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A Rapid Isolation Technique for Drugs from Tissues and Fluids: Use of the Du Pont Prep 1 System

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ABSTRACT: A microprocessor-controlled, automated extraction/concentration device, the Prep 1 (Du Pont Clinical Systems), was evaluated for application to the isolation of drug substances from postmortem fluids and tissue homogenates. Two classes of materials were investigated: barbiturates and the benzodiazepine, diazepam. With as little as 200 mg of tissue, barbiturate derivatives were successfully isolated and measured by gas chromatography using nitrogen-phosphorus detection with a coefficient of variation of 2 to 5%. Diazepam was measured in a similar fashion with a coefficient of variation of 4.4%. Preliminary investigation indicates that this system is applicable to a wide range of drug substances of toxicological interest.

KEYWORDS: toxicology, drug identification

XAD-2 resins have been used to extract drug substances from biological fluids and tissues. The methods employ macro samples, and generally thin-layer chromatography is the detection method. These previous applications of XAD-2-type resin have been considered to be of a qualitative nature.

A new, automatic processing technique, known as the "Prep 1," manufactured by Du Pont Instruments, is described in this report. This instrument has previously been applied to the isolation of drug substances quantitatively from biological fluids [*1-3*] for clinical purposes. Now, this report details the application of this technique to the isolation of drug substances of toxicological interest from postmortem fluids and tissues.

Materials and Methods

Instrumentation

The Prep 1 is an automated sample-processing device that uses the advantages of an XAD-2 type nonionic adsorption resin to facilitate the isolation of drug substances from the aqueous biological matrix to a convenient organic extract. This solid phase extraction pro-

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cess is carried out under centrifugal force with a twelve-position rotor attached to a multidirectional motor. Changes in rotor rotation rate and direction effect the requisite extraction and elution into the appropriate receivers (Fig. 1). Further information regarding the operation of this instrument follows, but complete information is best obtained from Du Pont Instruments, Wilmington, DE.

Gas Chromatography

Several gas chromatographic systems were used to develop the procedures described here. Separate systems are maintained for barbiturate derivatives (GC-System I) and for basic-neutral components (GC-System II).

The GC-System I for barbiturate derivatives was a Perkin-Elmer 3920A gas chromatograph equipped with a nitrogen-phosphorus detector. The column was 3% OV-1 on Chromosorb WHP, 100-120 mesh, maintained at 180°C, with helium as the carrier gas. Injection port and manifold temperatures were 250°C each. Barbiturate derivatives were chromatographed as their methyl derivatives, generated on-column by co-injection with trimethylphenylammonium hydroxide (0.1*M* in methanol).

The GC-System II for basic-neutral compounds was a Perkin-Elmer 900A gas chromatograph equipped with a nitrogen-phosphorus detector. The column was 3% S.P. 2250 (Supelco Clinical Packing 1-1767), with helium as the carrier gas. The column oven was temperature-programmed for 185 to 250°C at 6°C per minute. Injection port and manifold temperatures were 250 and 280°C, respectively.

The gas chromatography/mass spectrometry/computer system employed during this study was a Hewlett-Packard 598A gas chromatograph-mass spectrometer system coupled to a Hewlett-Packard 5933A 16K Data System. The chromatographic conditions were similar to those described for the GC-System I and GC-System II procedures.

Reagents

Standard analytical-grade reagents were used throughout this project. Typically, organic solvents such as methanol, acetone, and methylene chloride were the eluting solvents, and the buffers included 0.1*M* sodium acetate (pH 4.0), 0.1*M* sodium borate (pH 9.5), and ammonium chloride-ammonium hydroxide (pH 9.0).

Samples

Postmortem specimens of blood, urine, bile, gastric content, and tissues such as brain and liver were obtained as part of the normal laboratory function for toxicological analysis. These specimens were processed by standard manual laboratory procedures prior to their use with the Prep 1 Systems.

Extraction Procedures

The cartridge assembly for the Prep 1 consists of a prefilled polypropylene column containing a specially prepared XAD-type resin along with the necessary effluent and recovery cups. The typical processing procedure is as follows:

1. Set up and label the cartridge assembly (twelve per full run).
2. For biological fluids such as whole blood, add 1 g of glass beads to the resin cartridge.
3. For tissue homogenates (20% in water), add a plug of glass wool to the resin cartridge.
4. Make aliquots of 0.2 to 0.5 mL of whole blood or 1 mL of tissue homogenate (equivalent to 200 mg tissue).

