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Note

Quantitative gas chromatographic analysis of barbiturates and hydantoins with quaternary ammonium hydroxides

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Derivatization with quaternary ammonium hydroxides such as trimethylanilinium hydroxide (TMAH) is at present the most widely used gas chromatographic (GC) procedure for the routine determination of anti-convulsant drugs of the barbiturate and hydantoin classes, of which phenobarbital (PB) and diphenylhydantoin (DPH) are the most important examples[1–6]. However, it has been shown[4] that PB undergoes partial hydrolytic cleavage during these on-column methylation procedures with the formation of N-methyl-α-phenylbutyramide, which, in gas chromatograms, appears as the "early phenobarb" (EP) peak[4] in addition to the desired fully methylated product, N,N-dimethylphenobarbital. The fact that PB appears as two different peaks on gas chromatograms under these conditions is highly undesirable from the analytical point of view, especially as they arise from uncontrolled and simultaneously occurring competing reactions at high temperatures. Claims that measurement of one of these compounds ("EP") can be used successfully and reproducibly as a measure of the total amount of PB present[4] could not be substantiated in our laboratory and apparently also in others[6]. N,N-Dimethylphenobarbital appeared to be a better compound than "EP" to use for the determination of PB in view of the known favourable chromatographic properties of methylated barbiturates[7] and the observed "tailing" and lower detector response of "EP" during GC separation[8].

Numerous published variations of the original flash-heater methylation procedure, including a recently published hexylation procedure[9], confirm the inadequacy of the procedure in its present form. Moreover, the hexylation procedure suffers from the same basic disadvantages associated with the other procedures and which are related to the high alkalinity of the solutions injected into the flash-heater compartment of the gas chromatograph. As an alternative approach, therefore, we have studied conditions that lead to the suppression of the hydrolytic cleavage reaction while favouring the methylation process, and these we have found to be of two types. Firstly, we were able to confirm the important role played in this respect by the reagent (TMAH) concentration previously studied and reported by others[4,6], and this suggested that the pH of the solution injected into the chromatograph could be of importance in determining the nature and extent of the various pyrolytic decompositions and other reactions that occur in the injection port of the instrument. We found that the hydrolytic reaction was in fact drastically suppressed by decreasing the pH of the solution injected when buffer solutions (pH 8–10) were used for the extraction

of the drugs from the initial toluene extract. However, under these conditions, DPH (pK_a 8.3) was not completely extracted. Optimal extraction efficiency as well as satisfactory GC separation was achieved by extracting the drugs with unbuffered TMAH solutions in the usual manner followed by the addition of a suitable buffer to the extract immediately before injection into the gas chromatograph.

Under these conditions, the PB peak height was increased while the EP peak was greatly reduced or absent in the chromatograms (Figs. 1 and 2). A similar but less pronounced effect was observed when methyl iodide and dimethyl sulphoxide were added to the unbuffered TMAH extracts immediately before injecting the extracts into the gas chromatograph[8]. The presence of these two compounds presumably creates a strongly methylating environment in the gas phase, which also tends to enhance the formation of the fully methylated product. However, in practice it is unnecessary to include such a step if the correct buffer system is used.

EXPERIMENTAL

Buffer system

Solution A: to a solution of 16.0 g of citric acid and 4.0 ml of 85% orthophosphoric acid in 100 ml of water were added 7.0 g of boric acid and 340 ml of 2 *N* sodium hydroxide solution and the mixture shaken to dissolve the boric acid. The solution was finally diluted to 500 ml. Solution B was concentrated hydrochloric acid.

The buffer system was prepared by dissolving 2.5 g of sodium fluoride in 400 ml of solution A and adjusting the pH of the mixture to 9.0 (glass electrode) by the addition of solution B. The solution was then diluted to 500 ml. The "acid buffer" solution was prepared by adjusting the pH of 20 ml of this solution to 4.0 with 6 *N* hydrochloric acid immediately before use.

Gas chromatographic conditions

A Varian Aerograph 1400 gas chromatograph fitted with a glass column (length 1 m, I.D. 3.0 mm, O.D. 6.0 mm) packed with 3% OV-17 on Gas-Chrom Q (100–120 mesh) and fitted with a flame-ionization detector was used. The detector temperature was 270° and the inlet block temperature 270°. The gas flow-rates were carrier gas (nitrogen) 30 ml·min^{-1}, hydrogen 20 ml·min^{-1} and air 300 ml·min^{-1}. The temperature was programmed from 150° to 280° at 15°·min^{-1}. The attenuation was 32 and the range 10^{11}.

