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Note

Gas chromatographic determination of indole and 3-methylindole in ovine plasma and rumen fluid using a nitrogen–phosphorus selective detector

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In ruminants suffering from certain pulmonary disorders, such as fog fever in cattle[1] and acute respiratory distress syndrome in sheep[2,3], it is of interest to determine indole and 3-methylindole in plasma and rumen fluid. Similar analyses are necessary in experimental poisoning with tryptophan or 3-methylindole[4–6] and in metabolic studies of these compounds[7].

In this paper, a simple, sensitive and precise gas chromatographic (GC) method for the determination of indole and 3-methylindole in plasma and rumen fluid is described.

MATERIALS AND METHODS

Chemicals and reagents

Indole, 3-methylindole and 1,2-dimethylindole were obtained from Koch-Light (Colnbrook, Great Britain). Sodium borate (borax) and benzene were of reagent quality.

Stock solutions

Indole, 3-methylindole and 1,2-dimethylindole were dissolved in benzene. The concentration of the internal standard (1,2-dimethylindole) was 2.0 μg/ml. In addition, aqueous solutions of indole and 3-methylindole were prepared by dissolving 100 mg of each substance in 15 ml of ethanol and then diluting to 100 ml with water. These solutions were thereafter diluted 1:1000. All solutions were kept in the dark and refrigerated. The saturated sodium borate solution was prepared at room temperature.

Samples

Some of the samples of plasma and rumen fluid originated from an experiment on 3-methylindole and *dl*-tryptophan poisoning in sheep[6] and some from sheep with the acute respiratory distress syndrome[3].

Extraction procedure

Depending on the concentration, 0.1–2.0 ml of plasma or rumen fluid was diluted to 2 ml, 0.5 ml of saturated sodium borate solution was added and the mixture

was extracted with 0.5 ml of benzene containing 1.0 μg of 1,2-dimethylindole as internal standard. The extraction was performed in 10-ml centrifuge tubes with ground-glass stoppers. After centrifugation for 5 min, 5 μl of the benzene extract was injected into the gas chromatograph.

Gas chromatography

All analyses were carried out on a Carlo Erba Fractovap Linea 2100 gas chromatograph equipped with a nitrogen–phosphorus selective detector (NPSD)[8]. A 2 m × 4 mm I.D. glass column packed with 3% OV-17 on 80–100-mesh Supelcoport was used and the column temperature was 145°. The injector and detector temperatures were 200°. The helium flow-rate was adjusted to 44 ml/min and the hydrogen and air flow-rates to 29 and 250 ml/min, respectively.

The potassium chloride ring in the detector was adjusted daily to the position at which the alkali salt gave maximal flame ionization and the polarization electrode was adjusted to give a standing current of 10–15%. The electrometer attenuation was ×1 ×32 or ×1 ×16.

Calibration graphs

Standard calibration graphs were established by adding suitable amounts of the aqueous solutions of indole and 3-methylindole to blank plasma samples. These samples were then extracted with 0.5 ml of benzene containing 1.0 μg of the internal standard and 5 μl were injected into the gas chromatograph. The peak-height ratios of indole and 3-methylindole to the internal standard were calculated.

In order to detect possible variations in the detector response, all extracts were chromatographed together with a standard benzene solutions containing 0.5 ng/μl of indole, 1.0 ng/μl of 3-methylindole and 1.0 ng/μl of 1,2-dimethylindole.

Recovery studies

In order to determine the recovery, indole and 3-methylindole were added to plasma and extracted with 0.5 ml of benzene without an internal standard.

RESULTS AND DISCUSSION

Some chromatograms of representative extracts are shown in Fig. 1. The calibration graphs were straight lines up to a peak-height ratio of about 1.0 for indole and 1.5 for 3-methylindole. Above these ratios, the calibration graphs curved slightly. The recovery varied from 95 to 130%, which indicates that some of the benzene had evaporated during the centrifugation, but also that a satisfactory extraction recovery was obtained. However, the results were highly reproducible when the internal standard was used. When the calibration graphs were established, the same peak heights were obtained both when indole and 3-methylindole were added to the plasma as aqueous solutions or when they were present in the benzene prior to the extraction.

