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CHEMICAL IONIZATION MASS SPECTROMETRY FOR RAPID ASSAY OF DRUGS IN SERUM

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SUMMARY

A rapid procedure has been devised for analyzing anti-epileptic drugs in serum by chemical ionization mass spectrometry. An internal standard, 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH), is added to serum diluted in buffer at pH 12. The mixture is washed with diethyl ether to remove neutral lipids, acidified, extracted with chloroform and the chloroform extract evaporated to dryness. The residue is then dissolved in methanol and an aliquot, corresponding to approximately 2% of the original mixture, is deposited in the glass capillary sample cup of the solid probe inlet of the mass spectrometer. The sample is then volatilized by heat into the ion source of the mass spectrometer, where it reacts with ionized methane reagent gas.

As the temperature of the probe is increased, quasimolecular ion peaks of the protonated anticonvulsants appear, rise and fall on the oscilloscope tracing, indicating similar but not identical rates of volatilization. We recorded these peaks photographically by opening the shutter of the oscilloscope camera for the entire heating cycle. The concentrations of the anticonvulsants were estimated from the ratio of the height of the peaks of the drug to that of the internal standard on the photograph. The peak-height ratios were proportional to concentration within, above and below the therapeutic range. Other drugs, including barbiturates, carbamazepine, nicotine and caffeine, were readily identified when present. With one solid probe inlet, an assay could be performed every 2 min.

INTRODUCTION

Compared with the complex fragmentation patterns that result from electron impact ionization, the mass spectra produced by chemical ionization (CI) contain very few peaks. For each compound there is generally a characteristic "quasi-molecular" ion peak, corresponding to the molecular weight of the compound plus one, which predominates, and a lower yield of a few fragment ions. Identification of compounds by their molecular weight is thus simplified, although the high specificity of the identification given by electron impact fragmentation patterns is not available. Compounds that have different molecular weights generally yield spectra with minimal overlap. CI thus makes more feasible the analysis of mixtures that contain only

a few compounds, or only a few compounds that are present in appreciably higher concentration than other compounds, by introducing all of the compounds in the mixture into the mass spectrometer at one time.

The detection of sedative drugs in blood serum is this kind of analytical problem. Milne *et al.*[1] demonstrated the use of CI for identifying drugs in the serum of patients who had attempted suicide. Their report prompted the study described here, in which we devised a similar technique for the quantitative assay of anticonvulsant drugs in serum. Knowledge of the concentrations of these drugs is considered helpful in the management of patients with epilepsy. These analyses have become popular applications of gas–liquid chromatography (GLC) in the clinical laboratory, but the time required for each assay often limits the rapidity with which data can be reported and, therefore, the clinical utility of the information. Our objective was to devise a means for assaying several of these drugs simultaneously with a rapidity comparable to that with which other, more simple, clinical assays are now available.

EXPERIMENTAL

The mass spectrometer used was a Finnigan Model 3200 F GC–MS (Finnigan Corp., Sunnyvale, Calif., U.S.A.) operating in the chemical ionization mode at 1.0 torr with methane as the reagent gas.

Stock solutions of 5,5-diphenylhydantoin (DPH) (Aldrich, Milwaukee, Wisc., U.S.A.), 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH) (Aldrich), primidone (Pr) (Ayerst Labs., New York, N.Y., U.S.A.) and phenobarbital (PB) (Mallinckrodt, St. Louis, Mo., U.S.A.) in methanol were prepared to contain 200 μg/ml in each instance.

Standard solutions containing 0.5 μg/ml, also in methanol, for calibrating the response of the spectrometer, were prepared by dilution.

Procedure

Diphenylhydantoin, phenobarbital and primidone were extracted from blood by a modification of the methods described by Chang and Glazko[2] and Evenson *et al.*[3]. To 1.0 ml of serum were added 65 μl of methanol containing 26 μg of the internal standard, MPPH. The solution was made basic with 0.3 ml of 0.1 *M* phosphate buffer (pH 12) and was then washed with 2-ml aliquots of diethyl ether to remove neutral lipids; the combined ethereal washings were back-extracted with 3 ml of 0.1 *M* phosphate buffer (pH 12). The basic aqueous fractions were then combined, acidified with 1 ml of 10% hydrochloric acid and extracted with two 5-ml aliquots of chloroform. The chloroform extracts were then evaporated to dryness and the residue was taken up in 65 μl of methanol. A 1-μl volume of the methanolic solution was transferred into a glass capillary cup contained in the heater coil of the solid probe inlet of the mass spectrometer. The sample was volatilized into the ion source of the mass spectrometer by applying a sufficient voltage to the heater coil of the inlet to increase the temperature of the capillary from 40° to 200° in approximately 40 sec. The spectrometer was set to scan continuously from 155 to 340 a.m.u. To record the evolution of the compounds, the shutter of the oscilloscope camera was opened for the entire heating period, producing peaks comparable in anayltical value to the

