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Comparative study of sample preparation methods; supported liquid membrane and solid phase extraction in the determination of benzimidazole anthelmintics in biological matrices by liquid chromatography–electrospray–mass spectrometry

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Abstract

Supported liquid membrane (SLM) and solid phase extraction (SPE) have been applied as clean-up and/or enrichment techniques for a mixture of five benzimidazole anthelmintics compounds, namely albendazole, fenbendazole, mebendazole, oxibendazole, and thiabendazole. Two biological matrices, mainly urine and milk, and ultra high purity (UHP) water were spiked with a mixture of these five compounds. Waters Oasis® MCX and International Sorbent Technology (IST) HCX SPE sorbents were used. The liquid membrane used for clean-up and/or enrichment of these compounds was 5% tri-*n*-octylphosphine oxide (TOPO) dissolved in *n*-undecane/di-*n*-hexyl ether (1:1). The SLM extraction efficiencies and SPE percentage recoveries ranged between 60 and 100%. The detection limits (DLs) for different benzimidazole compounds by SPE/LC–ES–MS for thiabendazole, oxibendazole, and albendazole was 0.1 ng/L, for fenbendazole and mebendazole was 1 and 10 ng/L, respectively. Similarly, the detection limits of SLM/LC–ES–MS for thiabendazole, oxibendazole, and albendazole was 0.1 ng/L and for fenbendazole and mebendazole was 1 ng/L. The results of optimization of various parameters of the SLM method are reported.

Keywords: Solid phase extraction; Supported liquid membrane; Liquid chromatography; Electrospray; Mass spectrometry; Benzimidazole anthelmintics

1. Introduction

Sampling and sample preparation is one of the most important aspects of the analytical science procedures. About 60–80% of the work activity and operating cost in an analytical chemistry laboratory is spent in preparing samples for introduction into analytical system or instrument of detection [1–3]. The importance of sample preparation in analytical analyses can never be over estimated. Frequently, it has been observed that, the component(s) of interest is/are in many cases present in trace levels too low to be detected easily by many analytical systems and instruments hence, the need to preconcentrate [1,2]. In most cases the analytes are present in either biological or environmental matrices that are too complex and often contain interfering elements which can mask or interfere with the analysis of the component(s) of interest such that direct analysis may not be possible [1–3]. Therefore, sample preparation is without doubt a prerequisite step to most analytical procedures.

Benzimidazole anthelmintic drugs are commonly used in the veterinary practices to treat gastro-intestinal infections and also for animal fattening purposes [4–6]. However, their use in worm treatment in animals, for example, is mostly through multi-component dosage forms containing combination of well established cocktail of anthelmintics [4–6]. Generally, the presence of drug residues can be detected in various target organs/tissues, for example, liver, kidney, fat, skin, milk, eggs, and blood; or in the metabolic by-products, such as, urine, faeces, bile, and sweat [4]. The European Union (EU) has regulated the maximum residue limits (MRLs) for some of these compounds, ranging from 0.010 to 1.00 μg/L depending on the compound of interest and the type of food (type of tissue) [6–8].

Solid phase extraction (SPE) is commonly used as a sample preparation method for a variety of analytical procedures [5–7]. However, the use of supported liquid membrane

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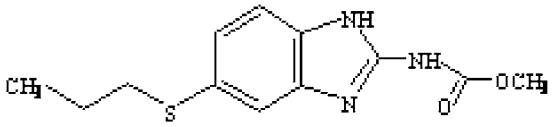
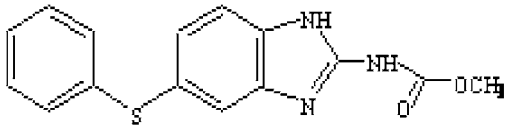
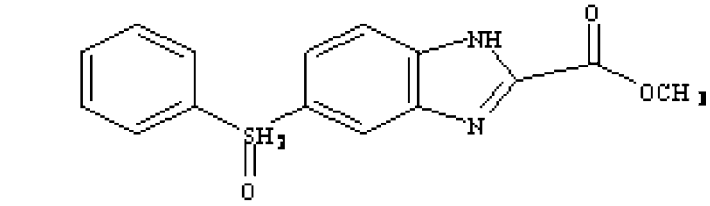
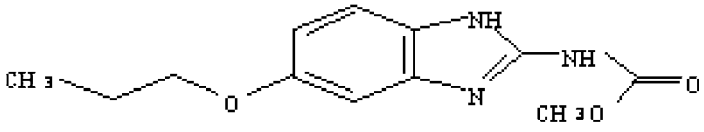
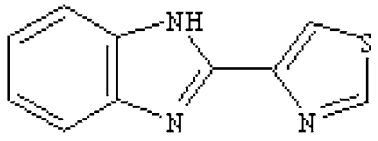
Table 1
The structures and molecular weights of benzimidazole anthelmintics under this study