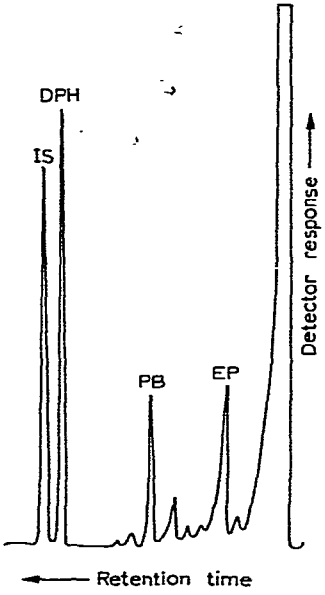
Method

The general procedure for the determination of barbiturates and diphenylhydantoin was similar to that previously described by McGee[10] and Kananen *et al.*[11] in which the primary toluene extract was vortexed with 100 µl of 0.2 *M* TMAH solution and 20 µl of water. After centrifugation, the lower, aqueous phase (approximately 50 µl) was transferred into a second nipple tube (10-ml ground-glass stoppered tube with the bottom portion drawn out) and 20 µl of "acid buffer" (freshly prepared) were added. After thorough mixing with a syringe, 1.5 µl of the mixture were injected slowly (6 sec) into the gas chromatograph. The appearance of turbity in the solution after the addition of acid buffer was ignored.

RESULTS AND DISCUSSION

Fig. 1 illustrates a gas chromatogram obtained in the analysis of a serum sample containing 15 $\mu g \cdot ml^{-1}$ of diphenylhydantoin (DPH) and 30 $\mu g \cdot ml^{-1}$ of PB using the conventional methylation procedure of McGee[10]. The result demonstrates the very prominent EP peak that may be encountered in practice in this type of analysis.

Fig. 2 illustrates a similar analysis carried out in the presence of methyl iodide and dimethyl sulphoxide (20 μl of the latter and 15 μl of the former added to 50 μl of the TMAH extract immediately before injection into the gas chromatograph). The result clearly shows the depressing effect of the methylation agents on the formation of N-methyl-α-phenylbutyramide (EP).



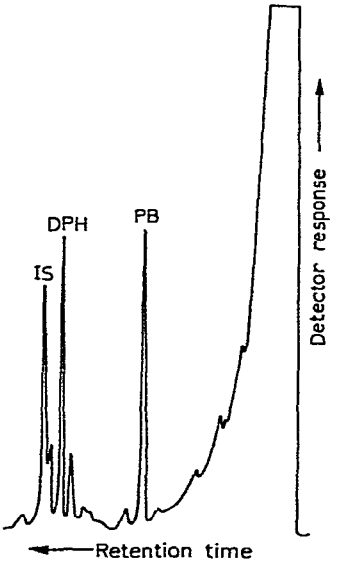


Fig. 1. Gas chromatogram obtained in the analysis of a mixture of PB (30 $\mu g \cdot ml^{-1}$) and DPH (15 $\mu g \cdot ml^{-1}$) showing "early phenobarbitone" (EP) peak.

Fig. 2. Gas chromatogram obtained in the analysis of a mixture of PB (30 $\mu g \cdot ml^{-1}$) and DPH (15 $\mu g \cdot ml^{-1}$) after the addition of methyl iodide (10 μl) and dimethyl sulphoxide (20 μl) to the TMAH extract (50 μl).

Fig. 3 shows a similar result obtained by the addition of acid buffer (20 μl) to 50 μl of TMAH extract immediately before GC analysis. The excellent peak form and separation as well as the low level of serum background interference in this chromatogram are notable.

A serum sample containing 15 $\mu g \cdot ml^{-1}$ of DPH and 30 $\mu g \cdot ml^{-1}$ of PB was analyzed ($n = 6$) and relative standard deviations of 0.79% for PB and DPH for 0.45% were found.

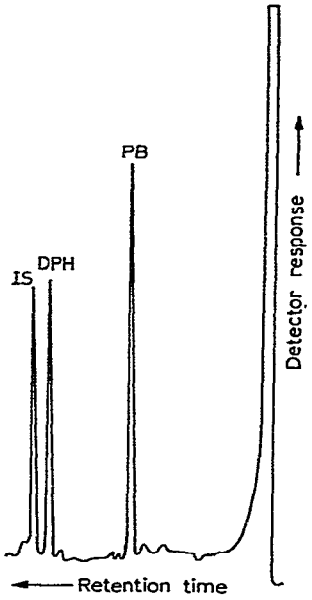


Fig. 3. Gas chromatogram obtained in the analysis of a mixture of PB (30 $\mu g \cdot ml^{-1}$) and DPH (15 $\mu g \cdot ml^{-1}$) after the addition of acid buffer (20 μl) to the TMAH extract.

This new procedure has been in use for several months in this Institute for the routine analysis of DPH and PB in patients' serum samples with excellent results and day-to-day reproducibility.

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