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ANALYSIS OF THERAPEUTIC AND COMMONLY ABUSED DRUGS IN SERUM AND URINE BY GAS–LIQUID CHROMATOGRAPHY USING A PHOTOIONIZATION DETECTOR

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SUMMARY

A simple, rapid and sensitive gas chromatographic procedure using the photoionization detector (PID) was developed for the detection and quantitation of several drugs in serum and urine. In order to evaluate the performance of the PID, the results were compared with those of the flame-ionization detector (FID).

The data indicate that the PID is 8–16 times more sensitive than the FID for the drugs studied in the barbiturate group. Excellent reproducibility was found for samples quantitated with the PID on a routine basis. The PID and FID produced statistically similar results on extracted serum samples. The correlation coefficient was 0.99. The PID also produced chromatograms with less background than those obtained with the FID for many extracted serum samples.

The advantages of the PID for drug analysis in biological fluids include simplicity of operation, lack of solvent response, universal drug response, non-destructive character and stability.

INTRODUCTION

Owing to the wide use and subsequent abuse of drugs, therapeutic monitoring and drug overdose analysis has become one of the primary tasks in a clinical toxicology laboratory. In the case of therapeutic monitoring, the therapeutic agent or metabolites are usually known and their concentration levels in the biological fluid analyzed are generally low. In a drug overdose situation, however, any drug becomes a suspect, and its concentration is usually high. In order to perform both types of analyses, the chromatographic method must have good resolving power and the detector should respond from nanogram to microgram levels and have a universal response.

Gas chromatography (GC) has successfully met the requirements for the separation of therapeutic drugs and the causative agents in most drug overdose situations. The GC detectors commonly used for these applications can be divided into either selective or non-selective groups. In the latter, the flame-ionization detector (FID) has

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generally fulfilled the requirements for drug analysis. However, two undesirable features of this detector are its destructive character, and large solvent response.

Other non-selective detectors, such as the electron-capture[1] and the microcoulometric detectors[2] have only found limited usage due to their susceptibility to detector overload, poor linearity, etc. The use of selective detectors such as the Hall electrolytic conductivity detector (element selective) and the alkali flame-ionization detector[3–6] have alleviated sample cleanup which is one of the most cumbersome problems related to the analysis of biological fluids. Since all abused drugs present in a patient sample must be known, for obvious reasons, these detectors are not always the best choice.

Despite the advantages of GC for the analysis of drugs in biological fluids, several drawbacks still remain due to the detectors and/or the chromatographic process. This has triggered an intensive search in two areas, detector technology and/or alternative chromatographic methods. Detectors which are more sophisticated, such as a mass spectrometer[7–9], an infrared detector[10–11], etc., have been used to overcome some of the drawbacks of the GC methods. Unfortunately, their high prices and complexity have prevented their widespread usage. The trend, however, has been oriented toward alternative chromatographic methods; for instance, high-performance liquid chromatography (HPLC). The advantages claimed for HPLC include: (1) elimination of drug derivatization, (2) micro as well as macro capabilities, (3) simplified sample preparation, (4) shorter analysis times, (5) drug collection for further analysis, (6) simultaneous analysis of drugs and metabolites, (7) direct analysis of biological fluids, and (8) higher sensitivity. These advantages are either the result of the chromatographic process per se, the detector only, or a combination of both. Previously, many of these advantages have not been obtained by GC using simple inexpensive detectors.

However, the use of GC coupled with the unique features of the photoionization (PID), have shown significant advantages for drug analysis. The purpose of the present work was to evaluate the performance of the GC–PID system for the analysis of drugs in biological fluids.

