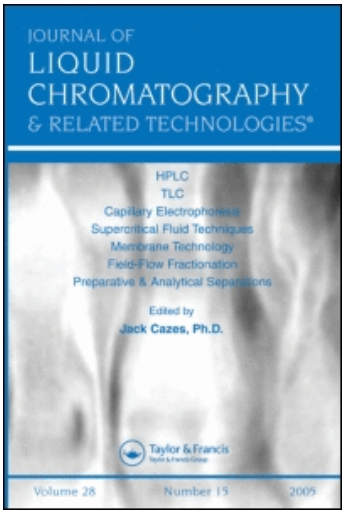

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

An Improved LC-MS Method for the Determination of Lasalocid, Monensin, Narasin, and Salinomycin in Feed

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To cite this Article Hormazábal, Víctor , Østensvik, Øyvin and Kaldhusdal, Magne(2005) 'An Improved LC-MS Method for the Determination of Lasalocid, Monensin, Narasin, and Salinomycin in Feed', Journal of Liquid Chromatography & Related Technologies, 28: 17, 2769 — 2776
To link to this Article: DOI: 10.1080/10826070500225119
URL: http://dx.doi.org/10.1080/10826070500225119

Journal of Liquid Chromatography & Related Technologies®, 28: 2769–2776, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070500225119

An Improved LC-MS Method for the Determination of Lasalocid, Monensin, Narasin, and Salinomycin in Feed

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Abstract: An improved liquid chromatographic-mass spectrometry method for the simultaneous determination of four anticoccidial drugs in feed is described. Samples were homogenized with methanol-acetone-tetrahydrofuran. The samples were mixed and centrifuged. After centrifugation, 100 μL supernatant was extracted with hexane, evaporated to dryness, diluted with acetonitrile-water, filtered through a Spin-X micro-centrifuge tube, and injected into the LC/MS. The calibration curves were linear in the investigated areas. The limit of quantification varied from 0.2 to 0.6 μg/g. The method described was used for the determination of anticoccidial agents in medicated feeds and to examine the degree of anticoccidials contamination of unmedicated feeds (carry over).

Keywords: Anticoccidials, Lasalocid, Monensin, Narasin, Salinomycin, Feed, LC-MS

INTRODUCTION

The anticoccidial drugs lasalocid (LAS), monensin (MON), narasin (NAR), and salinomycin (SAL) are frequently used in the prophylaxis and treatment of coccidiosis, a parasitic disease that causes enteric disease and impaired productivity in poultry.[1,2]

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In addition, monensin and salinomycin are used as growth promoters in cattle and swine, respectively. Monensin, given to cows before calving, has also been found to increase milk production.[3]

Concern has been raised on the toxic and lethal effects of the misuse of these drugs,[4–6] which can occur when the drugs are present in feed not intended for a specific species or when given at higher levels than recommended. For these reasons, a method for the analysis of anticoccidial drugs in both medicated feed and the possible cross contamination of nonmedicated feed is desirable.[7,8]

The most used anticoccidial drugs are members of a group of compounds known as the carboxylic acid ionophores. The group includes among others, LAS, MON, NAR, and SAL. In Norway, the approved concentration (mg/kg) ranges in broiler feed of LAS, MON, NAR, and SAL are 75 to 125, 90 to 125, 60 to 70, and 50 to 70, respectively.

Several methods based on fluorescence or UV- detection with post-column derivatization have been reported for analysis of the drugs.[9,10] One method[11] describes simultaneous detection of LAS, MON, NAR, and SAL by LC/MS. These methods are, however, time-consuming and require relatively large amounts of reagents. More recently, a simple method for the determination of six anticoccidial drugs in feed using LC/MS has been published,[12] with limits of quantification of 4 μg/g for LAS, MON, NAR, and 5 μg/g for SAL. However, the sensitivity does not satisfy the demands for analysis of possibly cross-contaminated feed.

