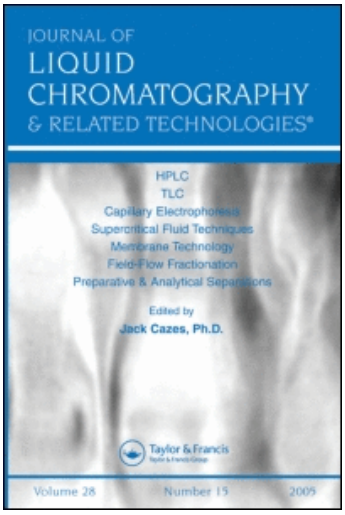

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Screening of Benzoylecgonine in Urine by Cyclobond Solid Phase Extraction and High Performance TLC

Joseph Sherma[a]; Joseph E. Bernardo[a]; Matthew H. Higgs[a]
[a] Department of Chemistry, Lafayette College Easton, Pennsylvania

To cite this Article Sherma, Joseph , Bernardo, Joseph E. and Higgs, Matthew H.(1988) 'Screening of Benzoylecgonine in Urine by Cyclobond Solid Phase Extraction and High Performance TLC', Journal of Liquid Chromatography & Related Technologies, 11: 15, 3135 — 3143
To link to this Article: DOI: 10.1080/01483918808076784
URL: http://dx.doi.org/10.1080/01483918808076784

JOURNAL OF LIQUID CHROMATOGRAPHY, 11(15), 3135-3143 (1988)

SCREENING OF BENZOYLECGONINE IN URINE BY CYCLOBOND SOLID PHASE EXTRACTION AND HIGH PERFORMANCE TLC

JOSEPH SHERMA, JOSEPH E. BERNARDO AND MATTHEW H. HIGGS
Department of Chemistry
Lafayette College
Easton, Pennsylvania 18042

ABSTRACT

A method is described for screening of the cocaine metabolite benzoylecgonine in urine at 0.2 µg/ml using a Cyclobond cyclodextrin solid phase extraction cartridge and high performance thin layer chromatography. Results are compared with those obtained using liquid-liquid and diatomaceous earth column extraction and C-18 solid phase extraction.

INTRODUCTION

Thin layer chromatography (TLC) is one of the most frequently used methods to screen urine for drugs of abuse (1). Because of rapid and extensive in vivo metabolism of cocaine (2), screening analysis for illegal use of cocaine is usually based on detection of the matabolite benzoylecgonine (BE) in urine. This paper

presents a method for screening benzoylecognine at a sensitivity level of 0.2 µg/ml with a 5 ml urine sample using a cyclodextrin solid phase extraction (SPE) column and high performance preadsorbent silica gel TLC plate. The results of cyclodextrin SPE are compared to those obtained with the same samples using traditional liquid-liquid extraction, a diatomaceous earth liquid-solid extraction cartridge, and a C-18 SPE cartridge.

EXPERIMENTAL

Thin layer chromatography was carried out on 10 x 20 cm Whatman LHPKD channeled, high performance silica gel plates with preadsorbent spotting area. The following two mobile phases were used: (a) methanol-chloroform-concentrated ammonium hydroxide (60:60:1), and (b) ethyl acetate-methanol-water-ammonium hydroxide (85:13.5:1:0.5). Benzoylecgonine was detected by spraying Whatman Dragendorff TLC reagent followed by 8% aqueous sulfuric acid (2).

Drug-free urine was collected, refrigerated for storage, warmed, and filtered through glass microfiber paper (Whatman GF/F) before use, and fortified by addition of an appropriate volume of a 1.0 mg/ml methanolic solution of BE. TLC standards of the metabolite (0.1 to 5 µg/µl) were also prepared in methanol.