EXPERIMENTAL

Reagents and chemicals

All solvents were analytical-reagent quality and were used as received. Carbon tetrachloride and chloroform were obtained from Fisher Scientific (Pittsburgh, Pa., U.S.A.). Pesticide-residue grade acetonitrile (J. T. Baker, Phillipsburg, N.J., U.S.A., was used to reconstitute the drug extracts. The analytical column was deactivated with phosphoric acid (Fisher Scientific). The drug standards were obtained from Applied Science Labs. (State College, Pa., U.S.A.). Urine and blood samples were donated from a local toxicology laboratory. A Lederle diagnostics serum toxicology control (American Cyanamid Company, Pearl River, N.Y., U.S.A.) was used for calibration purposes. Mass-Skreen columns (Brinkmann, Westbury, N.Y., U.S.A.) containing Amberlite XAD-2 resin was used for urine extractions.

Apparatus

The HNU Systems Model 401 gas chromatograph equipped with an FID and a

built-in PID (HNU Model PI-52-02) with a 10.2-eV lamp was used. The column was inserted directly into the detector base. The analytical column was designed to permit rapid analysis of the sixteen drugs chosen. A 6 ft. × 2 mm I.D. silanized glass column was prepared and packed with 3% OV-17 on 80–100 mesh Gas-Chrom Q. The column was deactivated with phosphoric acid. Samples were injected with a 10-μl Hamilton syringe throughout these studies.

Extraction procedure and GC analysis

Serum samples. Serum was separated from whole blood by centrifugation. To 10 ml of serum were added 10 ml of carbon tetrachloride or chloroform, 1 ml of a known internal standard solution (10 μg/ml of aprobarbital in 0.1 *N* HCl) and the liquids were shaken for 1 min and then centrifuged for 8 min. The upper aqueous layer was carefully removed and the organic solvent evaporated with a stream of nitrogen at 40°. The extract was later reconstituted for the GC analysis. The controls and standards were prepared by diluting 1 ml of the stock standard (1 mg/ml in methanol) to 100 ml of 0.1 *N* HCl and extracted in the identical manner as the serum samples.

GC analysis. The standard and patient samples were analyzed using temperature programming from 200 to 285°. Nitrogen was used as the carrier gas at a flow-rate of 19 ml/min. For the GC analysis, the extract was reconstituted in 10 μl of acetonitrile. Finally, 2 μl of the reconstituted extract (underivatized drug) was reproducibly injected into the analytical column and response data were obtained as a function of time.

Two aspects were considered in chosing the solvent to reconstitute the drug extract; first, good drug solubility and second, low PID response. Acetonitrile was the best choice since its ionization potential is higher (12.2 eV) than the energy of the UV source (10.2 eV) and consequently is not ionized. Recovery of the drug redissolved in acetonitrile was better than 95%.

RESULTS AND DISCUSSION

The PID was used previously[12] to determine the sensitivity and lower limits of detection (LLD) for a variety of unextracted barbiturate standards dissolved in acetonitrile. The PID was found to be at leas. an order of magnitude more sensitive than the FID. We found also that under optimum chromatographic conditions, the LLDs for these barbiturate standards were 20–70 times lower than those obtained with the FID. The LLDs are more than a decade better than the sensitivity values, since the small solvent response of the PID allows trace enrichment techniques (such as temperature programming, injection volumes) to be applied more successfully.

A major question which had to be answered was whether the increased sensitivity of the PID would be a problem (*i.e.*, more interference by impurities) for the detection of extracted drugs from standards or biological samples. For this current evaluation the extracted drugs were detected with the PID and the FID under identical chromatographic conditions.

Fig. 1 shows the separation of an extracted standard sample containing sixteen frequently abused or therapeutic drugs detected with the PID and the FID. As observed in this figure, no interference was encountered from solvent impurities or other

