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Determination of drugs from urine by on-line immunoaffinity chromatography–high-performance liquid chromatography–mass spectrometry

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ABSTRACT

A method for rapid extraction and identification of drugs in urine is described. The system utilizes a high-performance protein G immunoaffinity column coupled to a reversed-phase analytical column by use of a trapping column and switching valve. A small amount of antibody (5 μg drug-specific) is used for each analysis to extract either propranolol or lysergic acid diethylamide (LSD) from human urine. Urine diluted with phosphate-buffered saline is pumped directly through the protein G column thus eliminating time- and solvent-consuming sample preparation procedures. On-line ultraviolet or mass spectral analysis provides the means of drug detection and identification. With ultraviolet detection propranolol may be detected in spiked urine at the 250 pg/ml level. A Hewlett-Packard mass spectrometer modified for atmospheric pressure ionization and equipped with an ion spray source allows detection of propranolol in urine at 2.5 ng/ml and LSD at 500 pg/ml using single ion monitoring. The potential applicability of the technique for drug confirmations is discussed.

INTRODUCTION

The determination of drugs at trace levels in biological matrices has seen growing demand, especially in the area of forensic and athletic urine drug testing. Generally, preliminary screening tests are performed to detect a foreign substance in the blood or urine, followed by gas chromatography–mass spectrometry (GC–MS) to provide unequivocal identification of the analyte(s). GC–MS in the selected ion monitoring (SIM) mode is currently the most widely used method for drug confirmation with the correct chromatographic retention time, the presence of the molecular ion, and at least three fragment ions, in the correct relative abundance ratios, usually being the minimum requirement [1]. Certain drugs and metabolites present problems for GC–MS confirmation either because of their low volatility, high polarity, or thermal lability. Also, many sample preparation procedures require involved liquid–liquid or solid-phase extractions and chemical derivatization before GC–MS analysis of the sample extract can be performed. By coupling the extraction and purification capabilities of immunoaffinity chromatography with the analytical potential of ion-spray (pneumatically assisted electrospray) liquid chromatography–mass spectrometry (LC–MS) [2] to detect polar and non-volatile compounds, some of these shortcomings can be surmounted.

There are a number of publications in which immunoaffinity chromatography (IAC) has been used for on-line purification of analytes from bi-

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ological samples [3,4]. Samples or extracts from urine, bile, serum, and tissue have all been introduced onto these immunoaffinity systems with little or no sample preparation. The analytes are then eluted from the immunoaffinity column and analyzed directly by on-line high-performance liquid chromatography (HPLC). Other workers have utilized immunoaffinity columns for sample preparation prior to GC–MS analysis [5–7] and for automated sample preparation and HPLC analysis [8]. Generally, these columns are packed with chemically activated Sepharose beads, and antibodies are then covalently bound to these beads. These columns use a relatively large amount of antibody (1 ml neat antiserum equivalent or more) which is used for repeat analyses. These columns, while having a large binding capacity for drugs, are somewhat limited by pressure and flow restrictions. In one recent report an immunoaffinity system was directly coupled to a gas chromatograph with a flame ionization detector for analysis of β-19-nortestosterone in urine [9].

An alternative to covalently bound antibody columns is the use of a system in which a small amount of antibody is held on a column by non-covalent interactions. Only enough antibody is used to capture sufficient analyte for detection. Assuming two active sites per immunoglobulin G (IgG) molecule it is calculated that 5 μg of drug-specific antibody should be sufficient to capture 20 ng of a compound with relative molecular mass 300. Recent work by Janis and Regnier [10] has shown that a column containing a *Staphylococcus*-derived protein (protein G) can be used for the capture of human transferrin (HTr) from human serum by first priming the column with anti-HTr antibody. Protein G binds exclusively to the F_c domain of IgG [11] thus leaving the F_{ab} binding regions free. The captured transferrin and antibody are then desorbed from the protein G and separated on a C_4 reversed-phase HPLC column using column-switching techniques [12]. This method allowed quantitation of transferrin directly from 20-μl injections of human serum.

