TECHNICAL NOTE

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Direct Analysis for Cocaine in Urine by High-Performance Liquid Chromatography (HPLC) Using a Column-Switching Technique

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ABSTRACT: The analysis of urine for the presence of drugs generally requires that the drug be first extracted from relatively large concentrations of endogenous compounds. By replacing the injection loop with a loop incorporating a cyano precolumn, urine can be injected directly onto the high-performance liquid chromatograph. Interfering compounds are washed off of the precolumn, with the valve in the load position. When the valve is then switched to inject, the mobile phase elutes the cocaine from the precolumn onto the analytical column. A preliminary identification of cocaine and benzoylecgonine can be made using this technique, which requires 100 μL of sample and has a detection limit of 10 ng/mL.

KEYWORDS: toxicology, chromatographic analysis, cocaine, urine, column switching, high-performance liquid chromatography

Forensic science analysis often involves the use of body fluids to determine the presence of exogenous substances, particularly controlled substances which the individual may have ingested. Urine, which is easily obtainable, even if sometimes in small volumes, is composed of water and a matrix of filtered components, including the drug and its metabolites. Under normal physiological conditions, only 9% of an intravenously administered dose of cocaine is excreted unchanged in the urine, and only 5% following intranasal administration [*1*]. Any chromatographic analysis of urine for cocaine therefore requires the use of an efficient extraction procedure, together with a sensitive analytical technique.

Column switching has been previously reported to be useful for preliminary identification of drugs in biological samples, such as plasma and vitreous humor [*2–5*]. This approach involves the use of a precolumn placed in the injection loop of the high-performance liquid chromatograph (HPLC) in such a way that a sample can be injected

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onto the precolumn and then flushed with another solvent. The precolumn retains the compounds of interest, while the endogenous compounds run to waste. The sample can then be washed by injecting, typically, 0.5 to 1 mL of water, buffer, or organic solvent through the injection system. After flushing, the injector is switched to the inject position, and the mobile phase passes through the precolumn and takes the relatively pure compounds of interest onto the analytical column.

The technique essentially removes the need for lengthy extraction procedures prior to the chromatographic analysis. The speed of this method of analysis and the small sample size required make it an attractive procedure for both forensic and clinical laboratories.

Materials and Methods

HPLC-grade acetonitrile, *o*-phosphoric acid, and diethylamine were obtained from Fisher Scientific. Cocaine hydrochloride was supplied by Merck, and benzoylecgonine and mepivacaine by Alltech. Postmortem urine samples were frozen at −20°C until required for analysis. The HPLC system was a modification of that previously described [*6*]. A Waters Model 510 liquid chromatograph pump was used to deliver the mobile phase at a flow rate of 1.6 mL/min. The mobile phase consisted of 0.025*M* aqueous potassium phosphate (monobasic)/acetonitrile/diethylamine (88:10:2) adjusted to pH 3 with *o*-phosphoric acid.

The column was a 150 by 4.6-mm C-8 column on 5-μm silica (IBM). The precolumn was a Resolve CN Guard Pak precolumn (Waters), incorporated in the loop of a Rheodyne injection system. A Kratos Spectraflow 773 ultraviolet (UV) detector was used to monitor the eluent at 230 nm. A Hewlett-Packard 3380A integrator was used for quantitative determinations.

Analytical Procedure

Preliminary solid-phase extraction of cocaine and benzoylecgonine was performed to determine the optimum precolumn to incorporate into the HPLC system. Cyano, C8, and C18 cartridges (Analytichem) were used for this purpose, together with a Vac-Elut system (Analytichem). Cartridges having a capacity of 1 mL were placed in the Vac-Elut, activated by passing 1 mL of methanol followed by 1 mL of water through the columns. One hundred microlitres of urine spiked with 500 ng of cocaine and 500 ng of benzoylecgonine were then applied to the cartridges and allowed to pass through under vacuum. The drugs were then washed by passing 0.25 to 2.0 mL of water through the columns. Finally, the drugs were eluted from the column with 1 mL of HPLC mobile phase. The extraction recoveries of both cocaine and benzoylecgonine were compared following extraction with each of the described solid-phase cartridges and after washing with different volumes of water.

