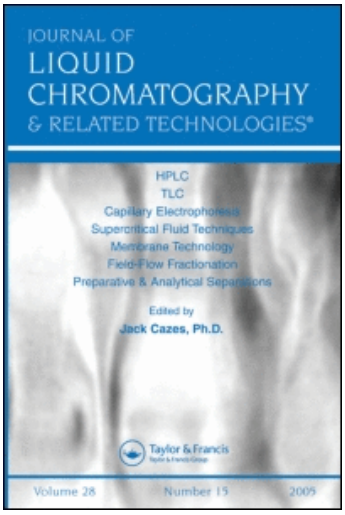

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Unique Solid Phase Extraction Column for Isolation of 11-Nor-Δ-9-Tetrahydrocannabinol-9-Carboxylic Acid in Human Urine

Vandana Dixit[a]; Vyas M. Dixit[a]
[a] Analytichem International, Harbor City, CA

To cite this Article Dixit, Vandana and Dixit, Vyas M.(1990) 'A Unique Solid Phase Extraction Column for Isolation of 11-Nor-Δ-9-Tetrahydrocannabinol-9-Carboxylic Acid in Human Urine', Journal of Liquid Chromatography & Related Technologies, 13: 16, 3313 — 3325
To link to this Article: DOI: 10.1080/01483919008049103
URL: http://dx.doi.org/10.1080/01483919008049103

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(16), 3313-3325 (1990)

A UNIQUE SOLID PHASE EXTRACTION COLUMN FOR ISOLATION OF 11-NOR-Δ-9-TETRAHYDROCANNABINOL-9-CARBOXYLIC ACID IN HUMAN URINE

VANDANA DIXIT AND VYAS M. DIXIT
Analytichem International
24201 Frampton Avenue
Harbor City, CA 90710

ABSTRACT

A sensitive, specific, qualitative, and quantitative extraction procedure followed by an hplc assay of 11-nor-Δ-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) from human urine is developed. Using a new, "mixed mode", bonded silica gel solid phase extraction (SPE) column , the analyte was selectively isolated from the urine component. Following extraction, the presence of THC-COOH was confirmed and quantitated by a UV detector on a Varian 15cm C18 column using 35:65 v/v 50 mM phosphoric acid:acetonitrile at a flow rate of 1.5 mL/min. The limit of detection was 10 ng/mL at a signal to noise ratio of 2.5. The method showed linearity in the 10-300 ng/mL range (r=0.999) with good precision (RSD 1.4%) and accuracy (87% absolute recovery).

INTRODUCTION

Marijuana and related cannabis products are used by a significant portion of our society. The

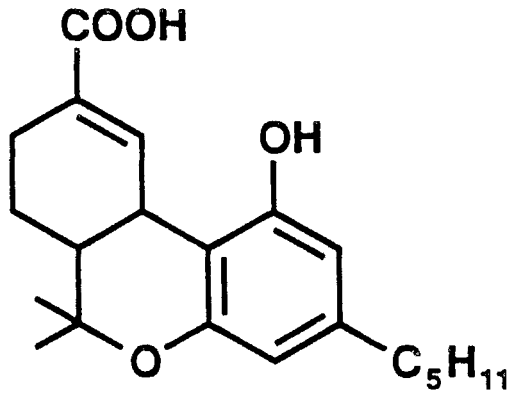

FIGURE 1. Structure of THC-COOH.

major psychoactive component in marijuana is tetrahydrocannabinol which is highly lipophilic. In humans, tetrahydrocannabinol is accumulated in tissues rich in lipids and then slowly released from these tissues in such a way that it is barely detectible in urine. The major metabolite of tetrahydrocannabinol is 11-nor-Δ-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH, figure 1) which is extracted in urine mainly as a glucoranic acid conjugate (1,2).

Radioimmunoassay (3-5), gas chromatography (5), high pressure liquid chromatography (7-8), thin layer chromatography (9-11), and gas chromatography-mass spectrometery (12-14) procedures have been developed for monitoring urine for the drug metabolite. Current methods for sample preparation, including liquid-

liquid extraction (15,16) and other Solid phase extraction techniques (7,9,12,8) either yield low drug recovery and provide incomplete removal of interfering urine components, or require long preparation times.

This paper presents a sensitive, specific, qualitative, and quantitative extraction procedure and an hplc assay that will simultaneously analyze for the drug metabolite (THC-COOH) in human urine using Bond Elut Certify II™, a new bonded silica gel solid phase extraction column. Bond Elut Certify II is a chemically modified silica gel material exhibiting three different types of interactions; hydrophobic, polar, and ion exchange. Due to mixed mode properties and the selective nature of Bond Elut Certify II, the THC-COOH extracts are extremely clean.

