*M* in each case. Three injections were made at each condition.

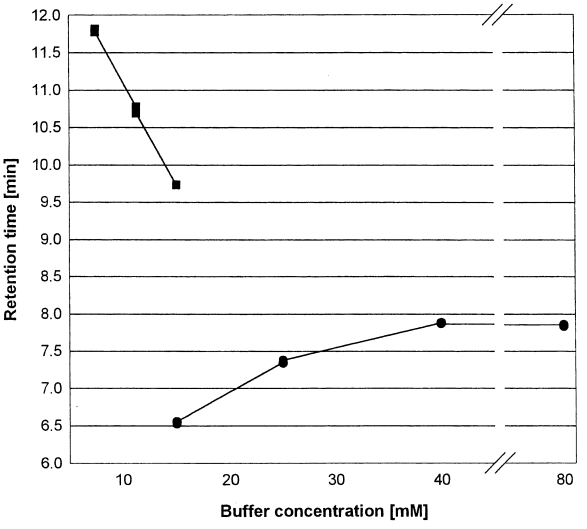

Fig. 2. The effect of the amount of TEAP (●) and ammonium acetate (■) buffers in the mobile phase on the retention time of 10 μg mL^{-1} avoparcin during HILIC. The total amount of aqueous component was 53% for TEAP experiments and 55% for ammonium acetate experiments. Three injections were made at each TEAP condition; two were made at each ammonium acetate condition.

ammonium acetate since this buffer is quite volatile and analyte peak shape was also poor.

For all future experiments, a mobile phase containing 47% aqueous component and 15 m*M* TEAP was used to provide adequate and reproducible retention and good peak shape. As shown in Fig. 3, there is no apparent resolution between α- and β-avoparcin, which allowed for easy quantitation. The