Structure	Molar mass	CAS#
 Albendazole (Methyl-5[propylthiol-2-benzimidazole-carbamate)	265.34	54965–21–8
 Fenbendazole (Methyl-5-[phenylthiol]-2-benzimidazole-carbamate)	299.35	43210–67–9
 Mebendazole (5-Benzoyl-2-benzimidazole-carbamic acid methyl ester)	295.30	31431–39–7
 Oxibendazole (Methyl[5-propylthiol]-2-benzimidazole-carbamate)	249.27	20559–55–1
 Thiabendazole (2-[4-Thiazolyl]benzimidazole)	201.25	148–79–8

(SLM) technique as clean-up and/or enrichment technique for anthelmintic compounds is limited. Recently, we demonstrated the potential of SLM as sample preparation techniques for benzimidazole anthelmintic compounds [9]. Several other sample preparation techniques have been reported for the analysis of one or more benzimidazole anthelmintic compounds in a variety of matrices [10–14]. There are several methods of analysis that incorporated sample preparation and/or clean-up techniques that have been reported in the literature. Many of these methods involve some form of separation such as gas chromatography (GC), and high pressure liquid chromatography (HPLC) using a variety of detectors. Mass spectrometry is the most sensitive and selective detectors used for GC and HPLC. However, GC–MS requires derivatisation of the compounds prior to detection. This additional step is time consuming as well as labour intensive. HPLC method, on the other hand, is direct and the analytes can be analyzed in the same matrix without the need for derivatisation. LC–MS has the potential to be the most useful multi-residue method of analysis for a variety of compounds. When LC–MS is combined with the sample preparation and/or clean-up technique the sensitivity is extended at least two orders of magnitude.

A comparative study of SPE and SLM enrichment of a five anthelmintic compounds mixture in water, urine, and milk biomatrices is presented in this study. LC–ES–MS was used as an analytical method.

2. Experimental

2.1. Standards and chemicals

The benzimidazole anthelmintics (albendazole, fenbendazole, mebendazole, oxibendazole, and thiabendazole), tri-*n*-octylphosphine oxide (TOPO), *n*-undecane, and ammonium hydroxide used were from Sigma (St. Louis, USA). Di-*n*-hexylether (99%) was from Aldrich (Steinheim, Germany), sulphuric acid (99%), sodium hydroxide pellets (98%), sodium bicarbonate (98%), and acetic acid were from Saarchem (Krugerdorp, South Africa). Formic acid was purchased from N.T. Laboratory Supplies (Johannesburg, South Africa). HPLC grade methanol and acetonitrile were from BDH Laboratory (Poole, UK) and ethyl acetate was from LAB-SCAN (Stillorgan, Dublin, Ireland). Ultra high purity (UHP) water was processed through a Millipore Quantum Ultrapure Ionex Gradient A10 purification system (Millipore, Molsheim, France). The aqueous solvents were filtered through cellulose nitrate membrane with 0.45 μm pore size and 47 mm diameter while the organic solvents were filtered through 0.45 μm organic membrane filter, type HVLP, Millipore (Dublin, Ireland). The structures and molecular weights including the CAS numbers of the benzimidazole anthelmintic compounds studied are shown in Table 1.

2.2. Stock solutions and preparation of working standards

Stock solutions for a mixture of all benzimidazole anthelmintics were dissolved in methanol/water (1:1) containing 5% formic acid to make a stock solution of 1000 mg/L. The working standards were each prepared by diluting with water the 1000 mg/L master standards to the required volume of the stock solution to make appropriate concentrations. The solutions mixtures of benzimidazole anthelmintics were prepared by diluting the 1000 mg/L master standards by UHP water, urine, and milk, respectively. The spiked mixtures were stored at 4 °C until needed.

