Rapid Qualitative Identification of Barbiturates by Mass Spectrometry

JAMES D. McCHESNEY▲, DEANNA K. BEAL, and RICHARD M. FOX

Abstract □ A rapid method of qualitative identification of barbituric acids, which does not require prior isolation of the barbiturate from the pharmaceutical dosage form, was developed utilizing mass spectrometry. This is achieved by the introduction of a portion of the powdered preparation directly into the mass spectrometer *via* the direct inlet system for solid samples and the fractional sublimation of the barbiturate by slowly increasing the solid inlet system temperature. The method is equally applicable to preparations of the sodium salts of the barbiturates as to the free acids.

Keyphrases □ Barbiturates, rapid identification—mass spectroscopy □ Barbituric acids—rapid identification by mass spectroscopy □ Mass spectroscopy—rapid identification of barbiturates

Many methods have been developed to determine the presence of barbituric acid derivatives in drug preparations, urine samples, and blood (1). Published analytical tests and assay procedures include the use of: extractions; spot tests (specific color reactions); thin-layer, paper, or gas chromatography; and IR and UV spectroscopy. Bogan and Smith (2) reviewed investigations of barbiturate poisonings in which these methods were discussed.

In view of the many deliberate and accidental deaths resulting daily from barbiturate poisoning, speed and ease of analysis are of major importance in any analytical method for barbiturate identification. However, demonstration or recognition that the clinical symptoms are due to barbiturate intoxication is not sufficient, since several different barbiturates having somewhat differing pharmacological effects are commonly prescribed. The specific agent must be identified so that correct treatment measures can be initiated.

Historically, mass spectrometry has occupied a position of importance in the petroleum industry in the solution of analytical problems associated with complex mixtures. The ability of mass spectrometry to supply answers to those complex problems prompted the investigation of its potential for rapid, specific, direct analysis of drug formulations containing various barbiturate derivatives.

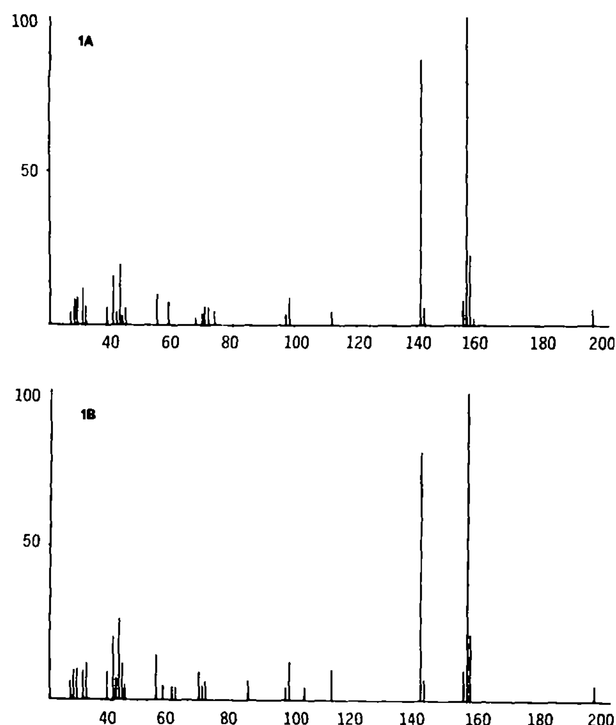


Figure 1—*Comparison of mass spectra of purified pentobarbital and a formulation of sodium pentobarbital. The spectra are essentially identical. Key: 1A, pentobarbital, 25°; and 1B, sodium pentobarbital, 30 mg., 50°.*

EXPERIMENTAL

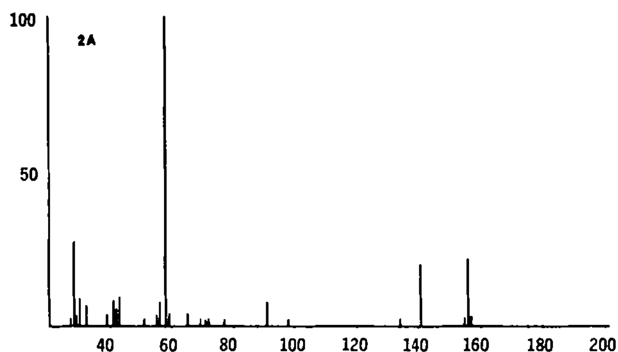
Materials—The standard barbiturates utilized in this study were purchased from commercial sources. These barbiturates were determined by chromatography to be homogeneous and to have reported melting points and correct elemental analyses. Pharmaceutical preparations were obtained as gifts from several drug houses[1]. These preparations included phenobarbital, pentobarbital, secobarbital, amobarbital, and talbutal, either alone in formulation or together with other medicinals.

