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Modifications of the XAD-2 resin column method for the extraction of drugs of abuse from human urine

The present requirement for rapid analytical methods for the detection and identification of drugs subject to abuse from biological materials has resulted in the development of mass screening techniques utilizing styrene divinylbenzene copolymers (primarily Amberlite XAD-2 as the adsorbent resin)[1–5]. As a result of a continuing research effort to evaluate and improve the use of polymeric adsorbents for the analysis of psychoactive drugs, it now appears that certain conditions must prevail to obtain optimal results with these resins. This communication contains modifications of the XAD-2 method previously published[3] including the results of studies on hydration and aging the resin, the use of other nonionic resins, the effect of urinary pH, and the stability of drugs adsorbed on the XAD-2 resin over various time periods.

Materials and methods

The urinalysis laboratory requires a 50 ml urine sample from which a 2 ml aliquot is analyzed by the ATS method[6] and a 25 ml aliquot analyzed by the XAD-2 resin column method[3]. Whenever the ATS results are positive, a 15 ml aliquot of urine is subjected to acid hydrolysis and analyzed by TLC[7] for confirmation.

Resins. The Amberlite XAD-2 resin (20–50 mesh) was obtained from Rohm and Haas Co., Philadelphia, Pa. and treated as described previously[3]. The XAD-2 resin was stored for a period of 7 to 14 days under water in the refrigerator and washed twice with one bed volume of distilled water prior to being transferred to the columns for subsequent urine analysis.

The effectiveness of recently developed resins, BRX-SM-1, -2, and -4 obtained from Bio-Rad laboratories, Richmond, Calif. and Porapak Type Q (50–80 mesh) obtained from Waters Assoc., Inc., Framingham, Mass., were compared with the XAD-2 resin. The original washing procedure[3] was followed except for the washing of the Porapak Type Q resin where the resin was poured into the column after the last methanol wash and then 100 ml of distilled water was poured through the column.

Columns. The polypropylene columns (135 × 10 mm) without flow regulator were obtained from the Whale Scientific Co., Denver, Colo. The columns were plugged with a circular piece of "Zitex" porous Teflon®, made by Chemplast Inc., Wayne, N.J. A $^{5}/_{16}$ in. punch obtained from P. J. Mieth Co., Point Pleasant, N.J. was used to produce the required size pieces from a sheet of the Teflon®. The Teflon® sheet was washed with chloroform before the pieces were punched out in order to remove an impurity which interfered with the TLC procedure. An aqueous slurry of the XAD-2 resin was poured into the column to provide a final quantity of about 1.8 g of dry resin. The columns were placed in distilled water until transferred to the metal troughs prior to analysis.

Procedure. The columns containing the XAD-2 resin were transferred to the hydraulic flow control apparatus (HFCA) and partially submerged in water while 25 ml of urine was poured into each of the columns and extracted as described previously[3], except for the following changes: (1) the drugs were eluted from the resin

by application of two 10 ml aliquots of chloroform–isopropanol (3:1); (2) four drops (about 200 μl) of 6 N HCl in methanol were added to each tube and the organic solvent evaporated by heating in a water bath set at 80° under a stream of air.

Results and discussion

Effective modifications. Minor modifications of the original procedure have provided an appreciable increase in the recovery of representative drugs from urine. The modifications, tested independently, were: (1) an increase in the hydration of the resin; (2) an improvement in column packing technique; (3) an increase in resin bed volume; and (4) an increase in the volume of the eluting solvent.

Drugs with relatively poor recoveries ranging from 56 to 65% with the original procedure were now recovered to the extent of 84–86% using the modified techniques (see Table I). The total recovery of phenobarbital was also increased from 83 to 96%. Other drugs which provided high recoveries (88–91%) with the original procedure, *e.g.*, caffeine, cocaine, meperidine and pentobarbital, would in all probability be almost completely extracted with the modified technique.

