Studies on the Use of XAD-2 Resin for Detection of Abused Drugs in Urine

M. Peter Kullberg and Charles W. Gorodetzky

We describe a procedure for extracting weakly acidic, neutral, and basic drugs from urine by using a column of XAD-2 resin. Adsorption of drugs from 20 ml of urine buffered at pH 8.5 ± 0.5 at a controlled flow rate of 2.5 ml/min was greater than 89% for all drugs tested except aspirin. On eluting the drugs tested with acetone and methanol/chloroform, recoveries ranged from 75 to 93%. Overall recoveries of drugs from urine to a thin-layer chromatography plate were between 63 and 78%. The concentration of morphine added to normal urine that can be detected 99% of the time (95% confidence limits) by this method was 80 (65–100) μg/liter. We evaluated three methods for recovering morphine from morphine glucuronide added to urine, by using appropriate modifications of the XAD-2 resin extraction method. Hydrolysis of urine, hydrolysis of urine extracts adsorbed on XAD-2 resin, and hydrolysis of urine extracts from the XAD-2 resin followed by a solvent extraction gave 75%, 40%, and 10% recoveries of morphine, respectively.

Additional Keyphrases: toxicology • thin-layer chromatography • liquid/liquid extraction • drug concentration before analysis • morphine and its conjugated metabolites

Use of XAD-2 resin to extract drugs from urine is becoming increasingly popular in screening for drugs of abuse (1-7). The first two reports in this series of studies (1, 2) described the characteristics and control of variables for efficient drug extraction with XAD-2 resin. In this report, we describe its use for removing a variety of drugs from urine (adsorption) and then eluting them from the resin into an organic solvent. Our object was to further investigate how effectively the resin adsorbs various drugs from urine at a single pH and flow rate, and to find a solvent that would elute them from the resin together with a minimal amount of the naturally occurring urinary compounds. Such a preliminary treatment of specimens would facilitate identification of drugs and drug metabolites by thin-layer chromatography. Specifically, we were interested in a sensitivity study to determine the amount of morphine detectable on a thin-layer plate after such an extraction from urine with the XAD-2 resin. Finally, we investigated such methods for recovery of morphine from morphine conjugates in urine, since most of the morphine excreted by man is in conjugated form not detectable by commonly used extraction and thin-layer chromatographic methods (8).

Materials

Apparatus

The controlled-flow apparatus used has been described (1). A two-speed Proportioning Pump II (Technicon Instruments Corp., Tarrytown, N. Y. 10591), capable of controlling the flow through 23 tubes simultaneously, was used to control the flow of urine through pre-packed 5.5 × 1.0 cm (bed dimensions) columns containing about 2.1 g of XAD-2 resin (Brinkman Instrument Co., Westbury, N. Y. 11590). Solvaflex tubing (0.090 inches i.d.) was obtained from Technicon Instruments Corp. Scintillation counting was done in a “Tri-Carb Scintillation Spectrometer,” Model 3003 (Packard Instrument Co., Downers Grove, Ill. 60515).

Radioactive Compounds

The following compounds were purchased from Mallinckrodt Chemical Works, St. Louis, Mo. 63160: acetyl salicylic acid-(carboxyl-14C) (1.5 Ci/mol); amphetamine (propyl-1-14C) sulfate (6.02 Ci/mol); amobarbital-2-14C (5.03 Ci/mol); caffeine-1-methyl-14C (5.43 Ci/mol); cocaine-(methoxy-14C) (4.20 Ci/mol); meperidine-(N-methyl-14C) hydrochloride (1.51 Ci/mol); meptohamate-(carbonyl-14C) (6.00 Ci/mol); mescaline-8-14C hydrochloride (2.19 Ci/mol); methadone-(heptanone-2-14C) hydrochloride

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(4.22 Ci/mol); nalorphine-(allyl-1,3-14C) hydrochloride (8.87 Ci/mol); naloxone-(allyl-1,3-14C) hydrochloride (8.51 Ci/mol); pentobarbital-2-14C (6.79 Ci/mol); secobarbital-2-14C (10.00 Ci/mol). Codeine-(N-methyl-14C) hydrochloride (56 Ci/mol) was obtained from Amersham/Searle Corp., Arlington Heights, Ill. 60005.