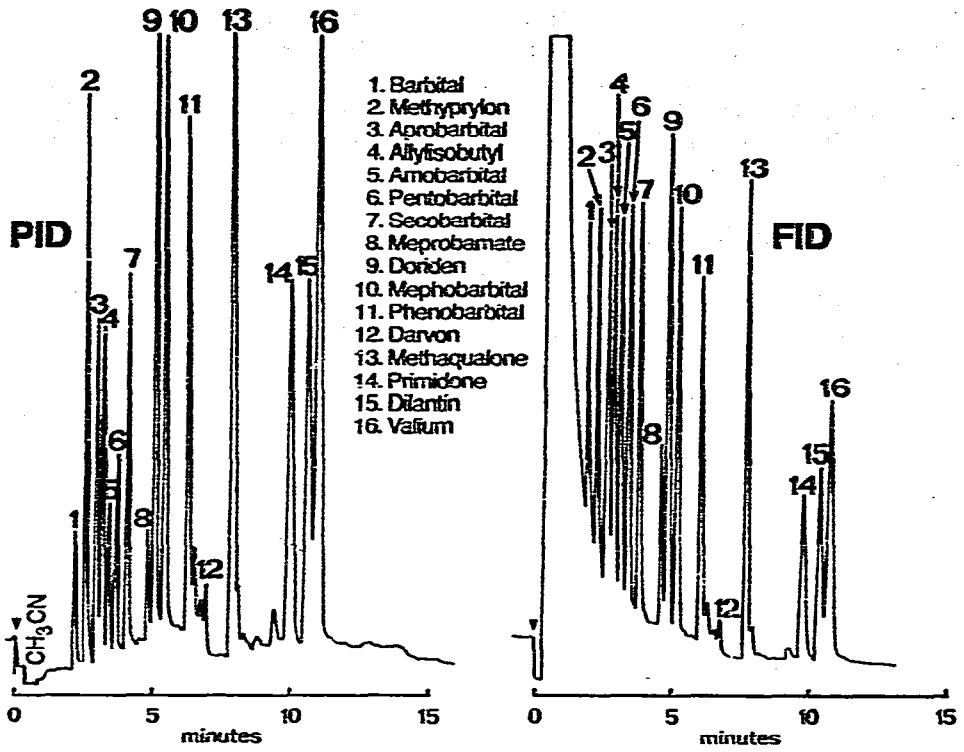

Fig. 1. Gas chromatograms of acidic and neutral drug standard detected by the PID and the FID. Column, 6 ft. × 2 mm I.D. glass packed with 3% OV-17 on 100–120 mesh Gas-Chrom Q deactivated with phosphoric acid. Nitrogen flow-rate, 19 ml/min. Solvent, acetonitrile. Column temperature, programmed 200–285°, 12°/min.

constituents with either the PID or the FID. Furthermore, the chromatogram for the PID shows a very small solvent front (negative peak) lasting about 1 min. By contrast, the FID produced a solvent front that lasted about 7 min and interfered with detection even at high levels of the early eluting drugs.

It should also be noted that the chromatogram with the PID was obtained with an electrometer setting eight times more attenuated than the FID. This indicates that, for these drugs, the PID is at least eight times more sensitive than the FID. For some of the drugs, *e.g.*, valium and dilantin the PID is sixteen times more sensitive than the FID, while for others, such as secobarbital, it is eight times more sensitive.

A typical chromatogram of a patient serum sample obtained with the PID is shown in Fig. 2. This patient had an overdose of phenobarbital. Note that the change in background during the run was less than 2% for the PID. When the same sample was run on the FID (Fig. 3), the change in background level was more than 12%. This is a consequence of the fact that the higher sensitivity of the PID to these drugs allows the use of a higher attenuation (for the PID) and therefore, less background. A comparison of the chromatograms in Figs. 2 and 3 clearly demonstrates that no additional impurities in the biological samples are observed as a result of the increased sensitivity of the PID compared to the FID. Peak I observed on both the PID