TABLE I

COMPARISON OF THE PERCENTAGE RECOVERY OF DRUGS FROM URINE IN THE ORIGINAL AND MODIFIED PROCEDURE OF THE XAD-2 RESIN METHOD

Drugs	*Concentration in urine (µg/ml)*	*Recovery (%)*	
		Original procedure (ref. 3) ±S.D.	*Modified procedure ±S.D.*
[^{14}C]Amphetamine[a]	1.0	65.0 ± 3.2[a]	84.0 ± 4.7
[^{14}C]Morphine	1.25	64.0 ± 6.5	84.0 ± 5.6
[^{3}H]Methadone	10.4	55.6 ± 2.4	86.3 ± 4.2
[^{14}C]Phenobarbital	2.0	83.1 ± 2.8	95.7 ± 2.8

[a] The recovery percentage reported for amphetamine (49.3) in the original procedure[3] was low primarily because of impurities in the tritiated product.

Washing and storage procedures. Storage of the resin under distilled water, following the washing steps with the organic solvents, allows for an increased hydration of the resin and drug recovery is enhanced. The total recoveries for morphine and phenobarbital increased 20 and 12.6%, respectively, when the hydrated-stored resin was compared to the freshly washed resin. The resin must be washed once or twice with distilled water prior to use after hydration storage. This prevents the appearance of a viscous material at the solvent front of the TLC plates following development.

Column preparation. Packing techniques may effect the amount of resin loaded in the column, the uniformity of the resin bed, and the flow rate of the eluting solvents. All of these factors effect the recovery of the drugs. The porous Teflon® plug "Zitex", now used at the bottom of each column in place of glass wool, was selected after a series of unsuccessful experiments with cotton, polyester and nylon wool. "Zitex" Teflon® avoided the difficulty of handling glass wool and further helped to provide a consistently uniform column.

Effect of urinary pH. The urine samples were adjusted to pH 8–9 with 10%

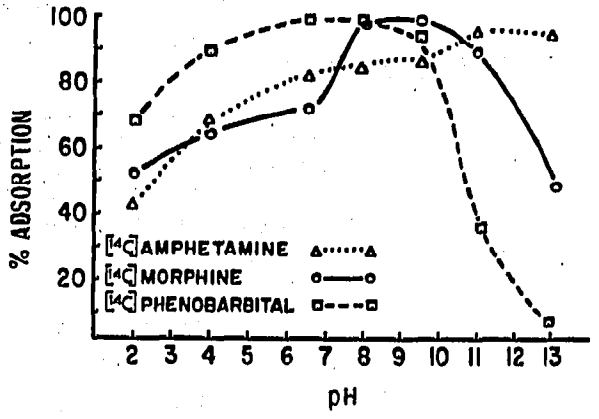

Fig. 1. Effect of urinary pH on the adsorption of drugs on the XAD-2 resin. Twenty-five ml of urine containing 35 μg of [^{14}C]amphetamine, 31 μg of [^{14}C]morphine and 50 μg of [^{14}C]phenobarbital were passed through the XAD-2 resin columns and extracted as described previously[8]. The percentage of drug adsorbed on the column was determined by adding the quantity eluted to the quantity remaining on the column following elution. Each value is the mean of three experiments.

NaOH prior to resin extraction and thus provided purer extracts and better adsorption of basic drugs on the resin. Morphine (see Fig. 1) was adsorbed most efficiently between pH 8 and 10, while the increase in adsorption of amphetamine between pH 6 and 10 was not significant. Phenobarbital was most effectively adsorbed between pH 4 and 9, with a subsequent significant decrease at higher pH values. Making the urine alkaline did not seem worthwhile due to an increase in the effort involved, and the probable loss of some drugs that are relatively unstable in an alkaline medium, *e.g.*, glutethimide (Doriden) and methylphenidate (Ritalin). However, should the urinary pH be very acidic (4.0–5.0) a significant loss of basic drugs would occur.

Comparison of various resins. The XAD-2 resin was compared with several recently developed Bio-Rad resins and with Porapak Type Q resin. The Porapak resin is used in gas chromatographic columns and recently has found use in the extraction of amino acids from urine[8,9]. In Table II appears the recovery data obtained using several representative drugs and the different resins, as well as the physical characteristics of the resins. The recovery of [^{3}H]methadone was somewhat similar for each resin, with a recovery range from 45.4 to 56%. [^{14}C]morphine and [^{14}C]phenobarbital were more effectively recovered using the Porapak Type Q resin then with the XAD-2 or BRX-SM resins, but the handling characteristics and the cost of the Porapak resin make it unsuitable for general use. The BRX-SM-2 resin and SM-4 resin were similar to the XAD-2 resin and the SM-1 resin was distinctly inferior. These results confirm other observations[10] that although the capacity of the columns for the adsorption of compounds increases by increasing the surface area of the adsorbent, no direct relationship exists between these two parameters. However, the rate of elution of the column with organic solvents is inversely proportional to both the surface area and the mesh size of the resin. Thus, all factors considered, none of these resins were fully superior to the XAD-2 resin.

