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Application of Empore C-8 Extraction Disks for Screening Urine in Systematic Toxicological Analysis

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ABSTRACT: Solid-phase extraction (SPE) by means of disposable columns has become a widely accepted technique for sample pretreatment in toxicology, both for directed analyses and for screening analyses. However, the sample capacity in SPE is usually limited to a few millilitres.

Therefore, we have investigated to what extent these problems can be overcome by using Empore extraction disks, consisting of chemically modified C-8 reversed-phase silica, embedded in an inert polytetrafluoroethylene (PTFE) matrix. Human urine was selected as the matrix and dexetimide and mepyramine were initially used as test drugs because these drugs were available in tritiated form. Additional drugs investigated included codeine, hexobarbital, imipramine, methamphetamine, and nitrazepam.

In these investigations, the sample capacity for untreated urine was at least 25 mL, and analyte quantities up to 250 μg could be retained by these filters. Washing with water/methanol mixtures was successful in removing substantial amounts of endogenous interferences, and methanol proved to be an acceptable eluent. Thus, these disks seem to have interesting potential for toxicological analysis in that sample concentration and cleanup can be achieved at the same time.

KEYWORDS: toxicology, extraction, urine, drug identification

The analysis of drugs and metabolites in biological matrices usually necessitates a purification step to eliminate endogenous compounds that might interfere with the analytical technique selected. If only a single compound of interest has to be quantified, the purification step should have a large impact on the selectivity, so the determination limits depend solely on the signal-to-noise ratio of the detector [*1,2*].

However, in systematic toxicological analysis, the nature of a drug present in a biological sample is unknown, and therefore a sample cleanup step should be primarily directed towards concentrating the drug, with simultaneous elimination of endogenous compounds [*3,4*].

Urine, which can be obtained in a noninvasive manner in relatively large volumes, is potentially an important matrix for toxicological analysis. However, liquid-liquid extrac-

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tions of large volumes of urine will result in large volumes of organic extracts. Regardless of the cost and safety of testing, this does not allow screening of many samples per working day.

Solid-phase extraction (SPE) with disposable columns seems to be an alternative. Yet, serious problems may arise when more than 2 mL of urine per 100 mg of sorbent are to be extracted because of clogging of the columns [5].

Recently, a new type of sorbent carrier for adsorbents has been developed in the form of a polytetrafluoroethylene (PTFE)-based filtration material. These Empore™ extraction disks contain chemically modified C-8 reversed-phase silica, embedded in an inert PTFE network [6]. These extraction disks have successfully been applied for extraction and concentration of pesticides from groundwater [7].

In the present study, the applicability of these extraction disks for urine samples has been studied. Special attention has been paid to sorbent capacity, breakthrough volumes, elution, recovery/losses and washing potentials. Tracking of the drug during adsorption and during desired and undesired elution was initially achieved by the use of two radiolabeled drugs.

Materials and Methods

Materials

Methamphetamine hydrochloride, mepyramine, dexetimide, metharbital, imipramine hydrochloride, hexobarbital, codeine hydrochloride, nitrazepam, and prazepam were obtained from commercial suppliers and were of pharmacopoeial quality. Stock solutions of these compounds (25 mg/mL) were prepared in ethyl acetate/methanol, 1:1 (v/v). Tritium-labeled dexetimide (12.6 curies (Ci)/mmol) and mepyramine (29 Ci/mmol) were obtained from Janssen (Beerse, Belgium) and NEN (Boston, Massachusetts), respectively. Stock solutions containing 3000 and 7800 becquerel (Bq)/mL for dexetimide and mepyramine, respectively, were prepared in ethanol. Dilutions of the unlabeled compounds were prepared in ethyl acetate/methanol, 1:1 (v/v), giving concentrations of 2 mg/mL and 20 μg/mL.

The Empore™ C-8 25-mm extraction disks were a gift of Analytichem International (Harbor City, California).

The organic solvents were of analytical grade (Merck, Darmstadt, Germany).

