conversion of AA to DHAA.

This HPLC procedure provides a relatively fast and sensitive technique for the simultaneous determination of AA and DHAA in foodstuffs and beverages. The method is simple and requires a minimum of sample preparation during the simultaneous determination of AA and DHAA. Further, this HPLC method measured AA and DHAA directly, which eliminates the need for the oxidation of AA to DHAA or the reduction of DHAA to AA prior to the analysis. The procedure was also found to be very useful for measurement of AA and DHAA in browned samples of orange juice where many interferring compounds limited the use of the dye titration and the microfluorometric methods.

Registry No. AA, 50-81-7; DHAA, 490-83-5.

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Qualitative and Quantitative Analysis of Monensin A Sodium Salt in the Low-Nanogram Range by Thin-Layer Chromatography and Fast Atom Bombardment Mass Spectrometry

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Fast atom bombardment mass spectrometry (FAB-MS) was used for quantitation of monensin A sodium salt in samples of chicken fat after separation using thin-layer chromatography. The method used offers the possibility to measure amounts of monensin A sodium salt at levels below 10 ng, corresponding to concentrations below 10 ng/g in chicken fat. The low values (10% or lower) obtained for the relative standard deviation using FAB-MS give a clear advantage over the conventional thin-layer bioautographic method.

INTRODUCTION

Monensin A, the major component of four polyether antibiotics produced by *Streptomyces cinnamonensis*, is frequently used as a feed supplement to control coccidiosis in chickens (Haney and Hoehn, 1968; Shumard and Callender, 1968). Methods have been developed for monensin determination at the milligram/kilogram (ppm) level in feeds based on microbiological (Kline et al., 1970; Kavanagh and Willis, 1972; Martinez and Shimoda, 1983), colorimetric (Golab et al., 1973), thin-layer chromatographic (TLC) (Asukabe et al., 1984), and high-performance liquid chromatographic (Macy and Loh, 1983) procedures. A semiquantitative thin-layer bioautographic assay procedure has been reported for the determination of monensin A residues at the microgram/kilogram (ppb) level in chicken tissues (Donoho and Kline, 1968). However, the identity of monensin is normally not confirmed.

The molecular formula of monensin A sodium salt (in the rest of this paper referred to as monensin A) has been determined by high-resolution mass spectrometry (Chamberlin and Agtarap, 1970). In a recent report (Chang et al., 1984), the mass spectra of three coccidiostats, including monensin, obtained by fast atom bombardment mass spectrometry (FAB-MS) are given. A method for quantitative analysis of corticosterone with progesterone as internal standard using FAB-MS has been described (Tanaka, 1983). In this paper we have used TLC and FAB-MS for quantitative determination of monensin A in the low nanogram/gram (ppb) range in chicken fat samples with monensin B sodium salt (in the rest of this paper referred to as monensin B) as internal standard.

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MATERIAL AND METHODS

Chemicals. Monensin A and B sodium salts were gifts from Dr. M. Beran, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia. Monensin A sodium salt standard was also supplied by Lilly Research Laboratories (Indianapolis, IN). All other chemicals were of analytical reagent grade.

Extraction. A 20-g chicken fat sample was extracted by methods similar to those reported earlier (Martinez and Shimoda, 1983; Donoho and Kline, 1968).

Thin-Layer Chromatography. The samples, dissolved in methanol, were applied to precoated silica gel TLC plates (20 × 20 cm; E. Merck, Darmstadt, FRG). The TLC plates were developed in a solvent system of carbon tetrachloride–benzene–methyl cellosolve (80:10:10). The TLC plates were air-dried, and a small band with the same R_f

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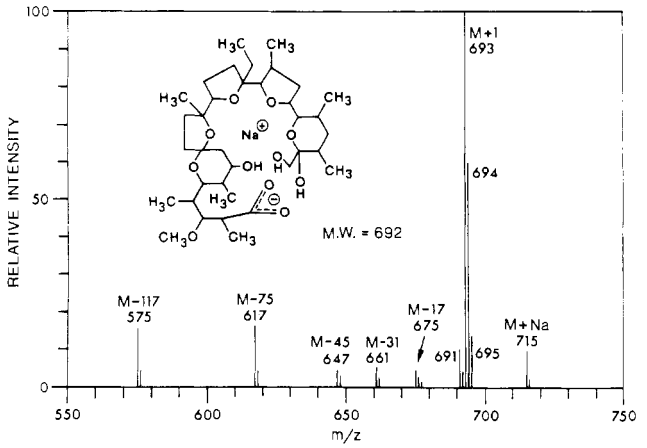

Figure 1. High-mass region of FAB mass spectrum of monensin A sodium salt.