The investigation described in this report sought to utilize a commercially available protein G column (Chromatochem, Missoula, MT, USA) primed with either anti-propranolol or anti-lysergic acid diethylamide (anti-LSD) antiserum to capture the corresponding drugs from human urines. Desorption and column-switching techniques could then be used directly to confirm drug presence by LC–MS. The combination of these techniques is hereafter referred to as IAC–LC–MS. The potential advantages of such a system include the possibility of performing automated sample extractions and trace enrichment with on-line mass spectral identification of drugs directly from urine. The determination of non-volatile, heat-labile drugs can also be done without the complicating derivatization procedures often required for GC–MS confirmation of these drugs. For this study propranolol and LSD were used as model analytes to test the feasibility of this approach.

EXPERIMENTAL

Materials

All solvents were HPLC grade or better and purchased from Fisher Scientific (Pittsburgh, PA, USA). Sodium salts, ammonium acetate, and glacial acetic acid were also purchased from Fisher Scientific. Triethylamine was purchased from Aldrich (Milwaukee, WI, USA) and formic acid from EM Science (Cherry Hill, NJ, USA). Water was distilled and deionized before use with a Millipore (Bedford, MA, USA) Nanopure purification system.

Propranolol antiserum (sheep) was purchased from Biodesign International (Kennebunkport, ME, USA) and purified rabbit IgG from Sigma (St. Louis, MO, USA). LSD antiserum (rabbit) was kindly supplied by Roche Diagnostic Systems (Belleville, NJ, USA). Phosphate-buffered saline (PBS) containing 0.02% sodium azide was used to dilute antisera 9:1 (PBS–antiserum) for storage (at −57°C) and use. DL-Propranolol was obtained from Sigma and LSD was obtained from Alltech Assoc. (Deerfield, IL, USA) as a 25 ng/μl solution in methanol.

Apparatus

A diagram of the IAC–LC–MS system used for the capture of propranolol and LSD from urine is shown in Fig. 1. The system consists of two Waters pumps (Division of Millipore Corporation, Milford, MA, USA), the IAC column, trapping column and analytical column. Pump 1 is a Model 6000A and pump 2 is a Model 510. Additional hardware includes a Waters automated switching valve (ASV), a Rheodyne Model 7125 injector (Cotati, CA, USA) with a 100-μl Tefzel loop (Upchurch Scientific, Oak Harbor, WA, USA) and a Kratos Analytical Spectroflow 783 variable-wavelength UV detector (Ramsey, NJ, USA) connected to a Hewlett-Packard 3390A recording integrator (Palo Alto, CA, USA).

The analytical HPLC columns used included: a Keystone Scientific, Spherisorb phenyl column, 50 mm × 2 mm I.D. with 3-μm particles (Bellefonte, PA, USA); a Spherisorb cyano column, 100 mm × 4.6 mm I.D. with 3-μm particles or a Du Pont Zorbax cyano column, 150 mm × 4.6 mm I.D. with 5-μm particles (MAC–MOD Analytical, Chadds Ford, PA, USA). The trapping columns were either Brownlee Labs. RP-18 or RP-8 NewGuard columns, 15 mm × 3.2 mm I.D. with 7-μm particles (Santa Clara, CA, USA), or Regis Chemical Company internal surface reversed-phase (ISRP) C_8 and C_{18} columns,

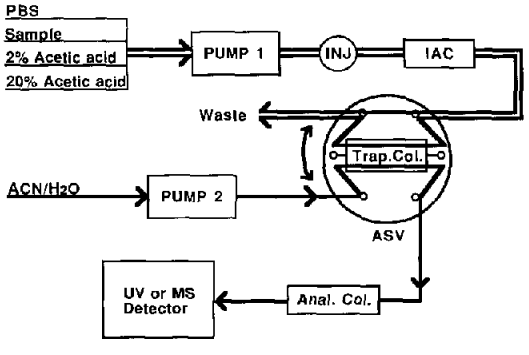

Fig. 1. Diagram of IAC–LC–UV–MS system in the backflush mode. The automated switching valve (ASV) is shown with flow going through the trapping column in both directions, though in actuality only one direction is possible at a time. ACN = acetonitrile. For further explanation of column-switching techniques see text, Table I and ref. 12.