Methods

For the filtration of samples through the extraction disks, a vacuum filtration device and a 25-mm stainless steel filter holder were used (Schleicher and Schuell, Dassel, Germany). The radioactivity in the samples was determined after the residues of the organic extracts were dissolved in 6 mL of RiaLuma (Lumac, Olen, Belgium) in 20-mL glass counting vials (Packard, Groningen, The Netherlands) by means of a Beckman LS 1800 liquid scintillation counter (Irvine, California). The samples were counted for 5 min or 10 000 counts, whichever came first.

A Hewlett-Packard (Avondale, Pennsylvania) Model 8550 gas chromatograph, equipped with a flame ionization detector, split/splitless injector system, and an automatic injector was used. The injector and detector temperature were 275 and 310°C, respectively. A Scientific Glass Engineering (Victoria, Australia) DB-1 0.53-mm internal-diameter capillary column with a length of 25 m and a film thickness of 3.0 μm was used. The flow rate of the helium carrier gas was 9 mL/min and the temperature program was as follows: 4 min at 135°C, 13°C/min to 200°C, 8°C/min to 240°C, 6°C/min to 312°C; and 6-min hold at the final temperature.

Procedure

An Empore extraction disk was placed in the filter holder on top of the filtration device under vacuum. The disks were preconditioned by being washed with 10 mL of methanol and 10 mL of water under partial vacuum (approximately 3.4×10^{-2} bar). Then, 10 mL of urine, adjusted to pH 7.0 with 1*N* sodium hydroxide (NaOH) and diluted with methanol (final methanol concentration, 5%) was pipetted onto the disk and filtered. During these steps the sorbent must not run dry. The filter was washed with 2 mL of 20% methanol/water (v/v). The disk was dried for 5 min under full vacuum; then 5 mL of hexane was filtered, followed by 5 min of full vacuum. The outlet of the filtration device was dried with tissue and an evaporation tube was placed below the outlet. The disks were then eluted with two 1.5-mL volumes of methanol under partial vacuum (0.5 mL/min). The eluates were evaporated under nitrogen in a water bath at 40°C. The residues were dissolved in 100 μL of ethyl acetate spiked with 200 μg/mL of the chromatographic standards prazepam and metharbital. One to four microlitres were injected into the gas chromatograph for quantitative analysis.

Recoveries

Calibration curves were prepared with standard solutions of each drug. The concentrations were 107, 159, 190, 214, 242, 262, and 321 μg/mL ethyl acetate. The concentration of prazepam and metharbital (chromatographic standards) was 214 μg/mL.

The peak-height ratios of the drug to the chromatographic standards were calculated, and the calibration curves were obtained from least-squares linear regression. The regression lines were used for the calculation of recoveries of the test drugs from urine.

Results

The drugs investigated in this study represent various physicochemical classes and demonstrate a large variation in the properties of compounds with toxicological relevance. The drugs dexetimide and mepyramine were chosen for reasons of convenience, as they were available in tritiated form and provided valuable information on the individual steps of the sample preparation procedure. The chemical structures of the substances are shown in Fig. 1.

In the evaluation of matrix effects and of the capacity of the extraction disks, losses of the drugs were quantified and expressed as a percentage of the amount of radioactivity pipetted on the disks. After loading the disks with 5 mL of water or urine containing 200 Bq/mL of radiolabeled dexetimide or mepyramine in the absence or presence of 0.1 and 10 μg/mL of the unlabeled compounds, the effect of washing the disks with the blank sample matrix (up to 50 mL) was determined. Virtually no losses of radioactivity could be observed. Then the capacity of the extraction disks was determined by subsequent filtrations of 5-mL urine or water sample aliquots spiked with 20 Bq/mL of the radiolabeled dexetimide in the presence or absence of 0.1 and 10 μg/mL of the unlabeled drug. After filtration of more than 30 mL of water with the highest dexetimide concentration, the first small losses were observed, but they were below 5%. However, when urine was used as the matrix, the filtration time increased exponentially with the volume, so that not more than 35 mL of urine could be processed within 30 min. No losses of dexetimide were observed when 35 mL of urine was processed.

