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Journal of Chromatography A, 835 (1999) 243–246

JOURNAL OF CHROMATOGRAPHY A

Short communication

Liquid chromatography with ultraviolet detection of lasalocid, monensin, salinomycin and narasin in poultry feeds using pre-column derivatization

Guglielmo Dusi*, Valentina Gamba

Department of Chemistry, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Via Bianchi 9, 25124 Brescia, Italy

Received 27 October 1998; received in revised form 19 November 1998; accepted 4 January 1999

Abstract

A rapid and very effective analytical procedure for the simultaneous determination of four polyether antibiotics (PEs) lasalocid, monensin, salinomycin and narasin in poultry feeds was tested. PEs were extracted from samples using methanol and without any clean-up derivatized with 2,4-dinitrophenylhydrazine (DNP) in an acidic medium at 55°C. The derivatization mixture was analyzed directly on an ODS column (150×4.6 mm, 5μm) with methanol–1.5% aqueous acetic acid (90:10, v/v) as eluent and UV detection was carried out at 305/392 nm. The recoveries of the PEs from spiked samples were 85–100 % with RSDs of 4–10 % in a concentration range of 50–150 mg/kg.

Keywords: Lasalocid; Monensin; Salinomycin; Narasin; Polyether antibiotics

1. Introduction

Polyether antibiotics (PEs) are produced by *Streptomyces* spp. and structurally characterized as the sodium salt of a carboxylic acid ionophore and a number of cyclic ether moieties [1]. These compounds possess unique structural properties making them capable of acting as cation carriers, especially sodium, across biological membranes [2]. They include salinomycin (SL), monensin (MN), lasalocid (LA) and narasin (NA) which have microbiological activities against Gram-positive bacteria, fungi and protozoa, and have been used all over the world as feed additives for poultry to prevent coccidiosis [1]. In addition, MN is used as a growth promoter in cattle and when given to cows before calving has also been found to increase milk production [3]. They are normally added to medicated feeds as sodium salts, from 50 to 125 mg/kg of finished feed, depending on the PE type and animal species.

Owing to the possibility of PE toxicity resulting from feeding higher than the recommended levels, administration to species for which they were not intended, adverse interaction with simultaneously administered drugs, and also to avoid residue build-up in poultry products (tissues and eggs) for human consumption, finished feeds need to be monitored regularly for PE levels.

Several screening and confirmatory analytical methods have been published for the determination

*Corresponding author.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00044-8

of one or more PEs in feeds since the early 1970s. Most confirmatory methods published use liquid chromatography (LC). Since the PEs, apart from LA, do not possess any significant UV or fluorescence response, derivatization is required. Many methods use post-column derivatizations with vanillin [4–6] while others use benzaldehyde reagents and UV detection [7,8]. Pre-column derivatization has been reported in a few studies with 2,4-dinitrophenylhydrazine (DNP) [9] and UV detection or 1-bromoacetylpyrene [10] and fluorescence detection.

The purpose of this study was to modify the pre-column derivatization published procedure [9] to analyze four PEs and to quantify them at their normal medicated feed concentrations.

Experimental

2.1. Chemicals

Trichloroacetic acid (TCA) and DNP were purchased from Carlo Erba (Milan, Italy). Acetic acid (100%) was purchased from Prolabo (Fontenay s/Bois, France). Methanol HiperSolv for high-performance liquid chromatography (HPLC) was obtained from BDH (Poole, UK). Water for LC analysis was prepared with a Barnstead Nanopure Ultrapure Water System from International PBI (Milan, Italy).

2.2. Standards and spiked samples

Monensin sodium salt hydrate and lasalocid sodium salt were obtained from Riedel-de Haen (Seelze, Germany). Hoechst (Frankfurt, Germany) and Ely Lilly (Greenfield, USA), respectively, generously donated salinomycin sodium salt and narasin.

The stock standard solution, prepared in methanol, contained all PEs each at 1 mg/ml. The working standard solution was prepared by diluting the stock standard solution to 100 μg/ml in methanol.

For the recovery studies, feed samples spiked with PEs in a concentration range of 50–150 mg/kg were prepared on the day used by spiking aliquots of blank control feed with the stock standard solution and were processed as described in Section 2.5.

