Journal of Chromatography, 216 (1981) 350–354
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,137

Note

Analysis of xylazine in biological material by gas chromatography using packed and capillary columns

ASTRI ROGSTAD* and MAGNE YNDESTAD
Department of Food Hygiene, Veterinary College of Norway, P.O. Box 8146, Dep, Oslo 1 (Norway)
(Received June 26th, 1981)

Xylazine is a widely used tranquillizer for both domestic and wild animal species. The compound's sedative, muscle relaxant and analgesic properties are due to its action on the autonomic and the central nervous system. In Norway, xylazine hydrochloride is available commercially as a colorless 2% solution for intramuscular injection (Rompun®, Bayer, Leverkusen, G.F.R.). It is approved for use in cattle, horses, dogs and cats. It is thought to have a relatively short excretion time, with residues below 0.1 ppm in 20 h in all edible tissues except for the injection site, liver and kidney[1,2]. On medication of cattle only two days are recommended in Norway between the treatment and the slaughtering of cattle or the delivery of milk for human consumption. Liver and kidney should be discarded if slaughtering has taken place less than four days after medication.

The only study on residues of xylazine in carcasses has been reported by Pütter and Sagner[2], who did not find any residues of the active compound in the muscles after 24 h. The detection limit of the method was 90 ppb*. The analyses were performed by a spectrophotometric method. The same detection limit was obtained by gas chromatographic (GC) analysis of xylazine in biological fluids[3]. More recently a simplified extraction procedure and a GC method based on sulphur-specific detection of xylazine were described[4].

We found it necessary, however, to modify the extraction procedure and apply a nitrogen-specific detector on the gas chromatograph, which is very sensitive and more common in most analytical laboratories.

MATERIALS AND METHODS

Reagents

All reagents used in the extraction procedure were of analytical grade. Methanol was dried over molecular sieves (3 Å). The purity of the solvents was tested by GC. Xylazine hydrochloride was donated by Bayer. Diphenhydramin hydrochloride was used as internal standard and was supplied by Norsk Medisinaldepot, Oslo, Norway. Stock solutions of xylazine (2 mg/ml) and diphenhydramin (0.5 mg/ml) were prepared in methanol and stored in the refrigerator at 4°C in dark stoppered flasks.

* Throughout this article, the American billion (10^9) is meant.

0021-9673/81/0000–0000/$02.50

Extraction procedure

Biological tissues. 10 g of tissue (liver, kidney or meat), 8 g dry sodium sulfate and 10 ml acetonitrile were homogenized 1 min with a Sorvall Omni-mixer. The homogenate was centrifuged for 5 min 2980 *g*) and the solution was transferred to a round-bottomed flask. The homogenization was repeated twice with portions of 10 ml acetonitrile. The combined extracts were evaporated under vacuum at 45°C on a rotary evaporator. The dry residue was dissolved in 2.5 ml of 1 *M* hydrochloric acid. The round-bottomed flask was rinsed three more times with portions of hydrochloric acid.

Internal standard was added to the combined aqueous phases which were alkalinized with conc. ammonia, and extracted three times with diethyl ether. The combined ether phases were extracted with 1 *M* hydrochloric acid (2 × 6 ml). After addition of 1.5 ml conc. ammonia the solution was extracted three times with chloroform. The extracts were combined, and the chloroform evaporated under a gentle stream of nitrogen in a water bath at 50°C. The dry residue was dissolved in 50 μl methanol, and *ca.* 2 μl were injected into the gas chromatograph.

Biological fluids. Serum (2 g) was alkalinized by conc. ammonia and internal standard was added. The aqueous phase was extracted with diethyl ether (2 × 4 ml). The combined ether phases were washed with distilled water, which was discarded. After extraction with 0.05 *M* sulphuric acid (2 × 2 ml) the combined aqueous solutions were alkalinized and extracted twice with 3-ml portions of chloroform. The chloroform was dried off under a gentle stream of nitrogen, and the residue was dissolved in 50 μl methanol; *ca.* 2 μl were injected into the gas chromatograph.