2.3. Sample preparation of anthelmintics mixtures in water, urine, and milk

A 1.5 mL of milk, urine or UHP water was transferred into 15 mL centrifuge tubes and then spiked with appropriate volume to make solutions between 0.01 ng/L and 1 μg/L concentration levels [9,10]. Four milliliters of acetonitrile was added and the contents shaken for 15 min, and then centrifuged for 5 min at 1800 rpm. The supernatant solution was transferred into another tubes containing 1.5 mL of *n*-octane, and further ultrasonicated for 15 min and then centrifuged for 5 min at 1800 rpm. The top layer was discarded and the tubes contents were mixed with 5 mL ethyl acetate and centrifuged for 5 min at 1800 rpm. The separated upper aqueous layer was removed followed by addition of 3 mL of 0.1 M phosphate (KH_2PO_4) buffer (∼pH 10) and the resulting solutions were shaken and then centrifuged for 5 min at 1800 rpm. The bottom organic layer from each tube was transferred into another tube and was evaporated with nitrogen. The residue was dissolved in appropriate solvent for either SLM or SPE clean-up. In all cases, blank samples were prepared in each biomatrix in the same way without spiking.

Table 2
Procedure for solid phase extraction (SPE) of anthelmintics compounds

Step#	Procedure	IST HCX (mL)	Waters Oasis® MCX (mL)
1	Condition with acetonitrile/acetic acid (95:5)	1.5	0.75
2	Application of sample in 5% formic acid in methanol	6.0	3.0
3	Acetone wash of the cartridge	1.5	0.75
4	Methanol wash of cartridge	3.0	1.5
5	Acetonitrile wash of cartridge	3.0	1.5
6	Elution of analytes with acetonitrile + 29.3% aqueous ammonium	3.0	1.5

2.4. Solid phase extraction of benzimidazole anthelmintic mixtures

A 12 port model Visiprep solid phase extraction vacuum manifolds from Supelco (Bellefonte, USA) was used for the extraction. This consisted of Visiprep large volume sampler tubes, which enabled transfer of samples directly from sample container to the solid extraction tubes and a Visiprep SPE vacuum manifold. Two types of SPE cartridges were used for sample preparation, mainly International Sorbent Technology (IST), Isolute HCX 130 mg, 6 mL cartridges (Midglamorgan, UK), and Waters Oasis® MCX, 60 mg, 3 mL cartridges (Massachusetts, USA). The cartridges conditioning, application of analyte, washing and elution are given in Table 2 for two types of SPE. For extraction procedure modification of the method given by Waters Oasis® MCX manufacturers' extraction products instruction sheet and Rose [10] was used. The analytes were eluted with a mixture of acetonitrile and 29.3% aqueous ammonium (95:5) into a test tube, 3 mL for HCX and 1.5 mL for MCX.

2.5. Enrichment of benzimidazole anthelmintics by SLM

The membrane solvent was prepared by dissolving 5% TOPO in *n*-undecane + di-*n*-hexylether (1:1). When the TOPO was completely dissolved, a Millipore filter, FG type with a pore size of 0.2 μm made of Teflon was immersed into the liquid membrane. The membrane was immersed for 24 min into membrane solvent, turning its side every 12 min. The impregnated membrane was placed between the two PVDF blocks, with the rough side of the membrane facing the donor side and the whole construction was clamped together tightly and evenly with six screws. The donor (feed) and the acceptor (receiving/stripping) channels of the SLM unit, were separated from each other by a liquid membrane [15]. After installation of the impregnated

Table 3
Gradient elution for five benzimidazole anthelmintic compounds

Time (min)	Flow rate (μL/min)	*A*	*B*
0	150	20	80
6	150	20	80
10	150	40	60
11	130	50	50
15	130	50	50
25	130	60	40

A = 25 mM acetic acid in acetonitrile, *B* = 25 mM acetic acid in water.

liquid membrane in the separator, both channels were flashed with UHP water to remove excess of the organic solvent from the surface of the membrane. Two peristaltic pumps Minipuls 3, Gilson (Villiers-Le-Bel, France) were used to control the flow rates of the donor and acceptor phases independently. The tubes used for pumping solutions were acid-resistant (acid-flexible), Elkay Products (Shrewsbury, MA, USA) with an internal diameter of 1.2 mm for the donor and 0.60 mm for the acceptor. The various parts of the flow system were connected with 0.5 mm internal diameter PTFE tubing and Alex screws fittings. The sample and buffer in the donor stream were emerging in a PTFE tee connection and then mixed in a coil (1.0 m × 0.5 mm i.d. coiled tubing) before entering the donor channel of the membrane device. The donor buffer used was NaOH/$NaHCO_3$ at pH 9.60 [9].