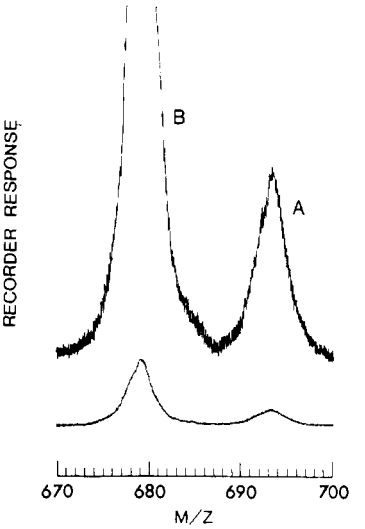

Figure 2. High-mass region of the unresolved FAB mass spectrum of monensin A (27 ng) and monensin B (92 ng).

value as monensin standard was scraped off the TLC plate and the monensin was extracted with 2 mL of methanol. After evaporation of the solvent, the samples were redissolved in 50–100 μL of methanol containing 92 ng/μL of the internal standard (monensin B).

Mass Spectrometry. An LKB 2091 mass spectrometer equipped with a water-cooled FAB source was used. Xenon atoms with an energy of 6 keV were used to bombard the compounds. The FAB target was made of copper and mounted onto the direct-inlet probe to obtain an angle of incidence for the atom beam of about 60°. Standard solutions were prepared with methanol as solvent. These, as well as the sample solutions, were applied to the FAB target in amounts varying from 1 to 3 μL. After the solvent had evaporated, about 1 μL of the FAB solvent (matrix) was transferred to the target. In this work four matrices have been used: glycerol, thioglycerol, triethanolamine, 1,1,3,3-tetramethylurea. The FAB target was washed after each analysis by immersion in ethanol in an ultrasonic bath.

To obtain maximum sensitivity, the ion source exit slit and the collector slit were fully opened. The molecular ion region, m/z 670–700, was monitored on a UV–recorder. In order to increase the signal-to-noise ratio the amplifier was equipped with a low-frequency filter.

RESULTS AND DISCUSSION

Figure 1 shows the high-mass region of the FAB mass spectrum of monensin A. The spectrum shows a base peak at m/z 693 $(M + H)^+$ and an additional peak at m/z 715 $(M + Na)^+$. Fragment ions are observed at m/z 691 $(M - 1)^+$, 675 $(M - 17)^+$, 661 $(M - 31)^+$, 647 $(M - 45)^+$, 617 $(M - 75)^+$, and 575 $(M - 117)^+$. All these fragments except $(M - 45)^+$ are present in the mass spectrum obtained by using a direct inlet system and an electron impact ion source (Chamberlin and Agtarap, 1970). However, using the latter technique, the MS peak at $(M - 75)^+$ appears as the base peak and the intensity of the molecular ion M = 692 is only a few percent. Monensin A has recently been run by FAB-MS (Chang et al., 1984), and the spectrum shown has M + 1 as its base peak and fragmentations similar to our results.

To be able to detect monensin A in the low-nanogram range it was necessary to decrease the resolution of the mass spectrometer. A gain in intensity of about 20 was obtained for a change of unit resolution from $m/\Delta m = 700$ to $m/\Delta m = 250$ at 10% valley.

Of the four matrices tested, we found that a mixture of triethanolamine and 1,1,3,3-tetramethylurea in the ratio 1:1 gave the best results. A relative calibration method was used to determine the amount of monensin A down to 5 ng with acceptable accuracy. As internal standard we have used monensin B (M = 678) which differs from monensin A by replacement of the ethyl group by a methyl group. However, the monensin B standard solution was found to contain about 6% of monensin A. The calibration curve and other measurements were corrected for this impurity. There are no peaks in the mass spectrum of monensin A that contribute to the maximum intensity of the molecular ion of monensin B at m/z 679 $(M + 1)^+$ and m/z 680 $(M + 2)^+$. None of the mass spectra of the matrices showed ion peaks in the current mass range.

It is important that the decrease in intensity with time is of the same order for the internal standard and the compound studied. It is also important that the kinetic energy of the xenon atoms is constant during the measuring periods. Optimal conditions were obtained at a xenon atom energy of 6 keV, and an increase of the atom energy did not improve the accuracy of the measurements.