2.3. LC system

The LC system consisted of a Hewlett-Packard (HP) 1100 Series Quaternary Pump, a HP 1100 Series variable-wavelength UV–Vis detector, a HP 1100 Series autosampler all controlled by a Vectra XA 5/200 computer using HP CHEMSTATION software.

Separation was carried out on a 5 μm Supecosil LC-18 (150×4.6 mm) column, at room temperature, with a 5 μm Supelguard LC-18 (20×4.6 mm) guard column (both the columns from Supelco, Bellefonte, PA, USA). The operating conditions were: eluent, methanol–1.5 % (v/v) aqueous acetic acid (90:10, v/v); flow, 1 ml/min; injection volume 10 μl. Between 0–6.5 min the wavelength was 305 nm (LA detection) and from 6.5 to the end of the run 392 nm (detection of MN, SL and NA derivatives).

2.4. Linearity

Different aliquots (10, 20, 50, 100, 200 and 400 μl) from the working standard solution (100 μg/ml) were placed in a 10-ml tube equipped with a screw-stopper, diluted to 700 μl with methanol and derivatized as described in Section 2.5. All the concentrations, from 1 μg/ml to 40 μg/ml were found to be linear.

2.5. Sample preparation

The feed sample was pulverised using a domestic grinder to obtain a homogeneous powder. A portion (10 g) was weighed into a 200-ml glass jar and 50 ml of methanol was added. The mixture was shaken with a horizontal shaker for 1 h. About 10 ml of supernatant was centrifuged for 5 min at 3000 rpm. Seven hundred μl of clear solution was transferred to a 10-ml centrifuge tube equipped with a screw-stopper and 100 μl of aqueous TCA solution (500 mg/ml) was added.

The tube was then tightly capped, vortex-mixed for 20 s, and kept at room temperature for 10 min. A 200 μl volume of DNP methanolic solution (1 mg/ml) was added to the tube. It was then tightly capped, vortex-mixed for 20 s and heated for 20 min at 55°C. Then the tube was cooled and 10 μl was injected into the LC–UV system.