10 mm × 3 mm I.D. with 5-μm particles (Morton Grove, IL, USA). The HiPac protein G immunoaffinity column, 33 mm × 2.1 mm I.D. with 30-μm silica particles was kindly supplied by Chromatochem.

Chromatography

The mobile phase used for HPLC in the 2 mm I.D. analytical phenyl column consisted of 25% acetonitrile in pH 3.0 water (adjusted with formic acid) at a flow-rate of 0.4 ml/min. For the 4.6 mm I.D. cyano columns 50% acetonitrile in water (acetic acid adjusted to pH 5.0) at a flow-rate of either 1.0 or 1.2 ml/min was used. Mobile phases contained either 3 or 5 m*M* ammonium acetate. For UV detection applications, 1 m*M* triethylamine was added to improve chromatographic peak shape.

PBS consisted of 0.01 *M* sodium phosphate buffer with 0.15 *M* NaCl (pH 7.4). The solution used for desorption of IgG from the protein G column (desorption solution) was either acetic acid (2%) or formic acid (0.2%) in distilled, deionized water (pH 2.65). To remove residual IgG from the IAC column after analysis a solution of 20% acetic acid in distilled, deionized water (stripping solution, pH 2.1) was pumped through the protein G column for 1 min at 3 ml/min before returning to a flow of PBS.

Detection

Detection of the drugs following HPLC was done using UV absorption at 220 nm for propranolol, 214 nm for LSD, or with a Hewlett-Packard 5985B mass spectrometer modified in this laboratory for atmospheric pressure ionization–mass spectrometry in a fashion similar to that published by Duffin *et al.* [13]. The ion spray source used was similar to that described elsewhere [14]. Nitrogen at 0.4 MPa was used as the nebulizing gas and a potential of 3–4 kV applied to the sprayer tip to effect the ion spray process. The potentials on the ion sampling capillary and skimmer, of the mass spectrometer interface, were adjusted to maximize the abundance of the protonated molecule ion of propranolol (MH^+, m/z 260) or LSD (MH^+, m/z 324) for single ion

monitoring, or to optimize fragment ion abundance for the three LSD ions monitored (*m/z* 324, 223, and 208). This optimization was done while infusing a 10 ng/μl solution of each of the drugs at 10 μl/min in the acetonitrile–water (50:50) mobile phase used for chromatography on the analytical column. All analyses were done in the SIM mode using an HP 59970 MS Chemstation data system. The high-energy dynode detector was operated at 2200 V with negative 6–8 kV on the conversion dynode.

Optimization of IgG desorption from protein G

Throughout the remainder of this report protein G and IAC columns are referred to interchangeably. Optimization of desorption conditions for IgG was done with the protein G column coupled directly to the UV detector where IgG antibodies were detected at 280 nm. Investigations were carried out to determine the desorption effectiveness of both acetic and formic acids by gradually decreasing the pH of the desorption solution with these acids. Experiments to determine the effect of sodium chloride concentrations (0.05, 0.15, and 0.25 *M*) in 2% acetic acid on IgG desorption were also conducted. Injections of 10 μl of diluted antiserum solution were made directly into a 3 ml/min flow of PBS delivered through the protein G column. Eluent flow-rate through the protein G column was maintained at 3 ml/min of PBS while unretained proteins eluted from the column. After 1 min, the solvent selection valve on the pump was switched to introduce the desorption solution to the IAC column. Following elution of the IgG peak, the 20% acetic acid stripping solution was selected to remove residual IgG from the IAC column. The area of the chromatographic peak obtained with the desorption solution being tested and that obtained with the stripping solution were used to evaluate the effectiveness of various salt concentrations and pH values on IgG elution. To determine the amount of time required for IgG elution during an actual urine analysis, 100 μl of diluted antiserum solution were injected, and the time required for a return to baseline was measured after selecting the 2% acetic acid desorption solution.

