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Note

Determination of α-chloralose in rodenticide formulations by gas–liquid chromatography

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α-Chloralose was prepared and its structure elucidated by Pictet and Reichel[1]. It has been employed as a hypnotic but is not now normally recommended because of its uncertain physiological action.

α-Chloralose is currently used as a narcotic in the control of pest birds and as a rodenticide for mouse control[2]. Formulated as a 4% bait (oatmeal coated with α-chloralose, dye and other inert ingredients) it is extremely effective even against warfarin-resistant mice. α-Chloralose has been determined in various matrices by titrimetry[3] and spectrophotometry[4], but neither of these methods is suitable for bait formulations. Gas–liquid chromatography (GLC) has been used (column of 3% E-301 + 0.3% Epikote resin on Celite; electron capture detector) to determine α-chloralose in the tissues of narcotised pigeons[5].

Manufacture of rodenticide bait formulations incorporates a mixing stage where oatmeal is coated with a fine powder containing α-chloralose. In order to improve the homogeneity of the product and establish quality control procedures, a reliable analytical method had to be devised. The GLC technique was thought most applicable, since interference effects can be minimised.

This paper reports a successful analytical method using this technique.

EXPERIMENTAL

Reagents and materials

Pure α-chloralose (Koch-Light, Colnbrook, Bucks., Great Britain), pure γ-1,2,3,4,5,6-hexachlorocyclohexane (abbreviated as γ-HCH; Phase Separations, Queensferry, Flints., Great Britain), Trisil and trifluoroacetylimidazole (Pierce, Rockford, Ill., U.S.A.) and "AnalaR" pyridine (BDH, Poole, Dorset, Great Britain) were used in these experiments. The internal standard was γ-HCH in pyridine (20 g/l).

β-Chloralose was prepared from chloral and glucose by stirring together in sulphuric acid[6]. After recrystallisation from ethanol, the product had a m.p. of 228° compared to a literature value[7] of 227–230°.

Apparatus

A Pye Unicam 104 gas chromatograph fitted with a flame ionisation detector

and a Smiths Servoscribe recorder was used. The column was constructed of 5 ft. × 4 mm I.D. coiled glass and packed with 2% neopentyl glycol succinate (NPGS) and 0.2% Epikote 1001, on 80–100 mesh Gas-Chrom Q.

The chromatographic conditions were as follows: column temperature, 185°; detector temperature, 250°; carrier gas (nitrogen) flow-rate, 60 ml/min.

Determination

Internal standard solution (15 ml) was added to accurately weighed samples of bait (2.5 g) or pure α-chloralose (0.1 g). After vigorous shaking, the samples were left for 5 min to settle. An aliquot (1 ml) of the extract was transferred to a dry test tube and Trisil (1 ml) added using a hypodermic syringe. The tube was stoppered and shaken. Using a 10-μl syringe, 1.5-μl aliquots of solution were injected on to the column, taking care not to inject any silica which might have been formed.

Calculation

A calibration graph was constructed (from the chromatograms of the standards) by plotting the peak area ratio between α-chloralose and γ-HCH against the weight ratio between α-chloralose and γ-HCH. The slope of the calibration graph was used to calculate the α-chloralose contents of the bait samples.

RESULTS

A recovery test was carried out by accurately weighing pure α-chloralose (0.1 g) into a test tube, adding blank bait (2.4 g) and mixing thoroughly. This was then treated as a normal sample and internal standard solution (15 ml) was added. The results are given in Table I.

TABLE I

RESULTS OF A RECOVERY TEST ON PURE α-CHLORALOSE MIXED WITH BLANK BAIT

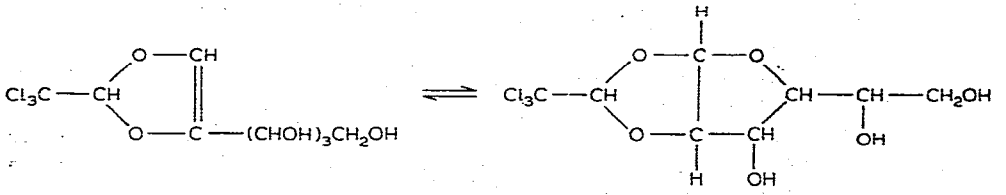
For experimental details, see text.

Concentration of α-chloralose (%)		*Recovery (%)*
Prepared	*Found*	
4.16	3.85	92.6
4.27	4.02	94.2
4.17	3.86	92.6
4.27	4.22	98.8
4.68	4.25	90.8
4.28	4.20	98.1
4.98	4.87	97.8
4.97	4.99	100.4
4.73	4.78	101.6
4.35	4.27	98.2
Mean recovery, %		96.5

DISCUSSION

The silylation procedure and GLC conditions (polar, NPGS column) give two well-separated peaks for a sample of α-chloralose claimed to be 98% pure. When a non-polar dimethyl silicone column (3% OV-1) was used, only a single skew peak was observed. Bailey[5] reported a similar result using a non-polar E-301 column (dimethyl silicone). He was, however, only interested in a qualitative procedure.

There are several possible reasons why two peaks might be formed from the α-chloralose standard sample. The two most probable are that; (i) the material supplied was impure and contained a small amount of the β-isomer, or (ii) two silyl derivatives were formed by reaction with Trisil (these would correspond to derivatives of I and II).



I II

To establish the reason for the two peaks, β-chloralose was prepared and silylated. This derivative of β-chloralose gave only one peak on the NPGS column corresponding to the smaller peak observed with the α-chloralose. A comparison was also made on a 3% OV-1 column. Here again, the peak for the silylated β-chloralose corresponded to the smaller partially resolved peak produced by α-chloralose. The above two findings were confirmed by the GLC of the trifluoroacetyl derivatives of both α- and β-chloralose (prepared using trifluoroacetylimidazole), clear assignment of the two peaks being established.

CONCLUSIONS

The GLC conditions herein reported allow a rapid method for separating α-chloralose from its β-isomer and for the determination of α-chloralose in formulated rodenticide bait samples.

REFERENCES

1 A. Pictet and F. H. Reichel, *Helv. Chim. Acta*, 6 (1923) 621.
2 P. B. Cornwell, *Munic. Eng. (London)*, 143 (1966) 2371.
3 *Pharmacopée Français*, L'Ordre National des Pharmaciens, Paris, 8th ed., 1965.
4 M. Mirjolet, M. Semars, G. de Ren and G. Petiet, *Bull. Soc. Pharm. Nancy*, 83 (1969) 7.
5 S. Bailey, *SAC Meeting: Microchemical Methods in Analytical Chemistry, March 28th and 29th 1969.*
6 H. W. Coles, L. D. Goodhue and R. M. Hixon, *J. Amer. Chem. Soc.*, 51 (1929) 519
7 H. Martin and C. R. Worthing (Editors), *Pesticide Manual*, British Crop Protection Council, 4th ed, 1974.