ELSEVIER

Food Chemistry 75 (2001) 345–354

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

The simultaneous determination of the ionophore antibiotics in animal tissues and eggs by tandem electrospray LC–MS–MS

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Received 23 October 2000; received in revised form 3 March 2001; accepted 3 March 2001

Abstract

A method has been developed for the simultaneous extraction and determination of the four most currently used Ionophore antibiotics (lasalocid, monensin, narasin and salinomycin) by LC–MS–MS from different animal tissues and eggs. Results show good repeatability, and mean spiked recoveries for lasalocid, monensin, narasin and salinomycin in animal livers are in the average range 93–103, 96–103, 93–102 and 97–106%, respectively, and in eggs the mean spiked recoveries are 101, 103, 98 and 102% for lasalocid, monensin, narasin and salinomycin, respectively. The detection limit is at 1 ng ml^{-1} for all the named ionophorous compounds. A quantitation level of 50 ng g^{-1} for lasalocid, monensin at 2.5 ng g^{-1}, and 10 ng g^{-1} for narasin and salinomycin is achieved which represents half the action limit prescribed by the UK Regulation in compliance with the European Council Directive 96/23/EC. A high throughput of samples is achievable using this method which allows the analysis of 30–40 samples by one analyst in a day.

Keywords: Lasalocid; Monensin; Narasin; Salinomycin; Egg; Liver

1. Introduction

Veterinary drugs have become an integral part of the livestock production and play an important role in maintenance of animal welfare, mainly for the prevention of disease, the curing of infection, controlling the risk of disease transmission to man and also increasing the productive capacity of animals. The use of veterinary medicines may induce the presence of drug residues in animal food products if appropriate withdrawal times are not respected or through the use of contaminated feed, therefore methods must be available for the determination of any residues in animal products used as food.

The ionophore antibiotics are the most successful anticoccidial agents in use today due primarily to their effectiveness in preventing coccidiosis in different animal species (i.e. all classes of poultry and ruminants) and they cause little or insignificant drug resistance problem. In addition to their anticoccidial activity, the ionophores have growth promotant properties because they improve feed efficiency and increase the rate of weight gain because they selectively inhibit some bacteria, including the methane producers, which are less efficient at feed conversion. The presence of the ionophores in ruminant feeds also reduces the problem of bloating by inhibiting the growth of bacteria typically responsible for excess mucuous production and acidosis in these animals.

Ionophores readily form complexes with polar cations, i.e. K^+, Na^+, Ca^{2+} and Mg^{2+}, and are lipid soluble. The transport of cations across the cell membrane by the ionophores is the main feature of their pharmacological activity. The activity of the ionophores against the coccidiosis causing parasites (*Eimeria* spp.) is best summarised as follows. The ionophores have a high affinity for the *Eimeria* cell membrane and are readily incorporated into the cell and they are capable of transferring cations across the membrane into the cytoplasm. This process upsets the osmotic balance of the *Eimeria* spp. which must expend energy in displacing the cations and excess water to counteract the effect caused by the ionophores, leading to the depletion of the stored energy level of the cell. The net effect results in the inability of the cell to control the osmotic imbalance caused by the ionophores leading to its death.

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0308-8146/01/$ - see front matter
PII: S0308-8146(01)00174-1

The ionophores exhibit a coccidiocidal action against the coccidia in that the parasite is killed in contrast to a coccidiostatic action where the coccidia is only prevented from further development, i.e. ''non-killing'' within the intestinal mucosa in the presence of the coccidiostatic drugs as long as an effective dosage is fed to the host animal; and the effectiveness of the coccidiostatic drugs decreases as soon as they are withdrawn or the drug is consumed below the required level.

Table 1 shows the different types of the *Eimeria* species causing coccidiosis in different animals which are all inhibited by the use of the ionophore antibiotics and some typical dosages required for coccidiosis inhibition or for improved weight gain.

Suitable methods must be available for monitoring the presence of ionophore antibiotics in animal tissue and foodstuffs resulting from improper use or from feed cross contamination. The UK Legislation controlling the presence of residues in animal products has set a differential action limit (DAL) for the ionophores in animal tissues and eggs as shown below in compliance with the EU Regulation:

Lasalocid	100 ng g^{-1}
Monensin	5 ng g^{-1}
Narasin	20 ng g^{-1}
Salinomycin	20 ng g^{-1}

Several methods (Elliot, Kennedy, & McCaughey, 1998; Oka, Nakazawa, Harada, & Macneil, 1995) have been reported for the determination of the ionophore antibiotics in matrices such as feeds, eggs, tissue and

