Journal of Chromatography, 134 (1977) 178–181

CHROM. 9835

Note

Estimation of chlorbutanol by electron capture gas chromatography

HAMILTON SMITH and J. W. THORPE
Department of Forensic Medicine, Glasgow University, Glasgow (Great Britain)
(First received September 30th, 1976; revised manuscript received November 22nd, 1976)

The estimation and identification of chlorbutanol and some related substances is of great importance in the sport of greyhound racing. A very large number of samples need to be analysed rapidly with accuracy. The available methods were found to be too slow or only applicable to non-specific qualitative analysis. The method of gas chromatography with electron capture detection was therefore chosen as a technique which might fulfil the requirements. This paper describes a method which uses simple extraction with a solvent containing an internal standard and subsequent quantitation by means of electron capture gas chromatography. The electron capture detector was chosen as the basis for the method because it was sensitive to drugs such as chlorbutanol which contain chlorine atoms and was relatively insensitive to the other organic materials which might complicate the analysis since only simple extraction procedures were used.

EXPERIMENTAL AND RESULTS

The requirement was that the column should separate chlorbutanol, chloral hydrate, trichloroacetic acid, and trichloroethanol from aqueous solution. From a review of the literature and manufacturers' lists several column packings were selected for study. These included Apiezon L[1], Carbowax 20M[2], free fatty acid phase[3], PEG 400[4], neopentyl glycol adipate[5] and Chromosorb 102, which were tested under a variety of conditions. Apiezon L on glass beads and Carbowax 20M and PEG 400 on Chromosorb W and Chromosorb 102 were found to be unsuitable as chlorbutanol was either not eluted from the column at all, or only eluted after a very long time in a broad peak. Neopentyl glycol adipate did not give the required separation. Apiezon L and FFAP on Chromosorb W did separate chlorbutanol from water but the water peak was broad and masked peaks from the other drugs of interest.

The major problem was the separation from water and as this was not accomplished on the column a solvent extraction procedure was required. The FFAP column tested gave rather sharper water peaks than the Apiezon L column, so it was tested further. The separation of the water was great enough so that it was not necessary to dry the solvent after extraction. Also, the various related drugs of interest were reasonably well separated from each other. The solvent used for the separation must be capable of extracting chlorbutanol and some related materials including trichlorethanol. The two most useful solvents were found to be diethyl ether and amyl alcohol.

The latter was selected because the volatility of diethyl ether could give rise to handling problems. The time required for shaking during extraction was also tested and recoveries were found to reach the maximum values within 2 min. To allow for variations in the efficiency of shaking, a time of 5 min was selected.

The internal standard must be a compound which can be detected when using an electron capture detector. This suggested a halogenated or possibly an oxygenated compound. In addition, under the gas chromatographic conditions employed the compound must be completely separated from the solvent and the trichloro materials. The internal standard must procude only one peak when chromatographed but absolute purity would not be essential as the estimation would be by means of a comparison with an aqueous solution of the trichloro material. A large number of suitable materials were tested under various conditions and it was found that benzaldehyde was the most suitable.

Method

The developed method is as follows. A 2-ml volume of urine is extracted with an appropriate volume (2 ml) of a 300 mg/100 ml solution of benzaldehyde in amyl alcohol by shaking for 5 min. Simultaneously 2 ml of a standard solution of chlorbutanol (about 0.2 mg/100 ml) in water is extracted.

A 1-μl volume of each extract is injected into the gas chromatograph and the peak areas are estimated for chlorbutanol and benzaldehyde. The concentration in the urine is calculated by comparison with the chlorbutanol standard.

The column is glass (5 ft. $\times$ 1/8 in. I.D.) and is packed with 15% FFAP on Chromosorb W AW DMCS. This is conditioned for 24 h at 225° before use, and is operated at 95° with a nitrogen or argon–methane (10:1) carrier gas flow-rate of 40 ml/min. The injector temperature is 125° and the electron capture detector is maintained at 150°.

The results of the experiment (Table I) showed that the method was suitable for qualitative analysis.