EXPERIMENTAL

Materials

Bond Elut Certify II extraction columns and a Vac Elut® vacuum manifold (AI6000) were provided by Analytichem International (Harbor City, CA). A Vortex mixer was obtained from Scientific Industries Inc. (Bohemia, NY). A Reacti Therm™ heating module

and a Reacti Vap™ evaporator were purchased from Pierce (Rockford, IL).

Reagents and Chemicals

THC-COOH was purchased from Alltech Applied Science (Deerfield, IL). HPLC grade methanol, acetonitrile, hexane, and ethyl acetate were purchased from EM Science (Cherry Hill, NJ). Certified negative human urine was obtained from Fisher Scientific (Tustin, CA) and certain volumes of urine were spiked with known amounts of THC-COOH. All other chemicals were purchased from Fisher Scientific.

Instrumentation

The chromatographic separation was developed and performed on a high pressure gradient HPLC System Varian Model Vista 5500 (Walnut Creek, CA), a Rheodyne Model 7126 injector (Burbank, CA) equipped with a 50 uL injection loop and a Varian Model diode Array 9060 polychrom programmable UV detector. Chromatographic separation was accomplished on a 15cm C18 Column (4.0 mm i.d., 4.5 um particle size). The mobile phase consisted of 35:65 v/v 50 mM phosphoric acid:acetonitrile.

METHODS

Specimen Preparation

Three mL spiked urine and 300 uL of 10 M potassium hydroxide solution were added to a large test tube. The mixture was vortexed and hydrolyzed for 15 minutes at 60 °C. The sample was cooled and 165 uL of glacial acetic acid and 2 mL of 100 mM sodium acetate buffer (pH 7.0) with 5% methanol (95:5) were added. The specimen was adjusted to pH 6.0-7.0 with glacial acetic acid and vortexed.

Extraction

Bond Elut Certify II tubes were connected to a Vac Elut and conditioned with 2 mL of methanol. Excess methanol was removed by washing with 2 mL of 100 mM sodium acetate buffer (pH 7.0) containing 5% methanol. The hydrolyzed urine specimen containing THC-COOH was added to each column and passed through the bed at a slow flow rate by applying vacuum at approximately 2-3 inch Hg. The column was washed with 10 mL of 50% aqueous methanol and the sorbent was dried for 10 minutes under full vacuum (15 inch Hg). The column was further washed with 2 mL of ethyl acetate and dried under full vacuum for 30 seconds. All washes were discarded.

The tips of the Vac Elut delivery needles were wiped and a rack with labeled collection tubes was placed in the Vac Elut. The drug metabolite was eluted with 2 mL of hexane/ethyl acetate (75:25) with 1% acetic acid. The Vac Elut was disassembled and the labeled test tubes removed and placed in the Reacti Therm evaporator. The solvent was evaporated to dryness under a slow stream of nitrogen at room temperature and reconstituted in methanol (100 uL) for further hplc analysis. A flow chart of the extraction procedure from human urine is shown in figure 2.

Preparation of Standard curve

A standard curve was generated from drug-free human urine spiked at 10-300 ng/mL. Table 1 lists the results from the analysis of THC-COOH. Quantitation was based on linear regression analysis of peak areas of the extracts versus peak areas of the standards.

RESULTS AND DISCUSSIONS

The solid phase extraction procedure described here provides rapid, reliable, and reproducible isolation of THC-COOH from human urine. The bonded