5. Add 10 mL of the appropriate buffer for the extraction desired (pH 4.0 acetate for barbiturates and pH 9.5 borate for basic-neutral derivatives).

6. Assemble these materials (the extraction column and the effluent and recovery cups) in their appropriate positions in the Prep 1 rotor.

7. Place the rotor in position in the Prep 1 instrument.

8. Add the appropriate solvents to their respective reservoirs (generally water wash as Solvent I and methylene chloride as the organic extractant in Solvent II). Use approximately 10 to 12 mL per solvent, resulting in 0.6 to 0.8 mL per cartridge.

9. Select the appropriate program sequence (for rotor speeds and duration of spin, there is a total of 15 possible programs).

10. Close the lid and press the start switch.

At this point the system will dispense solvents and generate a dry extract in the recovery cup according to the generalized sequence shown in Fig. 2. The total run time is typically about 20 min for a dry extract.

Recovery and Precision Data

Precision data regarding the reproducibility of the extraction process for the drugs investigated were obtained by assaying replicate samples of a prepared tissue homogenate, both with the addition of the drugs of interest to the homogenate and the assay of tissues known to contain those drugs by prior analysis. Our initial investigation, part of which is reported here, deals mainly with tissue barbiturates and diazepam.

Results and Discussion

Initial attempts to assay whole blood and tissue homogenates with the Prep 1 System failed in flow dynamics in the columns. With the modifications described in the Methods section, flow failures are kept to a minimum. The occasional failures that do occur result from small portions of the tissue homogenate or whole blood components reaching the upper polypropylene column support and plugging it. Care in the placement of the glass wool plug is emphasized.

The first compounds chosen for evaluation with the described isolation technique were the barbiturate derivatives. The initial consideration was the chromatographic quality of the blank extract and the negative tissue extract as it pertains to potentially interfering chromatographic peaks. In Fig. 3, one can easily see that no problems were experienced here. It is important to note that all the chromatograms were obtained from nitrogen-phosphorus detection instruments. Experiences with a gas chromatographic system based on flame ionization detection were less satisfactory.

Recovery estimates of the barbiturate derivatives indicated absolute recoveries greater than 90% from fluid samples and greater than 75% from homogenates. The use of the internal standard, either hexobarbital or mephenytoin, was found to compensate for variable recoveries. Relative recoveries ranged from 95 to 100%.

The precision of the barbiturate analysis was estimated by two methods: standard addition to blank tissue homogenates and assay of case material known to be positive for barbiturates. Table 1 describes the precision data obtained. An example of typical barbiturate positive results is presented in Fig. 3.

A second component evaluated was diazepam. For precision estimates, the standard addition approach was used because case material containing diazepam was not available. The data obtained are given in Table 1. A typical chromatogram is presented in Fig. 4.

Other substances of toxicological interest that were evaluated (on a preliminary basis) for application of this sample processor included tricyclic antidepressants (Fig. 5), glutethimide