Gas chromatography

Two Carlo Erba gas chromatographs were used. A Fractovap Model 2101 AC was equipped with a nitrogen–phosphorus-selective flame ionization detector (FID) and a packed column, whereas the Fractovap Model 4160-01 was equipped with an ordinary FID and a capillary column. Model 4160 has both a split–splitless injector and an on-column injector.

Silanized glass columns (2.0 m × 2 mm I.D.) were packed with GP 3% SP-2250 DB or 5% SP-2401 DB, both on 100–120 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). The oven temperature was programmed from 190 to 225°C at 6°/min, and the injector/detector temperature was 250°C. Another silanized glass column (0.5 m × 2 mm I.D.) was packed with 10% Carbowax 20M–2% KOH on 80–100 mesh Chromosorb W AW (Supelco). For this column the oven temperature program was from 190 to 220°C at 8°/min, and the injector/detector temperature was 225°C. For all columns three successive injections were performed, followed by 20 min at 225°C or 220°C, respectively, to elute contaminant buildup prior to the next series of injections. Helium was used as carrier gas and the flow-rate was 35 ml/min.

An open tubular glass capillary column (20 m × 0.32 mm I.D.) was coated with SP-1000 and supplied by H. & G. Jaeggi (Trogen, Switzerland). The operating conditions for Model 4160 were as follows: oven temperature 110–235°C, programmed at 15°/min; injector temperature 250°C; detector temperature 270°C; and carrier gas (helium) flow-rate 3.5 ml/min. The sample was injected at 44°C, and the oven temperature quickly raised to the initial programming temperature.

The peak areas were integrated by a LDC Model 308 integrator and calculated relative to the internal standard.

Calibration curve and recovery studies

Calibration curves were prepared in the range 10–1000 ng xylazine per sample of serum and in the range 40–8000 ng xylazine per sample of meat. Linear equations from regression analysis of the calibration points were determined and are given in Table I.

TABLE I

CALIBRATION GRAPH REGRESSION EQUATIONS USED FOR XYLAZINE SAMPLE CONTENT DETERMINATIONS

Biological sample	*Internal standard (ng per sample)*	*Rompun range (ng per sample)*	*Regression equation* $y = ax + b$*		*Correlation coefficient*	
			a	*b*	*r*	*n*
Meat	2500	800–8000	2.065	−0.324	0.972	24
Meat	250	40–800	1.575	−0.263	0.976	20
Serum	250	100–1000	1.728	0.001	0.986	29
Serum	25	10–100	1.353	0.215	0.974	17

* Peak area ratio; peak area internal standard = 1.0.

The recovery was estimated by repeated extraction of xylazine and internal standard added to meat and serum samples. The xylazine was added to the sample before the homogenization and taken through the extraction procedure as described.

The precision of the extraction procedure was estimated by using multiple replicates at various concentrations and calculating the standard deviation.

RESULTS AND DISCUSSION

When applying the simplified extraction procedure of Laitem *et al.*[4] the GC analysis of the extracts of biological tissues, particularly meat, showed strong impurities. The fat dissolved easily in the mixture of acetone and sulphuric acid, and fat and other contaminants reduced the sensitivity of the nitrogen selective detector. However, insignificant quantities of the fat dissolved in acetonitrile and did not disturb the GC analysis.

Based on previous studies of GC separations of drugs containing amine groups, the GC properties of xylazine on a variety of stationary phases were studied. Tailing, a common observation with underivatized amines, and thus low peak intensity were the major problems. Analysis with a 2-m 10% Carbowax 20M–2% KOH column resulted in symmetrical peaks and good separation, but unacceptably long retention times and a significant broadening of the peaks. A 0.5-m column with the same phase, however, provided excellent chromatographic properties for xylazine and the internal standard when extracted from serum. When analyzing a meat extract on this column the xylazine peak was not separated from impurities. Analysis of the meat extract on semi-polar phases such as SP-2250 DB and SP-2401 DB showed that SP-2250 DB gave the best separation. Due to some tailing of the xylazine peak the intensity was reduced compared to that on the 10% Carbowax 20M–2% KOH column.

The higher resolution obtained with the capillary column makes glass capillary GC a superior technique for determination of low concentrations of xylazine and good separation from interfering contaminants. The intensity on the FID with capillary column was comparable to that on the alkali FID with a packed column system. However, analysis with the conventional packed column is more simple and less expensive and time consuming. The packed column is more suitable for routine analysis in the food control of residues of xylazine in meat, liver and kidney. Both the split–splitless injector and the on-column injector were applicable. No discrimination was observed.