Figure 2 shows an unresolved mass spectrum of a solution containing 27 ng of monensin A and 92 ng of monensin B. The mass range m/z 670 to m/z 700 was recorded in about 15 s. The main contributions to the intensity of the peaks are m/z 693, 694 and m/z 679, 680 for monensin A and monensin B, respectively. The signal-to-noise ratio for monensin A is approximately 10:1.

The peak heights were measured, and the ratios were calculated. Several mass spectra were collected after each insertion of the probe, and the average of the peak height ratios was determined. The peak height ratios ($h(m/z$ 693–694$)/h(m/z$ 679–680), mean of at least three separate determinations) for the standard solutions were plotted as a function of the concentration of monensin A. The resulting calibration curve ($y = 0.027 + 0.0081x$, correlation coefficient $r = 0.999$) is shown in Figure 3. The relative standard deviations were less than 10%, except for the two lowest concentrations, where somewhat higher values were obtained. As can be seen, the linearity is good over the concentration range from 3 to 200 ng/μL (0.004–0.29 nmol/μL) of monensin A.

Two samples of chicken fat were analyzed for their monensin A content by both the FAB-MS assay and by the semiquantitative thin-layer bioautographic assay earlier reported (Donoho and Kline, 1968). One was a pooled fat sample from chickens fed 90 ppm of monensin A and sacrificed at zero withdrawal and the other a spiked chicken fat sample (400 ng/g) taken through all steps of analysis. Unfortunately, no background samples from

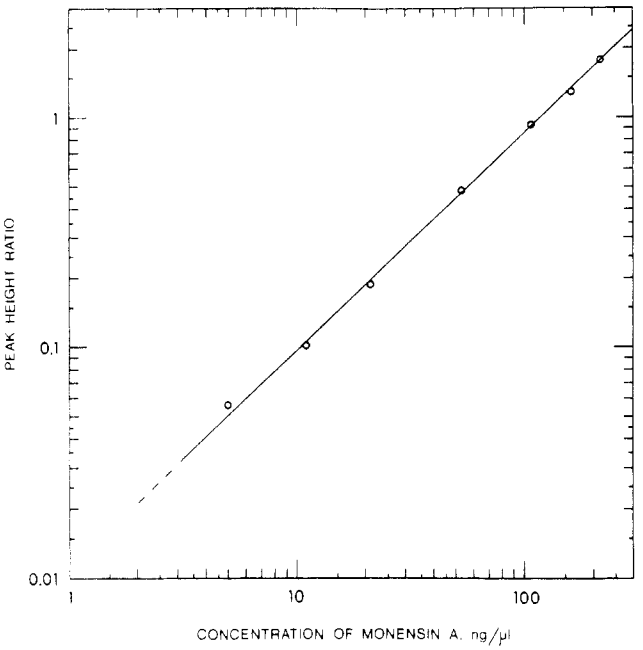

Figure 3. Calibration curve for monensin A. The ratio of the height of m/z 693–694 (representing monensin A) to the height of m/z 679–680 (representing monensin B) as a function of the concentration of monensin A.

Table I. Comparison of FAB-MS and Thin-Layer Bioautographic Methods for Monensin A Determination in Chicken Fat

	monensin A, ng/g	
sample	FAB-MS	thin-layer bioautographic
chicken fat	6	9
spiked chicken fat	75	>45

chickens not fed monensin A were available to us. The sample extracts were analyzed by both methods. Before analysis by FAB-MS, the extracts were further purified by preparative thin-layer chromatography.

The results in Table I show that the agreement between the two methods is very good. The lower value in the unspiked chicken fat sample obtained by the FAB-MS assay may be caused by the additional purification by thin-layer chromatography.

The precision of the FAB-MS method was again demonstrated by the relative standard deviations calculated for replicate measurements on the sample solutions. Values around 10% (four and three measurements, respectively) were obtained even at low concentration levels. These values are better than those usually expected from microbiological methods.

The instrumentation used in this study only permitted single-ion monitoring, and resolution had to be decreased in order to increase sensitivity. Thus, the identity of monensin A was tentatively established. However, the mass spectrometric method offers increased specificity compared to the conventional bioautographic assay.

The results clearly show that FAB-MS has great potential for confirmation and quantitation of monensin A in the low nanogram/gram (ppb) range in chicken fat samples.

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Registry No. Monensin A sodium salt, 22373-78-0.

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