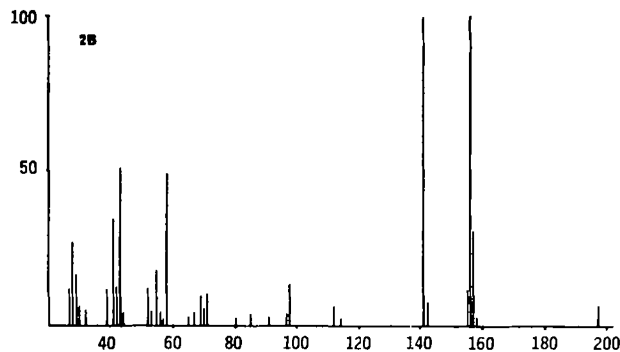
Sample Preparations—Pure standards of the free barbituric acids, including phenobarbital, butabarbital, amobarbital, hexobarbital, allobarbital, 5-allyl-5-isopropylbarbituric acid, and 5-allyl-5-phenylbarbituric acid, were run for comparison.

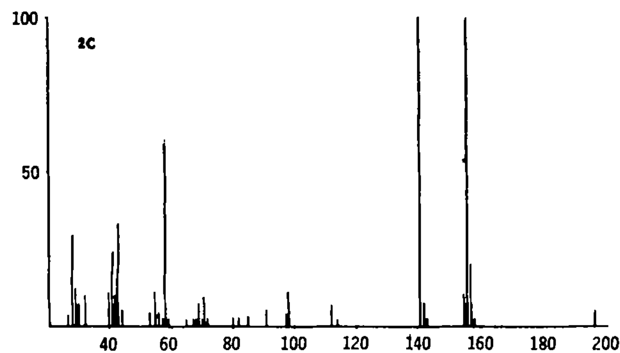
Pharmaceutical dosage forms were powdered and used directly for analysis. In no case was it necessary to modify the formulation other than by powdering the sample. For comparison the barbiturates were isolated from the formulation by the method of Coutts and Locock (5). A portion (5–10 mg.) of the powdered formulation was dissolved or suspended in water (2 ml.), concentrated hydrochloric acid (0.1 ml.) was added, and the whole was extracted with ether (2 × 5 ml.). The combined ether extracts were extracted twice with saturated sodium bicarbonate solution (2 × 2 ml., discarded), washed with water (2 ml.), and dried (Na_2SO_4). The ether solution was evaporated to dryness, and the residue was used for mass spectral determination. Homogeneity of the barbiturate was checked by TLC.

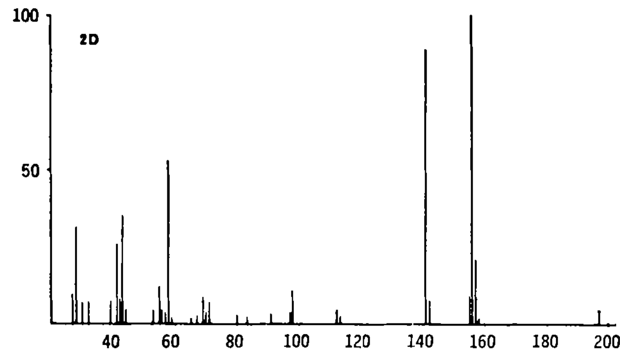
Equipment—Mass spectra were measured at low resolving power with a CH-5 single-focusing mass spectrometer. The samples were admitted to the source *via* a direct insertion probe whose temperature could be precisely regulated between 10 and 500°. It was determined that slow increases in the temperature of the probe to 150° resulted in volatilization of the barbituric acids, even when formulated as sodium salts. The electron impact source was adjusted to