Stability of drugs adsorbed on the XAD-2 resin. The possibility of sending the resin rather than the urine itself to a central laboratory, or using the same resin for

TABLE II

PHYSICAL CHARACTERISTICS OF RESINS AND RECOVERY DATA OF SOME DRUGS

Characteristics	*Physical properties*				
	SM-1	*SM-2*	*SM-4*	*Porapak Q*	*XAD-2*
Weight of the resin (g)	1.33	1.33	0.96	0.78	1.10
Mesh size	20–50	20–50	20–50	50–80	20–50
Surface area (m^2/g)	100	330	750	600	330
Average pore diameter (Å)	200	90	50	75	90
Flow rate of urine (min/25 ml)	20	20	20	20	20
Flow rate of chloroform-Isopropanol (3:1) (min/15 ml)	50	30	16	30–50	6–10
Handling characteristics	fair	good	good	poor	good
Drug	*Percentage recoveries*[a] ± *S.D.*				
	SM-1	*SM-2*	*SM-4*	*Porapak Q*	*XAD-2*
[^{3}H]Methadone	56.5 ± 2.2	44.9 ± 6.4	45.4 ± 1.2	48.8 ± 1.4	55.6 ± 0.1 (86.3 ± 4.2)[b]
[^{14}C]Morphine	28.1 ± 1.1	73.4 ± 1.7	72.8 ± 4.9	96.7 ± 0.8	64.0 ± 6.5 (84.0 ± 5.6)
[^{14}C]Phenobarbital	65.1 ± 3.6	97.9 ± 0.5	95.7 ± 0.8	95.7 ± 0.8	83.1 ± 2.8 (95.7 ± 2.8)

[a] The data was obtained with the original procedure reported previously[3].
[b] Recovery data obtained with the modifications described under *Methods*.

sequential extractions of the urines over a period of weeks may be desirable. However, one must assume that the drugs will remain stable on the resin over various periods of time. In order to test the effectiveness of this technique, commercially available columns (Brinkmann Product Bulletin No. 141-B, 5/71) were used. Briefly, 20 ml of urine containing radioactively labeled cocaine, morphine, amphetamine and phenobarbital and 2 ml of pH 9.5 buffer (satd. NH_4Cl solution plus aq. NH_3) were passed through the Brinkmann XAD-2 columns (5–10 min). The columns were washed with 20 ml of distilled water and then disconnected from the sample reservoir, capped and maintained in the refrigerator (4°) for 0, 4 and 10 days. Following each time period, each column was eluted with 15 ml of dichlorethylene–ethyl acetate (4:6). A drop of 0.1 *N* HCl was added to the solvent and the solvent evaporated to dryness on a Fisher slide warmer at 65° and the radioactivity estimated as described previously[3]. Cocaine remained stable during the 10 day storage period. After 4 days the recovery of morphine was the same, but the recovery of amphetamine and phenobarbital decreased to 80 and 52%, respectively, with regard to the 0 day 100% reference values. After 10 days storage, the relative recovery values for morphine and amphetamine dropped to 56 and 84%, respectively, with apparently an increase in the recovery of phenobarbital.

Additional studies were conducted with non-labeled drugs adsorbed on the XAD-2 resin and also with drugs allowed to age in urine for periods of 4 to 20 days at 4° and room temperature. No apparent loss was observed with methadone, chlorpromazine, meperidine and quinine whether adsorbed on the XAD-2 resin or ex-

tracted from urine (after 20 days). Phenobarbital, morphine, codeine, methamphetamine and pentobarbital showed reductions after 20 days whether stored on the resin or allowed to age in urine. The loss was somewhat less for the refrigerated samples in comparison to those stored at room temperature. Greater losses were observed for codeine and morphine after 20 days in the urine samples as compared to the results with the resin. The opposite was observed for methamphetamine, amphetamine, phenobarbital and pentobarbital, *i.e.*, greater losses with the resin in comparison to storage in urine.

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