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## Note

# Gas chromatographic determination of tranquillizer residues in body fluids and in the meat of slaughtered animals

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Tranquillizers are used extensively in order to subdue cattle being transported from the farm to the slaughterhouse. This practice also permits the mortality of stress-sensitive animals to be reduced. These tranquillizers are butyrophenone or phenothiazine derivatives and, of the latter, propionylpromazine or Combelene (I) and chlorpromazine or Largactil (II) (Fig. 1) are commonly used to subdue horned cattle before slaughter. Xylazine or Rompun (IV), which is not a phenothiazine derivative, is also often utilized for this purpose. Phenothiazines are also important drugs in human medicine, and much work has been done on the detection and determination of the residues and metabolites of these compounds in human body fluids. It is not our purposes to review systematically all of the procedures which have been proposed; examples are thin-layer chromatography (TLC)[1], reversed-phase TLC[2], TLC–mass spectrometry (MS)[3], high-performance liquid chromatography[4–6] and gas chromatography (GC)–MS[7]. Riedman[8] proposed a GC method based on the specific detection

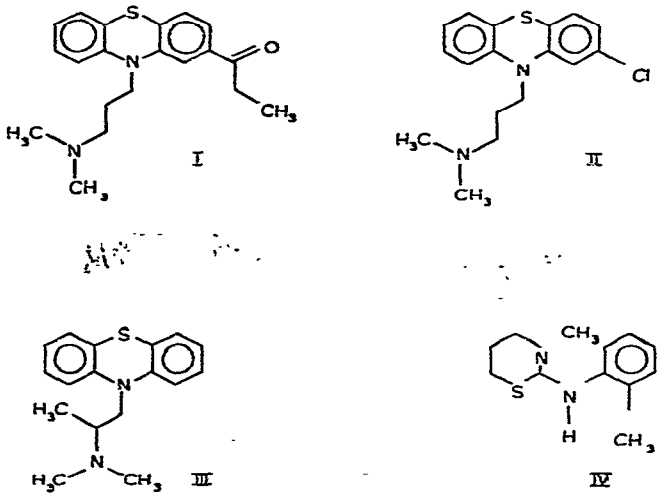

Fig. 1. Structures of propionylpromazine (I), chlorpromazine (II), promethazine (III) and xylazine (IV).

of nitrogen compounds by using an alkali flame-ionization detector. We have developed a more specific and reliable GC method for the determination of phenothiazine and xylazine residues in body fluids (bile, plasma and urine) and in animal tissues, involving the use of a flame-photometric detector fitted with a 394-nm filter, giving a specific response to sulphur compounds.

## EXPERIMENTAL

### *Apparatus*

A Tracor Model 560 gas–liquid chromatograph was used, equipped with a flame-photometric detector (394-nm filter and response linearizer) and a glass column (2.0 m × 2 mm I.D.) packed with 3% OV-1 on Chromosorb W HP (80–100 mesh). The operating conditions were as follows: oven temperature, 250°; detector temperature, 240°; inlet temperature, 260°; carrier gas (nitrogen) flow-rate, 35 ml/min; hydrogen flow-rate, 40 ml/min; air flow-rate, 170 ml/min.

### *Solvents and reagents*

Analytical-reagent grade solvents were purchased from Merck (Darmstadt, G.F.R.) and were redistilled in an all-glass apparatus. Reference compounds were extracted from pharmaceutical preparations.

### *Extraction and clean-up*

*Body fluids (bile, plasma and urine).* To a 5-ml volume of fluid were added 2 ml of 1 *N* sodium hydroxide solution. The tranquilliser residues were extracted three times with 10 ml of *n*-hexane, the combined organic extracts were dried under a stream of nitrogen on a water-bath at 50°, the residues were dissolved in 0.1 ml of benzene and 1–5 $\mu$l of the solution obtained were injected into the chromatograph. For the analysis of samples of bile, after evaporation of the extraction solvent 1 ml of 1 *N* sulphuric acid was added to the residue. This solution was washed by shaking three times with 3 ml of *n*-hexane, the aqueous solution was neutralized by addition of 2 ml of 1 *N* sodium hydroxide solution and extracted four times with 3 ml of *n*-hexane and, after evaporation of the solvent, the tranquillizer residues were treated as described above.

*Meat.* A 5-g sample of meat was homogenized in 10 ml of a mixture of acetone and 1 *N* sulphuric acid (10:1). After centrifugation, the supernatant was decanted, the acetone was evaporated and the extract was dissolved in distilled water to give a final volume of about 5 ml. To this solution was added 0.3 ml of 2 *N* sodium hydroxide solution and the tranquillizer residues were extracted by shaking three times with 3 ml of *n*-hexane. The combined hexane extracts were dried under a stream of nitrogen and the residues were finally dissolved in 0.1 ml of benzene.

## RESULTS AND DISCUSSION

The recovery of these tranquillizers was of the order of 90–95% from urine samples, 95–100% from plasma samples, 70–80% from bile samples and 50–60% from meat samples. The limit of detection of phenothiazine derivatives was 1.0 ng and of xylazine residues 0.5 ng. In spite of the very simple clean-up procedure, the

chromatograms of body fluids or meat extracts were very clean because of the high specificity of the flame-photometric detector. Under the described conditions (with an oven temperature of 250°), the phenothiazine derivatives were well separated; the mean retention times were 3.3 min for promethazine (III), 5.0 min for chlorpromazine (II) and 9.0 min for propionylpromazine (I). The mean retention time of xylazine (IV) was 4.8 min at an oven temperature of 200°.

We tested the efficiency and reliability of the procedure. A pig (weight 95 kg) was injected with 4 ml of Combelene (10 mg/ml of propionylpromazine) and slaughtered 2 h later. The urine, plasma and bile were analysed using promethazine and chlorpromazine as internal standards and 0.37 ppm of the free tranquillizer in the urine, 0.030 ppm in the plasma and 0.2 ppm in the bile were determined. These results were confirmed by mass spectrometry using the single-ion scanning mode. As shown in Fig. 2, the recorded ion (molecular ion: $C_{20}H_{24}N_2OS^{+\cdot}$, $m/z$ 340) in the mass spectrum (b) of the urine extract was similar to the mass spectrum of the standard solution (a).

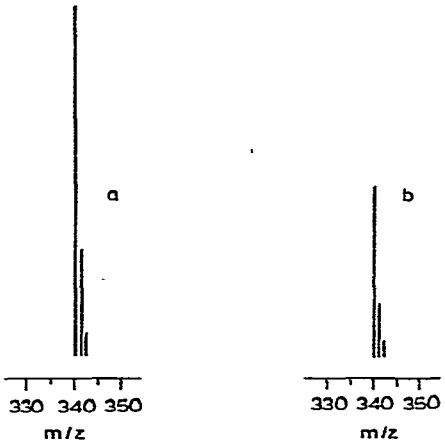


Fig. 2. Molecular ion of (a) standard propionylpromazine and (b) propionylpromazine extracted from the urine of a pig that had been injected with Combelene.

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