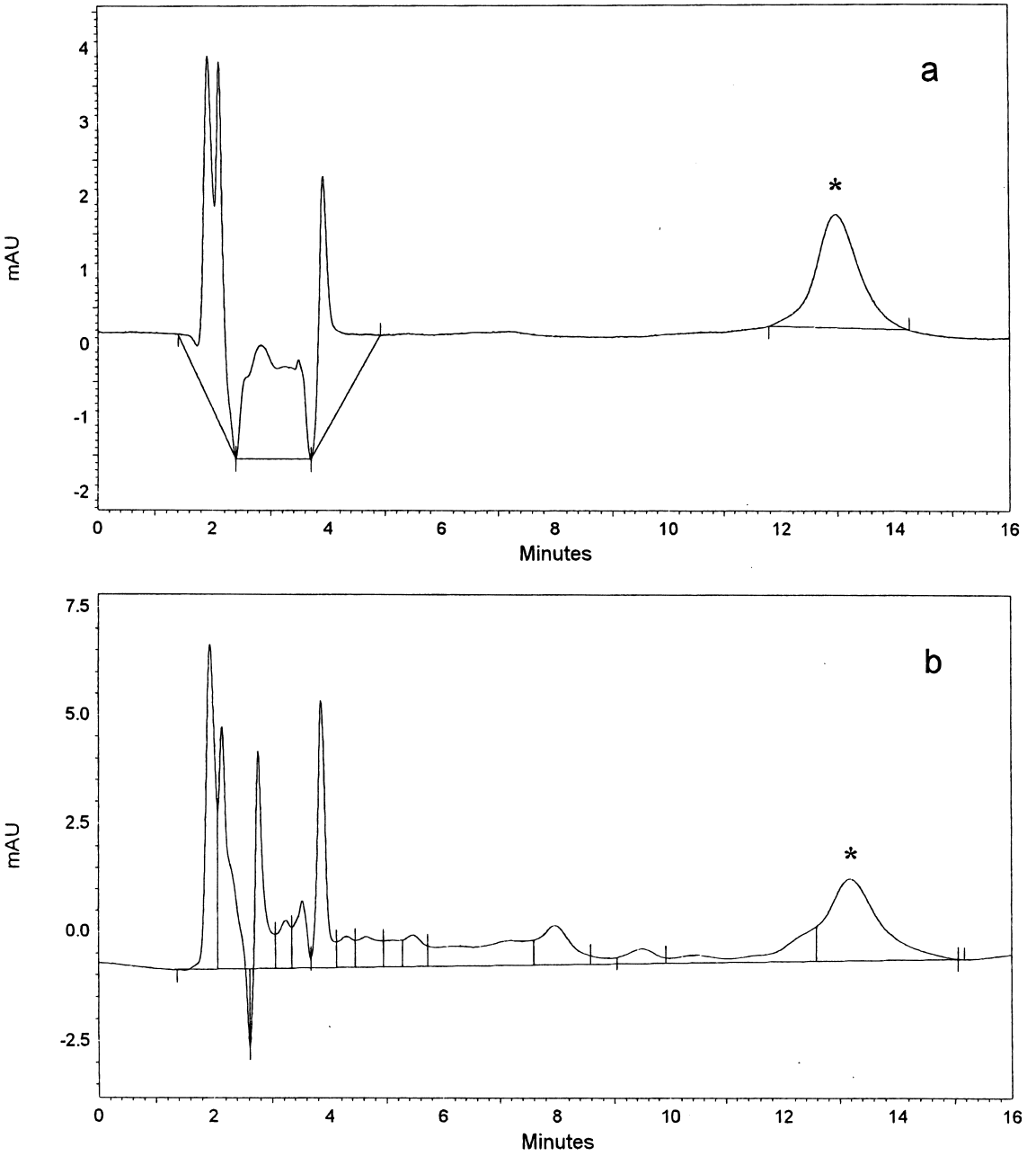

Fig. 3. Chromatograms of (a) pure avoparcin standard and (b) avoparcin prepared in a blank kidney extract. Standard concentrations are 1.0 μg mL^{-1}.

retention time of about 13 min also allowed for resolution from earlier-eluting kidney matrix coextractives. The additional chlorine atom on the β form appears to have minimal effect on compound hydrophilicity.

3.2. Concentration of aqueous extracts by HILIC solid-phase extraction

It was determined that the HILIC material is also well suited to the concentration of avoparcin from aqueous kidney extracts, since avoparcin is strongly retained by the stationary phase, for example in an acetonitrile eluent containing less than 45% aqueous component. However, since ethanol was utilized as modifier during extractions, it was necessary to determine the amount of ethanol that was required to obtain complete retention of avoparcin in solid-phase extraction cartridges. This was done by concentrating 10 mL solutions containing varying amounts of ethanol and that were uniformly spiked with 25 μg of avoparcin. Following the concentration step, the cartridges were eluted with neat water to ensure complete recovery of the analyte. The final extracts were diluted 1:1 with acetonitrile before being injected to the LC, so that the sample composition would not differ greatly from the HILIC mobile phase.

The data in Fig. 4 indicate that kidney extracts should contain no more than 40% aqueous component in ethanol (by volume) in order to achieve complete retention of avoparcin by SPE. This means that additional dilution of the extracts with ethanol may be necessary prior to the SPE concentration step.

3.3. Kidney extractions

Before proceeding with hot water extractions, it was necessary to determine whether avoparcin would degrade in hot water. Avoparcin standards heated to 80 °C were unaffected; however, a change in the chromatographic profile was observed for an avoparcin standard heated to 90 °C when compared with a corresponding normalized standard. The analyte peak from the heated standard was noticeably shorter and broader, and there was also an unresolved shoulder

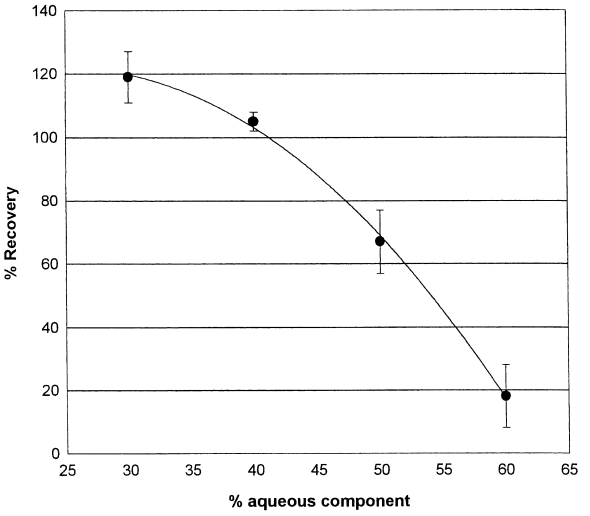

Fig. 4. Effect of the amount of aqueous component in ethanolic solutions on the retention of avoparcin in HILIC solid-phase extraction cartridges. Data are expressed as percent recovery of avoparcin. Concentration of TEAP in each solution is 15 m*M*. Data are averaged from duplicate injections of two separate samples. Error is expressed as standard deviation.

on the front end. It was therefore decided to perform all kidney extractions at 75 °C.