Urine samples were spiked with cocaine and benzoylecgonine at concentrations of 0, 0.1, 1, 25, and 50 μg/mL. One hundred microlitres of mepivacaine (50 μg/mL) were added as an internal standard. One hundred microlitres of spiked urine were injected onto the HPLC. Endogenous compounds in the urine were then eluted by flushing with 1.0 mL of water. The Rheodyne valve was then switched to the inject position (Fig. 1). A calibration curve was constructed for both cocaine and benzoylecgonine, based on an average of three determinations.

Results

The percentage recovery of cocaine and benzoylecgonine from urine, after extraction with various solid-phase extraction columns and after washing with different volumes of

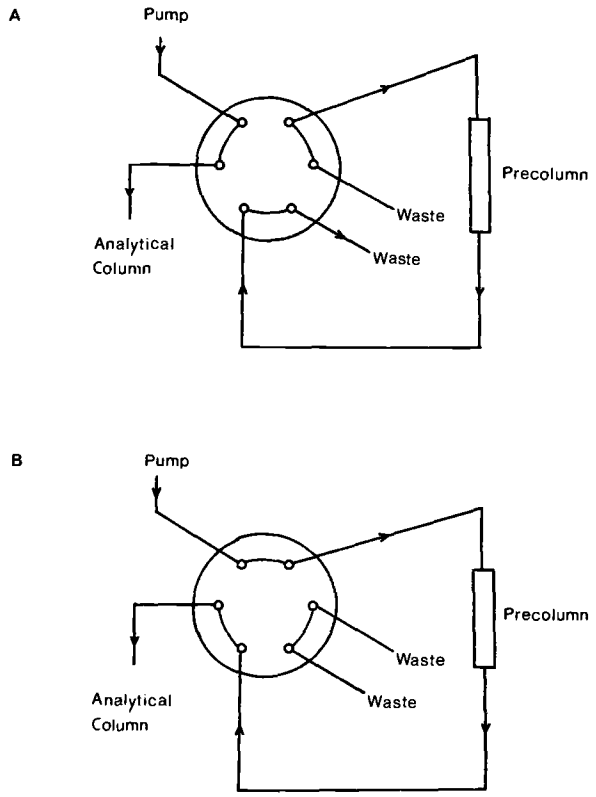

FIG. 1—*Rheodyne injection valve with precolumn in the* (a) *load and* (b) *inject positions.*

water, was determined. In each case, the drugs were eluted from the extraction column with 1 mL of HPLC mobile phase. A solid-phase cartridge with a silica-bonded cyanopropyl packing material was found to give the highest recovery (near 100%) and the cleanest extracts. A cyano precolumn was subsequently incorporated into the HPLC system. The optimum volume of water for flushing the extraction column was found to be 1 mL. Greater volumes caused some of the drugs to be eluted, with a subsequent reduction in extraction efficiency. A typical HPLC chromatogram of 100 μL of postmortem urine containing cocaine and benzoylecgonine, before and after washing with 1 ml of water, is shown in Fig. 2. No loss of the drug standards was seen with the incorporation of a 1 mL water wash stage in the process.

The chromatographic method is linear over a concentration range of 0 to 50 μg/mL, with a linear regression of $y = 1.455x - 0.8587$ for cocaine ($R = 0.998$) and $y = 1.274x - 0.5331$ for benzoylecgonine ($R = 0.999$). The minimum detectable level, with a signal-to-noise ratio of >2, was 5 ng/mL for a 100-μL sample injected onto the HPLC. The cyano precolumn was able to sustain 50 injections of urine before a significant rise in back pressure became apparent. At this point, it was necessary to replace the precolumn.