Reagents

\( \text{NH}_4\text{Cl} \) buffer. Add concentrated ammonia water (NH\(_4\)OH; 28% NH\(_3\)) to a saturated solution of NH\(_4\)Cl in distilled water until the pH is 10.0.

Dilute \( \text{NH}_4\text{Cl} \) buffer. Dilute the \( \text{NH}_4\text{Cl} \) buffer 10-fold with distilled water.

Modified Bray's scintillation fluid. Dissolve 240 g of recrystallized naphthalene (Eastman Kodak Co., Rochester, N. Y. 14650) in "Spectrograde" p-dioxane (Matheson, Coleman and Bell, Division Matheson Co., Inc., Norwood, Ohio 45212) to make 2 liters of solution. Make another solution with 32 g of "Omni-fluor" (New England Nuclear Corp., Boston, Mass. 02118), 400 ml of reagent-grade methanol (Fisher Scientific Co., Fairlawn, N. J. 07410), 80 ml of ethylene glycol (reagent-grade; J. T. Baker Chemical Co., Phillipsburg, N. J. 08865) and sufficient p-dioxane to make 2 liters of solution. Mix the 2-liter solutions and store in the dark at 4 °C.

Methods

Thin-Layer Chromatography

For all thin-layer chromatography we used "Quantum QLD" thin-layer plates, pre-scored into 19 columns, by the manufacturer (Quantum Industries, Fairfield, N. J. 07006). Develop the plates in a saturated tank containing ethyl acetate/methanol/ammonium hydroxide (180:17:7 vol) until the solvent front ascends about 12 cm. Dry the plate with a hair dryer for 15 min and place it for 5 min in an oven set at 100 °C. Remove the plate, place it in a spraying box and spray with iodoplatinate spray prepared by the method of Mulé (9). Allow the plate to air-dry for about 2 h before reading. Morphine appears as a distinct blue spot with an \( R_f \) of 0.25.

Drug-Extraction Efficiency Studies

Collect, pool, and store normal urine at 4 °C. “Normal” urine is defined as urine from subjects in good health and known not to have taken any drugs for at least one month.

Wet the pre-packed Amberlite XAD-2 resin columns with about 10 ml of the dilute \( \text{NH}_4\text{Cl} \) buffer about 5 min before use. Discard any columns that are not completely wetted or do not pass a continuous column of water through the resin column.

For each drug investigated, prepare six 20-ml urine specimens. To each 20-ml urine aliquot, add 40 \( \mu \)g of nonradioactive drug (2 \( \mu \)g/\( \mu \)l), 100 \( \mu \)l of radioactive drug (1 \( \times \) 10\(^{6}\) dpm/100 \( \mu \)l), and 1 ml of \( \text{NH}_4\text{Cl} \) buffer to adjust the final urinary pH to 8.5 to 9.0, as read with Brinkmann "Neutralite" (pH 5–10) indicator sticks (EM Reagents Division, Brinkmann Instruments Inc.). Place the XAD-2 resin columns into the controlled-flow apparatus and pour the urine into the top of the column. Start the flow pump and allow the urine to pass through the tubing at 2.5 ml/min (as regulated by the 0.090 inch i.d. tubing) into a 50-ml volumetric flask in the rack on the other side of the pump. Add 15 ml of the dilute \( \text{NH}_4\text{Cl} \) buffer to the resin columns and allow it to pass through the resin column at 2.5 ml/min into the same 50-ml volumetric flask containing the urine. Remove each volumetric flask from the rack and dilute the contents to 50 ml with distilled water. Stopper and invert the flask to obtain good mixing, and then pipet 1 ml of the contents into a counting vial. Add 10 ml of the modified Bray's scintillation fluid to the vial, cap it, and place it in the scintillation counter for 30 min before counting. These counts represent the amount of radioactive drug not adsorbed from the urine by the resin. Counting efficiency was about 75%; all count rates were corrected for quenching by the channel-ratio method. Place three 100-\( \mu \)l aliquots of each radioactive drug in counting vials with 10 ml of counting solution. Take the average of the three figures for dpm obtained for these samples as the amount of radioactivity initially added to each urine sample (i.e., 100%).