Table 1
Dosage and indications for ionophore uses

Animal species	Indication	Typical ionophore dosagein the feed
Beef cattle, Non-lactating cattle	Feed improvement efficiency	Lasalocid: 10–30 mg kg^{-1} Monensin: 5–30 mg kg^{-1}
	Increase in rate of weight gain	Lasalocid: 25–30 mg kg^{-1} Monensin: 25–100 mg kg^{-1}
	Prevention and control of coccidiosis caused by *E. bovis* and *E. zuernii*	Lasalocid: 70–130 mg kg^{-1} Monensin: 10–30 mg kg^{-1}
Goats	Prevention and control of coccidiosis caused by *E. crandallis* and *E. christenseni*	Monensin: 20 mg kg^{-1}
Sheep	Prevention and control of coccidiosis caused by *E. ovina, E. crandallis, E. ovinoidalis, E. parva* and *E.intricata*	Lasalocid: 20–30 mg kg^{-1}
Pigs	Feed improvement efficiency and increase in rate of weight gain	Salinomycin : 15–60 mg kg^{-1}
Broiler chickens	Prevention of coccidiosis caused by *E. necatrix, E. tenella, E. acervulina, E. brunetti, E. mivati* and *E. maxima*	Lasalocid : 60–120 mg kg^{-1} Monensin: 90–110 mg kg^{-1} Narasin : 55–75 mg kg^{-1} Salinomycin : 40–110 mg kg^{-1}
Chicken raised for laying	Prevention of coccidiosis caused by *E. necatrix, E. tenella, E. acervulina, E.brunetti, E. mivati* and *E.maxima*	Lasalocid : 60–120 mg kg^{-1} Monensin: 90–110 mg kg^{-1} Fed continuously until 16 weeks of age and not to laying hens.
Turkeys	Prevention and control of coccidiosis caused by *E. adenoides, E. meleagrimitis*, and *E. gallopavonis*	Lasalocid : 90–120 mg kg^{-1}
Pheasants	Prevention and control of coccidiosis caused by *E. colchici* and *E. phasiani*	Monensin: 60–90 mg kg^{-1} Lasalocid : 90–120 mg kg^{-1}

liver. These methods generally involve solvent extraction of the residues followed by a clean-up stage; determination is achieved using different types of chromatography which are outlined in Table 2.

The methods outlined in Table 2 have limitations for their application to the simultaneous determination of ionophore residues in animal tissues regarding the analysis time and the throughput of samples per working day which are important factors.

The aim of this study was to develop a rapid method which could be used to examine a large number of liver samples from different animal species and eggs, with specific detection and a limit of quantitation below the prescribed action level for the presence of lasalocid, monensin, narasin and salinomycin residues. It has been found that LC–MS–MS provides better specificity and improved sensitivity compared with conventional HPLC with ultra violet/fluorescence detection or LC–MS. A significant reduction in sample preparation and chromatographic development times is achievable with LC–MS–MS making it a cost effective and efficient tool for the screening and confirmation of the ionophore residues in animal tissues and eggs.

2. Experimental

2.1. Reagents and materials

Animal tissues and eggs were obtained from local retail outlets and were examined for the presence of lasalocid, monensin, narasin and salinomycin by the described method. The tissues and eggs were homogenised and stored at −30 and 4°C, respectively.

2.2. Reagents

Methanol, acetonitrile, tetrahydrofuran: HPLC grade, Aldrich, Riedel-de Haën, Seelze, Germany; Trifluoroaetic acid, Sigma, St Louis, Montana, USA; Sodium sulphate anhydrous, Analytical grade, Aldrich, Gillingham, Dorset, UK; Lasalocid sodium salt (approx. 97%), Narasin sodium salt (approx 97%), Salinomycin sodium salt (approx 96%) analytical standards, Sigma, St Louis, Montana, USA; Monensin sodium salt, (⩾95%) analytical standard, Calbiochem, La Jolla, California, USA; Silica — SPE columns (500 mg, volume 3 ml), ISOLUTE IST, Ystrad Mynach, Mid Glamorgan, UK.

2.2.1. Standards stock solutions (1 ml = 1 mg ionophore)

Lasalocid sodium salt (0.0258 g) 97% purity; 0.0263 g of monensin sodium salt 95% purity; 0.0258 g of narasin sodium salt 97% purity; 0.0260 g of salinomycin sodium salt 96% purity were dissolved separately in methanol and made to volume in four separate 25 ml volumetric flask. These solutions are stable for up to 3 months and are kept at 40°C.

2.2.2. Working solution (Ionophore mixture)

Lasalocid (100 μl), 5 μl of monensin, 20 μl of narasin and 20 μl of salinomycin stock solutions (1 ml = 1 mg) were pipetted into a 10-ml volumetric flask and made to the mark with acetonitrile. This solution is prepared weekly and kept at 40°C.

2.3. Spiking of samples

Five gram portions of blank material (i.e. material which had been shown to contain no detectable lasalocid, monensin, narasin and salinomycin by the developed method) were spiked with 25, 50 and 100 μl of the working standard solution (ionophore mixture; Table 3).

The spiked samples were left to stand for 1 h prior to the extractions.

2.4. Apparatus

Polypropylene tubes, Falcon tubes (15 and 50 ml), Becton Dickinson Labware, France; Long Flat-end spatula, (205×10 mm, stainless steel); Centrifuge up to 4000 ***g*** — Jouan CR422, St Herblain, France; Turbo-Vap Evaporator (40°C) with nitrogen supply, Zymark, USA; Mechanical shaker — JK IKA Labortechnik, Staufen, Germany.