peak heights on GLC records without the corresponding peak-width measure of the time rate of rise and fall of the peaks. The concentrations of the anticonvulsants were then estimated from the heights of the peaks of the drug and the internal standard on the photograph with the aid of a calibration graph.

The mass spectrometer was set to scan the mass range 155–340 a.m.u. The sensitivity was set at 10^{-7} A/V and the scope sensitivity was set at 2–5 V per division, depending on the concentration of a particular sample.

RESULTS

Washing the solution in serum–basic buffer with diethyl ether prior to extraction removed most of the fatty acids and their esters, thus reducing the interfering background.

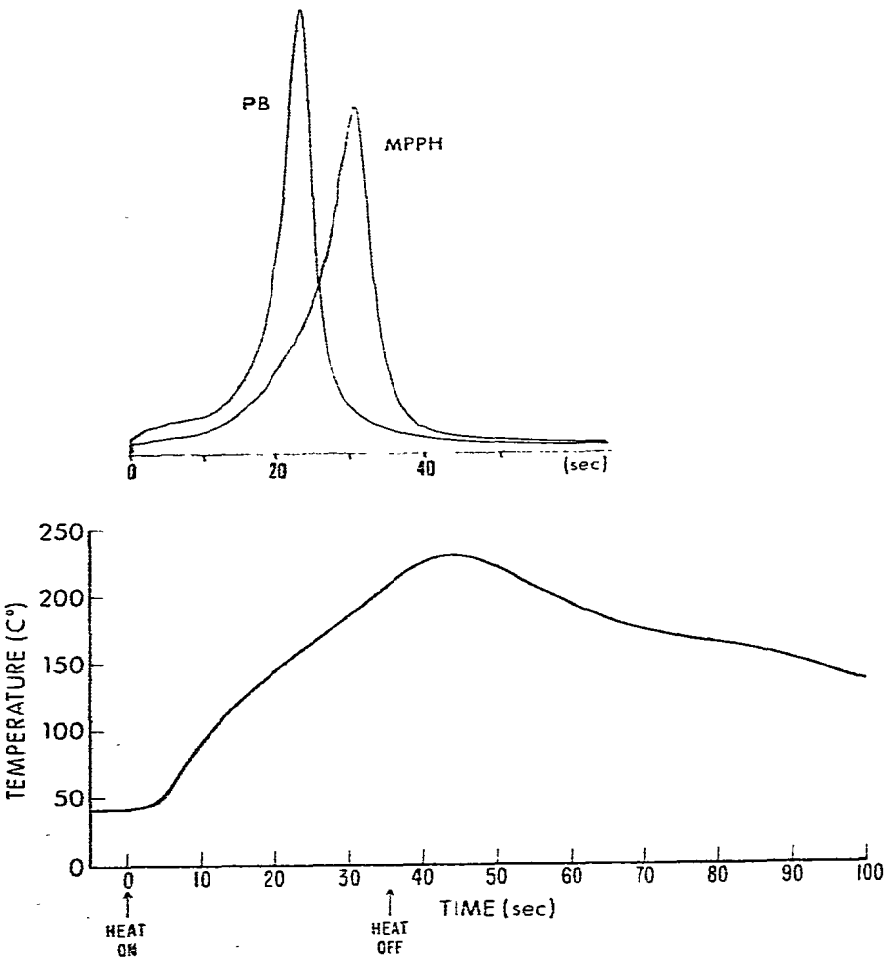


Fig. 1. Evaporation profiles (total ion current) of phenobarbital (PB) and 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH) as heat is applied to the sample capillary cup of the solid probe.

Diphenylhydantoin and phenobarbital added to human plasma were quantitatively recovered. This result had previously been confirmed by conventional GLC assays of these extracts. Only 60% of the primidone was recovered, possibly because of its poor solubility and its extremely weak acidic character[4].