Remove the XAD-2 resin columns from the controlled-flow apparatus and suspend each one over a 25-ml graduated cylinder containing 0.5 ml of the dilute \( \text{NH}_4\text{Cl} \) buffer. Replace the two cotton plugs from the top of the column with a small glass-wool plug. Add 1 ml of acetonitrile (AR grade, Mallinckrodt Chemical Works) to the top of each column and allow it to pass through the column for about 2 min. Then add 20 ml of methanol/chloroform (1:3, by vol) to the top of each column, and force about 2 ml of the solvent through the column with a slight air pressure exerted through glass tubing in a wide rubber stopper that is slightly larger than the column reservoir cups. In this way, the water and air trapped on the column are eliminated, and the remainder of the solvent always almost always passes through the column.

Record the volumes of the organic and aqueous phases in the graduated cylinder. Pipet a 1-ml aliquot of the aqueous phase into a counting vial, add to it 10 ml of the scintillation fluid, and count the radioactivity. These counts represent the amount of drug that back-extracts into the aqueous phase. Then aspirate and discard the remainder of the aqueous phase. Add methanol to the organic phase to bring the volume to 20 ml, and transfer the solution to a test tube in which the organic phase will be taken to dryness. Pipet a 1-ml aliquot of the organic phase into a counting vial, add 10 ml of scintillation fluid to it, and count the radioactivity. These counts represent the amount of drug desorbed from the resin by the solvent. Place the test tubes with the organic eluate into a water bath set at 65 to 70 °C, and evaporate under a stream of air. When the solvent
has evaporated, wash down the inside of the test tube with a thin stream of methanol and again evaporate to dryness in the water bath. Dissolve the residue in 70 μl of methanol and transfer it in 20- to 30-μl aliquots with a Pasteur pipet into a counting vial (in a manner similar to transferring the residue to a thin-layer plate). Add 10 ml of scintillation fluid to the counting vial, and count the radioactivity. These counts represent 95% of the drug that would ultimately reach a thin-layer plate for analysis (i.e., because of the 1-ml aliquot removed before evaporation). It includes all losses caused by transfer or adsorption onto glass.

Sensitivity of the Procedure for Detecting Morphine in Urine

To determine sensitivity, add nonlabeled morphine to normal urine (buffered at pH 8.5 with the NH₄Cl buffer) in concentrations of 40, 45, 50, 55, 60, 65, and 70 μg of morphine free base per liter. Make enough urine solution for at least 20-ml samples of each concentration. Randomize and code these samples, as well as 10 blank urine controls.

Prepare the XAD-2 resin columns for use as previously described and pass the urine through the resin columns at 2.5 ml/min, discarding the effluent. Wash the columns with 10 ml of the dilute NH₄Cl buffer, and discard the effluent. Remove the first cotton plug from the column, and place the resin column over a test tube containing 0.5 ml of dilute NH₄Cl buffer (as a buffer to prevent back-extraction) for collecting the organic effluent. Add 1 ml of acetone to each column, followed about 2 min later by 20 ml of methanol/chloroform (1:3, by vol). Force 2 to 3 ml of the organic solvent through the column by air pressure applied to the top of the column, and allow the rest of the solvent to drip through the column under the force of gravity. Aspirate and discard the aqueous phase. Place the test tubes in a water bath, and evaporate to dryness. Wash down the sides of the test tubes with a thin stream of methanol and evaporate the methanol to dryness. Dissolve the residue in 70 μl of methanol, and apply the residue to a thin-layer plate in 20- to 30-μl aliquots with an air stream blowing across the plate to hasten evaporation of the solvent. Develop and spray the plates as already described above. For each concentration of morphine, determine the ratio of the number of spots detected to the number of replicates analyzed, convert to percentage, and plot vs. concentration on log probability paper. Then use the method of Litchfield and Wilcoxon (10) to determine the concentration detectable 99% of the time (defined as IA99), and its 95% confidence limits. This method has been previously described in detail (11, 12).