2.5. Method

A 5 g portion of liver or egg was weighed in a 50-ml Falcon tube, then 15 g of anhydrous sodium sulphate was added to the tube. With the flat end of a long spatula, the tissue or egg was mixed until a powdery or granular mixture was obtained. This was best achieved by mixing the sodium sulphate with the sample for approx. 1 min and leaving to stand for approx. 5 min. When all the water was absorbed by the sodium sulphate, the solid mixture was dispersed with the spatula. The granular mixture was not allowed to set at the bottom of the tube; if this occurred, the tube was inverted and with a tapping action the solid was dislocated to allow maximum contact with the solvent. Ten milliletres of acetonitrile was added to the mixture and this was vortexed for 30 s. The tube was placed on a horizontal shaker for 30 min at 300 motions min^{-1}. The mixture was then centrifuged at 4000 ***g*** for 15 min.

A silica (SPE) cartridge was conditioned with 2 ml of acetonitrile, then a 5-ml aliquot of the extract was passed through under gravity and the eluate was collected in a 15-ml Falcon tube. The silica cartridge was washed with a further 2 ml of acetonitrile and this was collected into the falcon tube. The combined eluate was evaporated to 1 ml in a Turbo-vap at 40°C. The concentrated

Table 2
Methods for ionophores detection

Ionophore	Sample matrix	Method	Condition	Applicable level	Reference
Lasalocid	Chicken liver	Thermospray LC–MS–MS	Column: μ-bondasphere C18; mobile phase acetonitrile-methanol-0.1 M ammonium acetate (2:2:1)	>60 ng g^{-1}	Horii, Miyahara, and Maruyama, 1991
Monensin	Chicken fat	TLC–FABMS Thin layer chromatography-fast atom bombardment MS	TLC: silica gel as the stationary phase and carbon tetrachloride–benzene-cellosolve (80:10:10) as solvent	>10 ng g^{-1}	Blomkvist, Jansson, Ryhage, and Osterdahl, 1986
Monensin, narasin, salinomycin	Premix, feed	TLC–colour reaction with vanillin	Solvent: ethyl acetate–water (100:3)	3–100 mg kg^{-1}	Owles, 1984
Monensin, narasin, salinomycin	Feed	Post-column derivatisation with vanillin at 70°C	Column: Partisil ODS; mobile phase: methanol–water–acetic acid (940:59:1); detection at UV 520 nm	Monensin >250 ng g^{-1} Narasin and salinomycin >500 ng g^{-1}	Blanchflower, Rice, and Hamilton, 1985
Narasin, salinomycin	Feed, food	Post-column derivatisation with p-dimethylaminobenzaldehyde at 90°C	Column: Hypersil ODS; mobile phase: methanol–phosphate buffer pH 4 (90:10); UV detection at 600 nm	1–2 ng g^{-1}	Johannsen, 1991
Monensin, narasin, salinomycin	Turkey feed	Derivatisation with dintrophenylhydrazine at 55°C	Column: Supelco LC-18; mobile phase: methanol–1.5% aq. Acetic acid (90:10); UV detection at 392 nm	Monensin >40 mg kg^{-1} Narasin, salinomycin >40 mg kg^{-1}	Guglielmo Dusi and Gamba, 1999
Monensin	Chicken	Derivatisation with 9-anthryldiazomethane (ADAM) at room temperature for 60 min in a dark room	Column: Nucleosil C18; mobile phase: methanol–water (95:1); Fluorescence detection at Ex 365 nm and Em 418	Monensin >100 ng g^{-1}	Hoshino, Horie, Nose, and Iwasaki, 1985
Lasalocid, monensin, salinomycin	Feed	Derivatisation with 1-bromoacetylpyrene (BAP) at 50°C for 90 min	Column: Develosil ODS; mobile phase: methanol–water (97:3)	25–100 mg kg^{-1}	Asukabe, Murata, harada, Suzuki, Oka, and Ikai, 1994
Lasalocid	Animal blood	LC fluorescence detection Ex 310 nm Em 430 nm	Column: Partisil PXS-10/25; mobile phase: hexane-tetrahydrofuran-methanol-triethylamine-ammonoim hydroxide (810:140:20:20:10)	Lasalocid >5 ng g^{-1} –5 mg kg^{-1}	Kaykaty and Weiss, 1983
Lasalocid	Bovine liver	Pyrolysis GC–MS	Column: 3% SE-30 Gas Chrom Q; detection: FID	Lasalocid >51 ng g^{-1}	Weiss, Kaykaty and Miwa, 1983
Monensin	Feed	TLC-Bioautography	Stationary phase: Silica gel G, solvent methylene chloride methanol (9:1) Bioautography: Nobel agar inoculated with B.subtilis; visualisation of inhibition: 2-(4_ Iodophenyl)-3-(4-nitrophenyl-5-phenyl) tetrazolium chloride (INT dye)	Monensin >10 ng g^{-1}	Martinez, and Shimata, 1983
Lasalocid, monensin, narasin, salinomycin	Poultry feeds	MALDI-TOF MS	Clean-up Sep-pak C18	Standards Lasalocid: 251 fmol; nonensin: 22 fmol; salinomycin & narasin: 24 fmol Feeds Salinomycin & narasin: 2.4 μg g^{-1}	Wang and Sporns, 2000

extract was filtered through 0.45 μm syringe filter and transferred to a screw-cap amber vial. The determination of the ionophores was performed by LC–MS–MS.