2.6. HPLC separation of benzimidazole anthelmintic compounds

A ThermoQuest HPLC system with DAD detector, quaternary pump system, thermostated column compartment, and an auto-sampler (Finnigan, San Jose, USA), coupled to ThermoQuest LCQ Quadrupole ion trap mass spectrometry (Finnigan, San Jose, USA) were used for the detection of all separated benzimidazole anthelmintic compounds. Separation of the anthelmintic compounds was by gradient elution. The components of the mixture were all separated within 20 min using the given conditions (Table 3). A shorter elution time could be achieved by increasing the organic component of the mobile phase (Table 4), i.e. less than 10 min. The mobile phase composition was: *A* = 75% acetonitrile + 25% of 25 mM acetic acid in water; *B* = 75% of 25 mM acetic acid in water + 25% acetonitrile (Table 4). Waters XTerra® C_8 microbore column 50 mm × 2.1 mm and particle size of 3.5 μm, at a flow rate of 150 μL/min was used for all separations. Ten microliters of the anthelmintics was injected into the analytical column. Monitoring of the separation components was made either by ES–MS or UV–vis at 296 nm.

Table 4
Gradient elution for five benzimidazole anthelmintic compounds

Time (min)	Flow rate (μL/min)	*A*	*B*
0.00	150	0	100
5.00	150	100	0
12.00	150	100	0

A = 75% acetonitrile + 25% acetic acid in water, *B* = 75% of 25 mM acetic acid in water + 25% acetonitrile.

2.7. LC–ES–SIM–MS

The compounds were separated on a short C_8 reversed-phase microbore column with acetic acid in acetonitrile/water as specified above and detected with the electrospray ion trap mass spectrometer. Acetic acid was added into the acetonitrile/water mobile phase to assist the formation of the charged droplets and hence produce protonated molecular ions.

3. Results and discussion

3.1. Extraction from the matrices

Benzimidazole anthelmintics are known to possess basic hydrophobic character and thereby are soluble in polar organic solvents. This property was exploited during the extraction from urine, milk, and water, where ethyl acetate was used to recover these drugs from types of matrices [9,10].

3.2. Sample preparation for benzimidazole anthelmintics in biomatrices

3.2.1. SPE enrichment and/or clean-up

The chemical similarities between benzimidazole anthelmintics were exploited during clean-up and/or enrichment using two types of the solid phase extraction sorbents. These consisted of (i) Waters Oasis® MCX sorbent, this type of sorbent is classified as a mixed mode, it features two retention mechanism mainly strong cation exchange and reversed-phase and (ii) Isolute HCX is a strong cation exchange. The most attractive features of the Waters Oasis® MCX are its hydrophilic–lipophilic-balanced composition that is responsible for both strong reversed-phase retention and water-wetability. In addition, these sorbents are stable from pH 1 to 14 due to the use of pH wash systems, which also allows optimization of the method as well as high selectivity for the analyte. Hence, minimize the need to adjust the pH to limited pH ranges. On the other hand, the Isolute HCX cartridges are IST styrene–divinylbenzene based on strong cation exchange resins that retain charged or potentially charged compounds from a primarily aqueous matrix and which contains sulfonic acid cation exchanger in the hydrogen form. This property appeared to have been essential for retaining the basic and amphoteric benzimidazole compounds.

Figs. 1 and 2 show a comparative study of the recovery efficiencies of benzimidazole anthelmintics between the two types of SPE cartridges from water and milk. The data in Fig. 1 shows that both Isolute HCX and Waters Oasis® MCX reached their breakthrough volumes at about 1000 mL. At lower volume, i.e. from 25 to 500 mL, the Isolute HCX cartridges displayed higher percentage recovery, but for a volume greater than 500 mL, higher recoveries were obtained with Waters Oasis® MCX cartridges. It should be noted that the mass of the sorbent material for Isolute HCX (130 mg, 6 mL) is more than twice as much as

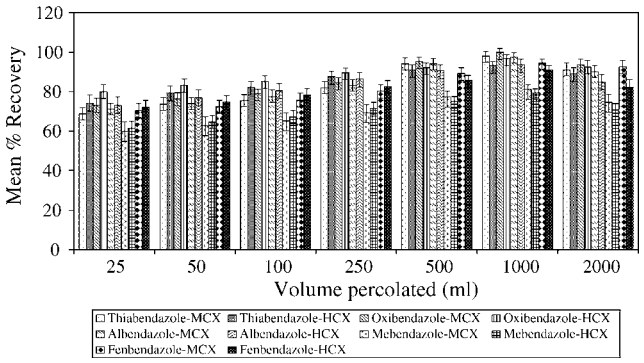


Fig. 1. Mean% recoveries for 1.0 ng/L concentration of benzimidazole anthelmintic mixture in water, with variation of percolated sample volume in the sorbents, $n = 5$.