We anticipated complete recovery of avoparcin from kidney utilizing pure hot water, due to the high aqueous solubility of the analyte. However, analyte solubility was not the only variable requiring consideration, since recovery was at or below the limit of detection utilizing pure water. Modifier was therefore required to disrupt analyte interactions with both the dispersing polymer XAD-7 HP and the kidney matrix.

To verify this hypothesis, samples of diatomaceous earth only and of diatomaceous earth combined with XAD-7 HP were spiked with avoparcin. The dispersed samples were then extracted with pure water. Although complete recovery of avoparcin was obtained from diatomaceous earth, recovery from the polymer sample was at or below the limit of detection. Complete recovery, however, was obtained once 10% ethanol was added to the water (v/v).

For kidney extractions, the organic buffer TEAP was also added to the water to facilitate sample clean-up, since there was incomplete retention of the matrix components when ethanol was present as

modifier. Inclusion of TEAP buffer in the extraction solvent produced significant reduction in sample turbidity and colour intensity, which had been attributed to the presence of lipids and proteins [12]. TEAP was also a judicious choice for clean-up, since this buffer was compatible with the subsequent HILIC–SPE step.

To assess the effect of ethanol on the recovery of avoparcin, kidney tissue was extracted with varying amounts of ethanol mixed with water containing 21.5 m*M* TEAP. (It should be noted that while the total amount of TEAP was different at each extraction condition, avoparcin recovery was not dependent on buffer concentration.) The kidney extracts were concentrated on SPE cartridges by diluting 5 mL of extract with 7 mL of ethanol, in order to bring the final amount of ethanol to approximately 70% by volume. The entire extracts (13–15 mL total) were not passed through the cartridges because of a build-up of minor matrix interferences that tended to cause very slow sample flow. Although the SPE cartridges were eluted with 5 mL of water, this volume can be reduced to 2 mL.

As shown in Fig. 5, up to 30% of ethanol is required to attain complete recovery from kidney.

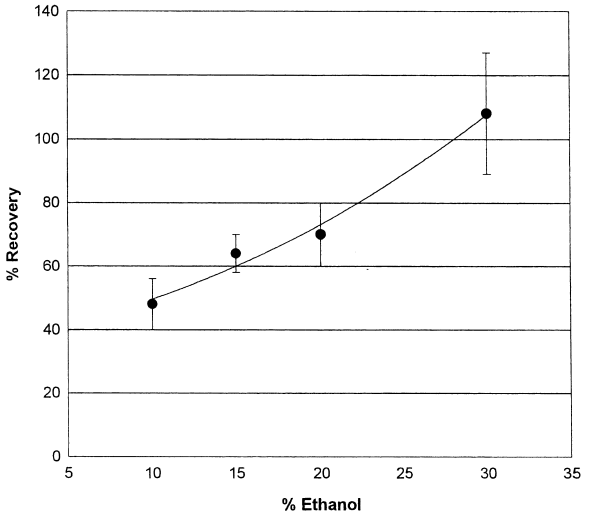

Fig. 5. Effect of the amount of ethanol in the aqueous ASE extraction solvent on the recovery of avoparcin from swine kidney. Extractions were performed at 75 °C. Data are averaged from the duplicate injections of three separate samples. Error is expressed as standard deviation.

This result is significantly different from that obtained for the previous polymer samples, which required only 10% ethanol. It is evident that avoparcin interacted directly with kidney after spiking. Avoparcin was quantified by constructing calibration curves using standards prepared from blank kidney extracts, which were analyzed in the same manner as kidney samples. The resultant calibration curves had good linearity with a minimum value of $r^2=0.98$. The chromatogram shown in Fig. 3 demonstrates that there was acceptable resolution of the analyte from matrix interferences, allowing the resultant peak to be easily quantified.