Four additional blank samples were extracted as described above and after solvent evaporation, were spiked with 0, 25, 50 and 100 μl of the working solution (ionophore mixture) to give post extraction matrix spikes.

Quantitation was performed against the post extraction matrix spikes which were used for the calibration curve. The linearity of the corresponding calibration curves by LC–MS–MS gave a correlation coefficient (R^2) $\geqslant$ 0.99.

The use of post extraction matrix spikes eliminates the problem of matrix suppression i.e. the MS–MS response of the analytes in pure solvent or mobile phase is usually stronger than the same concentration of analytes added to blank extracts.

Table 3
Spiking levels for the different ionophores

	Lasalocid (ng g^{-1})	Monensin (ng g^{-1})	Narasin (ng g^{-1})	Salinomycin (ng g^{-1})
25μl	50	2.5	10	10
50μl	100	5	20	20
100μl	200	10	40	40

Table 4
LC–MS–MS data for ionophores

Compound	Retention time (min)	Parent ion	Transition 1	Transition 2
Lasalocid	~ 4.2	613	613 > 377	613 > 359
Monensin	~ 5.1	693	693 > 461	693 > 479
Narasin	~ 6.3	787	787 > 431	787 > 531
Salinomycin	~ 5.2	773	773 > 431	773 > 265

2.6. Equipment for LC–MS–MS

The HPLC equipment consisted of a Waters Alliance 2690 system (Waters Ltd, Watford, Herts, UK), connected to a Micromass Quattro LC Tandem Mass Spectrometer with Z Spray™ API source operating in positive ion electrospray (ESP) mode (Micromass UK Ltd, Altrincham, Cheshire, UK).

2.7. Chromatographic conditions

The mobile phase had the following composition: acetonitrile, methanol, tetrahydrofuran, water and trifluoroacetic acid (67:10:10:13:0.1 v/v); column: Phenyl hexyl, Luna C18(2), 3 μm (150×2.1 mm i.d.), Phenomenex, Cheshire, UK, fitted with a 10 mm Hichrom C-18 guard column; flow rate: 300 μl min^{-1}; injection volume: 15 μl.

2.8. Optimisation of MS–MS conditions

A solution of lasalocid sodium, monensin sodium, narasin sodium and salinomycin sodium (10 μg ml^{-1} in mobile phase) was infused at a rate of 10 μl min^{-1} into the detector and the capillary/cone voltages were adjusted to yield the [M+ Na]+ion. Further MS/MS experiments were performed to generate the major product (daughter) ion fragments; both these ions are used for confirmatory and quantitation purposes, see Figs. 1–4.

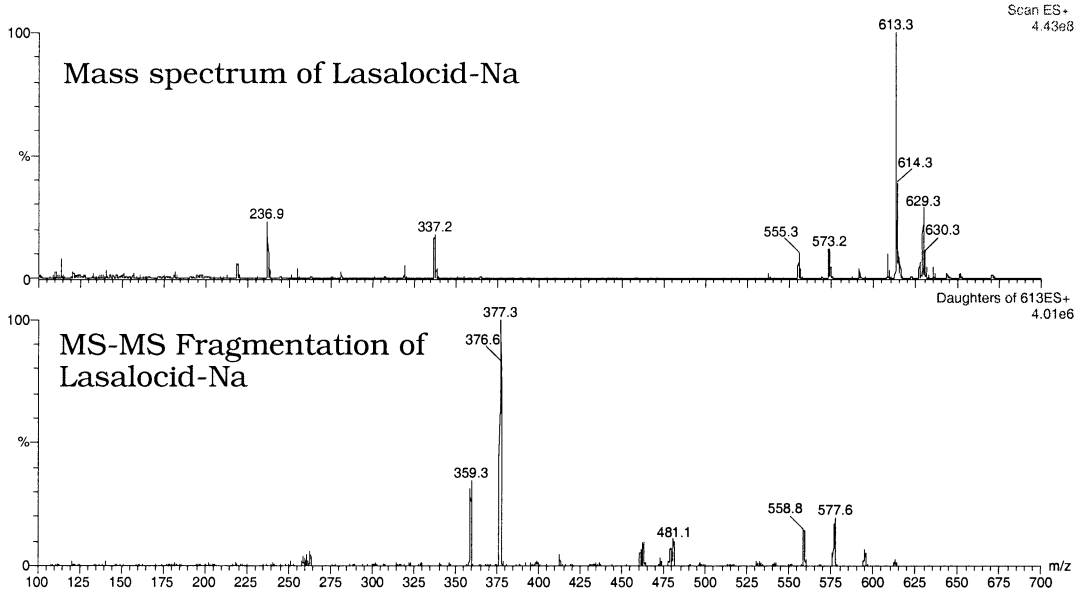

Fig. 1. Mass spectra of lasalocid.

The narrow bore column allows eluant to be transferred directly into the electrospray interface without the need for flow splitting.