The linear dynamic range for benzaldehyde was checked and a linear response was found for amounts up to 80 μg, *i.e.*, 1 μl of an 800 mg/100 ml benzaldehyde solution in amyl alcohol. The linear response for chlorbutanol was up

TABLE I

RELATIVE RETENTION TIMES OF DRUGS AT 95°

Compound	*Relative retention time*
Benzaldehyde	1.00 (5.5 min)
Chloral betaine*	0.40
Chloral hydrate	0.40
Chlorbutanol	1.40
Ethchlorvynol	0.40, 0.65, 1.20 (main peak), 3.43
Trichloroacetic acid	0.24
Trichloroethanol	2.36
Trichloroethyl phosphate**	2.36
Water	0.21

* Chloral betaine appears to decompose to chloral hydrate.

** Trichloroethyl phosphate appears to decompose to trichloroethanol.

to 2 ng, *i.e.*, 1 μl of a 0.2 mg/100 ml chlorbutanol solution in amyl alcohol. The expected concentrations of chlorbutanol and the machine responses lead to a choice of a concentration of benzaldehyde in amyl alcohol in the region of 300 mg/100 ml. The constancy of the relationship between chlorbutanol and benzaldehyde was investigated by injecting the same solution into the gas chromatography unit ten times. The concentration of the solution was 0.121 mg chlorbutanol/100 ml amyl alcohol and 300 mg benzaldehyde/100 ml. The mean peak area ratio was 0.75 and the standard deviation 0.019. A series of chlorbutanol concentrations ranging from 0.02–0.161 mg/100 ml in the above benzaldehyde solution was made and the resulting peak areas were plotted against concentration. The result was a straight line which passed through the origin. For each concentration the standard deviation was within the value quoted above. Benzaldehyde was therefore a suitable internal standard for the estimation of chlorbutanol by electron capture gas chromatography.

The efficiency of extraction of the chlorbutanol was tested by adding the internal standard after the separation of the solvents. A more convenient and effective use of the internal standard was extraction with a solution of benzaldehyde in amyl alcohol. A series of experiments showed that less than 1% of benzaldehyde was lost into the aqueous phase when the water-to-amyl alcohol volumes were 2:1. Further tests were made by comparing analyses which used the addition of the benzaldehyde after extraction with analyses using the benzaldehyde solution extraction technique. Eight experiments using the first method gave a mean value of 3.59 (S.D. = 0.27) arbitrary units. A similar number of experiments using the second method gave a mean value of 3.64 (S.D. = 0.15). The results are essentially the same but the second method gave a smaller range of variation. Quantitative analysis of a chlorbutanol solution (0.34 mg/100 ml urine) resulted in a mean recovery of 97.3% using the first method and of 99% using the second.

DISCUSSION

The response of the electron capture detector to water made the direct injection of water-based samples impracticable. The solvent extraction technique developed here solved this problem. The use of amyl alcohol, containing benzaldehyde as an internal standard, for the single extraction step resulted in a rapid accurate method. Any losses of solvent have a corresponding loss of standard and chlorbutanol which is automatically included in the calculation. Further, the use of an aqueous chlorbutanol standard as a means of direct comparison rather than a benzaldehyde/chlorbutanol concentration graph removes difficulties caused by deterioration of the benzaldehyde or by the day-to-day change in instrument response.

Urine samples may vary widely in chlorobutanol concentration but by varying the water-to-organic phase ratio it was possible to compensate for this within wide limits and still be within the linear range of the detector.

The application of the method to many hundreds of urine samples demonstrated the speed and ease of the estimation and the lack of interference by normal constituents. Related materials (Table I) and metabolites were easily detected when present and did not interfere with the chlorbutanol estimation.

CONCLUSIONS

A method was developed for the estimation of chlorbutanol in urine by electron capture gas chromatography with the calculation of results based on the use of benzaldehyde as an internal standard. The technique can also be applied to related materials such as trichloroethanol. Because of its speed, accuracy and freedom from interference, the method can be used where many samples require to be screened quickly.

ACKNOWLEDGEMENT

Dr. J. W. Thorpe wishes to thank the National Greyhond Racing club for the provision of a grant during the tenure of which this work was carried out.

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