The purpose of the present study was to develop a rapid, simple, specific, and more sensitive LC-MS method for the determination of LAS, MON, NAR, and SAL in feed. The sensitivity should meet the requirements set by the Norwegian Food Safety Authority.

EXPERIMENTAL

Materials and Reagents

In the recovery experiments, samples of broiler, cattle, swine, turkey, and horse feed declared drug-free feed by the manufacturer, were used as control material and for spiking with LAS, MON, NAR, and SAL. The horse feed was supplied by a feed mill that has never used anticoccidials (Warå Mølle AS, P.O. Box 141, N-1901 Fetsund, Norway).

All chemicals and solvents were of analytical or HPLC grade, (BDH Laboratory Supplies Poole, England). LAS, MON, NAR, and SAL was supplied by Sigma Co. (St. Louis, MO, USA). Stock solutions (1 mg/mL) and a mixed working standard (10 μg/mL) were prepared monthly by dilution with methanol and stored under refrigeration at +4°C. Spin-X micro-centrifuge filter units (0.22 μm nylon type, Costar, NY, USA) were used for filtration.

Chromatographic Conditions

The LC-MS instrumentation used for the method development, consisted of a Series 200 quaternary pump and a Series 200 autosampler (Perkin-Elmer, Norwalk, CT, USA) and an API 100 single quadrupole mass spectrometer (Applied Biosystems, Ontario, Canada) equipped with a Turbo-Ion Spray ion source. The turbo probe of the interface was maintained at 175°C and the probe air flow rate was 6 L/min. The N_2 nebulizer and curtain gas was 5 and 10 L/min, respectively. The MS was set to collect single-ion data in positive ion mode at the ions m/z 613.4, 693.4, 787.5, and 773.6 for LAS, MON, NAR, and SAL, respectively. The entrance electrode voltages and the position of the ion spray inlet were adjusted to provide optimum intensity for the molecular ions.

The analytical column (stainless steel 150 × 4,6 mm I.D.) and guard column (5.0 × 3 mm I.D.), were packed with 5 μm particles of PLRP-S 100Å (Polymer Laboratories, Amherst, MA, USA) and were operated at a constant temperature of 24°C. The guard column was connected to the autosampler with an A-318 precolumn filter on line with an A-102X frits (Upchurch Scientific, USA). The mobile phase consisted of a mixture of four solutions. Solution A consisted of 10 mM (0.77 g/L) ammonium acetate added 100 μL/L NH_3, solution B was methanol, solution C was acetonitrile, and solution D was tetrahydrofurane. The mobile phase operating conditions are shown in Table 1. After separation, the LC eluent was connected to a two position microelectric valve actuator (Vici, Valco Instruments Co. Inc. Texas, USA) and programmed in mode two by our Norwegian provider. Thereafter the LC eluent was split approximately 1 : 20 before entering the MS interface.

Sample Pretreatment

Five grams of feed sample was weighed into a 50 mL centrifuge tube with screw cap (Nunc, Roskilde, Denmark), and volumes of 10 mL methanol and 7 mL acetone-tetrahydrofuran (6 + 4) were added. The mixture was hom-

Table 1. Mobile phase operating conditions

Step	Total time (min)	Flow rate (μL/min)	A (%)	B (%)	C (%)	D (%)	TE#1
0	0.0	800	8	38	38	16	Open
1	1.8	800	8	38	38	16	Close
2	4.5	800	8	38	38	16	Close
3	8.0	800	8	38	38	16	Open

TE#1 = events.