that of Waters Oasis® MCX (60 mg, 3 mL) cartridges. Therefore, it is expected that the recovery should increase when IST HCX is used in comparison with Waters Oasis® MCX cartridges, especially when smaller sample volume is percolated. However, the Waters Oasis® MCX sorbents displayed greater capacity especially when larger volume were percolated probably due to the fact that, the sorbent material is made up of smaller particle size with larger pore diameters which enabled higher sample loading speed. Waters Oasis® MCX sorbent used in this work has particle sizes of 30 μm in comparison with 50 μm observed for IST HCX cartridges. The Waters Oasis® MCX had specific surface area of 810 m^2/g in comparison with 521 m^2/g for IST HCX, and average pore diameter of 80 Å compared to 54 Å of HCX sorbents. It should be noted also that, the percolation time was not the same for the different volumes percolated. The larger the volume the longer it took to elute the sample. Similarly, Fig. 2 shows the trend observed in Fig. 1. The optimum extraction efficiency was observed at concentration of 0.001 μg/L for all the compounds from milk and urine biological matrices.

3.3. SLM clean-up and enrichment

Several membrane liquids were tested in an attempt to identify the best trapping supported liquid membrane, mainly: (i) hexylamine; (ii) *n*-undecane; (iii) di-*n*-hexylether; (iv) *n*-undecane/di-*n*-hexylether (1:1); and (v) 5% TOPO in *n*-

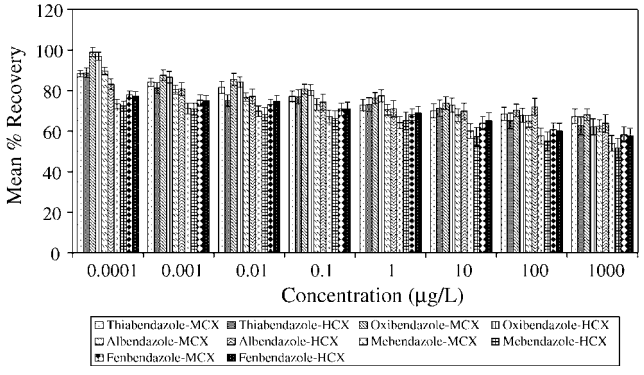


Fig. 2. Comparison of MCX and HCX sorbents in the extraction of anthelmintics mixture spiked in milk, $n = 5$.

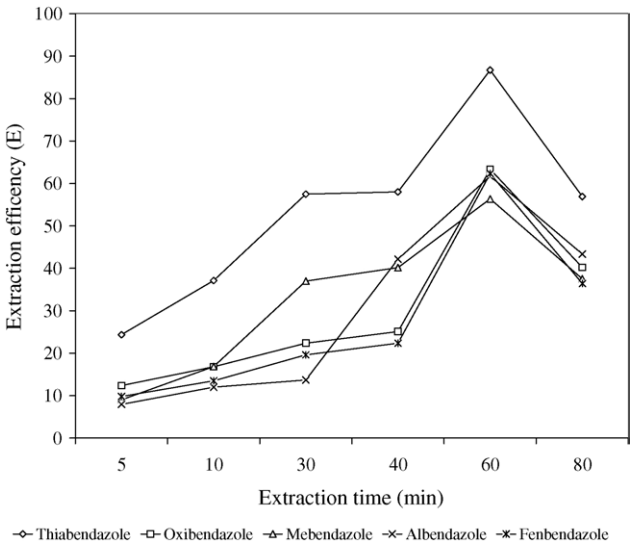


Fig. 3. Optimization of enrichment time using di-*n*-hexylether: *n*-undecane (1:1) + 5% TOPO membrane in SLM extraction of benzimidazole with LC/UV–vis detection.