For mepyramine, the experimental conditions were adjusted, in that the concentrations were doubled so that the sorbent capacity might be saturated after processing smaller volumes: 40 Bq/mL of radiolabeled mepyramine in the presence or absence of 0.2 and 20 μg/mL of the unlabeled drug. When more than 10 mL of the aqueous solution with the highest concentration was filtered, increasing amounts of the drug passed through

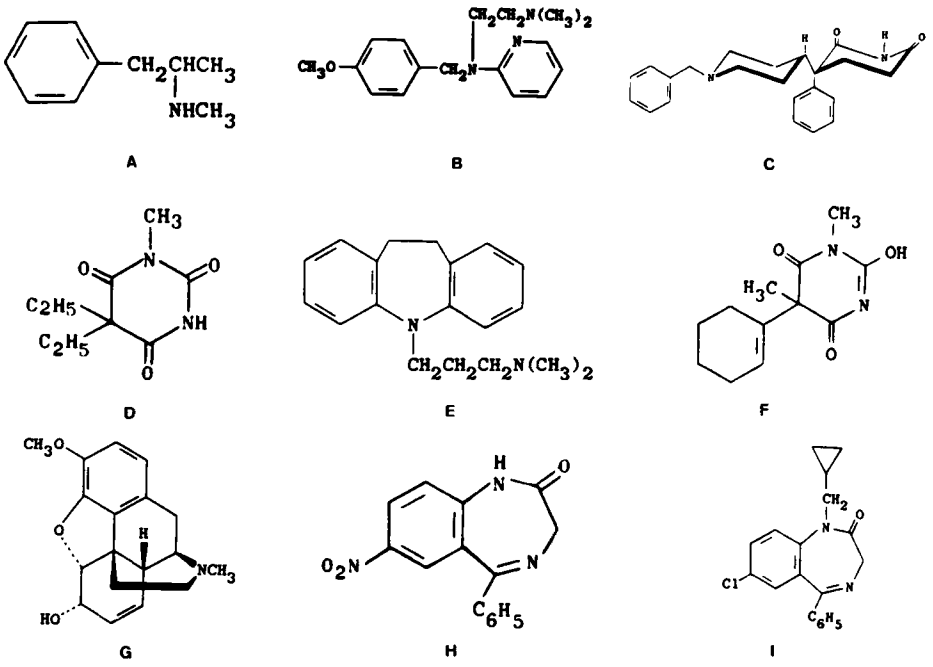

FIG. 1—*Chemical structures of the tested drugs and chromatographic standards:* a = *metamphetamine;* b = *mepyramine;* c = *dexetimide;* d = *metharbital;* e = *imipramine;* f = *hexobarbital;* g = *codeine;* h = *nitrazepam; and* i = *prazepam.*

the column. However, when urine was used, the losses were only 2 to 4% for volumes up to 25 mL. The results of the capacity experiments with mepyramine in water and urine are shown in Fig. 2. These data for the two drugs indicate that the capacity of these extraction disks exceeds 250 μg of the tested drugs when present in urine, which seems adequate for identification and semiquantitative determination procedures in toxicological analysis.

A second step to be evaluated was the elution of the drugs from the extraction disks. Ideally, elution is to be achieved with a small volume of organic solvent which can be easily evaporated with minimal interference from endogenous compounds. The disks were loaded with 25 mL of urine spiked with 1000 Bq of the radiolabeled compounds and 250 μg of the unlabeled analogs. The drugs were eluted with ten 1-mL volumes of either hexane, ethyl acetate, methylene chloride, or methanol. The ten fractions were collected and counted. Hexane eluted less than 1% of the drugs but removed the remaining water from the disks. The latter was considered advantageous for the speed of evaporation of methanol extracts and was incorporated in the final procedure. Over 90% of the dexetimide could be eluted with about 5 mL of methylene chloride; only 70% of the mepyramine could be eluted with 10 mL of methylene chloride. When the disks were eluted with ethyl acetate, 6 mL was required for complete elution of dexetimide, whereas 80% of the mepyramine was eluted with 10 mL of this solvent. Both drugs were eluted completely with only 3 mL of methanol. However, it is not surprising that many endogenous compounds are coeluted with methanol. In order to remove a substantial part of these endogenous compounds prior to elution, the use of a washing step was evaluated. After the extraction disks were loaded with urine, they were either washed with 5 times 1 mL of water, 20% methanol, or 40% methanol. The result was that 2 mL of 20%