3.4. Preliminary results and future considerations

The sample preparation, extraction, concentration, and detection parameters for the preliminary analysis of avoparcin in swine kidney are supplied in Table 1. At the 30% ethanol extraction condition, the average recovery for avoparcin was 108%, with a relative standard deviation of 19 (also shown in Fig. 5). Although the limit of detection for avoparcin standards prepared in pure solvents was 0.1 μg mL^{-1}, we were only able to detect 0.25 μg of avoparcin in a 0.5 g kidney sample. At present, better sensitivity is limited by the fact that the HILIC–SPE cartridges became plugged for large extracts when connected to a simple vacuum system. It was therefore necessary to analyze only about one-third of the tissue extracts. It is also difficult to achieve satisfactory limits of detection with the UV system, due to the elevated baseline noise at 225 nm. These limitations will be resolved with the development of a modified HILIC–SPE methodology that is better suited to large tissue extracts, and by incorporating a more analyte-specific detector. Despite these current limitations in our laboratory equipment, we believe the developed method can be used with confidence to detect trace levels of avoparcin.

4. Conclusions

Our investigation demonstrates that avoparcin can be recovered from kidney samples utilizing hot water extraction technology, and that the resultant aqueous extracts can subsequently be both concentrated and

analyzed by HILIC. This instrumental method utilizes non-toxic and inexpensive materials for the sample preparation, clean-up, and extraction steps, in addition to techniques that can be easily incorporated into the modern analytical laboratory. The retention of avoparcin on the HILIC stationary phase is easily controlled by the amounts of acetonitrile, water, or buffer in the mobile phase. The HILIC material can also be utilized for the concentration of avoparcin from aqueous extracts when ethanol is the organic component.

The disruption of analyte–matrix interactions is an important and necessary consideration during the pressurized hot water extraction of polar contaminants such as avoparcin from kidney. These interactions can be overcome with the use of ethanol modifier in order to facilitate complete recovery.

The inclusion of the buffer triethylammonium phosphate to the extraction solvent provides an additional means for the clean-up of kidney that is complementary to the technique of matrix solid-phase dispersion utilizing the acrylic polymer XAD-7 HP. The use of TEAP is a judicious choice since aqueous kidney extracts are amenable to the HILIC–SPE concentration step.

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References

[1] J. Bates, J.A. Jordens, D.T. Griffiths, J. Antimicrob. Chemother. 34 (1994) 507.
[2] K. Borgen, M. Sørum, Y. Wasteson, H. Kruse, Int. J. Food Microbiol. 64 (2001) 89.
[3] A. van de Kerk-van Hoof, A.J.R. Heck, J. Antimicrob. Chemother. 44 (1999) 593.
[4] F. Howarth, D. Poulter, Lancet 347 (1996) 1047.
[5] F. Bager, M. Madsen, J. Christensen, F.M. Aarestrup, Prev. Vet. Med. 31 (1997) 95.
[6] N. Woodford, J. Med. Microbiol. 47 (1998) 849.
[7] A. Kubátová, A.J.M. Lagadec, D.J. Miller, S.B. Hawthorne, Flavour Fragr. J. 16 (2001) 64.
[8] A. Basile, M.M. Jiménez-Carmona, A.A. Clifford, J. Agric. Food Chem. 46 (1998) 5205.
[9] L. Gamiz-Garcia, M.D.L. de Castro, Talanta 51 (2000) 1179.
[10] T.N. Pawlowski, C.F. Poole, J. Agric. Food Chem. 46 (1998) 3124.
[11] J.F. Lawrence, B. Niedzwiadek, P.M. Scott, J. Assoc. Off. Anal. Chem. Int. 83 (2000) 604.
[12] M.S.S. Curren, J.W. King, J. Agric. Food Chem. 49 (2001) 2175.
[13] J.A. Field, R.L. Reed, Environ. Sci. Technol. 33 (1999) 2782.
[14] A.J. Alpert, J. Chromatogr. 499 (1990) 177.
[15] M.A. Strege, Anal. Chem. 70 (1998) 2439.
[16] M.A. Strege, S. Stevenson, S.M. Lawrence, Anal. Chem. 72 (2000) 4629.
[17] F. Sztaricskai, J. Borda, M.M. Puskás, R. Bognás, J. Antibiot. 36 (1983) 1691.
[18] W.J. McGahren, R.A. Leese, F. Barbatschi, G.O. Morton, N.A. Kuck, G.A. Ellestad, J. Antibiot. 36 (1983) 1671.
[19] The Special Review of Avoparcin, National Registration Authority for Agricultural and Veterinary Chemicals, Canberra, Australia, January 2001.
[20] B.-Y. Zhu, C.T. Mant, R.S. Hodges, J. Chromatogr. 548 (1991) 13.