undecane/di-*n*-hexylether (1:1). The first four membranes were unsuccessful with regard to efficient recovering of investigated compounds from biological matrix. Membrane (i) and (ii) only successfully recovered thiabendazole, membrane (iii) only oxibendazole and membrane (iv) thiabendazole and to lesser extent albendazole Membrane (v), 5% TOPO in (1:1) undecane/di-*n*-hexylether was found to be the best membrane as all the compounds of interest were successfully recovered with high extraction efficiencies in comparison to other membranes. The presence of lone pairs of electrons on the oxygen atom in TOPO has been proposed to be responsible for the high efficiencies due to chelation formed with the molecules of the analytes [16]. Both acceptor and donor channels of the SLM were optimized to achieve optimal efficiencies [9]. Fig. 3 shows the optimization of enrichment time using optimal pH of acceptor and donor conditions. The trend observed is due to the increasing enrichment factor with respect to time. However, the increase reaches an optimal at about 60 min before it starts to decrease. The reason for this behaviour is not clear, but we believe that the decrease might be due to the equilibrium that is established after the saturation of the stagnant aqueous acceptor solution. This may bring about back extraction into the donor side of the membrane.

3.4. Comparison of SPE and SLM enrichment and/or clean-up

Fig. 4, shows a comparison between the two types of SPE cartridges and SLM in the extraction of the five benzimidazole anthelmintics compounds from water. The results show that there is no significant difference between these two techniques, except that different individual compounds performed differently when applied in these two techniques. Mebendazole, for example, extraction by the two SPE was noticeably lowest than the other five compounds. While as in SLM these compounds extracted as well as the other compounds.

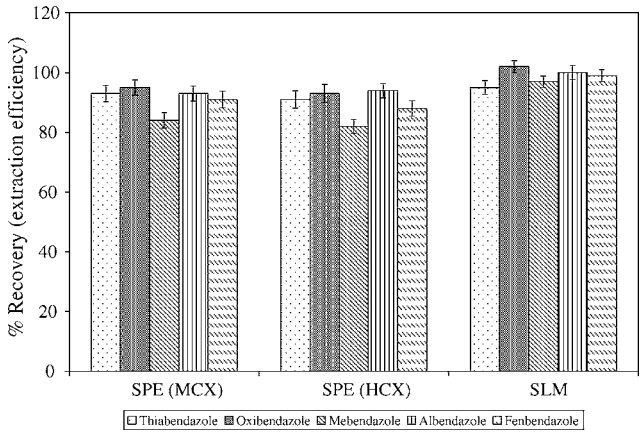


Fig. 4. Comparison of SLM with MCX and HCX sorbents in the extraction efficiency of anthelmintics mixture spiked in water samples, $n = 5$.

3.5. LC–ES–SIM–MS

The compounds were separated on a short C_8 reversed-phase microbore column and detected with the electrospray ion trap mass spectrometer. Fig. 5 shows a typical separation of a mixture of benzimidazole anthelmintics with a C_8 XTerra® column. The five compounds in the mixture were well resolved from each other and the separation was achieved within 21 min. A separation method using a shorter column was also developed using a gradient given in Table 4, the compounds were eluted within 17 min using this method. The last four compounds were not as well resolved from each other as was observed in Fig. 5. Separation of the 0.01 ng/L concentration of benzimidazole anthelmintics mixture from milk that had been processed through SLM (results not shown). Similarly, the same concentration was processed by SPE and the results were not significantly different (Figure not shown).

3.6. Linearity and linear range in the extraction of benzimidazole anthelmintics

Linearity was determined for membrane solvent (v) at different concentration levels between 0.1 ng/L and 1 mg/L mixture of benzimidazoles at constant enrichment time of 1 h. Linearity was determined for each membrane at different concentration level in five replicates. Similarly, for SPE each concentration level was processed with a different (new) cartridge. Correlation coefficients of the order 0.99 and 0.94–0.99 were achieved for SLM and SPE, respectively. Linear range of the order of 1 ng/L–0.01 μg/L for thiabendazole, oxibendazole, and albendazole; 0.1 ng/L–1 mg/L for fenbendazole and 1 μg/L–1 mg/L for mebendazole were achieved for Waters Oasis® MCX sorbents. On the other hand, SLM linear relationship of 0.01 μg/L–1 mg/L for oxibendazole, albendazole, and