Benzoylecgonine was extracted from fortified urine by the following four methods: (Method 1). 500 mg/3 ml Cyclobond I SPE column (Advanced Separation Technologies, Inc.) - 5 ml of urine was added to the unconditioned column in a vacuum manifold (Spe-ed

Mate-30, Applied Separations, Inc.) operated at 10 in. of mercury. The column was washed with 10 ml of water and dried by centrifuging (IEC Clinical Centrifuge, speed setting 6) or drawing vacuum for 10 minutes. The column was then washed with one column volume (ca. 2.5 ml) of acetone and dried with vacuum. The tube was removed from the manifold and BE was eluted using gentle pressure from a rubber bulb into a small tapered tube with 2 ml of chloroform-ethanol (8:2). The solution was evaporated just to dryness, reconstituted in a small volume of the mixed solvent, and the entire sample spotted onto the preadsorbent. (Method 2). Liquid-liquid solvent extraction - the procedure described earlier (2) was followed exactly for extraction of BE with chloroform-ethanol (8:2) from 5 ml of urine. (Method 3). Diatomaceous earth extraction column - 2 ml of pH 9.5 saturated ammonium chloride-ammonia buffer and 6 g of NaCl were added to 20 ml of urine. The urine was applied to a Spe-ed Screen N column (Applied Separations, Inc.), and after 3 minutes two sequential 16 ml volumes of methylene chloride-isopropyl alcohol (9:1) were added. The eluate was evaporated to dryness, reconstituted, and spotted for TLC. (Method 4). 500 mg/3 ml C-18 bonded silica gel SPE column (Applied Separations, Inc. and J. T. Baker) - 5 ml aliquots of urine were adjusted to pH 4.2 with 0.1 M sodium acetate-acetic acid buffer, to pH 7.6 with 0.5 M potassium phosphate dibasic buffer, to pH 9 with saturated ammonium chloride-ammonia buffer, or were analyzed unbuffered (pH about 5). The columns were conditioned with two 3 ml portions of methanol followed by 3 ml of pH 7.6 phosphate

buffer. Samples were added to the columns, and the columns washed with 1 column volume of phosphate buffer-acetonitrile (9:1). After drying the columns, BE was eluted with three sequential 0.5 ml portions of methanol- methylene chloride (1:1), and the combined eluate was evaporated, reconstituted, and spotted for TLC.

Minimum detectable concentration was evaluated by finding the lowest fortification level that allowed definite visual detection of the BE zone on the layer. This was determined by a combination of the recovery of BE, its sensitivity of detection by TLC, and the amount of background color from sample extracts at the same R_F value as the BE zone. Recovery was determined by scanning BE zones from spotted column eluates with a Kontes Chromflex densitometer and comparing peak areas to those of bracketing BE standards developed on the same plate.

RESULTS AND DISCUSSION

Thin Layer Chromatography

Mobile phase (b), which gave an R_F value of 0.080, was generally used to develop chromatograms when determining the minimum detectable concentrations of BE. Mobile phase (a) gave an R_F of 0.30 and was used when BE zones were quantified by densitometry to determine percentage recovery. Spraying with Dragendorff reagent followed by sulfuric acid (2) detected BE as a narrow orange zone on a light yellow background with a sensitivity of ca. 250-500 ng. Iodoplatinate TLC spray reagent (Whatman) detected only 1 μg

or more of BE as a purple zone on a slightly lighter purple background. The contrast between the colors of the zone and background was much better for Dragendorff reagent than for iodoplatinate, and the color of the zone faded much less quickly. It was superior both for visual detection and densitometric quantification of BE. Placing a plate sprayed with Dragendorff reagent in a chamber containing iodine vapors, as suggested earlier (2), did not improve detection.

The minimum detectable level of BE is 5 to 10 times smaller on high performance silica gel than on a conventional silica gel layer such as Whatman LK6DF because of the formation of a tighter zone. BE is separated from many drugs of abuse and their metabolites by TLC in solvent (b). Data for a selection of important drugs are shown in Table 1. The characteristic low R_F value of BE provides a high degree of selectivity for the proposed Cyclobond SPE/HPTLC method.

Isolation of BE on Cyclobond

Of the extraction methods tested, use of the Cyclobond column with water and acetone washes and chloroform-ethanol elution clearly gave the best results. The minimum detectable concentration was 0.2 µg/ml or less, which is below the 0.3 µg/ml detection cutoff level for the popular EMIT and RIA immunoassay screens for BE (1) and the guideline published in the Federal Register for screening federal workers (3). Densitometric analysis indicated that recovery of BE was approximately 50%, but the very clean plate background allowed the 0.2 µg/ml minimum detection concentration to be realized.