Procedure for the determination of drugs in urine

The sequence of events used for the extraction and analysis of propranolol and LSD in urine is shown in Table I. Diluted antiserum solution is injected into a 3 ml/min flow of PBS directed through the protein G column where the IgG class of antibody is bound by the F_c or "tail" region. By use of the solvent selection valve on pump 1, or by using an additional valve added for the purpose, one of four different solutions is selected to be pumped through the protein G IAC column (Fig. 1). A filtered solution of 20% urine in PBS is selected (t = 0.5, Table I) for the appropriate amount of time before returning (t = 5.5) to a PBS flush of the pump and IAC column. For these first steps the IAC column effluent goes directly through the automated switching valve to waste. This position for the automated switching valve is referred to as the starting position in Table I. By turning the switching valve to the alternate position (t = 8.5) and selecting the desorption solution (t = 9.0), flow is redirected from the IAC column through the trapping column, and dissociation of bound antibody and drug from the protein G occurs.

TABLE I

SEQUENCE OF TIMED EVENTS FOR A TYPICAL URINE ANALYSIS

Time (min)	Step
0.00	PBS flow (3 ml/min) through protein G (IAC column) to waste (Automated switching valve in starting position) Injection of diluted antiserum solution
0.50	Begin pumping of urine in PBS through column at 4 ml/min
5.50	Return to PBS at 3 ml/min
8.50	Switching valve turned to alternate position (flow directed from IAC column through trapping column)
9.00	Begin pumping desorption solution through protein G column and trapping column
12.00	Switching valve turned to starting position for backflush of trapping column and HPLC detection Pump stripping solution through protein G column for 1 min before returning to PBS

This allows collection of the drug on the trapping column following its release from the IAC column. Typically, 30 s are allowed to elapse before switching to the desorption solution in order to pre-condition the trapping column with PBS. After 3 min of desorption from the IAC column HPLC is performed in the usual fashion by turning the automated switching valve back to the starting position (t = 12.0). This effectively "backflushes" the trapped analyte from the trapping column and onto the analytical column with the acetonitrile–water mobile phase. The effluent from this column is then directed to either the UV or MS detector. During this time the 20% acetic acid solution is simultaneously pumped through the protein G column for 1 min, as recommended by the column manufacturer, to remove any residual IgG remaining on the column.

Sample flow-rate, urine, and urea experiments

A study was made to determine the effect of sample flow-rate through the protein G column on the recovery of drug from fortified urine samples. For this experiment 20 ml of 10% urine in PBS, containing 10 ng/ml propranolol in urine, was pumped through the protein G column after it had been primed with 50 μl of diluted antiserum solution. Only the flow during the sample introduction step was varied. All other steps were carried out as outlined in Table I, though at the appropriately adjusted times.

A study to determine the effect of the urea component of urine on drug recovery was carried out by comparing the recovery obtained with a 0.28 M solution of urea in PBS with that obtained from urine in PBS and PBS alone. The concentration of urea chosen is the same as that typically found in urine [15]. Urea and urine samples were diluted with PBS to 50 ml at the desired concentration, filtered, and spiked with 10 ng of propranolol prior to analysis. A 20-ml volume of each solution was then pumped at 4 ml/min through the protein G column after it had been primed with 50 μl of diluted antiserum solution. No attempt was made to adjust the pH of these samples either before or after dilution with PBS.

RESULTS AND DISCUSSION

The protein G column used in this study contains a recombinantly engineered protein G (Gammabind G, Genex) covalently bound to silica particles. Protein G displays broader reactivity to IgG of different species than does protein A [16]. The column has a 2-mg capacity for IgG and has not shown any deterioration in performance after nine months of use. Pressure on the column at a flow-rate of 10 ml/min of PBS was measured at 3.3 MPa.

In order to find a suitable desorption solution, several possibilities recommended by the protein G column manufacturer were evaluated. Both acetic and formic acids were found to be satisfactory, and each allowed refocussing of the desorbed analyte on the trapping column. Control experiments demonstrated that 2% acetic acid (pH 2.65) eluted more than 90% of the IgG from the protein G column based on peak areas obtained from sequential elution with desorption and then stripping solution (2 and 20% acetic acid solutions, respectively). Formic acid was also evaluated and found to be satisfactory at a concentration of only 0.2% (pH 2.65). It was found that the best desorption was obtained without the use of sodium chloride (not shown). Fig. 2 shows a typical UV (280 nm) chromatogram obtained with drug antiserum (rabbit) (A) and with commerically purified sheep IgG (B).