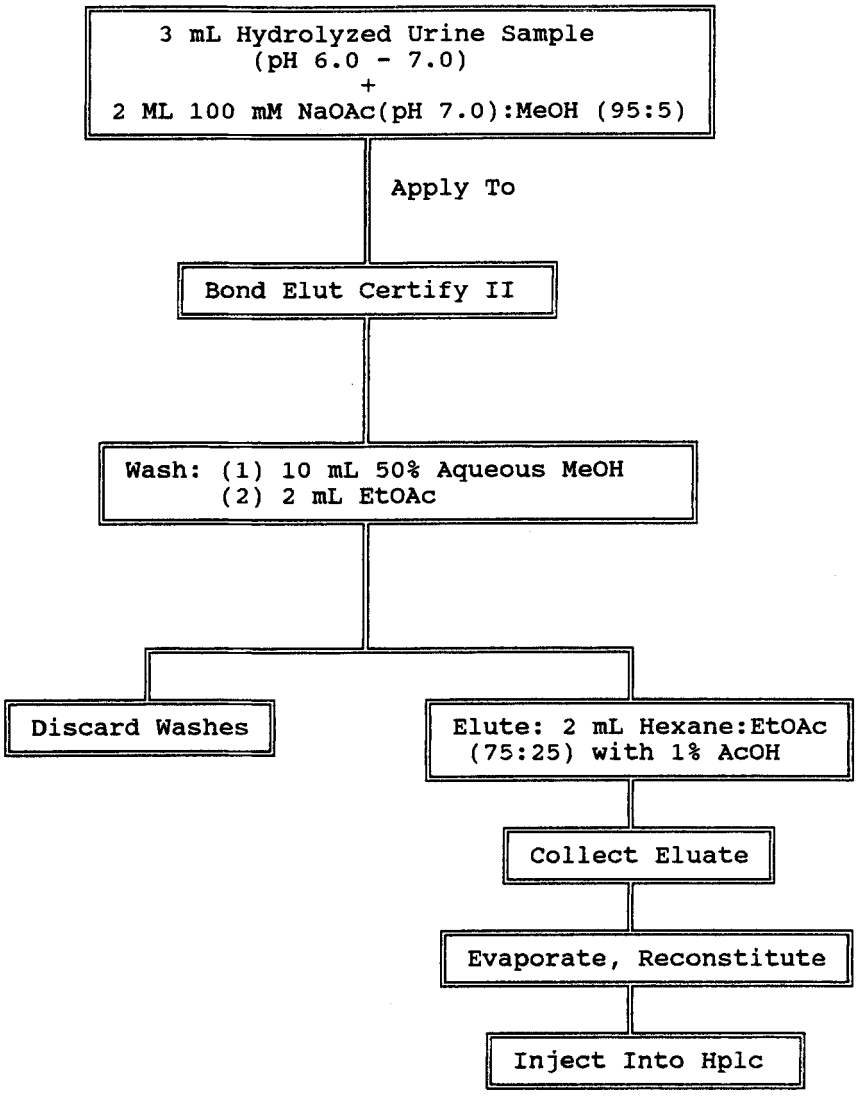

FIGURE 2. Flow chart of the extraction procedure of THC-COOH from human urine using Bond Elut Certify II.

TABLE 1
HPLC Determination of Absolute Recovery of THC-COOH From Urine

Initial Urine Concentration (Absolute % Recovery)			
10 ng/mL (n=5)	100 ng/mL (n=12)	200 ng/mL (n=12)	300 ng/mL (n=12)
87 ± 7.0	86 ± 7.8	89 ± 8.7	87 ± 7.5

phase selectively retains and elutes the drug metabolite by hydrophobic, polar, and ionic interactions. The use of silanized glassware is not necessary throughout the extraction procedure (17). The analysis of urine extracts by hplc shows the cleanliness of the extraction procedure. The National Institute on Drug Abuse (NIDA) requires a quantitative GC/MS confirmation of THC-COOH at 15 ng/mL. A 5 mL urine sample volume provided the detection of THC-COOH at 10 ng/mL.

The hplc chromatogram obtained from the blank urine sample (Figure 3a) was clean and no interfering peaks were found at the THC-COOH retention time. Figure 3b is a spiked urine sample analyzed by hplc at 100 ng/ mL. The detection was performed by UV set at 214 nm using a 15 cm C18 Varian column and a 35:65

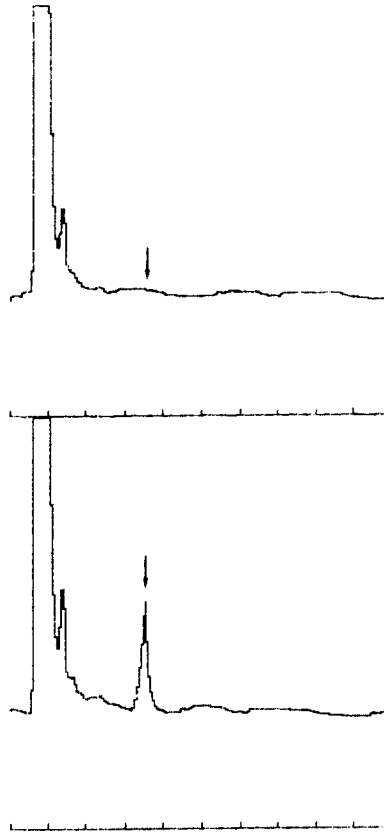

FIGURE 3. a) HPLC Chromatogram of blank urine, b) HPLC chromatogram of spiked urine sample at 100 ng/mL.

mixture of 50 mM phosphoric acid:acetonitrile as a mobile phase.

