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SPECIFIC GAS CHROMATOGRAPHIC DETERMINATION OF PHENOTHIAZINES AND BARBITURATE TRANQUILLIZERS WITH THE NITROGEN FLAME IONIZATION DETECTOR

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SUMMARY

The advantages of the use of the nitrogen flame ionization detector (N-FID), particularly for the analysis of phenothiazines, barbiturate tranquillizers and other nitrogen-containing organic drugs, are discussed and compared with the electron capture detector and flame ionization detector. The sensitivity, selectivity, and the response characteristics of the N-FID to organic nitrogen are independent of the chemical structure of a particular drug, so that the method requires biological sample volumes of only about 100 μl, simplifies extraction procedures and increases the detection specificity and accuracy of the analytical data.

INTRODUCTION

Drugs with psychosedative and depressant activity are for the most part phenothiazines and barbiturates. For the analysis of phenothiazines, barbiturates and their metabolites gas chromatography (GC) represents the most practical means to date. The normal methods of analysis of these drugs and their metabolites in biological media are non-specific, insensitive or time-consuming in the clean-up and concentration steps.

Nitrogen-containing organic drugs can more satisfactorily be detected and quantitated by the nitrogen flame ionization detector (N-FID) than by the electron capture detector (ECD) or by the flame ionization detector (FID). The ability of drugs to capture electrons is very dependent upon the type and position of atomic or group substituents, while the N-FID responds proportionally and selectively to the percentage nitrogen content of the drugs and their metabolites; this response is independent of the chemical structure, thus allowing the calculation in advance of the signal to be expected. The FID is a comparatively non-selective general-purpose detector without any detection property specifically related to the structure of organic compounds. The sensitivity of the N-FID, especially with the gate electrode modification[1–3], is far higher than the sensitivity of the ECD or of the FID, allowing use of small biological sample volumes of 100 μl for the determination of drugs at therapeutic levels.

EXPERIMENTAL

Analysis of phenothiazine derivatives

The chromatograms in Fig. 1a and b show the powerful GC separation and detection of phenothiazine derivatives and related drugs. The differing detector range settings indicate the hundred-fold ionization efficiency of the N-FID over the FID. The lowest detection limit in the application of the N-FID to the analysis of phenothiazines is about five hundred-fold over the FID. Due to the selectivity of up to a factor of 10^4 between organic nitrogen and carbon the N-FID does not detect any bleeding of the liquid phase even at elevated column temperatures. Bleeding is often the limiting factor in determining the lowest detection limits with the FID.

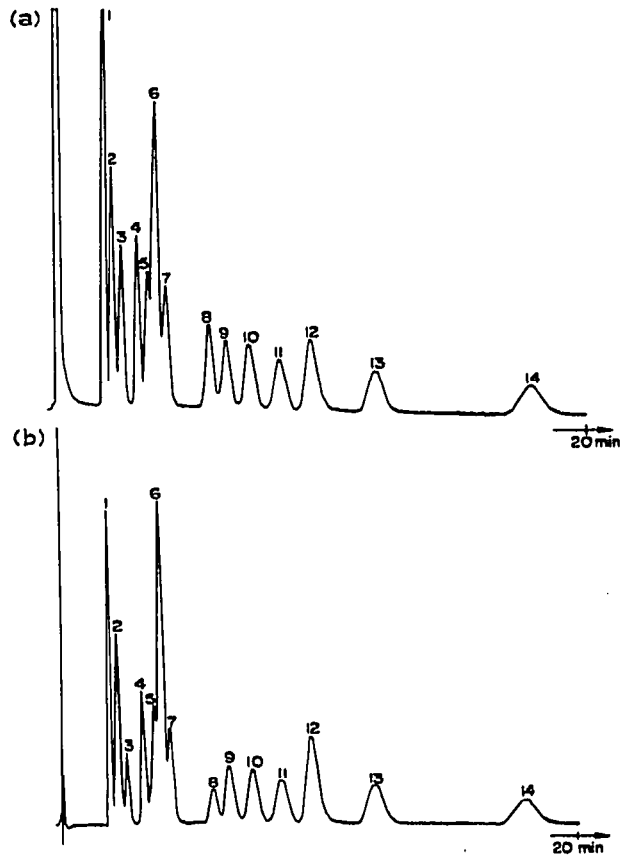

Fig. 1. Detection sensitivity difference between the FID (a) and the N-FID (b) in the separation of phenothiazines and related drugs. Instrument, Hewlett-Packard Model 5751 G gas chromatograph with an FID and a Model 15161 B N-FID; column, 6 ft × 2 mm I.D., glass; liquid phase, 4% OV-225; support, Chromosorb W AW-DMCS, 80–100 mesh; column temperature, 230°; range, 10^3 (FID) and 10^5 (N-FID); attenuation, 2. 1 = Amitriptyline; 2 = imipramine; 3 = doxepine; 4 = trimeprazine; 5 = medazepam; 6 = methaqualone; 7 = chlorimipramine; 8 = chlorprotixene; 9 = chlorpromazine; 10 = methotrimeprazine; 11 = chlorproethazine; 12 = dibenzepine; 13 = diazepam; 14 = acepromazine (approx. 1 μg of each).

The nitrogen proportional signal characteristics of the N-FID are demonstrated by peaks 8 (chlorprotixene) and 9 (chlorpromazine) of the chromatograms in Figs. 1a and b. The nitrogen content of chlorprotixene is about half that of chlorpromazine, resulting in the expected signal ratio N-FID/FID of about 0.5 between peaks 8 and 9.