[1] Abbott Laboratories, North Chicago, Ill. (Nembu-donna ½, Nembutal Na 30 mg., Nembugesic, Desbutal); Ciba Pharmaceutical Co., Summit, N. J. (Trasentine with phenobarbital); Lederle Laboratories, Pearl River, N. Y. (Pathilon with phenobarbital); Eli Lilly and Co., Indianapolis, Ind. (Tuinal 100 mg.); Searle and Co., Chicago, Ill. (Pro-Banthine with phenobarbital); Smith Kline & French Laboratories, Philadelphia, Pa. (Daprisal); Warner-Chilcott Laboratories, Morris Plains, N. J. (Peritrate 20 mg. with phenobarbital, Peritrate SA with phenobarbital, Tedral SA with phenobarbital, Tedral with phenobarbital, Tedral 25 with phenobarbital, Tedral Anti H with phenobarbital); and Winthrop Laboratories, New York, N. Y. (Lotusate).









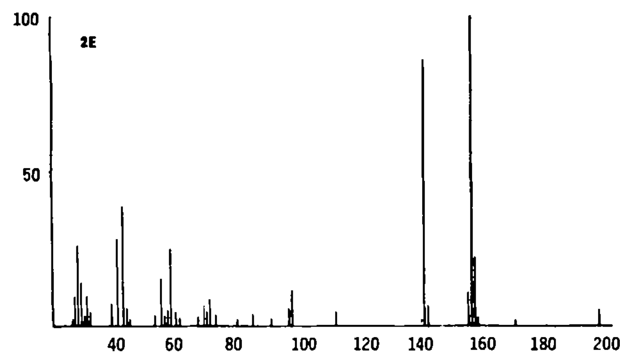


Figure 2—*Determination of pentobarbital in a combination formulation (Desbutal 10). The presence of the barbiturate is clearly shown by running the sample at several temperatures (at the higher temperatures the spectrum becomes very similar to spectra of pentobarbital alone). Key: 2A, 25°; 2B, 50°; 2C, 75°; 2D, 100°; and 2E, 150°.*

an electron beam energy of 70 ev. and an accelerating voltage of 3000 v. Line spectra were drawn from the mass spectra; in each instance the most abundant ion was set to 100%. Figure 1 presents a direct analysis of a formulation and a comparison with authentic standard. Figure 2 demonstrates the utilization of increasing analysis temperature for determining the presence of barbiturate in a combination formulation.

RESULTS AND DISCUSSION

Studies of the mass spectra of barbituric acids were reported previously (3–5). Very recently, Fales *et al.* (6) reported the utilization of chemical ionization mass spectrometry for the identification of barbiturates. These authors noted the superior nature of the chemical ionization method for the determination of barbituric acid derivatives. The necessity for a shortening of the usual identification procedure (1) so that rapid qualitative identifications might be made prompted this investigation of the feasibility of direct qualitative analysis of barbiturate formulations by mass spectrometry.

Mass spectra obtained directly using powdered pharmaceutical barbiturate formulations were identical with those obtained from preparations that had been carefully prepared by extraction of the free barbituric acid and its prior purification before the mass spectral determination. This was true even if the barbiturate was present as a sodium salt (Fig. 1). It is evident, therefore, that direct analysis is a feasible method of shortening the time required for a qualitative barbituric acid determination.

To determine the extent of interference by other drugs in the formulation, a number of combination pharmaceuticals were examined by the direct introduction technique. In every case, the presence of the barbiturate was demonstrable by the technique. For such combination formulations, it was found that improved determinations were obtained if spectra were run at several temperatures and then compared (Fig. 2). In this way the presence of a mixture was quite evident; when the fragment peak intensities were compared at the several temperatures, those corresponding to the components of the mixture were readily identified. As with pure barbiturate formulations, the presence and identity of the barbiturate were established.

The Fales *et al.* (6) demonstration of the super capability of chemical ionization mass spectrometry for barbiturate analysis as well as our demonstration of the suitability of direct analysis without prior purification, even with an electron bombardment ionization mass spectrometer, indicates that direct mass spectral analysis is the most rapid and diagnostic method available for barbiturates.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 3, 1971, from the *Department of Medicinal Chemistry, School of Pharmacy, University of Kansas, Lawrence, KS 66044*

Accepted for publication December 6, 1971.