Gravitational-Flow Adsorption and Elution

Make five 20-ml solutions in urine of labeled and nonlabeled morphine, methadone, pentobarbital, amphetamine, or naloxone as described above under “Drug Extraction Efficiency Studies.” Suspend the previously wetted resin columns over 50-ml volumetric flasks. Add the urine solution to the columns and record the time that elapses between addition of the urine to the column and spontaneous emergence of the last drop from the column under the force of gravity. Then add about 10 ml of the dilute NH₄Cl buffer to the resin columns, which also drips into the 50-ml volumetric flasks. The adsorption efficiency of the resin is determined as previously described.

Remove both cotton plugs from the top of the column, and place the columns over 25-ml graduated cylinders containing 0.5 ml of the dilute NH₄Cl buffer. Add the 20 ml of methanol/chloroform (1:3, by vol) to the resin columns in two 10-ml aliquots about 5 min apart, and allow the effluent to pass through spontaneously. Radioactivity in the aqueous and organic phases is measured as described under “Drug Extraction Efficiency Studies.”

Recovery of Morphine from Morphine Glucuronide

Urine hydrolysis followed by XAD-2 resin extraction. To six 20-ml aliquots of normal urine in 40-ml centrifuge tubes, add 40 μg of authentic morphine glucuronide (obtained from Dr. S. Y. Yeh, NIDA Addiction Research Center, Lexington, Ky. 40507) and 2 ml of concentrated HCl. Stopper the tubes and place them in a steam-jacketed autoclave (Barnstead Still & Sterilizer Co., Boston, Mass. 02132) at 104 kPa (15 lb/in²) and 120 °C for 45 min. Adjust the pH of the cooled hydrolysate to 8.5 ± 0.5 by adding 2 ml of NaOH (12 mol/liter) followed by NH₄Cl buffer. Similarly treat six 20-ml aliquots of normal urine. Then extract the samples with the XAD-2 resin as described already. After the urine concentrates from the six blank urines are spotted on a thin-layer chromatographic plate, add morphine (2, 5, 10, 15, and 20 μg) from a standard solution of morphine free base in methanol (1 g/liter) to one of the blank urine spots to act as standards against which the amount of morphine recovered from the urines containing the morphine glucuronide will be compared. The plates are then developed and sprayed as described above. On reading them, estimate the amount of morphine in each spot of hydrolyzed sample by comparison with the known morphine standards.

Hydrolysis of morphine glucuronide adsorbed on the resin. Add morphine glucuronide (as described above) to six 20-ml aliquots of normal urine, adjust the urine pH to 8.5 ± 0.5 with NH₄Cl buffer, and pass the urine solution through the resin columns at 2.5 ml/min. Remove the resin columns from the pumping rack, remove the two cotton plugs, and use air pressure to force the resin out of the column into a 40-ml centrifuge tube, washing out with distilled water any resin that adheres to the sides of the column. To the total of about 15 ml of water and resin, add 2 ml of concentrated HCl, stopper, and hydrolyze the urine as described above. Similarly treat six blank urines. Adjust the pH of the cooled hydroly-
sate to 8.5 ± 0.5 as described above. Pour the resin and aqueous solution back into the original columns placed in the pumping rack and pass the solution through the resin at 2.5 ml/min. The rest of the procedure is analogous to the procedure used in determining sensitivity. Add morphine to the concentrates of the blank normal urines, to serve as standards against which to judge the recovery of morphine.

**XAD-2 resin extraction of morphine glucuronide followed by hydrolysis and solvent extraction of morphine.** Prepare urine solutions containing morphine glucuronide at pH 8.5 ± 0.5 and pass through the resin columns at 2.5 ml/min, as described above. We investigated three different elution solvents by the elution techniques used in the previous two methods. The three eluting solvents were methanol, methanol/chloroform (3:1, by vol), and methanol/water (1:1, by vol). To each of the eluates in 40-ml centrifuge tubes add 2 ml of concentrated HCl, place the tubes in a water bath, and evaporate the organic phase. Stopper and autoclave the tubes. After adjusting the pH to 8.5 ± 0.5, add 20 ml of methanol/chloroform (1:3, by vol) to the tubes and shake for 30 s. When the phases separate, aspirate the aqueous phase, evaporate the organic phase to dryness, and apply the residue to a thin-layer plate as described above. Add morphine to the concentrates of blank normal urines to serve as standards.