The relative signal strengths of the N-FID and the FID for the single constituents of the chromatograms demonstrate the N-FID to be a detector generally applicable to the analysis of nitrogen-containing drugs without the disadvantage of a wide-spread lowest detection limit for drugs of similar or different chemical structures or for various types and positions of atomic or group substituents.

The main advantage of the N-FID selectivity in the application of the detector is a more uniform clean-up procedure, which is in many cases a single-step extraction[1,4]. Concomitant extracted material is largely suppressed by the N-FID, which in this way acts as a separating device between concomitant material and the compounds under study.

The sensitivity of the ECD to different phenothiazine drugs was found to range by a factor of about a thousand[5]. This extreme variation in the lowest detection limit makes the ECD valuable for only a limited number of these drugs in biological media and special clean-up procedures and large sample volumes are necessary in order to fit the sample concentration requirements with respect to the limited linear range and sensitivity of the detector.

Selective isolation of single phenothiazines and phenothiazine metabolites from urine[6] and plasma[7] specimens, isolation using ion-exchange resins[8], as well as methods for phenothiazine group extractions from urine, blood, plasma and tissue homogenates[7,9,10] have been reported.

Only for compounds having a chlorine atom at the 2-position of the phenothiazine nucleus does the electron absorption property provide a sensitivity ten- to fifty-fold higher than that of the FID, thereby permitting 2-chlorophenothiazine, prochlorperazine, chlorpromazine, and desmethylchlorpromazine to be determined at low therapeutic concentration levels in relatively small biological samples[5]. The electron affinity of the phenothiazines decreases rapidly in the order $Cl > CF_3 > SCH_3 > H > OCH_3$ for the substituent in the 2-position.

Analysis of barbiturate tranquillizers

Because of the tendency to mix two or more different barbiturates in tablets and the low specificity of chemical methods in distinguishing between the small structural differences, GC together with detection systems which are largely structure independent in sensitivity and selectivity are required both for the routine analysis of toxicological emergency cases and in metabolic studies of therapeutic doses.

Barbiturates tend to tail and adsorb on GC diatomaceous supports, thus limiting sensitivity and accuracy in their analysis. Derivative formation of the acidic barbiturates is therefore required for injected sample quantities below 0.1–1 μg. Reaction GC[11–13], using tetramethylammonium hydroxide or trimethylphenylammonium hydroxide solutions of the acidic barbiturates or reaction with diazomethane or dimethyl sulphate followed by the silylation of the hydroxyl groups of metabolites are the most common and efficient derivative formation procedures. A new alkylation technique for barbiturates extracted from biological fluids has also been recently reported[14].

The need to derivatize the acidic barbiturates is eliminated by using Haloport F instead of diatomaceous materials as the support for the liquid phase. Accurate determinations of thiopental and nembutal in foetal blood at very low nanogram quantities have been demonstrated using Haloport F[1].

The chromatogram in Fig. 2 shows the relative peak areas of four barbiturates with structurally differing substituents in position 5. The N-FID response (peak area) is independent of the barbiturate substituent structure and is proportional to the nitrogen equivalent, while the ECD response is widely structure dependent.

The ability of a particular barbiturate to capture electrons depends upon the type of substituent at position 5 of the barbiturate nucleus. The ECD sensitivity decreases in the order phenyl > allyl > vinyl > saturated hydrocarbon substituent. Mephobarbital (1-methyl-5-ethyl-5-phenylbarbituric acid) could be detected with ten-times higher sensitivity by the ECD compared with the FID[15].

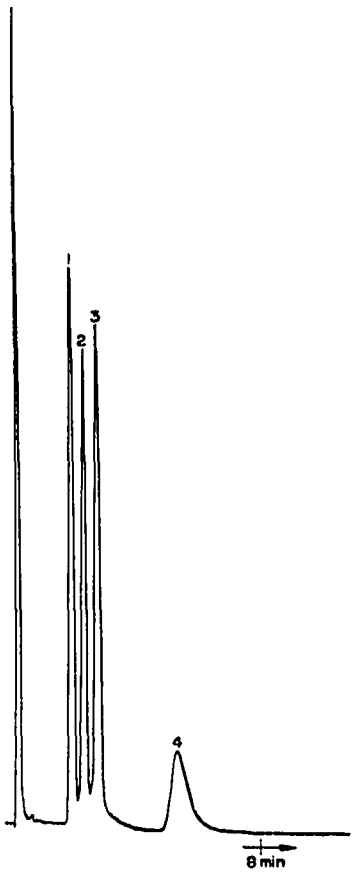

Fig. 2. Quantitative response of the N-FID to barbiturate tranquillizers as a function of the nitrogen content. Instrument, Hewlett-Packard Model 5751 G gas chromatograph with a Model 15161 B N-FID; column, 2 ft. × 2 mm I.D., glass; liquid phase, 2% Carbowax 20M; support, Haloport F, 40–60 mesh; column temperature, 200–260° at 60°/min; range, 10^2; attenuation, 32. 1 = 5,5-Diethylbarbituric acid; 2 = 5-ethyl-5-butylbarbituric acid; 3 = 5-allyl-5-(1-methylbutyl)barbituric acid; 4 = 5-ethyl-5-phenylbarbituric acid (approx. 50 ng of each).

CONCLUSION

The main advantages of the use of the N-FID in the analysis of phenothiazines and barbiturate tranquillizers by gas phase chromatography are: minimum sample preparation; the lowest detection limit is independent of chemical structure; detection sensitivity in the ppm to ppb range, even for sample volumes as small as 100 μl; high detection specificity; and accuracy of the analytical results.

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