TABLE I

R_F VALUES FOR DRUGS

D-Amphetamine	0.28
D-Methamphetamine	0.18
Phenylpropanolamine	0.20
Methadone	0.54
Meperidine	0.42
Codeine	0.19
Diazepam	0.69
Quinine	0.28
Morphine	0.13
Nicotine	0.43
Phencyclidine	0.72
Chlordiazepoxide	0.54
Amitriptyline	0.48
Pentazocine	0.58
Chlorpromazine	0.48
Promethazine	0.47
Prochlorperazine	0.34
Cocaine	0.67
Dextropropoxyphene	0.68
Davron	0.62
Librium	0.54
Demerol	0.47

Layer: Whatman Diamond LK6DF silica gel.

Mobile phase: ethyl acetate-methanol-water-conc. ammonium hydroxide (85:13.5:1:0.5).

Detection: plate sprayed with ninhydrin followed by iodoplatinate.

Recovery at the 93% level from 5 ml of urine was obtained using a Cyclobond SPE column with a 5ml deionized water wash and elution with 2 ml of anhydrous methanol when BE analysis was carried out by HPLC on a Cyclobond I (beta) column developed with methanol-0.1% aqueous TEAA (pH 4.0) (4:6) (4). This procedure could not be used successfully with TLC because heavy background streaking from matrix impurities obscured detection of BE and limited detection to concentrations greater than 5 µg/ml. Attempts to modify our method in order to increase recovery of BE above 50% without eluting more background interferences were unsuccessful. These included increasing the water wash from 10 to 25 ml, increasing or decreasing the volume of the acetone wash, and increasing the volume of chloroform-ethanol eluent. The background interferences were shown to be from urine and not from the unpreconditioned Cyclobond column because recovery of BE from spiked water using a water wash and methanol elution was 93%, and the thin layer chromatogram of the eluate had a light background.

Comparison of Other Isolation Methods

The results of the Cyclobond SPE-TLC method were compared to those obtained with other common drug extraction procedures using identical fortified urine samples. Both the liquid-liquid extraction method (2) and diatomaceous earth extraction column had 2-3 µg/ml minimum detection concentrations because of considerable background interference at the R_F of BE with both chromatographic solvents (a) and (b). We could not confirm the 0.25 µg/ml sensitivity reported for the extraction method (2) with our urine samples.

Bonded C-18 SPE columns gave a minimum detectable concentration of 1 µg/ml when urine was buffered to pH 4.2, and 3 µg/ml for unbuffered and pH 9 buffered urine. Two variations of the basic C-18 column method described above were tested, but neither improved detectability below 1 µg/ml. The first included adjustment of urine to pH 2.5 with 0.05 M phosphoric acid, conditioning the column with methanol and phosphoric acid, washing the column with phosphoric acid, and elution of BE with 3 x 0.5 ml of methanol. The second involved analyzing urine adjusted to pH 5 (acetate buffer) or 11 (carbonate buffer) using a column preconditioned with methanol and then the buffer. The column was washed with water, and BE eluted with 2 ml of methylene chloride-isopropanol (9:1). Our results with C-18 columns indicated that as a general rule, as the pH of urine was increased, more matrix interferences were eluted causing poorer plate backgrounds, but recovery of BE improved Conditions with C-18 columns could not be optimized so as to give detectabilities as low as with the Cyclobond column, but pH 4.2 gave the best compromise between recovery and background.

ACKNOWLEDGEMENT

The authors are indebted to Thomas E. Beesley of Advanced Separation Technologies, Inc., Richard J. Badesso of Applied Separations, Inc., Ruthann E. Kaiser of J. T. Baker Chemical Company, and Elaine Heilweil of Whatman, Inc. for helpful suggestions and samples of products that facilitated this research.

REFERENCES

1. Hanson, D. J., Chemical and Engineering News, June 2, p. 7 (1986).

2. Wallace, J. E., Hamilton, H. E., Schwertner, H., King, D. E., McNay, J. L., and Blum, K., J. Chromatogr. 114, 433 (1975).

3. Hanson, D. J., Chemical and Engineering News,, August 31, p. 13 (1987).

4. Cyclobond SPE Handbook, Advanced Separation Technologies, Inc., p. 4 (1987).