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Note

Probenecid, a possible interferent in the gas chromatographic determination of diphenylhydantoin

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The on-column alkylation of barbiturates and related compounds with reagents such as tetramethylammonium hydroxide (TMAH) or triethylanilinium hydroxide (TEAH) is a frequently used technique for the quantitative analysis of these compounds [1—6]. As these determinations are usually performed on sera from patients suffering from epilepsy with the object of helping the clinician to establish the optimal dose regimen, it is imperative that one should be aware of any drugs that may influence the quantitation of the antiepileptic drugs or produce a false positive result. We have found probenecid to be such a drug.

METHOD FOR THE ANALYSIS OF BARBITURATES AND DIPHENYLHYDANTOIN

Our laboratory is engaged in the routine analysis of phenobarbitone and diphenylhydantoin for an epilepsy clinic and we make use of the following method. To 1 ml serum are added secobarbitone and tolylphenylhydantoin as internal standards. The serum is acidified with 0.5 ml of 1 *M* H_3PO_4 and extracted with 3 ml of toluene by shaking for 2 min on a Whirlimixer. After centrifuging to separate the layers, the toluene phase is transferred into a conical centrifuge tube and 100 µl of 0.5 *M* TEAH solution in ethanol and 20 µl of water are added. After vigorous shaking for 2 min on a Whirlimixer, the tube is centrifuged and 4 µl of the bottom layer are injected during 7 sec into the gas chromatograph.

The ratios of the peak areas of N-ethylphenobarbitone and N-ethyldiphenylhydantoin to those of the two internal standards are used to quantitate the drugs.

EXPERIMENTAL

Conventional gas chromatography

A Hewlett-Packard 5700A gas chromatograph equipped with a flame-ionization detector was used. The peak areas were recorded on a Hewlett-Packard 3380A reporting integrator. Conditions: column length, 180 cm; temperatures: column, 150°, programmed at 8°/min to 270°; injector port and detector, 300°; stationary phase, 3% SE-30 on Chromosorb W (Supelco, Bellefonte, Pa., U.S.A.); carrier gas, nitrogen, at a flow-rate of 60 ml/min.

Gas chromatography–mass spectrometry (GC–MS)

A Varian CH5 mass spectrometer coupled to a Varian 2700 gas chromatograph and a Varian SS 100 computer was used for GC–MS analysis. A double-stage Watson-Biemann separator was used for enrichment of the eluting sample. Conditions: column length, 120 cm; temperatures: column, 120°, programmed at 8°/min to 270°; injector port, 270°; detector and separator, 280°; stationary phase, 3% Dexsil 300 on Supelcon AW DMCS, 80–100 mesh (Supelco, Bellefonte, Pa., U.S.A.); carrier gas, helium, at a flow-rate of 30 ml/min.

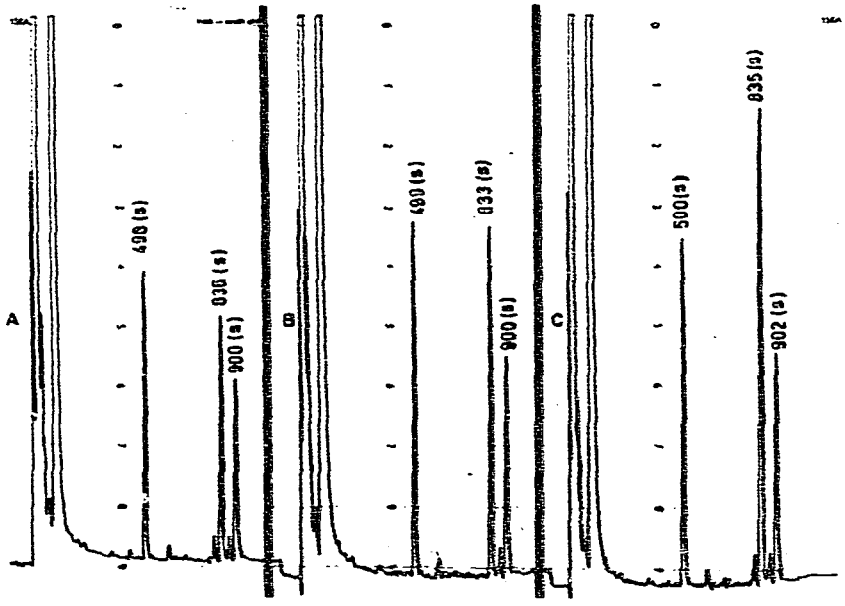

Fig. 1. A, Gas chromatogram of N-ethyldiphenylhydantoin (836 S), N-ethylsecobarbitone (498 S) and N-ethyltolylphenylhydantoin (900 S). B, Gas chromatogram of probenecid ethyl ester (833 S), N-ethylsecobarbitone (499 S) and N-ethyltolylphenylhydantoin (900 S). C, Gas chromatogram of a mixture of N-ethyldiphenylhydantoin and probenecid ethyl ester (835 S), N-ethylsecobarbitone (500 S) and N-ethyltolylphenylhydantoin (902 S). Column, 180 cm, glass, packed with 3% SE-30 on Chromosorb W, 80–100 mesh; column temperature, 150°, programmed at 8°/min to 270°; injector and detector temperatures, 300°; carrier gas (nitrogen) flow-rate, 60 ml/min; flame-ionization detection.

RESULTS AND DISCUSSION

During the investigation of a case of attempted suicide, we identified diphenylhydantoin as one of the drugs involved by using the method described above. As the gas chromatogram also showed peaks that could not be attributed to the more commonly used barbiturates, we decided to submit the sample to a GC—MS investigation. A 2-μl volume of the TEAH extract, obtained by the method described above, was injected and mass spectra were obtained during the entire analysis and the computer used to reconstruct the gas chromatogram. An investigation of the spectra of the eluates disclosed that the product identified previously as diphenylhydantoin was in fact some other substance, as its mass spectrum differed from that expected from N-ethyldiphenylhydantoin.

A computer-assisted library search identified the unknown product as probenecid ethyl ester, which was confirmed by subjecting an authentic sample of probenecid to the procedure described above, followed by GC—MS analysis.

A further investigation revealed that N-ethyldiphenylhydantoin and probenecid ethyl ester could not be separated gas chromatographically (Fig. 1) when 3% SE-30, 3% SP-2100, 3% Dexsil 300 or 3% OV-17 stationary phases were used.

We found that the detector responses for probenecid ethyl ester and N-ethyldiphenylhydantoin were virtually identical and also that probenecid taken therapeutically procudes blood levels equivalent to those expected to be found for diphenylhydantoin in therapeutic doses.

Probenecid is, therefore, a potential source of error when the method of on-column alkylation of diphenylhydantoin is applied, a fact which needs to be heeded when determining the latter drug for therapeutic or toxicological purposes.

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