**Results**

**Drug Extraction Efficiency Studies**

The percentages of the drugs adsorbed onto the resin from the aqueous (urinary) phase are shown in the first column of Table 1. Except for aspirin (acetylsalicylic acid) and caffeine, 89% or more of all drugs were adsorbed at the flow rate of 2.5 ml/min.

Table 1 (columns 2 and 3) contains data pertinent to drug desorption. When the organic solvent is passed through the resin column, some water that was in the interstices of the resin and (or) trapped in the cotton plug is liberated. This 3 to 4 ml of aqueous phase (buffered at pH 8.5 ± 0.5), which would normally be discarded, contained 0.1 to 7.0% of total drug. This aqueous phase was also highly colored, indicating that many urinary pigments that would otherwise appear on the chromatographic plate were eliminated from the drug extract.

Column 3 in Table 1 shows the percentages of drugs recovered from the resin in the organic phase. Except for aspirin, 75 to 93% of drug that was in the original urine specimen was recovered in the organic eluate.

Column 4 of Table 1 indicates the percentages of drugs that would ultimately reach a chromatographic plate. We did not try unduly to maximize the efficiency of the aspiration, concentration, and transferring processes, but proceeded just as one might in a routine laboratory. The results show that 10 to 25% of the drug was lost in the steps between recovery of the organic eluate and plating of the residue.

Thus, the overall recoveries of the drugs tested were between 63% and 78% of the drugs in the original urine sample.

**Sensitivity of the Procedure for Morphine**

The data show that morphine could be detected, by the described procedures, 99% of the time (IA99) at 80 ng/ml, with 95% confidence limits of 65 to 100 ng/ml.

**Gravitational-Flow Adsorption and Elution**

This experiment investigated the effects of uncontrolled flow methods on drug adsorption and the effects of batch elution methods on the desorption of drugs from the XAD-2 resin. The data in Table 2, column 1 show that the drug adsorption efficiency was from 1 to 12% less than those reported for the same five drugs in Table 1, column 1. The average time required for the urine to pass through the columns under the force of gravity was about 4.5 min (range, 3.2 to 6.8 min). This is a flow rate of greater than 5 ml/min, twice that used with the controlled-flow apparatus. The adsorption of morphine was most greatly affected by this increased flow rate, its adsorption efficiency being decreased by about 12%.

The significant result from this experiment was the marked decrease in drug recovery of the batch elution process (Table 2, column 3). In this method two 10-ml portions of solvent were used to elute the drug from the resin, and the resin was not restrained in the column, but floated to the top of the solvent. The efficiency of batch elution was decreased over 50% for all five drugs as compared to the method involving the preservation of the resin column (Table 1, column 3).

**Hydrolysis Methods**

Recovery of morphine glucuronide from urine as free morphine was found to be most effective by the traditional method of acid hydrolysis in dilute (1 mol/liter) HCl, followed by the resin extraction of the liberated morphine. As shown in Table 3, the overall estimated recovery of morphine by this method was 75% of the morphine originally present as morphine glucuronide.

The second method that appears in Table 3, acid hydrolysis of the morphine glucuronide adsorbed on the XAD-2 resin, was estimated to be about 40% effective in the recovery of liberated morphine. However, the background or the number of other compounds that appeared on the chromatographic plate from this urine concentrate was significantly less than that appearing on the thin-layer chromatogram of hydrolyzed urine. To test the effectiveness of the resin to adsorb drug before and after hydrolysis, we subjected resin from unused columns to the same hydrolytic conditions and then tested the resin as described for the drug recovery experiments in Table 1. Absorption and elution characteristics of the resin were unchanged by the hydrolytic treatment.