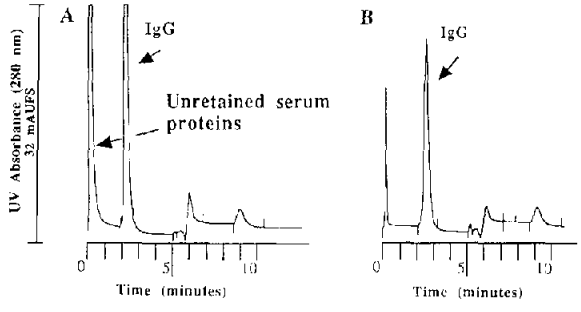

Fig. 2. Elution of IgG from the protein G column by UV detection at 280 nm. Flow: 3 ml/min of PBS at time (t) = 0, 2% acetic acid at t = 1, 20% acetic acid at t = 4 and PBS again at t = 7. (A) 100 μl diluted LSD antiserum solution; (B) pre-purified sheep IgG.

To determine the length of time necessary for IgG desorption during an actual urine analysis, an injection of 100 μl of diluted antiserum was made. This indicated that unwanted serum proteins are swept through the column to waste within 1 min of injection at a 3 ml/min flow-rate of PBS. Subsequent desorption with 2% acetic acid solution demonstrated that approximately 3 min at 3 ml/min were sufficient to obtain a return to baseline following IgG elution.

Initial experiments with the conventional reversed-phase trapping column (using the configuration in Fig. 1) indicated that 50 μl of diluted antiserum solution is sufficient to capture 6.5 ng from a total of 10 ng of propanol contained in 20 ml of PBS when pumped through the IAC column at 2 ml/min (not shown). It is estimated this amount of antiserum would contain approximately 5 μg of propranolol-specific IgG (personal communication with antibody supplier). Using the 2 mm I.D. phenyl column as the analytical column with the conventional C_{18} trapping column and UV detection, we were able to detect propranolol in urine at the 250 pg/ml level using 40 ml of a 50% solution of urine in PBS and 50 μl of diluted antiserum (not shown).

Unfortunately, there was a gradual accumulation of antibody on the C_{18} reversed-phase column that was originally selected as a trapping column. Attempts to elute the protein from the column using a high percentage of acetonitrile were not successful, and as a result there was a noticeable decline in recovery of drug from this column. The build-up of protein on the trapping column most likely reduces the interaction of drug with the stationary reversed-phase surface thus leading to the decline in recovery.

To overcome this problem an ISRP trapping column [17] was substituted for the conventional C_{18}-type reversed-phase column. These columns have a hydrophilic surface on their silica beads which does not retain large-molecular-mass proteins. The inside surfaces of the pores are coated with common reversed-phase coatings thus allowing retention of smaller-molecular-mass drug molecules. Using this type of column we obtained better reproducibility, though slightly lower overall recovery, compared with a new conventional trapping column. Direct injections of 9 ng of a propranolol standard solution (using the injector) onto the protein G column primed with 50 μl of diluted antiserum solution gave an average recovery of 30% (2.7 ng) with a peak-area R.S.D. of 8.6% from six analyses. One drawback of these columns was that excessive peak broadening occurred when used with 2 mm I.D. analytical columns because of the low flow-rate (0.4 ml/min) during backflushing.

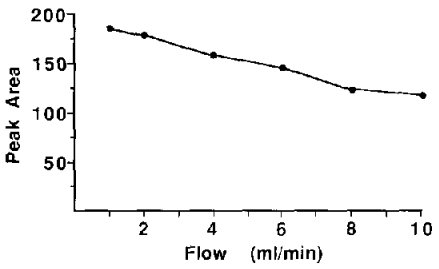

Fig. 3. Effect of flow-rate on capture of propranolol (10 ng) from 20 ml of 10% urine using 50 μl of diluted antiserum solution. Analyses were performed using the configuration as shown in Fig. 1 with the C_{18} ISRP trapping column and the 4.6 mm I.D. Spherisorb cyano analytical column maintained at a flow-rate of 1 ml/min. Each point represents a single analysis using UV detection at 220 nm (see text for details).