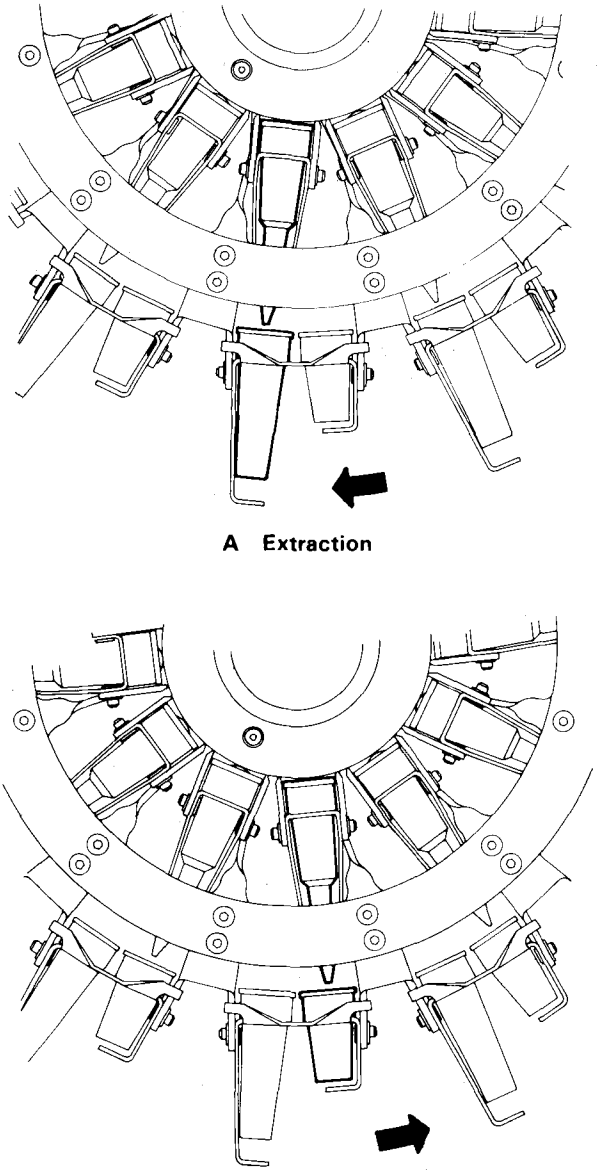

FIG. 1—*Extraction and elution sequence with the Prep 1 processor (reprinted with permission from Du Pont Clinical Systems).*

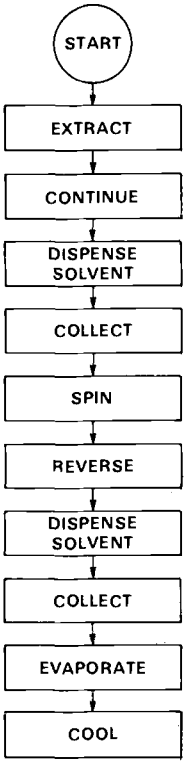

FIG. 2—*Extraction sequence with the Prep 1 system (reprinted with permission from Du Pont Clinical Systems).*

(Fig. 6), methadone (Fig. 7), and methaqualone (Fig. 8), as well as cannabinoids from urine and blood. Other analytical techniques that have been coupled to Prep 1 extracts include high-pressure liquid chromatography (barbiturates, xanthine derivatives), enzyme immunoassay (phencyclidine EMIT®), and gas chromatography-mass spectrometry-computer systems (Δ^9-tetrahydrocannabinolic acid). In each case, the coupling of the analytical technique to a Prep 1 extract was successful.

Amberlite XAD-2 was first described as a nonionic extractant for drugs by Fujimoto and