The third method (Table 3) involved the extrac-
Table 1. Percent Recovery of Various Drugs at Different Stages of XAD-2 Resin Extraction

<table>
<thead>
<tr>
<th>Drug Type</th>
<th>Adsorption*</th>
<th>Aqueous effluent*</th>
<th>Organic eluate*</th>
<th>Final recovery*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narcotic analgesics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codeine</td>
<td>95.0 ± 1.4*</td>
<td>1.1 ± 0.2</td>
<td>88.9 ± 2.0</td>
<td>72.0 ± 1.9</td>
</tr>
<tr>
<td>Meperidine</td>
<td>95.8 ± 0.3</td>
<td>0.1 ± 0.0</td>
<td>88.9 ± 1.3</td>
<td>78.0 ± 1.1</td>
</tr>
<tr>
<td>Methadone</td>
<td>99.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>93.2 ± 1.46</td>
<td>78.1 ± 4.5</td>
</tr>
<tr>
<td>Morphine</td>
<td>89.3 ± 0.5</td>
<td>4.9 ± 0.8</td>
<td>85.0 ± 1.2</td>
<td>64.8 ± 3.2</td>
</tr>
<tr>
<td>Narcotic antagonists</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nalorphine</td>
<td>97.7 ± 0.2</td>
<td>3.2 ± 0.3</td>
<td>91.1 ± 1.8</td>
<td>65.4 ± 1.1</td>
</tr>
<tr>
<td>Naloxone</td>
<td>96.0 ± 0.3</td>
<td>5.0 ± 0.3</td>
<td>86.9 ± 1.2</td>
<td>72.7 ± 0.8</td>
</tr>
<tr>
<td>Stimulants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphetamine</td>
<td>95.6 ± 0.4</td>
<td>2.7 ± 0.3</td>
<td>90.5 ± 1.0</td>
<td>63.1 ± 1.3</td>
</tr>
<tr>
<td>Cocaine</td>
<td>97.0 ± 1.4</td>
<td>0.3 ± 0.1</td>
<td>90.6 ± 1.2</td>
<td>74.7 ± 0.9</td>
</tr>
<tr>
<td>Barbiturates and tranquilizers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amobarbital</td>
<td>94.3 ± 0.7</td>
<td>1.9 ± 0.3</td>
<td>89.2 ± 1.6</td>
<td>70.9 ± 1.7</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>94.2 ± 0.8</td>
<td>7.0 ± 0.2</td>
<td>83.8 ± 1.8</td>
<td>67.6 ± 0.4</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>90.6 ± 0.5</td>
<td>1.0 ± 0.1</td>
<td>86.4 ± 1.4</td>
<td>65.4 ± 1.0</td>
</tr>
<tr>
<td>Secobarbital</td>
<td>96.7 ± 0.4</td>
<td>1.9 ± 0.3</td>
<td>92.4 ± 0.4</td>
<td>68.5 ± 1.5</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>19.5 ± 1.9</td>
<td>15.6 ± 2.2</td>
<td>3.0 ± 0.3</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Caffeine</td>
<td>85.6 ± 1.1</td>
<td>2.7 ± 0.6</td>
<td>75.5 ± 1.9</td>
<td>61.7 ± 1.4</td>
</tr>
<tr>
<td>Mescaline</td>
<td>95.5 ± 0.4</td>
<td>6.5 ± 0.9</td>
<td>83.0 ± 1.4</td>
<td>63.7 ± 1.4</td>
</tr>
</tbody>
</table>

* Percent drug adsorbed on the XAD-2 resin (i.e., removed from the urine).
* Percent of drug in the aqueous phase of the effluent from XAD-2 resin (would normally be discarded).
* Percent drug in the organic eluate from XAD-2 resin column.
* Percent drug reaching thin-layer plate.
* Averages for 6 samples ± SEM.

Table 2. Percent Recoveries on Gravitational-Flow Adsorption and Batch Elution

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adsorption*</th>
<th>Aqueous effluent*</th>
<th>Organic eluent*</th>
<th>Adsorption time*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>77.6 ± 1.8*</td>
<td>3.1 ± 0.1</td>
<td>43.2 ± 3.2</td>
<td>5.2 ± 1.1</td>
</tr>
<tr>
<td>Methadone</td>
<td>98.2 ± 4.2</td>
<td>0.1 ± 0.1</td>
<td>27.9 ± 1.2</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>84.5 ± 0.8</td>
<td>1.4 ± 2.0</td>
<td>39.0 ± 2.0</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>90.1 ± 0.5</td>
<td>4.4 ± 0.6</td>
<td>39.5 ± 2.0</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>Naloxone</td>
<td>90.3 ± 1.1</td>
<td>1.0 ± 0.1</td>
<td>46.9 ± 5.9</td>
<td>4.5 ± 0.3</td>
</tr>
</tbody>
</table>