Experiments to determine the effect of flow-rate on capture of propranolol from a 10% urine solution in PBS were done using 50 μl of diluted antiserum solution to prime the IAC column. The results of the flow-rate study shown in Fig. 3

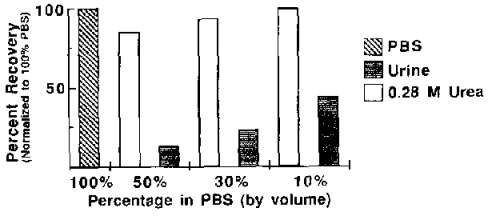

Fig. 4. Comparison of propranolol recovery from 20-ml dilutions of 0.28 *M* urea, and urine, in PBS, with that captured from 20 ml of PBS alone. Each 20-ml sample contained 4 ng of propranolol and was loaded at a flow-rate of 4 ml/min. Analyses were performed using UV detection at 220 nm with the conventional C_8 reversed-phase trapping column and the 2 mm I.D. Spherisorb phenyl column at a flow-rate of 0.4 ml/min.

indicate that there is a 35% decrease in drug capture with an increase in flow-rate from 1 to 10 ml/min through the protein G column. This is not surprising, considering that faster flow means less time for drug to interact with antibody. However, it is important to note that this decrease in recovery can be more than made up since more urine can be pumped through the column in a given time period while using a higher flow-rate.

The analysis of varying dilutions of urine in PBS indicated that lower drug recovery is obtained with increasing concentrations of urine. This finding can also be noted in the work of others [9]. To examine the possibility that the high urea concentration in urine may cause this loss, we compared the recovery of propranolol from solutions of urine and urea in PBS to that obtained from a solution of PBS alone (Fig. 4). The 0.28 *M* urea solution and a urine sample were diluted to the same concentration (v/v) with PBS to give approximately equivalent concentrations of urea in each. Fig. 4 indicates the presence of urea can only account in part for the decreased recovery obtained from the urine solutions, indicating that other urine components may be contributing factors. Again, it is important to note that the decrease in recovery with increasing urine concentration can be made up by virtue of the increase in the amount of drug loaded. While this study is not exhaustive, it does indicate an approach that can be used to determine which urine components cause the greatest decrease in recovery seen with these columns.

An example of propranolol purification from a urine analysed by immunoaffinity chromatography, and a comparison with an analysis performed by pumping urine directly onto the trapping column, is shown in Fig. 5. In each of Fig. 5A, B and C the analysis was performed as outlined in Table I except that in A and C the protein G column was omitted, and for C alone the switching valve was turned to the alternate position at time 0.5 instead of at time 8.5 min. As seen in Fig. 5B there appear to be no significant interferences that are extracted using protein G immunoaffinity chromatography when compared with the standard injection in A. In contrast, analysis of the same urine solution pumped directly onto the trapping column, shown in Fig. 5C, shows significant interference by endogenous material that elutes along with the drug that is captured. The relatively clean appearance of the UV chromatogram in Fig. 5B indicates that the IAC system may offer some advantages when large numbers of samples need to be analyzed or where reproducible retention times are desirable. Increased analytical column lifetime may be an additional benefit.