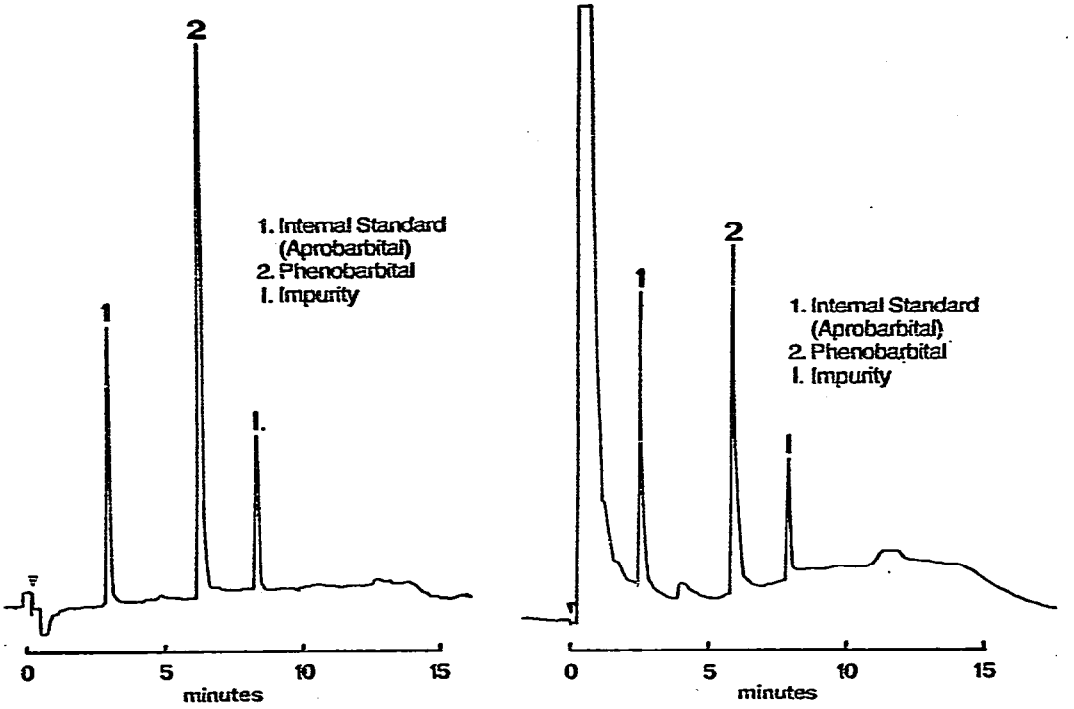

Fig. 2. Gas chromatogram of a serum patient sample detected with the PID. Conditions as in Fig. 1.

Fig. 3. Gas chromatogram of a serum sample detected with the FID. Conditions as in Fig. 1.

and FID is an impurity and does not interfere with any of the sixteen drugs chromatographed.

Fig. 4 illustrates typical calibration curves for phenobarbital with the PID and the FID. On the vertical axis, the PID response was normalized to keep both calibration plots on scale for comparison. The parallel nature of these two lines indicates that the PID produced a response equivalent to the FID in the 159 ng/μl to 15.9 μg/μl concentration range. At lower levels (15 ng/μl) the curve deviated from linearity for both detectors. This deviation was due to the partial irreversible adsorption of phenobarbital on this column.

The concentration of drugs in four patient serum samples was determined using internal standardization. Before the serum samples were run, a commercially available serum control was used to check the overall performance of the extraction and chromatographic procedure with the PID. As shown in Table I, the agreement between the concentration of the drug in the control and the results obtained with the PID were excellent. A linear regression of the PID and FID data on biological samples yields the following equation:

$$\text{PID} = 0.96\ \text{FID} + 0.48$$

with a correlation coefficient of 0.99 and an average uniformity of 1.01. The mean concentration values for the PID and FID were 6.78 and 6.70, respectively. Although