* Percent drug adsorption, cf. column 1, Table 1.
* Percent drug lost in aqueous phase, cf. column 2, Table 1.
* Percent drug elution, amount of drug recovered from the resin, cf. column 3, Table 1.
* Average time (minutes) for 20 ml of urine to pass through the resin column under force of gravity.
* Average of 3 replicates ± SEM.

tion of the morphine glucuronide from urine with the resin, elution of the morphine glucuronide (three different solvent systems), hydrolysis of the organic concentrate, and finally a liquid/liquid extraction with methanol/chloroform. Overall recovery of morphine by any of these methods was about 10%.

Discussion

Adsorption of morphine, amphetamine, and phenobarbital from urine by the XAD-2 resin was found to depend on urine pH, urine flow rate through the resin column, and the urine/resin ratio (1, 2). The adsorption efficiency of the XAD-2 resin for a number of compounds of widely different structures was examined to demonstrate the applicability of the resin to the adsorption of these compounds in a single pass through the resin. The pH of the 20-ml urine sample containing these drugs was 8.5 ± 0.5, and its flow rate through the resin was 2.5 ml/min. The only compound not efficiently adsorbed was acetylsalicylic acid (aspirin), probably because this compound is too acidic to be appreciably un-ionized at pH 8.5. Otherwise weakly acidic compounds (barbiturates), neutral (meprobamate) and amphoteric compounds (morphine), weak bases (tertiary amines) and strong bases (amphetamine and mescaline) were all efficiently adsorbed.

When a controlled-flow apparatus was not used, the gravitational flow rates (Table 2) observed were about twice as fast as the controlled-flow rate. The increase in flow rate is reflected in the decreased adsorption efficiency of the compounds studied. Adsorption efficiency was most decreased for morphine, a compound that has a lower affinity for the resin, and consequently adsorption efficiency for it fluctuates more with flow rate (1).

Factors governing the elution of drugs from the XAD-2 resin include solvent polarity, flow rate of the...
solvent through the column, and the degree of contact of the solvent with the resin beads. Another important consideration is the amount of background in the thin-layer chromatogram resulting when a particular eluate is used. In a previous report (2), a mixture of 25% isopropanol in the originally proposed solvent system of ethyl acetate/dichloroethane (3:2) was required to efficiently desorb amphetamine, a compound readily adsorbed by the resin. In the present studies, the solvent system of methanol/chloroform (1:3, by vol) satisfied the criteria of efficient drug desorption and a low background on the thin-layer chromatogram of the urine extract.

To attain satisfactory solvent flow rates and good contact between the solvent and all the resin beads in the column, we modified the elution procedures. Addition of about 1 ml of acetone to the resin column before the methanol/chloroform greatly facilitated the flow of water, which is displaced from the resin by the solvent and trapped in the cotton plug situated beneath the resin column, through the small opening in the bottom of the column. We also used positive air pressure to force about 3 ml of solvent (mostly water) through the column after the 20 ml of solvent had been added to the reservoirs above the resin column, increased the uniformity in solvent flow through the column, and increased the contact of solvent with the resin beads by forcing out water and air trapped on the resin column after the adsorption step.

The desirability of maintaining the resin column integrity during drug elution, as opposed to the removal of both cotton plugs and allowing the resin to float on top of the solvent in a batch-type elution as described in the Methods, is well documented by comparing elution data in Tables 1 and 2. When the resin is allowed to float on top of the solvent, much of the solvent does not come in contact with the resin, leading to poor drug elution from the resin.

The addition of NH₄Cl buffer to the test tubes that receive the organic eluate from the resin columns was another modification in elution procedures, which was necessary to prevent back extraction of drug from the solvent into the aqueous layer that forms on top of the organic eluate. In early experiments with labeled morphine, pentobarbital, mescaline, and methadone, in which such buffering of the aqueous layer was not used, 40, 25, 36, and 22%, respectively, of the above drugs back-extracted into the aqueous phase, and would have been discarded before concentration. The data in column 2 of Table 1 show that the amount of drug that would be so discarded when the buffering technique is used is negligible. Also, this aqueous phase contains much of the pigmentation present in the organic eluate. Removal of this aqueous phase greatly diminishes the background present in the thin-layer chromatogram, making for easier drug detection on the plate.