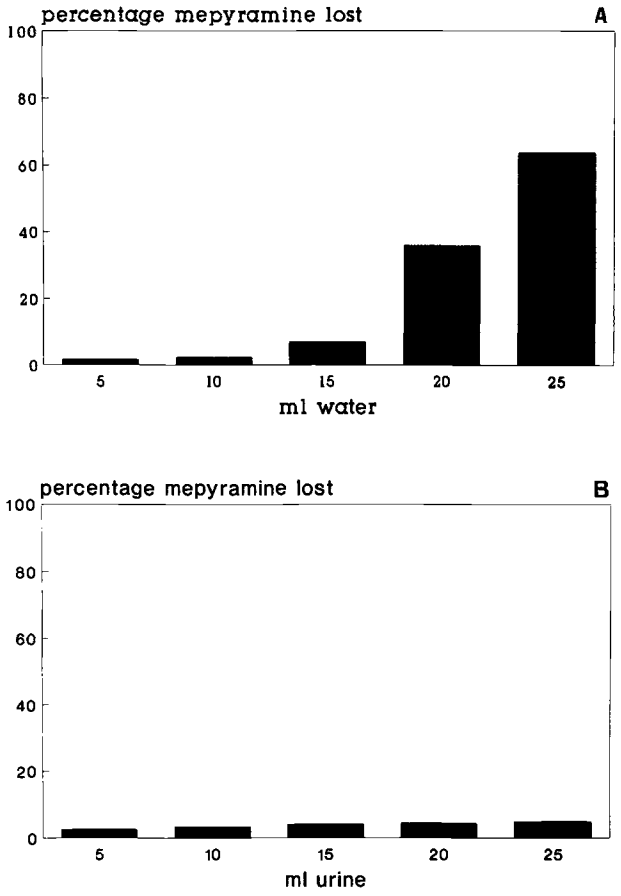

FIG. 2—*The capacity of the extraction disks tested with subsequent filtrations of 5-mL aliquots of water* (A) *or urine* (B) *spiked with 40 Bq/mL of* ^{3}H*-mepyramine and 20 μg/mL of mepyramine. Losses were calculated relative to each aliquot and expressed as a percentage of the applied amount of radioactivity.*

methanol removed most (colored) endogenous compounds without negatively affecting the recovery of the two drugs.

These experiments led to the following procedure: An Empore extraction disk was placed in the filter holder on top of the filtration device under vacuum. The disks were preconditioned by washing with 10 mL of methanol and 10 mL of water under partial vacuum (approximately 3.4×10^{-2} bar). Then 10 mL of the urine sample, adjusted to pH 7.0 with 1*N* NaOH and mixed with 0.5 mL of methanol was pipetted onto the disk and filtered. During these steps the sorbent must not run dry. The filter was washed with 2 mL of 20% methanol/water (v/v). The disk was dried for 5 min under full vacuum; then 5 mL of hexane was filtered, followed by 5 min of full vacuum. The outlet of the filtration device was dried with tissue and an evaporation tube was placed below the outlet. The disks were then eluted with two 1.5-mL volumes of methanol under partial vacuum (0.5 mL/min). The eluates were evaporated under nitrogen in a water bath at 40°C. The residues were dissolved in 100 μL of ethyl acetate spiked with 200 μg/mL of the chromatographic standards prazepam and metharbital. One to four microlitres were injected into the gas chromatograph for quantitative analysis.