The standard solutions chromatographed together with the samples showed very small variations in the peak-height ratios. Over a 3-month period the average indole to 1,2-dimethylindole peak-height ratio was 0.84 ± 0.04 and the average 3-methylindole to 1,2-dimethylindole peak-height ratio was 1.00 ± 0.03 when calculated from 56 measurements. On comparing these figures with the calibration graphs,

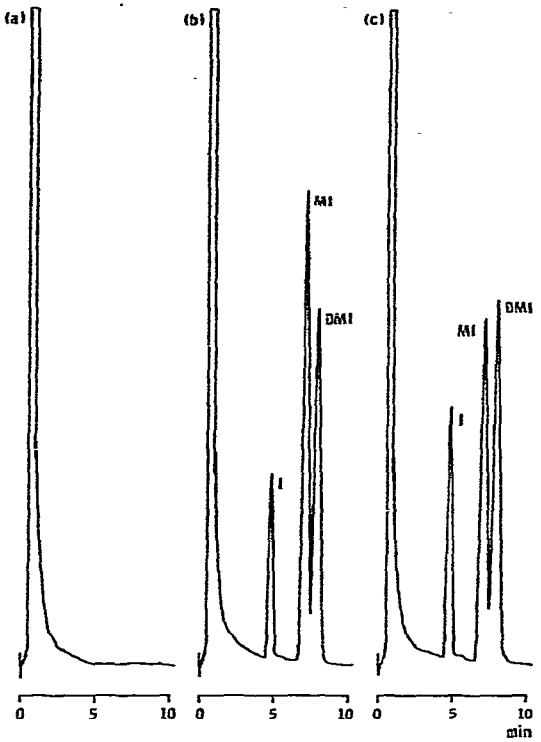

Fig. 1. Chromatograms of indole (I), 3-methylindole (MI) and 1,2-dimethylindole (DMI). (a) Blank plasma extract. (b) Extract of plasma sample. (c) Extract of rumen fluid sample. These samples originated from a sheep given *dl*-tryptophan.

the recoveries of indole and 3-methylindole relative to that of the internal standard were 79 and 96%, respectively.

A 4-mm I.D. column was chosen in order to give sufficient separation at the helium flow-rate used. The column gas flow-rate is very critical when helium is used as the carrier gas, but with proper adjustment a high sensitivity is obtained. The retention times for indole, 3-methylindole and 1,2-dimethylindole were 4.8, 7.1 and 7.9 min, respectively.

The present method was developed for the rapid, simultaneous determination of indole and 3-methylindole in sheep plasma and rumen fluid. The method has a high sensitivity and the detection limit for these two compounds under the present conditions was 0.005 μg/ml. No interfering peaks were detected in plasma or rumen fluid.

In comparison with previously described methods, the present method is very simple and has a high sensitivity. Bradley and Carlson[9] and Yokoyama and Carlson[7] extracted indole and 3-methylindole three times with dichloromethane. Under their conditions, the two compounds had retention times of about 28 and 32 min, respectively. Mackenzie *et al.*[1] mixed their samples with Celite 545 to form a dry powder and used column extraction with dichloromethane. Their GC conditions were similar to those in this work but they used a flame-ionization detector. In all of these in-

stances the final extracts were evaporated to a small volume, whereas this step is not necessary with the present method.

The present method has been found to be suitable for analyses in experimental poisoning with 3-methylindole and *dl*-tryptophan. After oral administration of 3-methylindole, large amounts of this compound have been found in plasma. After the administration of *dl*-tryptophan significant amounts of both indole and 3-methylindole have been detected in plasma and rumen fluid. In most instances where the acute respiratory distress syndrome was diagnosed, only trace amounts of the two compounds were detected. The detailed results of these investigations will be published elsewhere[3,6].

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