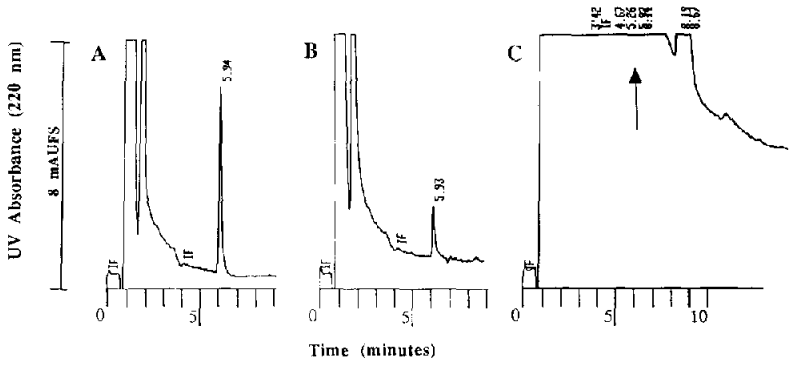

Fig. 5. LC–UV (220 nm) chromatograms demonstrating the effectiveness of IAC analysis in urine sample clean-up. Each analysis was performed with the configuration as shown in Fig. 1 (except were noted) and the C_{18} ISRP trapping column with the 4.6 mm I.D. Spherisorb cyano column at a flow-rate of 1.0 ml/min. (A) A 10-ng amount of standard propranolol was injected (using injector) directly onto trapping column, and without IAC column. (B) Analysis of 20 ml of 20% urine solution, containing 10 ng of propranolol, with IAC system using 100 μl of diluted antiserum solution. (C) Same as in B, but the urine solution was pumped directly through trapping column, and without IAC column (see text for details).

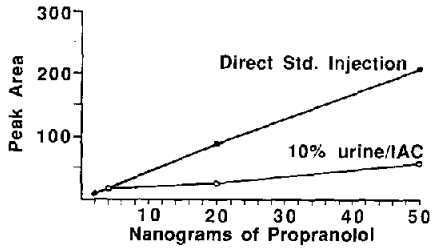

Fig. 6. Recovery of drug from 20 ml of 10% urine solutions, containing indicated amounts of propranolol, in comparison with direct injections of standard solution onto trapping column. Urine solutions were loaded at 5 ml/min, and 50 μl of diluted antiserum solution were used with the configuration as shown in Fig. 1. Analyses were performed with the C_{18} ISRP trapping column and the 4.6 mm I.D. Spherisorb cyano column at a flow-rate of 1 ml/min.

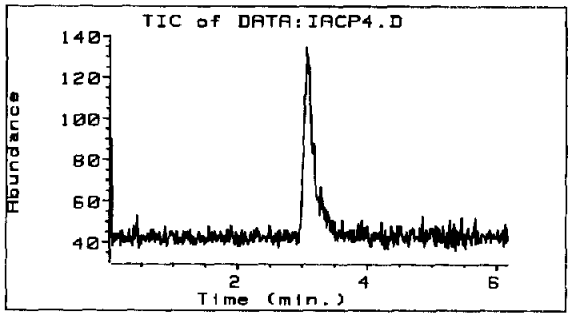

Fig. 7. Single ion chromatogram (*m/z* 260) from the IAC–LC–MS analysis of 20 ml of 20% urine (2.5 ng/ml propranolol in urine) for the protonated molecule ion of propranolol. Sample was loaded at 2 ml/min after priming the IAC column with 50 μl at diluted antiserum solution. Analyses were performed with the C_{18} ISRP trapping column and Zorbax cyano analytical column at a flow-rate of 1.2 ml/min.

Using a fixed amount of antibody it is found that the overall recovery of drug from urine decreases with increasing drug concentration (Fig. 6). For each analysis on the lower curve 50 μl of diluted antiserum solution was used. Each point represents one analysis of 20 ml of 10% urine in PBS. Each point on the upper curve represents an average of three analyses performed by making direct injections of a standard propranolol solution (using the injector) onto the trapping column. The washing and desorption steps in each case are as shown in Table I. The decrease in recovery at higher drug concentrations is due to high-affinity, drug-specific antibody sites becoming saturated with drug. At higher drug concentrations, only lower-affinity sites are available to retain excess drug, and these are not capable of doing so through the subsequent washing sequence. However, the use of a larger amount of antibody can improve the recovery for higher concentrations of drug. It is only for low concentrations of drug that the capture and recovery is near quantitative. For this reason quantitation of drugs at higher levels in urine may prove difficult using the non-covalent binding utilized in this work.