The following MS–MS parameters were used: Capillary Voltage: 3.5 kV, Cone: 50 V; Source temperature: 140°C; desolvation temperature: 400°C; Collision Energy: 36 eV; Collision gas pressure: 2.3 e^{-3} mBar.

Sample extracts and standard solutions were injected onto the system, and single peaks for lasalocid, monensin, narasin and salinomycin were observed. The product (daughter) ion fragments were monitored by MRM (multiple reaction monitoring) mode (Table 4).

3. Results and discussion

Table 5 presents recovery data for ionophore antibiotics from spiked samples of liver from different animal species and egg using LC–MS–MS. The results have been summarised in Table 6. The mean percentage recoveries by this method for lasalocid are 93, 97, 103, 101; monesin 103, 101, 96 and 103; narasin 101, 94, 95 and 98; salinomycin 98, 99, 106 102 for sheep liver, broiler liver, calf liver and eggs respectively. The consistency of the mean results from different matrices indicates the versatility and robustness of this method.

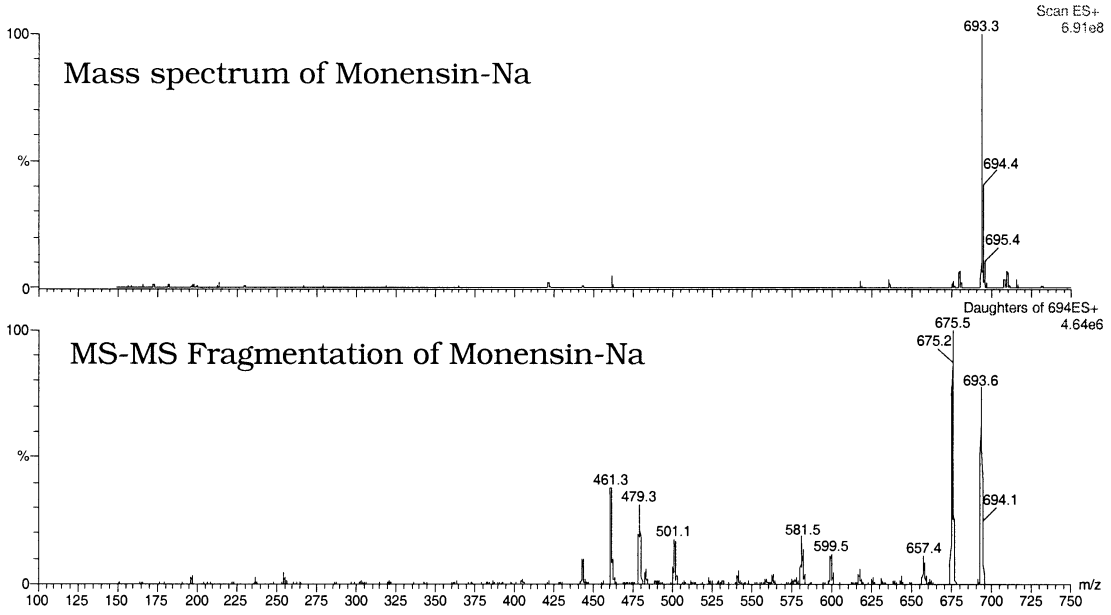

Fig. 2. Mass spectra of monesin.

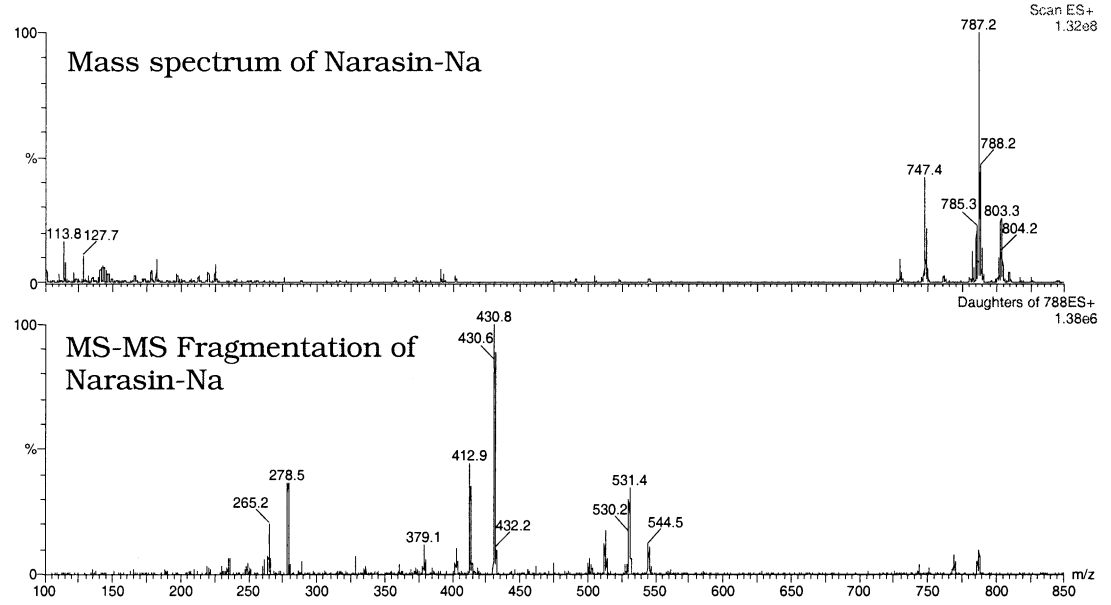

Fig. 3. Mass spectra of narasin.