ogenized for approximately 6 sec in an Ultra-Turrax TP 18/10 and left in an ultrasonic bath for 5 min. The sample was shaken for 3 sec and then centrifuged for 5 min (5000 rpm). To 100 μL of supernatant, 2 mL hexane was added and stirred with a wirlimixer for 10 sec and then centrifuged for 1 min (4000 rpm). The upper layer (hexane) was transferred into a glass-stoppered tube and the residue was reextracted with 1 mL hexane. The collected hexane fractions were evaporated to dryness under a stream of air at 60°C. To the dry residue, 3 mL acetonitrile-water (90 + 10) was added, stirred for 5 sec left in an ultrasonic bath for 6 min and stirred for 3 sec to reconstitute the residue. After addition of 100 μL water, the sample was mixed again. Approximately 500 μL were then filtered through a Spin-X centrifuge filter. Aliquots of 15 μL were injected into the LC-MS at intervals of 8 min for the determination of LAS, MON, NAR, and SAL. For analysis of samples with high concentrations of anticoccidial agents, further dilution of 100 μL filtrate was carried out by adding 1.8 mL acetonitrile-water (90 + 10) and 100 μL water. The dilution was mixed and injected into the LC-MS.

Calibration Curves and Recovery Studies

The precision, recovery, and linearity for LAS, MON, NAR, and SAL were determined by spiking horse feed samples with standard solutions to yield 100, 200, 400, 500, 600, 1000, 1500, 2000, 5000, 40000, 50000, 60000, 70000, 100000, and 125000 ng/g for LAS, MON, NAR, and SAL, respectively. The recovery rates were determined by comparing the analyses of spiked feed with those of standard solutions. The linearity of the standard curves for MON, NAR, and SAL in feed was calculated using peak height measurements, for LAS calibration was calculated using peak area measurements.

Under sample pretreatment, 10 mL methanol was added. To determine the correlation coefficients (standard curve), the methanol volumes were reduced according to the volumes of standards.

To compare the analyses of spiked feed with those of standard solutions (low concentrations), the corresponding standards were diluted to 20 mL with acetonitrile-water (90 + 10) in a Nunc centrifuge tube. To 100 μL from these solutions, 2.9 mL acetonitrile-water (90 + 10) and 100 μL water were added and mixed. To compare the analyses of spiked feed (high concentrations) with those of standard solutions, the corresponding standards were diluted further as described in the sample pretreatment.

RESULTS AND DISCUSSION

The standard curves were linear in the investigated areas from 0.6 to 5 μg/g, and from 40 to 125 μg/g for LAS, 0.2 to 5 μg/g and 40 to 125 μg/g for MON,

0.4 to 5 μg/g and 40 to 125 μg/g for NAR, and for SAL from 0.5 to 5 μg/g and 40 to 125 μg/g, in horse feed. The correlation coefficients were r = 0.999 for all four drugs in both low and high concentrations. The recovery and repeatabilities for LAS, MON, NAR, and SAL from feed are shown in Table 2. Chromatograms obtained from samples from drug-free feed and from the corresponding samples spiked with LAS, MON, NAR, and SAL are shown in Figure 1.

Table 2. Recovery and reapeatability for LAS, MON, NAR and SAL from spiked samples of horse feed

Added[a]	No.[b]	S.D.[c]	Rec.[d]
	LAS		
600	5	1.8	99.0
1000	5	0.9	104.0
2000	5	1.0	99
60000	5	1.9	101
70000	5	1.7	98
100000	5	0.7	104
	MON		
600	5	1.0	99
1000	5	0.6	100
2000	5	1.0	99
60000	5	3.1	103
70000	5	1.9	102
100000	5	0.7	106
	NAR		
600	5	2.3	100
1000	5	1.4	97
2000	5	0.6	98
60000	5	2.4	98
70000	5	3.3	100
100000	5	1.0	105
	SAL		
600	5	2.1	98
1000	5	1.2	99
2000	5	1.2	100
60000	5	2.5	101
70000	5	3.9	102
100000	5	1.1	106

[a]Concentration μg/g.
[b]No.of samples.
[c]Standard deviation.
[d]Recovery.