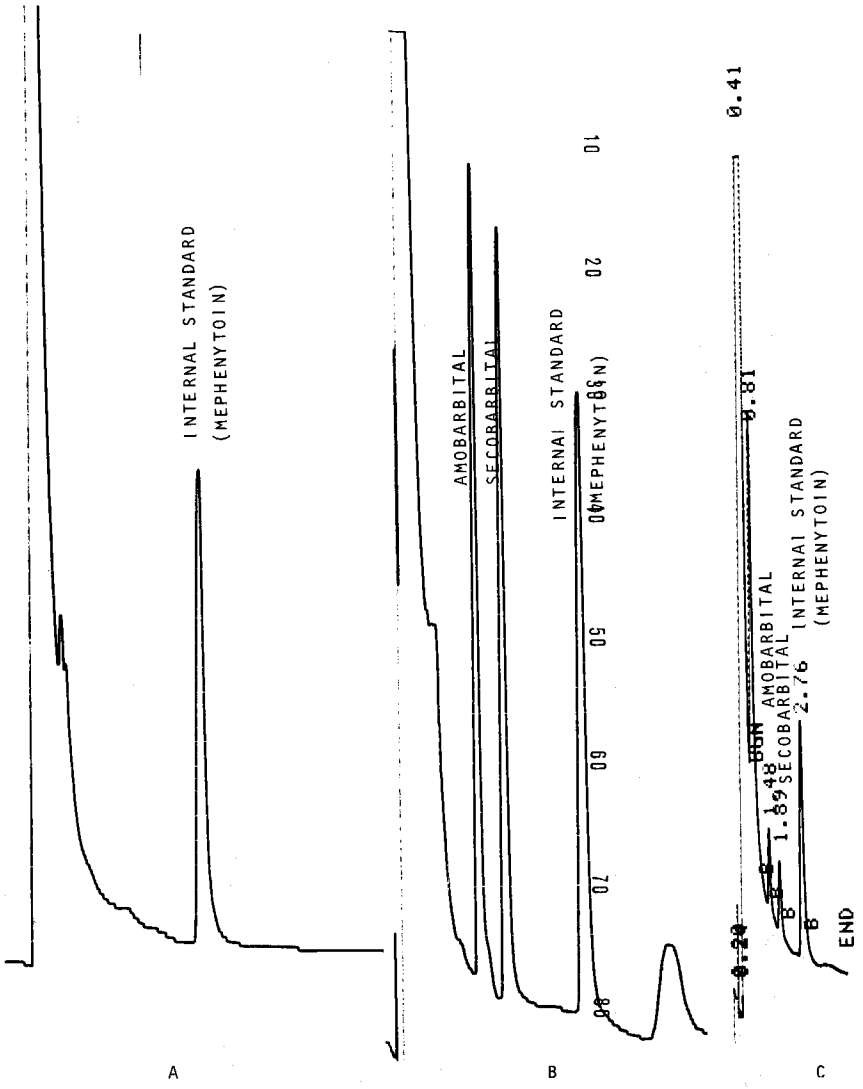

FIG. 3—*Gas chromatograms (nitrogen-phosphorus detector) of* (A) *known barbiturate-negative liver,* (B) *known Tuinal®-positive liver, and* (C) *known Tuinal-positive brain tissue.*

Wang [*4*] and Wiessman et al [*5*] in 1970 and 1971. Many modifications of those procedures have followed, including the differential elution process [*6*]. Other researchers [*7-12*] describe the optimization of the absorption-elution process. All of the reports that appear in the literature surveyed deal with qualitative and crudely semiquantitative estimates of drugs in urine.

Methods for the isolation of drug materials from blood, bile, and tissues have been described by various groups including Pranitis et al [*6*] and Caplan et al [*13,14*]. Generally, in those reports, XAD-2 was added to the biological fluid, the material mixed, and the XAD-2 isolated by filtration. Elution of the drugs of interest was then accomplished by washing the XAD-2 resin with organic solvents. Sample size was on the order of grams of tissue, and the analytical technique was commonly thin-layer chromatography. Little, if any, information was provided describing quantitative application of the resultant extract.

TABLE 1—*Precision data for the measurement of selected barbiturates and diazepam with the Prep 1 system and gas chromatography with a nitrogen-phosphorus detector.*

	Coefficient of Variation, %			
	Diazepam, 2 μg/g	Phenobarbital, 50 μg/g	Amobarbital, 20 μg/g	Secobarbital, 20 μg/g
Within-day precision (n = 10), liver matrix	4.45	2.69	4.78	9.65
Day-to-day precision (n = 10), liver matrix	7.10	4.89	3.53	9.21
Known positive samples assayed in replicate (n = 10), amobarbital = 16 μg/mL and secobarbital = 19 μg/mL	...	...	2.2	1.13

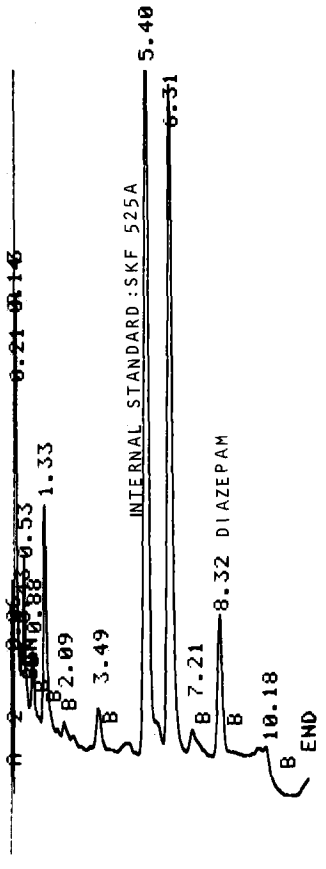

FIG. 4—*Chromatogram of a Prep 1 extract of a known diazepam-positive liver.*

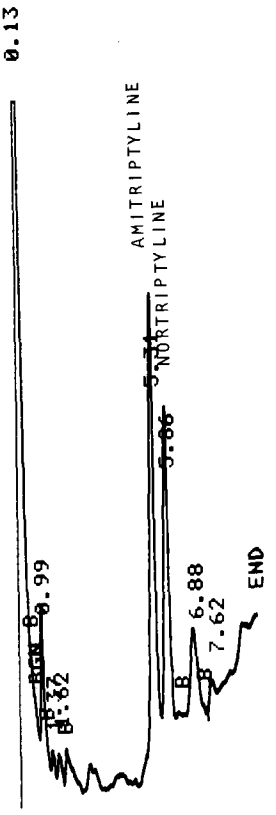

FIG. 5—*Chromatogram of a liver sample known to be positive for a tricyclic antidepressant. Amitriptyline and nortriptyline were isolated by the Prep 1 processor.*