This procedure was further evaluated with a test mixture of methamphetamine hydrochloride (HCl), imipramine, nitrazepam, hexobarbital, and codeine HCl. Urine was spiked with this test mixture and extracted with the Empore disks. A second chromatographic standard, prazepam, was applied for nitrazepam because of the large difference in the retention index of the latter compound in comparison with the other test compounds.

Evaporation of the extract to complete dryness resulted in cleaner chromatograms in comparison with evaporation, after the addition of the chromatographic standards, to about 100 μL of the organic extract, although a slight reduction in recovery for more volatile compounds is anticipated.

A representative chromatogram for the entire procedure with 50 μg of each of the test substances in 25 mL of urine is shown in Fig. 3. Recoveries of the individual compounds were calculated with the individual calibration curves and are presented in Table 1. These data are indicative of the potential of the extraction disks, although the number of observations is limited. In general, recoveries of at least 80% are considered acceptable for toxicological screening. This criterion is not fully met for codeine and methamphetamine. Nevertheless, one should take into account the large volume of urine that can be processed.

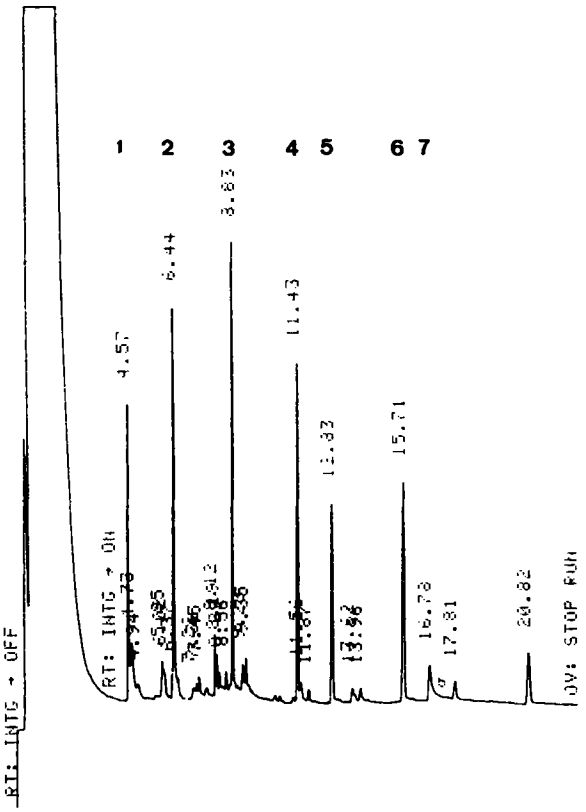

FIG. 3—*Chromatogram of urine spiked with the test drugs; the concentration of each drug was 2 μg/mL. The peaks are as follows: 1 = metamphetamine; 2 = metharbital; 3 = hexobarbital; 4 = imipramine; 5 = codeine; 6 = prazepam; 7 = nitrazepam. The chromatographic standard metharbital was applied for Peaks 1, 3, 4, and 5 and prazepam for Peak 7.*

TABLE 1—*Recovery (N = 2) of drugs after extraction from urine by means of Empore C-8 extraction disks.*

Drug	Mean Recovery, %
Methamphetamine	69.0 ± 1.1
Hexobarbital	95.6 ± 5.5
Imipramine	96.6 ± 9.9
Codeine	76.0 ± 3.9
Nitrazepam	89.1 ± 1.0

Conclusions

In this preliminary study, the potential application of extraction disks for toxicological analysis has been studied. The disks are able to retain various drugs almost completely from large volumes of urine. Cleanup with water/methanol mixtures is equally effective, as has been previously observed for C-8 extraction columns (unpublished results). Elution of the tested drugs can be achieved with methanol. Further experiments, also with different sorbents, should be directed to a larger number of drugs at lower concentrations to prove the expected applicability of these extraction disks for toxicological analysis.

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