Table 5
Recovery of Ionophores from spiked samples using LC–MS–MS

Spike (ng g^{-1})	Sheep liver	Broiler liver	Calf liver	Egg
	Determined (ng g^{-1})	Determined (ng g^{-1})	Determined (ng g^{-1})	Determined (ng g^{-1})
Lasalocid				
50	46.37	45.24	57.73	41.11
	45.80	50.10	62.13	54.40
	40.96	49.26	48.64	47.56
	46.29	55.22	62.29	54.70
	45.80	49.44	52.30	58.52
100	94.43	94.41	112.00	91.26
	99.45	86.91	112.01	120.53
	104.03	106.69	87.44	89.72
	94.39	102.52	95.68	95.06
	99.45	99.48	96.08	119.52
200	162.08	191.97	200.00	219.76
	186.28	162.10	190.96	185.24
	193.91	194.38	173.00	179.92
	162.09	191.40	201.13	188.76
	193.84	191.42	174.15	185.66
Monensin				
2.5	2.47	2.23	1.92	1.94
	2.46	2.43	2.52	2.25
	3.02	2.56	2.81	3.03
	2.59	2.37	2.44	2.66
	2.23	3.14	2.84	2.78
5	6.21	5.69	3.92	4.43
	6.20	6.40	4.54	5.54
	6.04	4.32	5.54	6.56
	5.78	5.03	4.62	4.56
	3.80	5.44	5.26	5.40
10	9.39	9.61	7.97	10.83
	9.40	9.40	8.57	9.77
	10.36	8.86	9.66	10.15
	9.70	9.96	10.35	10.08
	8.15			
Narasin		9.49	10.01	9.57
10	8.50	8.81	8.26	8.82
	11.16	9.43	9.86	8.16
	9.80	9.43	7.43	10.66
	10.36	9.31	10.67	10.88
	9.84	9.27	11.20	9.27
20	19.62	19.86	15.44	22.09
	22.28	16.85	21.04	16.66
	21.82	16.42	19.04	18.76
	21.95	20.46	18.57	20.82
	23.82	20.38	20.77	20.38
40	44.37	40.37	34.76	42.98
	34.37	33.57	37.04	39.44
	38.38	33.72	38.45	39.65
	34.19	41.50	41.04	39.85
	37.42	39.85	40.32	39.25
Salinomycin				
10	8.90	9.47	11.31	8.49
	10.98	10.73	8.95	10.37
	9.37	10.10	12.10	11.37
	10.54	9.95	10.24	11.18
	9.95	9.84	12.36	9.84
20	18.59	21.98	21.60	17.59
	23.07	19.44	21.99	23.05

(*continued on next page*).

Table 5 (*continued*)

Spike (ng g^{-1})	Sheep liver	Broiler liver	Calf liver	Egg
	determined (ng g^{-1})	determined (ng g^{-1})	determined (ng g^{-1})	determined (ng g^{-1})
	19.39	17.17	23.15	19.11
	21.99	19.96	20.82	21.35
	22.91	21.07	23.03	21.07
40	38.65	39.14	39.06	44.34
	34.64	37.29	36.65	38.38
	32.45	34.43	37.90	39.05
	34.57	39.48	39.54	38.83
	35.74	43.71	42.79	43.71

The samples examined to date have shown no significant matrix interferences.

Fig. 5 shows a chromatogram of an egg sample spiked with the ionophores at half the differential action level (DAL), i.e. with 50 ng g^{-1} lasalocid, 2.5 ng g^{-1} monensin, 10 ng g^{-1} salinomycin and narasin, respectively. The chromatogram does not show any interference. Fig. 6 presents a chromatogram of an egg sample with incurred lasalocid at about 20 ng g^{-1} which shows that levels significantly below the prescribed DAL can be easily determined.

This method was developed for the rapid screening of the different ionophores, (lasalocid, monensin, narasin and salinomycin) in animal tissues and eggs with a short extraction and minimal 'clean-up'. The main advantages of this method are that up to 40 samples can be assayed by one analyst in a working day, and the amount of acetonitrile used for the extraction of a sample is only 10 ml with a further 4 ml for the 'clean up' stage. The sample preparation takes <3 h, with the extraction, centrifugation and the evaporation taking a total of 90 min. The solvent evaporation process takes

Table 6
Summary of results for recovery of ionophores from spiked samples using LC–MS–MS