Coupling of the immunoaffinity system to the ion-spray mass spectrometer was straightforward and requires only disconnecting the analytical column from the UV detector and reconnecting it to the ion-spray interface. IAC–LC–MS determinations using SIM for both propranolol and LSD spiked into human urine were performed. While monitoring only the protonated molecule ion, propranolol was detected at 2.5 ng/ml of urine with a signal-to-noise ratio of 10:1 (Fig. 7). This figure shows an unsmoothed ion current profile for *m/z* 260 of propranolol, with a retention time of 3.06 min. This is comparable to the detection limits we have seen reported in the literature for the GC–MS determination of propranolol [18,19]. Fig. 8 shows the unsmoothed SIM ion current profiles for two analyses of urine containing LSD at the 500 pg/ml level (A) and the 1 ng/ml level (B). The two fragment ions (*m/z* 223 and 208) monitored in B are produced by "up-front" collision-induced dissociation (CID) within the first vacuum region of the atmospheric pressure ionization (API) MS interface [13]. The extent to which fragmentation occurs can be controlled by the potentials applied to the ion sampling capillary and the skimmer [20]. For the LSD analysis shown in Fig. 8B, a potential of 130 V was applied to the ion sampling capillary and 8 V to the skimmer. The coincidence of retention times for these three ions with those of an authentic standard, along with the close agreement of the relative abundance ratios (less than 25% difference), provide confirmation of identity. While levels of this drug in real samples are often ten or

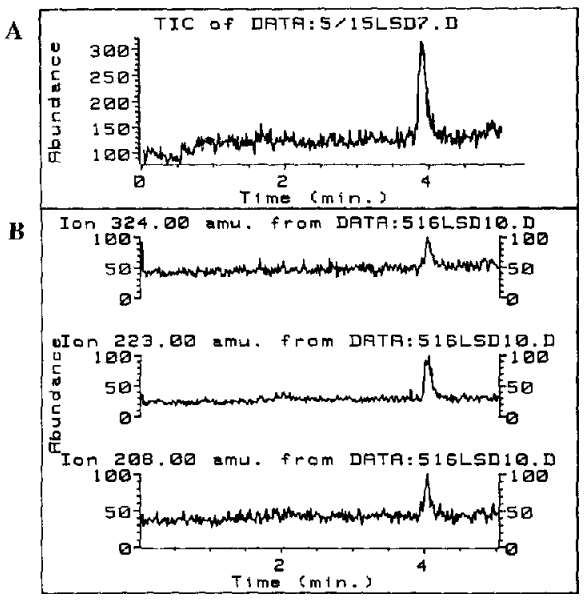

Fig. 8. Selected ion traces from analyses of 20 ml of 20% urine for LSD at (A) 500 pg/ml urine monitoring protonated molecular ion at m/z 324 only and (B) 1 ng/ml urine using collision-induced dissociation, and monitoring the three ions indicated. Samples were loaded at 4 ml/min, and 50 μl of diluted antiserum solution were used. Analyses were performed with the C_{18} ISRP trapping column and the Zorbax cyano analytical column at a flow-rate of 1.2 ml/min.

more times lower than the spiked urines shown here, it should be possible to lower the detection limit for the described method by using a smaller-I.D. analytical column and by further optimization of the column-switching system.

CONCLUSIONS

This paper presents for the first time the direct coupling of immunoaffinity chromatography to an ion-spray mass spectrometer. The advantage of the described method is that high flow-rates of up to 10 ml/min can be used on the protein G column thus allowing large sample volumes (4 ml of urine or more) to be analyzed in a short time (less than 20 min per sample for complete analysis). Antibody extraction using a protein G column affords excellent purification of drugs directly from solutions of urine in PBS as evidenced by UV detection. By coupling the IAC system directly to a mass spectrometer we have demonstrated the feasibility of performing automated sample preparation and trace enrichment directly from urine with mass spectral confirmation. Only a very small amount of antibody (less than 1 US $) is used, which is then desorbed during each analysis. This allows the IAC column to be reused for a number of different analytes by loading the appropriate antibody. While the method described here may not be useful for quantification, because of antibody saturation with drug at high drug concentrations, it could be very useful for situations in which confirming the presence of drugs in a rapid, inexpensive, and automated fashion is the primary objective.

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