The voltage applied to the heating coil increased the temperature from 40° to 200° in 40 sec. Compounds volatilize at different rates, but the volatilization of phenobarbital, the lowest boiling compound, and MPPH were both complete at the end of this period (Fig. 1).

The quasimolecular ion peaks corresponding to the molecular weight of the protonated drugs $(MH)^+$ appear at *m/e* 219 (primidone), *m/e* 233 (phenobarbital), *m/e* 253 (diphenylhydantoin) and *m/e* 267 (MPPH) (Fig. 2). The quasimolecular ion for each of the drugs is the base peak and accounts for more than 50% of the total ion current (Fig. 3).

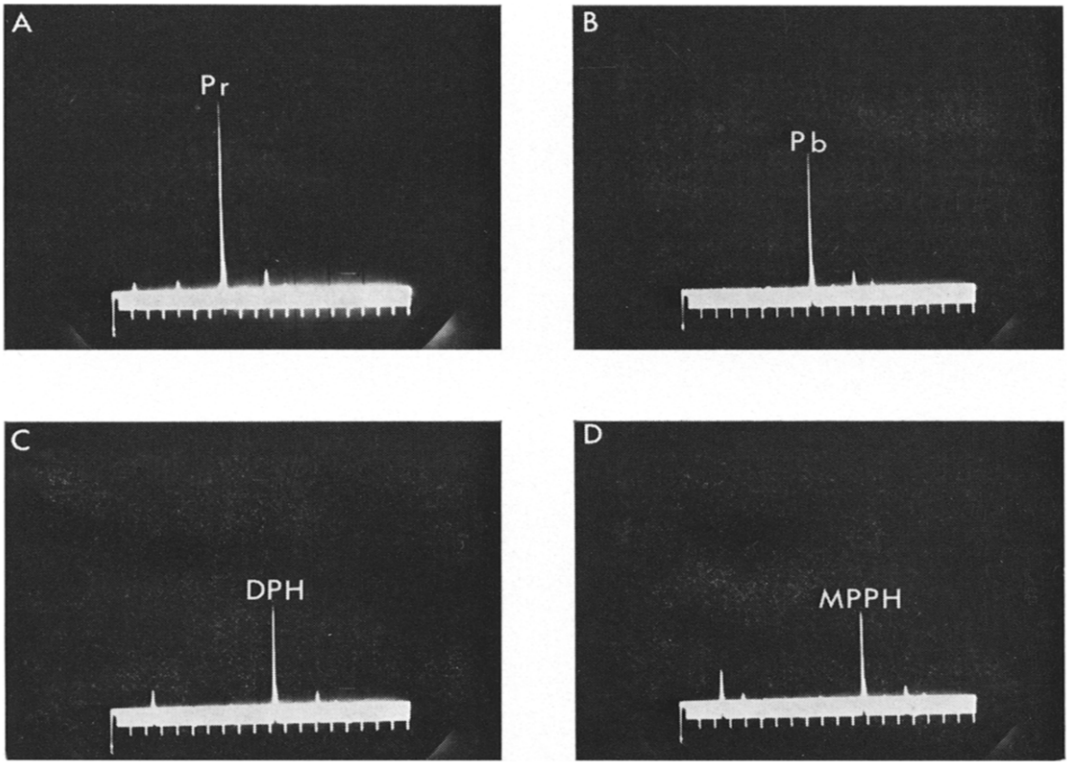


Fig. 2. Chemical ionization mass spectra of (A) primidone, (B) phenobarbital, (C) diphenylhydantoin and (D) the internal standard. The scanning range shown is 155–340 a.m.u.

In addition to those listed, fragment ions characteristic of the methane reagent gas appear at M + 15 $(MCH_3)^+$, M + 29 $(MC_2H_5)^+$ and M + 41 $(MC_3H_5)^+$.

When all four compounds are introduced into the mass spectrometer simultaneously, the four quasimolecular ions corresponding to the molecular weight of

CI FRAGMENTATION PATTERNS

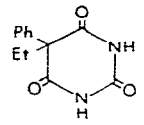
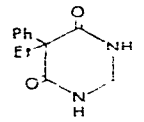
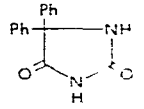
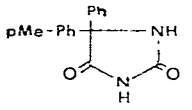
COMPOUND	m/e	RELATIVE INTENSITIES (%)	
a) Phenobarbital, MW 232	233	100	(MH^+)
	204	< 2	(Loss of Et)
b) Primidone, MW 218	219	100	(MH^+)
	190	8	(Loss of Et)
	163	10	(Loss of Et, CH_2NH)
c) Diphenylhydantoin, MW 252	253	100	(MH^+)
	225	3	(Loss of CO)
	175	15	(Loss of Ph)
d) MPPH, MW 266	267	100	(MH^+)
	189	2	(Loss of Ph)
	175	15	(Loss of CH_2Ph)

Fig. 3. Relative abundance of ions in the CI spectra of the anticonvulsants and the internal standard.

the protonated compounds are easily distinguished. None of the secondary ion-fragments interferes (Fig. 4).