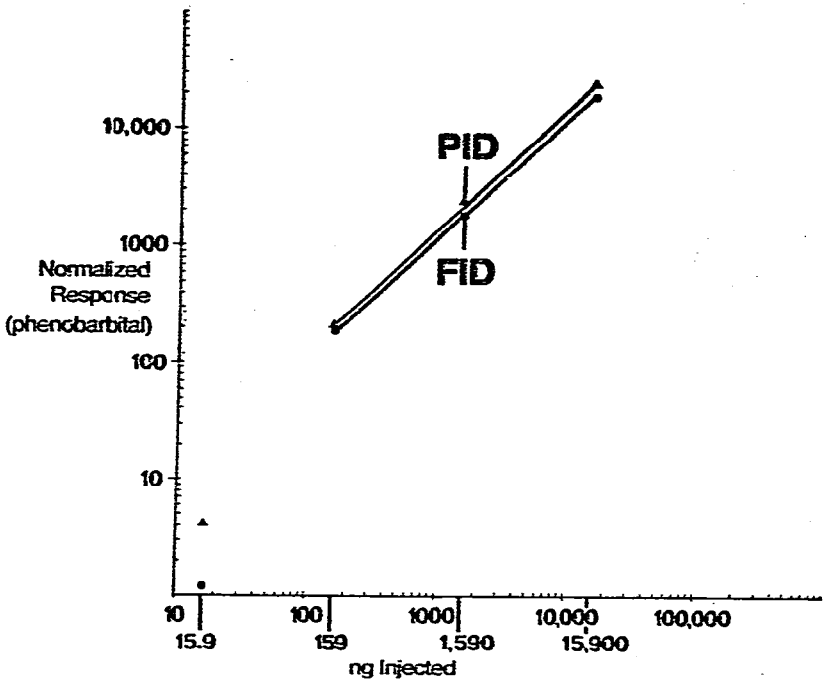

Fig. 4. Typical calibration curves for phenobarbital detected with the PID and FID.

TABLE I

GC DATA OBTAINED WITH THE PID AND FID ON CONTROL AND PATIENT SAMPLES

Patient sample No.	*Compound*	*Lederle serum control (μg/ml)*		*Patient samples (μg/ml)* *	
		Found (PID)	*Label*	*FID*	*PID*
1	Dilantin	2.1	2.0	6.7	6.4
2	Mephobarbital	—	—	13.9	14.0
3	Mysoline	—	—	0.9	1.1
4	Phenobarbital	2.0	2.0	1.3	1.4
	Dilantin	1.9	2.0	10.7	11.0

* $\frac{\text{Sample pH component}}{\text{pH internal standard}} \times \frac{\text{Standard pH internal standard}}{\text{pH component}} \times 10\ (\mu g/ml) = \text{concentration.}$

the sample population is small, these statistics indicate excellent agreement between the two detectors

Fig. 5 shows the chromatogram of the urine sample of patient No. 2 (Table I). A high level of mephobarbital was present in urine as expected from the high levels of this drug found in the serum sample. As can be noted, several impurities were detected in the first four minutes of the run. This indicates that the urine extraction procedure followed is not adequate for drugs eluting within this time frame. Although not shown, the FID produced a chromatogram with a large solvent peak, that lasted for 7 min partially masking mephobarbital.

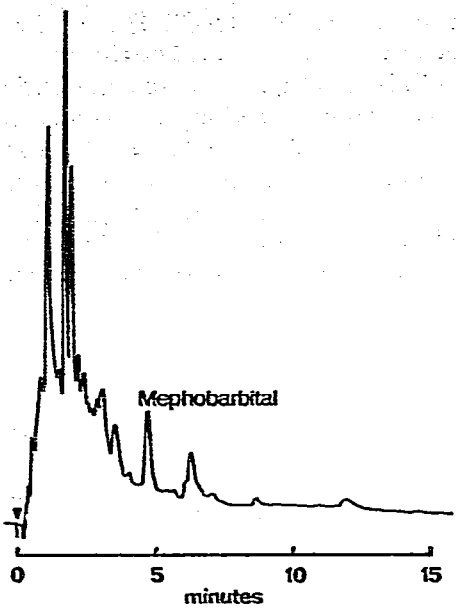

Fig. 5. Gas chromatogram of a urine patient sample detected with the PID. Column temperature, programmed 210–285°, 12°/min. Other conditions as in Fig. 1.