Chromatograms typical for extracted samples of biological tissues and fluids are shown in Fig. 1. Calibration curves were used for content determinations of xylazine in the biological samples (see Table I). The average recovery of xylazine was estimated to be 93% from meat samples and 100% from serum samples. The standard deviation of the extraction procedure was estimated to be 12.5% for meat samples and 9.3% for serum. The detection limit of xylazine extracted from meat samples was 40 ng or 4 ppb and from serum 10 ng or 5 ppb.

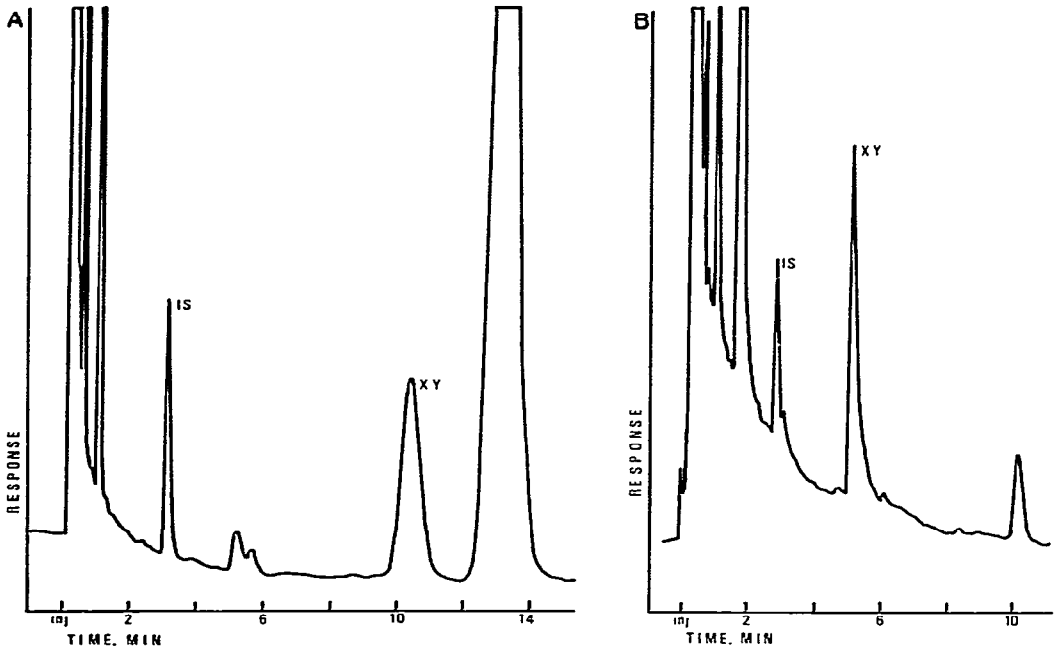

Fig. 1. Gas chromatogram of xylazine (XY) and internal standard (IS) extracted from serum (A) and meat (B). Conditions: A, 10% Carbowax 20M–2% KOH column; 400 ng xylazine added to 2 g serum. B. GP 3% SP-2250 DB column; 800 ng xylazine added to 10 g meat.

The described analytical methods are suitable both for determination of residues of xylazine in tissues of slaughtered animals and in biological fluids, and for pharmacokinetic studies of unchanged xylazine. It may also be possible to extend the method to the simultaneous determination of metabolites.

ACKNOWLEDGEMENTS

We are grateful to Tone Hauk Olsen for skilful technical assistance. Financial support from the Norwegian Research Council for Agricultural Science is gratefully acknowledged.

REFERENCES

1 A. P. Knight, *J. Amer. Vet. Med. Ass.*, 176 (1980) 454.
2 J. Pütter and G. Sagner, *vet.-Med. Nachr.*, (1973) 133.
3 D. Courtot, E. Blanchard and N. Fischer, *Ann. Rech. Vet.*, 5 (1974) 101.
4 L. Laitem, I. Bello and P. Gaspar, *J. Chromatogr.*, 156 (1978) 327.