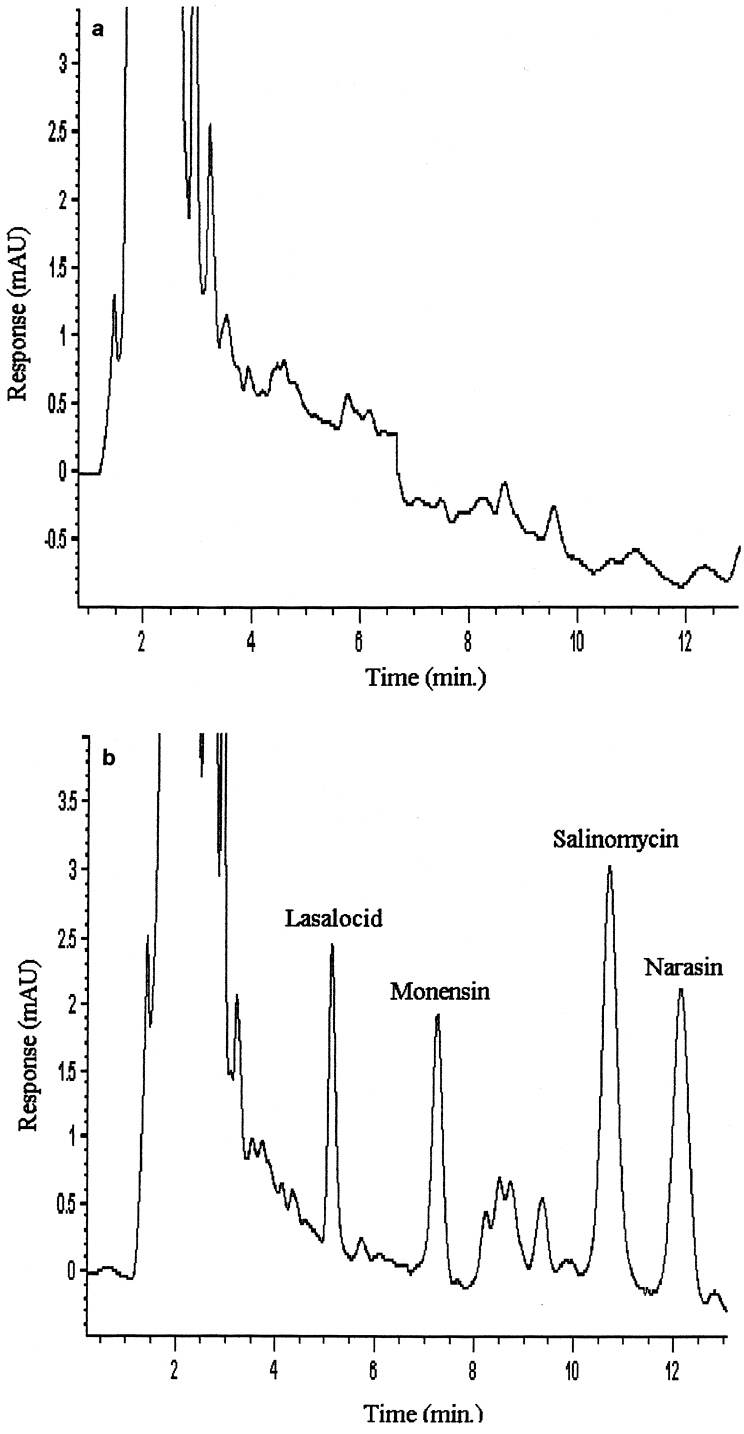

Fig. 1. Chromatograms after derivatization. (a) Blank turkey feed sample; (b) blank turkey feed sample spiked with 50 mg/kg of LA, MN, SA and NA.

3. Results and discussion

PEs are carbonyl-containing drugs. They should react, under acidic conditions, with hydrazine to form hydrazone; however the LA molecules that exhibit a free carbonyl group, did not react under our derivatization conditions.

In either the free acid or salt form LA assumes a cyclic structure [11] where the carbonyl group is directed inward and involved in metal ion chelation. In this conformation the initial attack of the hydrazine reagent is presumably not permitted because of steric hindrance. Fortunately LA detection is possible, though not derivatized, by exploiting the UV absorption at 305 nm of the benzenic group in the molecule.

MN does not have a free carbonyl group; nevertheless it exhibits a ketal ring that produces the carbonyl group, needed for derivatization, under our acidic conditions.

We optimized the derivatization procedure and the chromatographic conditions. LA and MN, NA and SL derivatives were resolved well and the response of the UV detection was linear in the working range of 10–400 ng injected for all the PEs. The stability of the PE derivatives at room temperature were checked by analyzing the same derivatized standard solution 24 h after derivatization; no degradation was observed. Moreover a sample extract injected every hour for 10 h after derivatization showed a negligible degradation.

Fig. 1a and b show the chromatograms of a blank turkey feed extract and of the same feed spiked with 50 mg/kg of each PE, respectively. The figure shows that the interfering peaks present in the chromatogram of the blank feed extract allowed the determination of all PEs at the concentrations normally used in medicated feeds and also that the four PEs were well resolved.

The chromatographic profiles of broiler and laying hen feed extracts were practically identical to those shown for turkey feed extract.

The detection limits ($S/N=3$), calculated from the chromatograms of blank feeds, were 40 mg/kg for MN and LA and 20 mg/kg for SL and NA.

For the method evaluation, different blank samples of turkey, broiler and laying hen feeds were spiked in a concentration range of 50–150 mg/kg with LA, MN, SL and NA and processed as described in Section 2.5. The PEs were quantified using the external standard method and the recoveries for the four PEs were 85–100% with relative standard deviations in the range of 4–10%.

4. Conclusions

The data reported showed that our method is suitable for routine analysis of ionophores in poultry feeds with good recovery and with acceptable specificity and repeatability results. The described assay offered, over previously pre-column LC–UV published methods, the possibility to analyze four PEs all at the concentration normally used in medicated feeds.

Acknowledgements

The authors wish to express their gratitude to Dr. Pradella of Ely-Lilly for his advice, to Dr. E. Faggionato and Mr. A. Baiguera of Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna for their assistance in the method development and to Dr. R. Coats of Centro Linguistico dell'Università di Brescia for his help during the translation of this paper into English.

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