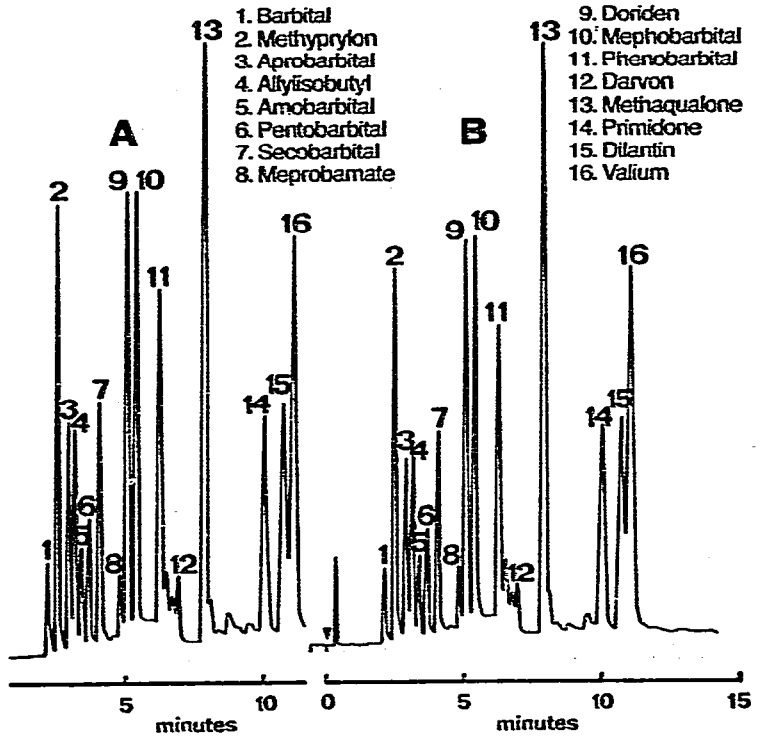

Fig. 6. Gas chromatograms of acidic and neutral drug standard obtained with the GC–PID system in a 100-h controlled stability test. (A) 0 h, (B) 100 h. Conditions as in Fig. 1.

The stability of the analytical method (chromatography plus PID) is an important consideration for routine monitoring of therapeutic drug levels. The chromatograms in Fig. 6 clearly illustrate the excellent GC–PID stability. An acidic and basic drug serum extract was repeatedly injected (2.0 μl) during a continuous 100-h controlled experiment. Chromatogram A corresponds to the initial run while B illustrates the final run at 100 h. The peak heights were measured at a number of time intervals during the 100-h span. The results indicate that the final data were within 10% of the values obtained initially. This small variation could be easily corrected with the lamp intensity control on the electrometer/power supply module if an absolute quantitation was necessary instead of internal standardization.

CONCLUSIONS

Several conclusions can be drawn from the above results, namely: (1) the sensitivity of the PID is 8–16 times greater than that of the FID for the drugs studied. (2) The GC–PID system has intrinsic characteristics, suitable for the detection and quantitation of drugs in biological fluids. In fact, chromatograms with lower background and fewer interferences were obtained with the PID even with its greater sensitivity. (3) The quantitative results of the serum samples produced by both detectors were identical within experimental error. (4) The PID is virtually a non-destructive detector, and consequently samples can be collected for further analysis. This represents an interesting alternative for quantitation in a GC–mass spectrometry system. (5) The detector does not respond to many common solvents. Due to this property, a very small solvent response (negative peak) is produced and lower background levels are generally found. Ultimately, the lack of detector response for certain solvents can result in shorter analysis times, higher accuracy and lower limits of detection.

Lastly, the system is simple to operate, stable and safe (no use of hydrogen and air). The latter feature makes the PID very attractive for use in hospital labs where flames or hydrogen usage is restricted.

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