The ratios of the peak heights of DPH, PB and Pr to that of the internal standard were directly proportional to the concentration of drugs added in the therapeutic range of interest (3–30 μg/ml in plasma) (Fig. 5).

Other acidic compounds present in the sample could be identified (Figs. 6–9). We noted no interference by any of these compounds with the quantification of the anticonvulsants.

Sensitivity

The sensitivity was such that $5 \cdot 10^{-9}$ g of diphenylhydantoin and phenobarbital produced peaks 3× and 5× the noise level respectively; $1 \cdot 10^{-9}$ g of primidone gave a signal-to-noise ratio of 4.

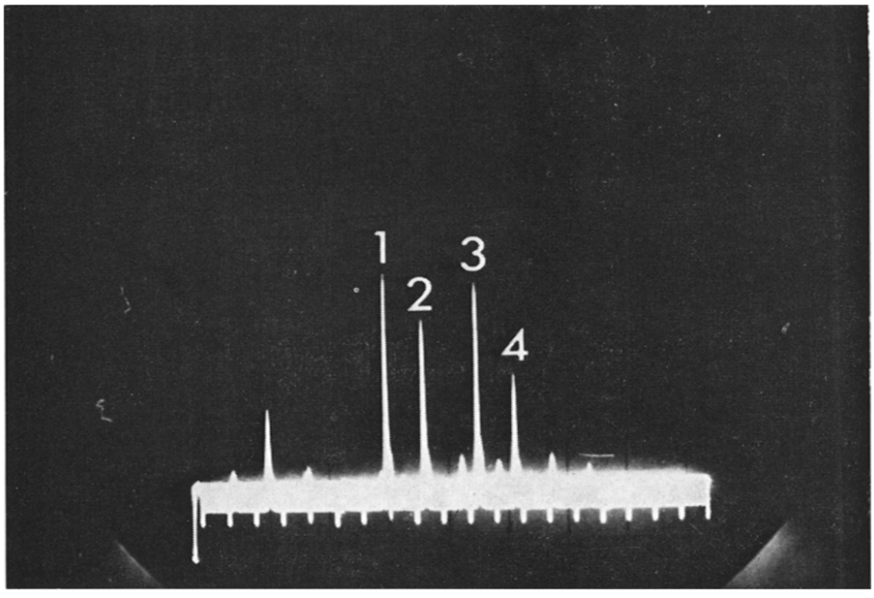


Fig. 4. Chemical ionization spectra obtained when all four compounds are introduced together. Quasimolecular ion peaks: (1) primidone, *m/e* 219; (2) phenobarbital, *m/e* 233; (3) DPH, *m/e* 253; (4) MPPH, *m/e* 267.

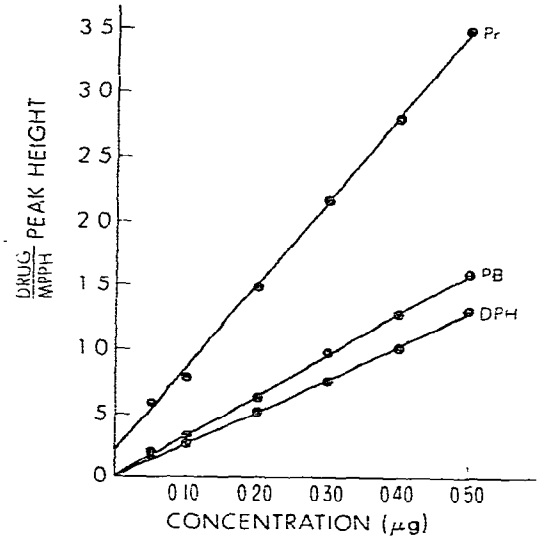


Fig. 5. Proportionality between the ratios of the heights of the quasimolecular peaks and the concentrations of each drug to that of the internal standard.