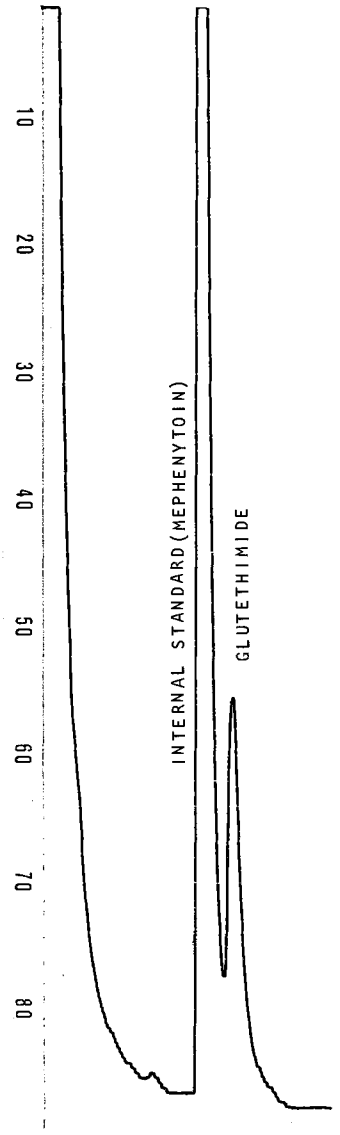

FIG. 6—*Chromatogram of a known glutethimide-positive blood sample processed by the Prep 1 processor.*

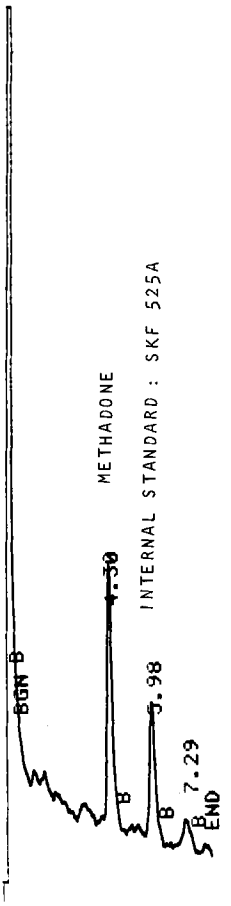

FIG. 7—*Chromatogram of a methadone-positive liver processed by the Prep 1 automatic extractor.*

With the Prep 1 sample processor equipped with Type W cartridges (containing XAD-2 resin), samples of as little as 200 mg of tissue and 100 μL of biological fluids are easily processed in approximately 20 to 30 min, yielding a dry extract. These extracts may be assayed by thin-layer chromatography, gas chromatography, enzyme immunoassay, gas chromatography/mass spectrometry, and liquid chromatography, with little concern for the cleanliness of the extracts, provided relatively nonpolar solvents are used for the isolation process.

The ability of the processor to handle up to twelve extracts simultaneously enables the

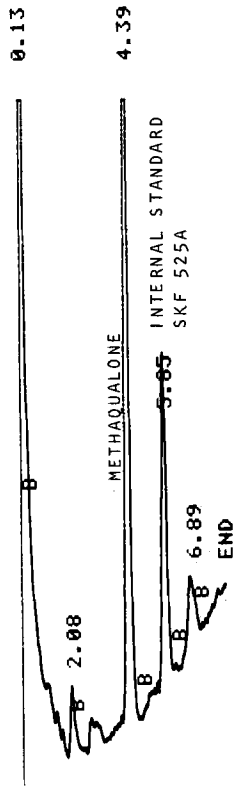

FIG. 8—*Chromatogram of brain tissue processed by the Prep 1 system. The tissue was positive for methaqualone.*

analyst to run simultaneously multiple cases for a particular class of components, or to extract a single case, analyzing a specific specimen or case composite of tissues along with a multipoint standard curve and perhaps even an assay control. The versatility of the technique plus the ability to handle small sample sizes make this an extremely valuable tool for the forensic toxicology laboratory.

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