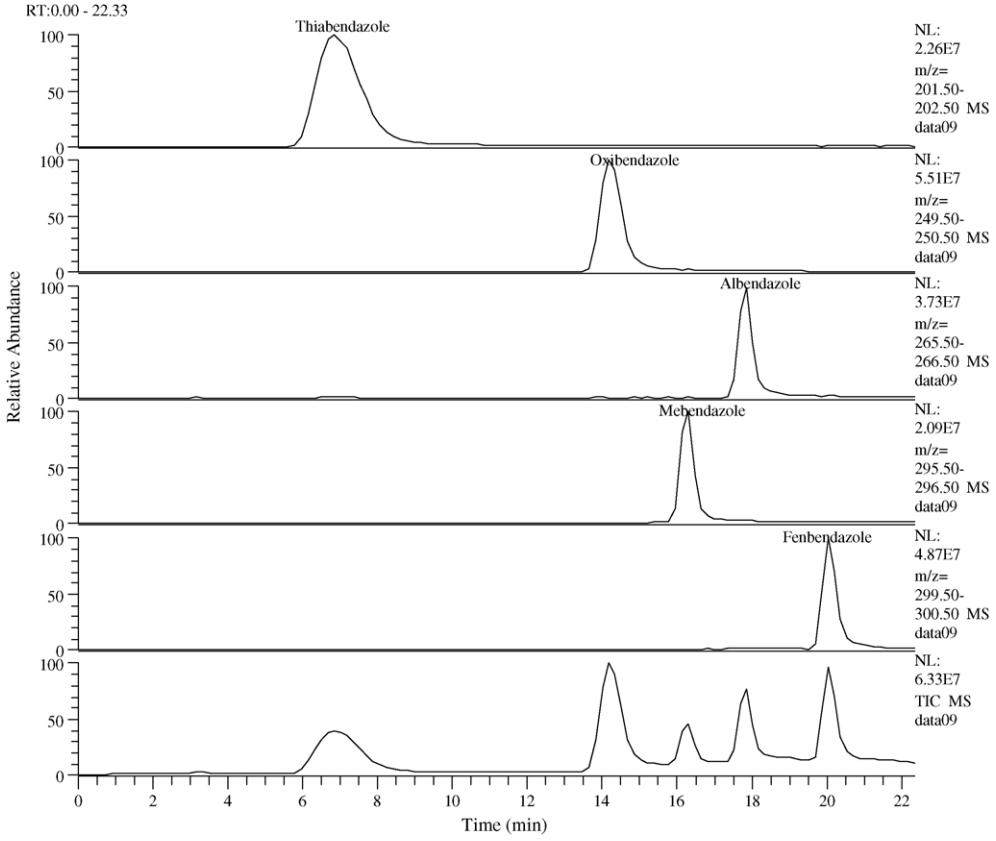


Fig. 5. SLM–LC–ES–SIM–MS for a 1 mg/L mixture of benzimidazole anthelmintics spiked in urine matrix and separated according to Table 3; column = XTerra® 150 mm × 2.1 mm × 3.5 μm.

Table 5
(a) Intra-day method validation for samples spiked at 100 μg/L[a] and (b) inter-day method validation for samples spiked at 100 μg/L[b]

Series from same day	Thiabendazole	S.D.	Oxibendazole	S.D.	Mebendazole	S.D.	Albendazole	S.D.	Fenbendazole	S.D.
Mean% recoveries										
Time 1	86.7	1.1	63.3	1.8	56.4	1.0	61.7	1.3	62.3	1.6
Time 2	88.2		65.8		55		63.5		60.0	
Time 3	87	1.2	62.7	1.6	56.1	1.0	62.8	1.8	62.4	1.1
Time 4	85.3		65		54.7		60.9		63.9	
Time 5	85.9	1.6	63.2	1.6	55.9	1.3	60.9	1.6	61.8	1.6
Time 6	88.1		60.9		57.8		63.2		64.1	
Series from 6 days	Thiabendazole	S.D.	Oxibendazole	S.D.	Mebendazole	S.D.	Albendazole	S.D.	Fenbendazole	S.D.
Mean% recoveries										
Day 1	87.2	1.5	64.1	2.3	58.1	1.8	63.5	2.3	65.8	2.5
Day 2	85.1		67.4		55.6		60.2		62.2	
Day 3	89.2	1.8	63.8	1.8	53.4	2.8	62.9	2.3	64.6	2.3
Day 4	86.6		61.2		57.3		59.7		61.3	
Day 5	84.2	2.3	65.7	1.6	54.1	2.5	64.2	2.4	63.7	1.6
Day 6	87.4		63.5		57.6		60.8		61.4	

[a] The blank samples were analyzed each time and were found negative throughout. S.D. = standard deviation = repeatability.
[b] The blank samples were analyzed each day and were found negative throughout. NB: S.D. = standard deviation = reproducibility.

thiabendazole; 0.1 μg/L–1 mg/L for fenbendazole and mebendazole were also achieved.