Spike (ng g^{-1})	Sheep liver			Broiler liver			Calf liver			Egg		
	Determined Mean (ng g^{-1})	S.D. dev	CV%	Determined Mean (ng g^{-1})	S.D.	CV%	Determined Mean (ng g^{-1})	S.D.	CV%	Determined Mean (ng g^{-1})	S.D.	CV%
Lasalocid												
50	45.0	2.299	5.1	49.9	3.559	7.1	56.6	6.043	10.7	51.3	6.912	13.5
100	98.4	4.054	4.1	98.0	7.649	7.8	100.6	10.931	10.9	103.2	15.469	15.0
200	179.6	16.323	9.1	186.3	13.558	7.3	187.8	13.619	7.2	191.9	15.913	8.3
Overall Mean % Recovery	92.8			96.9			102.6			100.6		
Monensin												
2.5	2.6	0.291	11.4	2.5	0.353	13.9	2.5	0.371	14.8	2.5	0.435	17.2
5	5.6	1.024	18.3	5.4	0.772	14.4	4.8	0.638	13.4	5.3	0.860	16.2
10	9.4	0.802	8.5	9.5	0.399	4.20	9.3	1.004	10.8	10.1	0.481	4.8
Overall Mean % Recovery	102.8			101.3			96.3			102.7		
Narasin												
10	9.9	0.971	9.8	9.3	0.256	2.8	9.5	1.598	15.8	9.6	1.177	12.3
20	21.9	1.503	6.9	18.8	1.990	10.6	19.0	2.244	11.8	19.7	2.094	10.6
40	37.7	4.136	11.0	37.8	3.842	102	38.3	2.536	6.6	40.2	1.551	3.9
Overall Mean % Recovery	101.1			93.7			95.2			98.3		
Salinomycin												
10	9.9	0.843	8.5	10.0	0.461	4.6	11.0	1.408	12.8	10.3	1.162	11.3
20	21.2	2.070	9.8	19.9	1.828	9.2	22.1	0.983	4.4	20.4	2.117	10.4
40	35.2	2.262	6.4	38.8	3.393	8.7	39.3	2.308	5.9	40.9	2.906	7.1
Overall Mean % Recovery	97.8			98.9			106.3			102.3		

approx. 45 min and does not involve analyst time. The run time on the LC–MS–MS is about 8 min. This represents a simple, rapid, efficient and versatile method which is adapted to the routine analysis of lasalocid, monensin, narasin and salinomycin in animal tissues and eggs.

To date very few methods have been reported that can simultaneously detect lasalocid, monensin, narasin and salinomycin in animal tissues and eggs. Martinez and Shimoda (1985) have reported a method which can simultaneously assay for these four ionophores, but the detection level of 150 ng g^{-1} and the lengthy extraction and acetylation (i.e. overnight) of monensin, narasin and salinomycin, followed by the derivatisation with 9-anthryldiazomethane (ADAM) and clean-up of the derivatised compounds, limit the use of this method for

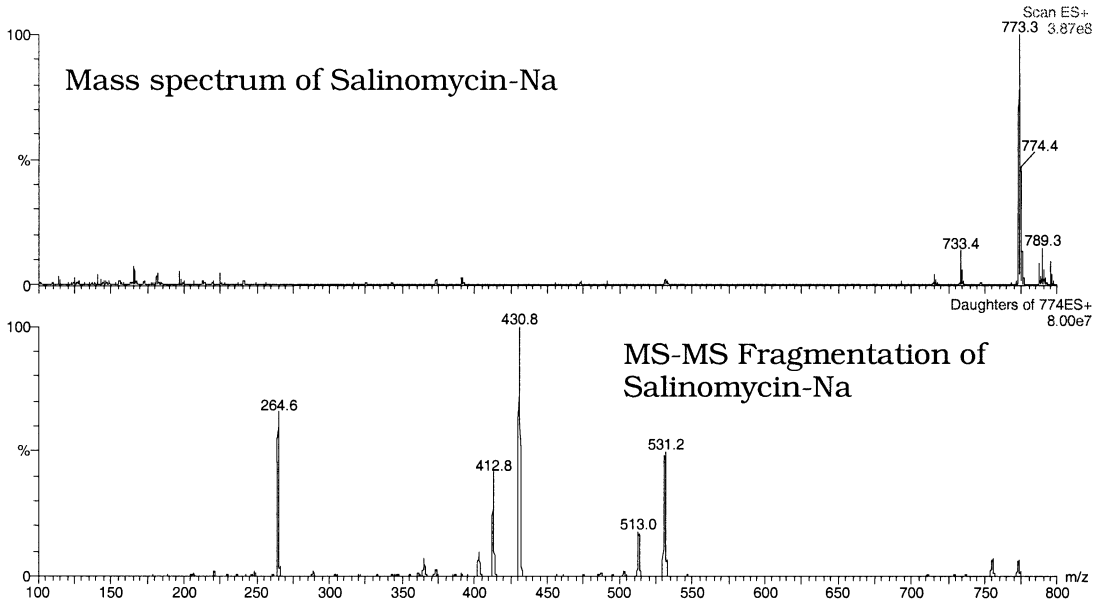

Fig. 4. Mass spectra of salinomycin.