The above method was used for the screening of postmortem urine samples for the presence of cocaine and benzoylecgonine. No interference with the determination of these drugs was observed as a result of the presence of other commonly encountered drugs. Most—such as the benzodiazepines, antidepressants, and opiates—were either not extracted or not eluted from the analytical column with the mobile phase described.

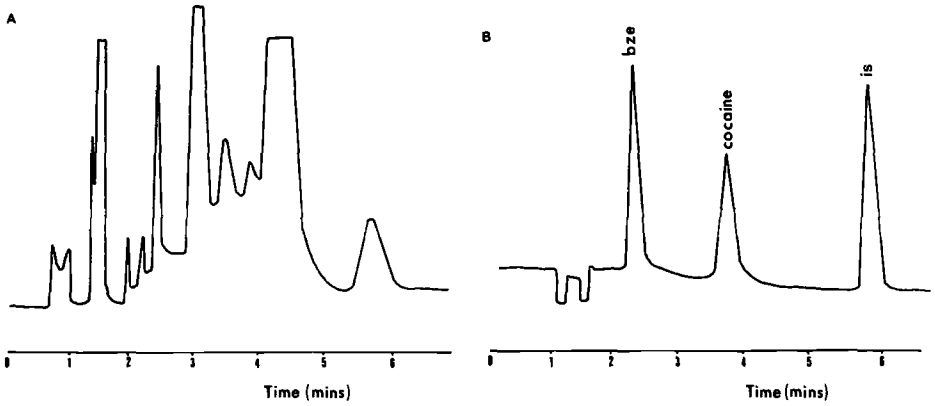

FIG. 2—*Chromatograms of a 100-μL injection of a urine sample containing cocaine and benzoylecgonine (1 μg/mL):* (a) *with no wash,* (b) *following a 1 mL water wash. Mepivacaine is the internal standard.*

Discussion

The use of HPLC with column switching offers a sensitive, reproducible, and efficient method for the rapid determination of drugs in urine. The advantage of this approach is that lengthy extraction procedures are eliminated, thus greatly reducing the analysis time. Recoveries of near 100% make this an attractive technique for the analysis of very low drug concentrations. The volume of urine injected onto the system can also be increased up to 2 mL, effectively concentrating the sample onto the precolumn as a further step to improve sensitivity.

References

[1] Clarke, E. G. C., *Isolation and Identification of Drugs*, A. C. Moffat, Ed., Pharmaceutical Press, London, 1986.

[2] Wahlund, K. G., "Separation of Acidic Drugs in the μg/ml Range in Untreated Blood Plasma by Direct Injection on Liquid Chromatographic Columns," *Journal of Chromatography*, Vol. 218, 1981, pp. 671–679.

[3] Roth, W., Beschke, T., Zech, K., and Huber, R., "Fully Automated High Performance Liquid Chromatography: A New Chromatograph for Pharmacokinetic Drug Monitoring by Direct Injection of Body Fluids," *Journal of Chromatography*, Vol. 222, 1981, pp. 13–22.

[4] Voelter, W. and Kronbach, T., "A Simple High Performance Liquid Chromatographic Precolumn Technique for Investigation of Drug Metabolism in Biological Fluids," *Journal of Chromatography*, Vol. 239, 1982, pp. 475–482.

[5] Logan, B. K. and Stafford, D. T., "Determination of Vitreous Cocaine and Benzoylecgonine Concentrations Using HPLC with Column Switching," presented at the 42nd Annual Meeting of the American Academy of Forensic Sciences, Cincinatti, OH, 19–24 Feb. 1990.

[6] Logan, B. K., Stafford, D. T., Tebbett, I. R., and Moore, C. M., "Rapid Screening for 100 Basic Drugs and Metabolites in Urine Using Cation Exchange Solid-Phase Extraction and High Performance Liquid Chromatography with Diode Array Detection," *Journal of Analytical Toxicology*, Vol. 14, 1990, pp. 154–159.

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