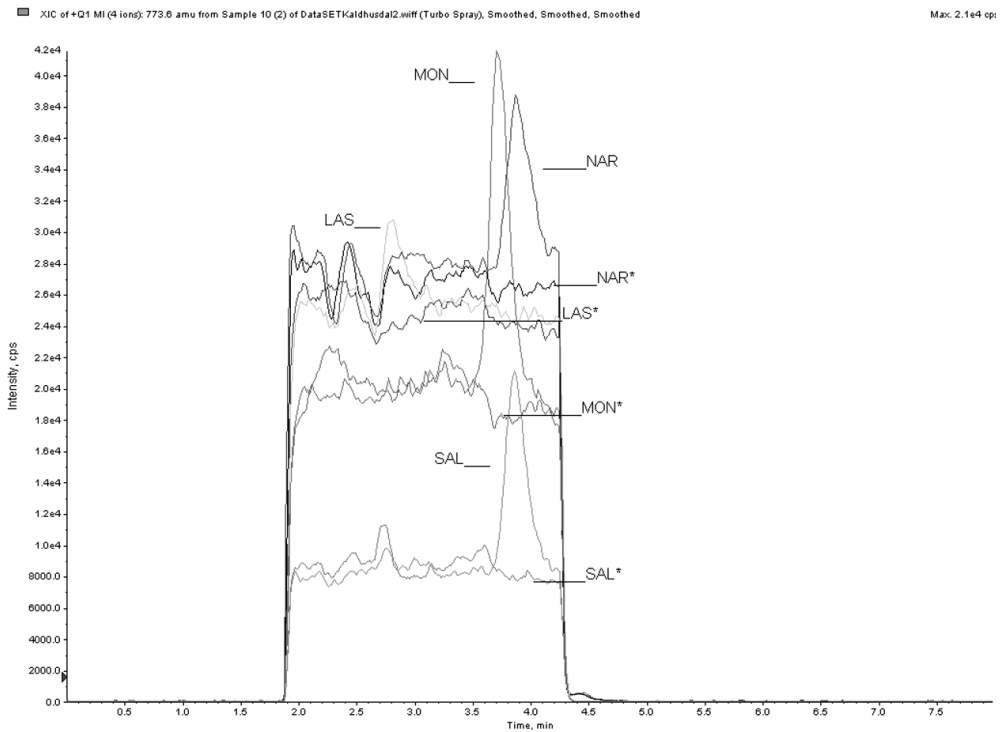

Figure 1. Chromatograms of extracts from horse feed. Drug free feeds are marked LAS*, MON*, NAR*, SAL* and feed spiked with LAS, MON, NAR, and SAL (1 μg/g) are unmarked.

The precision, recovery, and linearity of LAS, MON, NAR, and SAL in chicken, cattle, swine, and turkey feed were not validated in this study, but extracts from these matrixes showed very similar baseline resolution to horse feed.

The limits of detection were calculated as three times the peak-to-peak baseline noise (S/N = 3) from drug-free horse feed. They were 0.3 μg/g for LAS and SAL, 0.1 μg/g for MON, and 0.2 μg/g for NAR.

The described assay offers a number of significant advantages compared to previously published methods for the detection and quantification of LAS, MON, NAR, and SAL in feed. A simple clean-up step that protects the analytical columns is introduced in the sample preparation, and the detection limit is reduced from 2 μg/g for LAS and MON to 0.3 μg/g for LAS and 0.1 μg/g for MON. For NAR, the detection limit was reduced from 2 to 0.2 μg/g and for SAL from 2.5 to 0.3 μg/g.

The use of a two position microelectric valve actuator avoids use of unnecessary mobile phase and, thereby, possible contamination from sample extract streamed into the MS. The use of the microelectric valve actuator appears favourable in all MS analysis. It is essential that the actuator is programmed in mode two.

The method presented is selective, robust, and accurate and satisfies the levels given by regulatory authorities. The method is used in the routine control of feed for the Norwegian Food Safety Authority.

The advantage of the LC-MS technique lies in the combination of the separation capabilities of HPLC and the power of MS as an identification and confirmation method with high sensitivity, selectivity, and quantitative capability. While conventional HPLC methods may require long complex separations, the LC-MS method generally requires only a simple clean-up procedure and no derivatization. Thus, LC-MS seems to provide a better alternative than HPLC.

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Received November 1, 2004
Accepted May 19, 2005
Manuscript 6516