Comparison of the data in columns 3 and 4 of Table 1 gives some idea of drug losses that might be realized in seemingly unimportant steps in the procedures after the drug is in the organic phase. Losses of 10 to 25% of drug can be attributed to drug lost in the aspiration of a portion of the organic phase, adsorption to the sides of test tubes and capillaries, and drug not taken up by the capillaries in transferring the residue from the concentration test tubes to the thin-layer chromatographic plate. These losses might then be considered operational losses, as are encountered in any analytical procedure and could be minimized with careful laboratory technique.

One measure of a method's effectiveness was proposed by Gorodetzky (11), sensitivity in this case being defined as the amount of drug in urine that can be seen or detected 99% of the time (IA99) on a thin-layer plate. Previously Gorodetzky has published the sensitivities of two common screening methods for the detection of morphine in urine (12). These methods were an organic solvent extraction with isopropanol/chloroform (1:3, by vol), and a modified Dole method in which an ion-exchange resin paper (Reeve-Angel, No. SA-2) is used. The IA99 of the two methods with 95% confidence limits were 0.19 (0.14–0.25) mg/liter and 0.16 (0.07–0.35) mg/liter, respectively. The sensitivity of the XAD-2 resin extraction followed by thin-layer chromatography was 80 (range, 65–100) μg/liter, about twice as sensitive as the organic solvent method or the ion-exchange resin paper method. This increased sensitivity is most likely the result of two factors, (a) the larger amount of urine (20 ml vs. 15 ml) extracted with the resin as compared to the organic solvent extraction, and (b) an increased extraction efficiency of the resin method over the ion-exchange paper method [65% vs. 48% for the ion-exchange paper (12)].

Recently Yeh (3) has reported results of quantita-
tive measurements of the urinary excretion of morphine. In this study, the acid-hydrolyzable morphine conjugates comprised as much as 73% of the total morphine in the urine of men being chronically administered morphine. Therefore, methods to efficiently recover morphine from the morphine glucuronide in urine with the XAD-2 resin were investigated. In previous reports (1, 2) the recovery of morphine, phenobarbital, and amphetamine from hydrolyzed urine with the XAD-2 resin was decreased from 5 to 15% as compared to unhydrolyzed urine. This evidence, in addition to the appearance of a greater amount of material in the thin-layer chromatogram of a hydrolyzed urine extract, leads us to conclude that the decreased adsorption efficiency might be due to the greater number of extractable organic compounds in hydrolyzed urine. Therefore, we investigated two methods for isolating the morphine glucuronide before hydrolysis. The first involved the hydrolysis of the morphine glucuronide adsorbed onto the resin; the other was to extract the morphine glucuronide with the resin, hydrolyze the glucuronide, and extract the morphine with an organic solvent. Neither method proved to be as good as was acid hydrolysis of the urine followed by the resin extraction of the liberated morphine, which provided an estimated 75% recovery of morphine present in the morphine glucuronide.

The low recovery of morphine by the extraction of morphine glucuronide before hydrolysis conflicts with previous reports in which the morphine glucuronide has been extracted from urine with the XAD-2 resin (14). These data are also contradictory, but in the opposite direction, to a report by Mulé et al. (4), in which essentially no morphine glucuronide was recovered by the XAD-2 resin extraction procedure. The reasons for these discrepancies will be investigated further.

However, one real advantage of the acid hydrolysis of the morphine glucuronide on the resin, versus in the urine, is the low background that is obtained on the thin-layer chromatogram of the urine extract. Presumably many compounds liberated during hydrolysis are not readily adsorbed by the resin in their conjugated form in unhydrolyzed urine. This could make trace quantities of drugs present in a urine concentrate much easier to detect. The balance of these two factors will be determined in a heroin validity experiment currently in progress in our laboratory, in which both methods will be evaluated on identical urine specimens.

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References