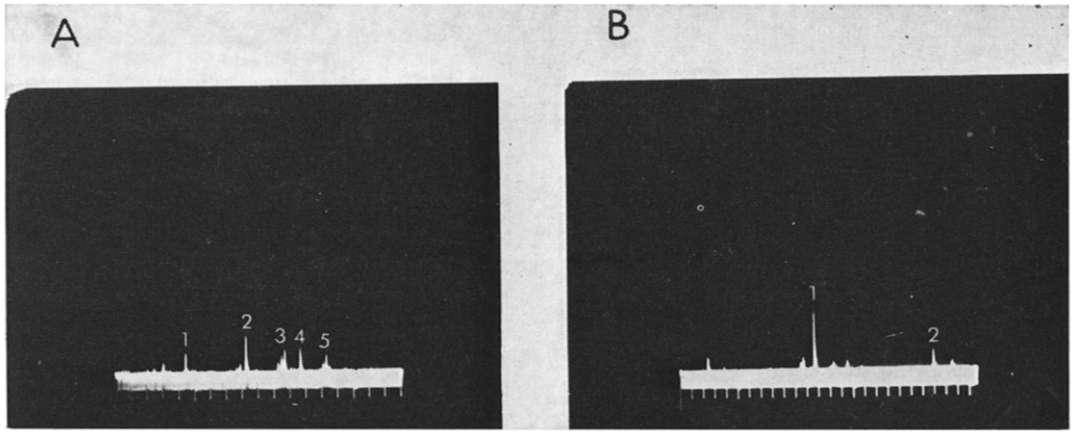


Fig. 6. Chemical ionization mass spectra of extracts of two sera submitted to the clinical laboratory for assay for diphenylhydantoin. Quasimolecular peaks identified: (A), (1) caffeine; (2) phenobarbital; (3) palmitic acid; (4) MPPH; (5) stearic acid. (B), (1) MPPH; (2) cholesterol.

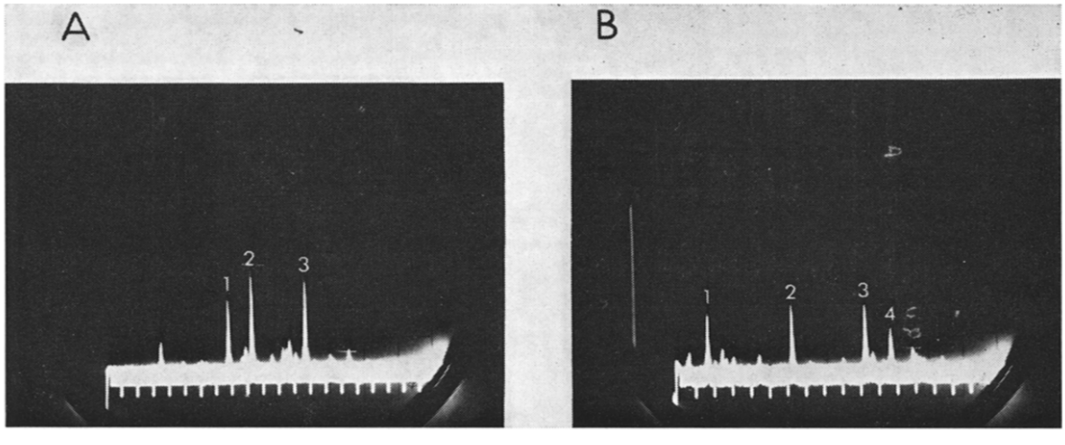


Fig. 7. Chemical ionization mass spectra of extracts of two sera submitted to the clinical laboratory for assay for diphenylhydantoin. Quasimolecular peaks identified: (A), (1) glutethimide; (2) phenobarbital; (3) MPPH. (B), (1) phthalate plasticizer; (2) butabarbital; (3) DPH; (4) MPPH.

DISCUSSION

Chemical ionization offers the possiblity of assaying simple mixtures without the preliminary separation by GLC that is so helpful in GC–MS with electron impact ionization. Milne *et al.*[1] suggested its use for detecting and identifying drugs taken in overdose amounts, in which the concentration of the compound of interest is generally appreciably greater than those of other compounds which might otherwise interfere.

The technique offers very rapid, simultaneous analysis with less than the ultimate in positive identification. We have not noted any significant interferences by compounds other than those we were attempting to assay.