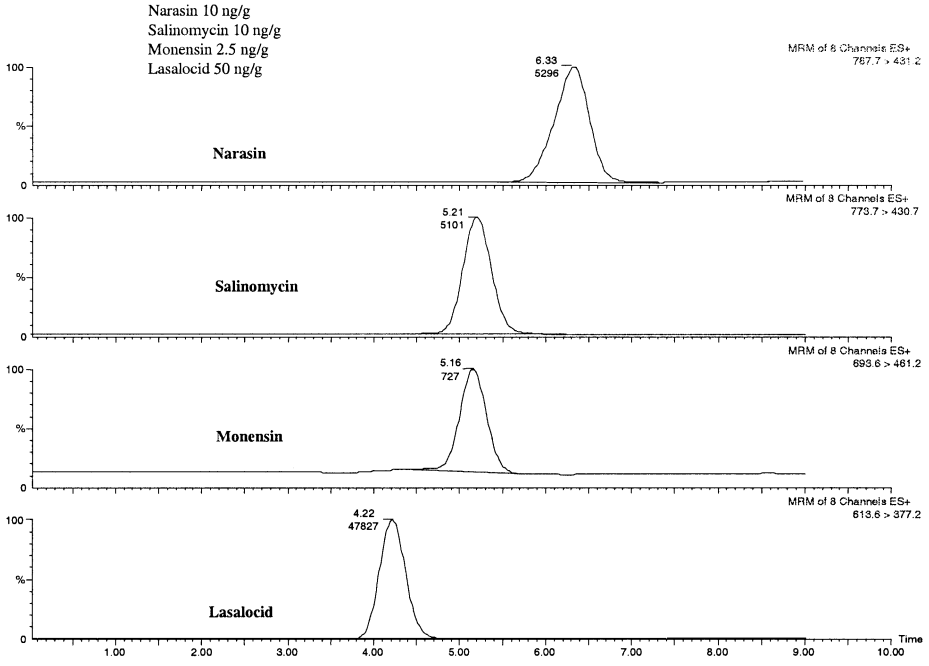

Fig. 5. Eggs spiked with ionophores.

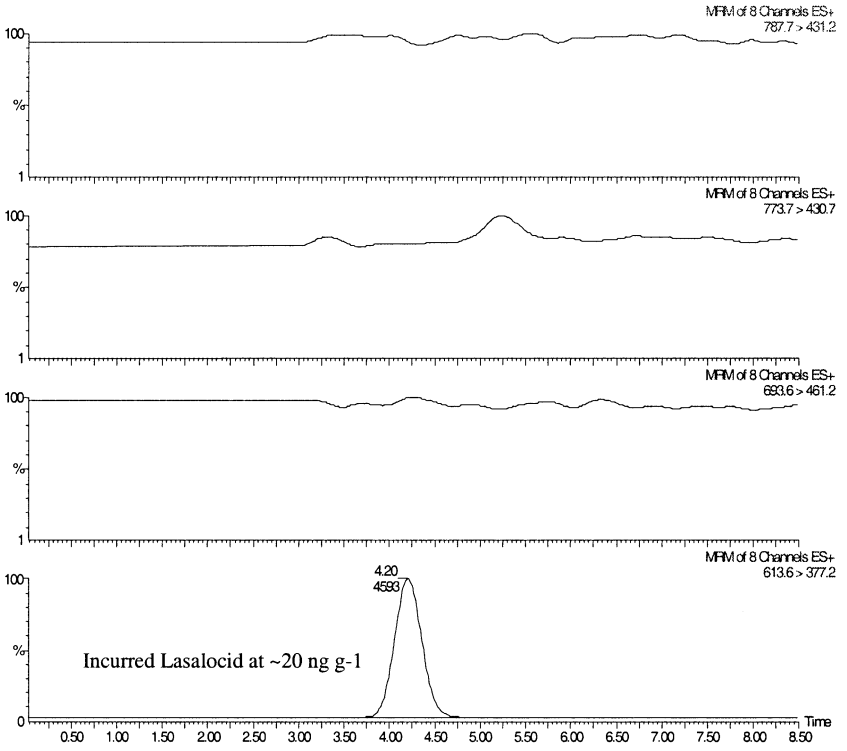

Fig. 6. Egg with incurred lasalocid residue.

routine applications, i.e. the ability to quantify lasalocid at 50 ng g^{-1}, monensin at 2.5 ng g^{-1}, narasin and salinomycin at 10 ng g^{-1} levels which are at half of the prescribed action limits by the UK Legislation.

4. Conclusion

The described method of extraction of the ionophores and quantification by LC–MS–MS has been used successfully by the Laboratory of the Government Chemist (LGC) for the screening and quantification of ionophores in livers and eggs submitted for examination over the last 12 months. The LC–MS–MS procedure has been used both for the primary screening and confirmation of ionophores in samples. Putative positive samples for the presence of ionophores were confirmed by using both the transitions (see Table 4). The chromatograms and results obtained by the described method have shown no significant interferences when applied to a variety of different biological matrices which demonstrates the robustness, sensitivity and specificity of the described method. This method has scope for the detection of the ionophores at very low levels in biological matrices as demonstrated by the low limit of detection of 1 ng ml^{-1} of the ionophores in standard solutions which is equivalent of approximately 0.4 ng g^{-1} in a sample based on working with a 5 g sample, as described in the method. A quantitation level of 1 ng g^{-1} of all the ionophores in different matrices is being validated and findings will be published at a later stage.

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