Supported in part by a grant from the National Science Foundation in support of undergraduate research participation.

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COMMUNICATIONS

Total Mathematical Resolution of Diffusion Layer Control of Barrier Flux

Keyphrases ☐ Diffusion layer control -mathematical derivation ☐ Membrane permeability -equations describing diffusion layer control ☐ Derivation- resolution of diffusion layer control, barrier flux

Sir:

Equations describing the flux of a penetrant through a membrane sandwiched between two liquid phases under the conditions where the chemical potential gradient is *virtually* entirely in the diffusion layers (diffusion layer control) were derived. With like solvent on each side of the membrane, these equations confirm that the steady-state flux is only dependent on the applied phase concentration, the diffusivity within the diffusion layers, and the reciprocal of the sum of the diffusion layer thicknesses.

Furthermore, a lag time expression was derived which relates the duration of the nonstationary state to the thicknesses of both the membrane and the diffusion layers, the membrane/solvent partition coefficient, and the reciprocal of the diffusivity within the solvent. This equation is of major theoretical significance because it, along with the relationship of partition coefficient to homolog chain length, indicates that at some point in a homologous series the lag time will begin to grow exponentially. This effect extrapolated to biological systems, *i.e.*, drug absorption and biodistribution, indicates that the activity of a long chain congener may not only be limited by the plateauing of steady-state transport but also by inability to break through the biological barrier(s) in sufficient time to exert an effect. The saliency of this point is heightened when it is realized that metabolism and elimination by filtration may not be similarly affected. Relative potency from biological assays with fixed, timed end-points, *i.e.*, vasoconstriction at 6-hr. postapplication, could be misleading if the time chosen is not relevant to actual drug usage. In short, the lag time dependency alone can suffice as the limiting factor in the structure–activity profile for a series of organic homologs.

Figure 1 describes the physical situation to be treated. It is a concentration profile of a membrane (III) positioned between two homogeneous solvent phases (I and V). Phase I is of relatively high concentration, C_0. C_0 is assumed to remain constant. Phase V is assumed to be a solute sink and thus is maintained at zero concentration. Regions II and IV represent solvent diffusion layers (Nernst layers) contiguous to the membrane surfaces (1–3). The thickness of the diffusion layers (II and IV) are h_{AQII} and h_{AQIV}, respectively, and the membrane is of thickness h_M.

By assuming the concentration curves C_0 to C_1 and C_3 to C_4 to be linear, the flow into (V_1) and out of (V_2) Compartment III, the membrane, may be represented, respectively, by:

$$V_1 = \frac{(C_0 - C_1)}{h_{AQII}} D_{AQ}A \quad \text{(Eq. 1)}$$

$$V_2 = \frac{(C_3 - C_4)}{h_{AQIV}} D_{AQ}A \quad \text{(Eq. 2)}$$

where D_{AQ} is the aqueous (solvent) diffusion coefficient, and A is the cross-sectional area available for diffusion. The rate of change of concentration in III is:

$$\frac{dC_{III}}{dt} = \frac{dC_2}{dt} = \frac{1}{h_M A}(V_1 - V_2) \quad \text{(Eq. 3)}$$

As diffusion layer control is approached, the membrane concentration at each membrane interface becomes imperceptibly different and, thus, $C_2 = (PC)C_1 = (PC)C_3$, where (PC) is the membrane/solvent partition coefficient. The identical assumption is made for the concentration across the diffusion layers under membrane control of flux. For convenience at this point, assume that $h_{AQII} = h_{AQIV}$; then:

$$V_1 - V_2 = \frac{2D_{AQ}A}{h_{AQII} + h_{AQIV}} \left\{ C_0 - \frac{2C_2}{(PC)} \right\} = \frac{D_{AQ}A}{h_{AQ}} \left\{ C_0 - \frac{2C_2}{(PC)} \right\} \quad \text{(Eq. 4)}$$

and:

$$\frac{dC_2}{dt} = \frac{D_{AQ}}{h_M h_{AQ}} \left\{ C_0 - \frac{2C_2}{(PC)} \right\} \quad \text{(Eq. 5)}$$

where the term h_{AQ} is the thickness of the individual diffusion layer. With the boundary conditions $C_2 = 0$