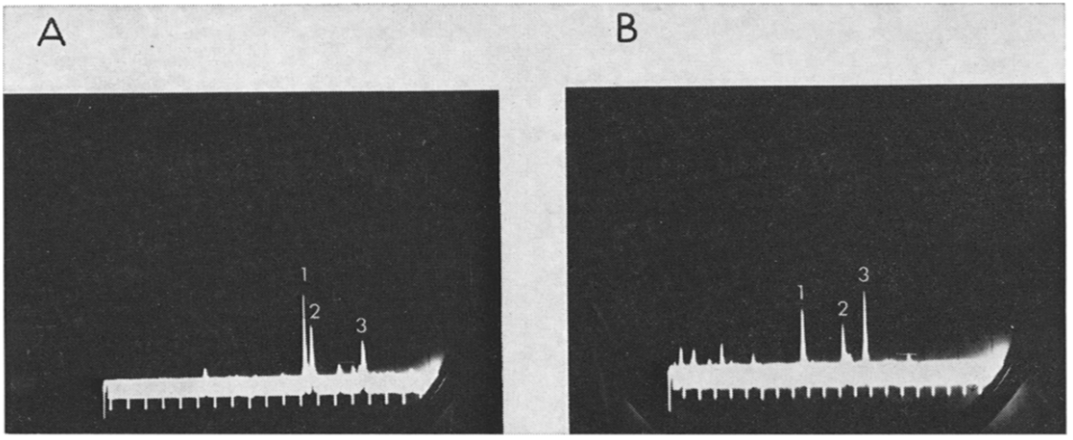


Fig. 8. Chemical ionization mass spectra of extracts of two sera submitted to the clinical laboratory for assay for diphenylhydantoin. Quasimolecular peaks identified: (A), (1) phenobarbital; (2) carbamazepine; (3) MPPH. (B) (1) amobarbital; (2) DPH; (3) MPPH.

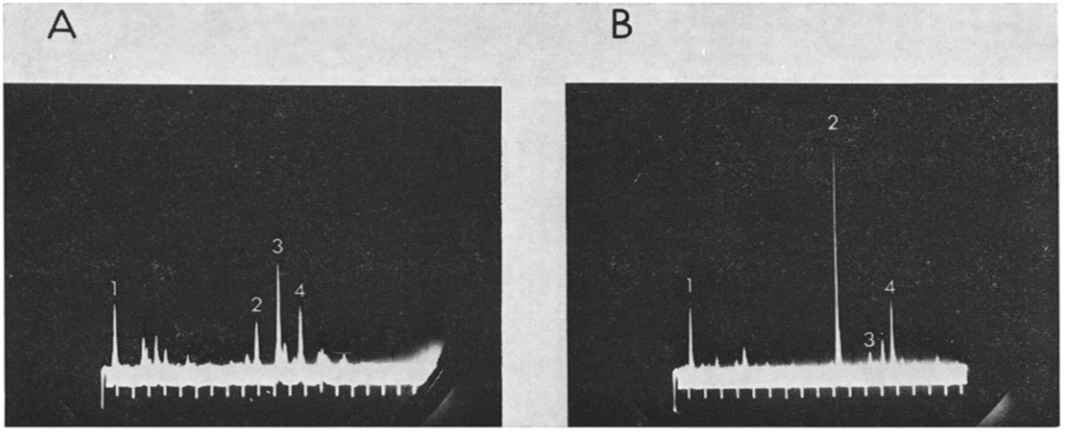


Fig. 9. Chemical ionization mass spectra of extracts of two sera submitted to the clinical laboratory for assay for diphenylhydantoin. Quasimolecular peaks identified: (1) (A), phthalate plasticizer; (2) secobarbital; (3) DPH; (4) MPPH. (B) (1) ethosuximide; (2) phenobarbital; (3) DPH; (4).MPPH.

Recording the spectra can be accomplished in several ways: (a) continuous sweep and recording of the complete mass spectrum in a computer memory; (b) photographic recording of the images displayed on the oscilloscope; and (c) multiple ion detection, *i.e.*, time sharing the detector among the expected quasimolecular ion peaks. Although the first two approaches offer less sensitivity than the third, they permit the detection of unexpected compounds. We chose photography only to study the feasibility of the approach we describe. We were surprised that the quantification was so good. The use of a computer should offer significant advantages.

Other acidic compounds present in the serum could be identified and quantified provided that a suitable calibration graph is obtained. The assay time of 2 min per sample could be shortened by the use of more than one solid probe inlet.

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