4. Method validation

To validate the method developed, bovine liver tissue was spiked with a mixture of benzimidazole anthelmintic standards of known concentrations ranging from 10 μg/kg to 1 mg/kg to verify the absence of benzimidazole anthelmintic residues. Batches of spiked samples together with the blank control samples were analyzed and checked for repeatability (intra-day variation) and reproducibility (inter-day variation) for six separate days. Four sets of samples and one blank were prepared and from each five replicates were extracted by both SLM and SPE and determined using LC–ES–MS. The results for inter- and intra-assay recoveries and precision are shown in Table 5a and b. The values of standard deviations (S.D.) obtained for within intra-day recoveries ranged between 1.0 and 1.8. The standard deviation values for within same day measurements gives the measure of repeatability of the results obtained. On the other hand, the standard deviation values for inter-day recoveries ranged between 1.5 and 2.5. This refers to the reproducibility of the results obtained. In all cases, the results show good repeatability and reproducibility and that blank samples tested negative throughout.

4.1. Detection limits (DLs)

Detection limits, were calculated as the analyte concentration giving a signal equal to the blank signal (y_B) plus three standard deviations (for $n = 5$) of the blank (s_B), that is, $y_B + 3s_B$ [17]. When SPE was used to enrich these compounds the DL obtained for urine spiked samples were 0.1 ng/L for thiabendazole, oxibendazole and albendazole, 1 ng/L for fenbendazole and 10 ng/L for mebendazole. For milk spiked samples, the detection limits were ∼2 ng/L for thiabendazole, oxibendazole, and albendazole, 4 ng/L for fenbendazole and 18 ng/L for mebendazole. Whereas for SLM the DL obtained from urine spiked samples was 0.1 ng/L for oxibendazole, thiabendazole, and albendazole and 1 ng/L for fenbendazole and mebendazole. For kidney and liver tissues the DL values were 1 ng/kg for oxibendazole, thiabendazole, and albendazole, 2 ng/kg for fenbendazole and 7 ng/kg for mebendazole. The DL values for milk spiked samples were 2 ng/L for thiabendazole, oxibendazole, and albendazole; 3 ng/L for fenbendazole and 17 ng/L for mebendazole. In both cases LC–ES–MS was used as a detection method.

The detection limits obtained in this work are far below the maximum residual limits as stipulated by the European Union (Table 6) [18]. DL values reported by other researchers [19–23] range between 0.05 and 60 μg/L whereas in this

Table 6
Maximum residual limits (MRLs, μg/kg) for benzimidazole anthelmintics as stipulated by the European Union [18]

Marker residue, pharmacol. active subst. and animal sp.	Milk	Muscle	Liver	Kidney	Fat
Oxibendazole (cattle, swine, chicken)		100	100	100	100
Mebendazole (cattle, chicken)	50				
Albendazole (cattle, chicken)	100	100	1000	500	100
Thiabendazole (cattle, chicken)	100	100	100	100	100
Fenbendazole	10	10	10	10	10

work the DL values are of one order of magnitude lower as compared to the previous reported values. This demonstrates that the method developed can be a better alternative for routine monitoring of these compounds in the matrices studied.

5. Conclusions

Sample pretreatment (clean-up and/or enrichment) has been developed using SLM and compared to the standard method, the SPE. When concentrations ranging from 0.1 ng/L to 1 mg/L were spiked in water and enriched using both SLM and SPE, the percentage recovery obtained for the five benzimidazole compounds under SLM ranged from 74 to ∼100% for thiabendazole, 72 to ∼100% for oxibendazole, 65 to 96.7% for mebendazole, 68 to ∼100% for albendazole, and 63 to 95% for fenbendazole, while as in comparison with SPE using Waters Oasis® MCX sorbents, the percentage recovery obtained was 78 to 99% for thiabendazole, 81 to 98% for oxibendazole, 67 to 93.25% for mebendazole, 75 to 95.40% for albendazole, and 70 to 92.88% for fenbendazole. This showed the validity and applicability of the SLM as a clean-up and enrichment method for these compounds. The limit of detection for spiked samples, were found to range from 0.1 to 10 ng/L and 0.1 to 1 ng/L with respect to SPE and SLM, respectively. The results also demonstrated the superior retention power of Waters Oasis® MCX against IST HCX sorbents.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2005.10.002.

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