Figure 4 shows the standard curve generated from the analysis of the spiked urine sample. The linear plot demonstrates the quantitative response for THC-COOH over a concentration range of 10 ng/mL to 300

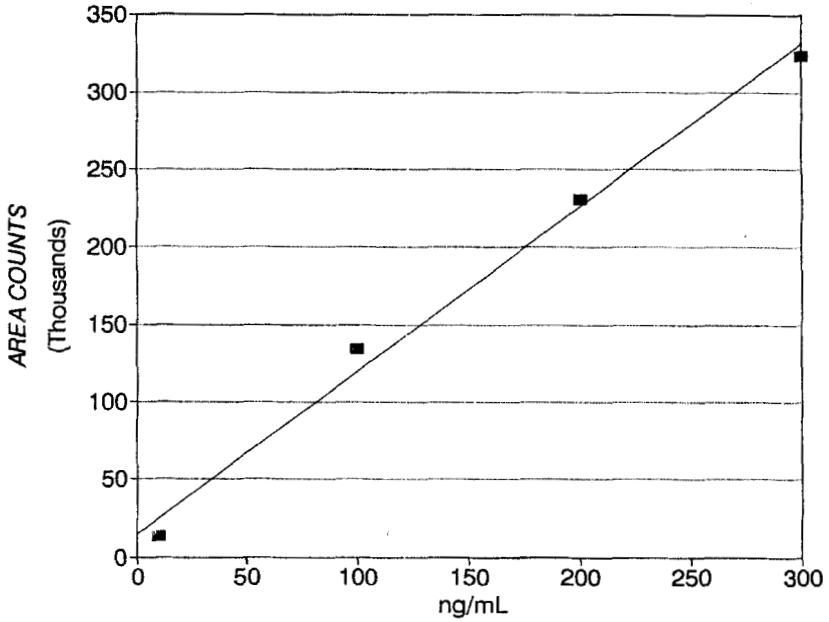

FIGURE 4. Standard curve generated from the analysis of spiked urine sample at 10-300 ng/mL.

ng/mL. The plot exhibits a correlation coefficient of 0.999.

The recoveries and precision data for THC-COOH are listed in Table 1. The data shows absolute recoveries calculated from the spiked urine sample at four concentration levels (10 ng/mL, 100 ng/mL , 200 ng/mL, and 300 ng/mL). The average absolute recovery was found to be 87% over four concentrations with a standard deviation of 1.26 and the correlation of

TABLE 2
HPLC Determination of Absolute Recoveries at 300 ng/mL of THC-COOH from Urine

(Absolute % Recovery)		
Lot 1 n=4	Lot 2 n=4	Lot 3 n=4
86 ± 3.7	84 ± 2.5	87 ± 3.2

variation was found to be 1.4%. Lot-to-lot reproducibility data also produced good results for three different lots of Bond Elut Certify II (Table II).

In summary Bond Elut Certify II, a new bonded phase provides high recovery and enhanced selectivity for the extraction of THC-COOH. Further evaluation of this bonded phase for the extraction of non steroidal acidic drugs from biological fluids is in progress.

ACKNOWLEDGEMENT

The authors wish to thank Dr. John Moore of Analytichem International and Ms. Debbie Kohnstamm of Varian Associates for their assistance in preparing this manuscript and Mr. Hung Nguyen for technical assistance.

REFERENCES

1. Green, D.E., Chao, F.C., Loeffler, K.O., and Kauter, S.L.; Quantitation of Δ-9-tetrahydrocannabinol and its metabolites in human urine by probability based matching GC/MS. In Cannabinoid Analysis in Physiological Fluids. ACS Symposium series No. 98, J.A. Vinson (Ed.). Am. Chem. Soc., Washington, D.C., 93-113 (1979).

2. Williams, P.L., and Moffat A.C., Identification in human urine of Δ-9-tetrahydrocannabinol-11-oic acid glucuronide: A tetrahydrocannabinol metabolite, J. Pharm. Pharmacol., 32, 445-48 (1980).

3. Rodgers, R., Crawl, C.P., and Eimstad, W.M.; Homogeneous enzyme immunoassay for cannabinoids in urine., Clin. Chem., 24, 95-100 (1978).

4. Chase, A.R., Kelley, P.R., and Rigby, A.T. Quantitation of cannabinoids in biological fluids by radioimmunoassay. In Cannabinoid Assays in Humans. NIDA Research Monograph No.7, R.E. Willette (Ed.). U.S. Government Printing Office, Washington, D.C., 1-9 (1976).

5. Gross, S.J., and Soares J.R., Separate radioimmune measurements of body fluid Δ-9-THC and 11-nor-carboxy-Δ-9-THC. In Cannabinoid Assays in Humans. NIDA Research Monograph No. 7, R.E. Willet (Ed.). U.S. Government Printing Office, Washington, D.C., 10-14 (1976).

6. Whiting, J.D., and Manders, W.W., Confirmation of a Tetrahydrocannabinol Metabolite in Urine by Gas Chromatography., J. Anal. Toxicol., 6, 49-52 (1982).

7. Elsohly, M.A., Elsohly, H.N., Jones, A.B., Dimson, A.B., and Wells, K.E., Analysis of the Major Metabolite of Δ-9-Tetrahydrocannabinol in Urine II. A HPLC Procedure., J. Anal. Toxical., 7, 262-264 (1983).

8. Bourquin, D., and Brenneisen, D., Confirmation of Cannabis Abuse by the Determination of 11-Nor-Δ-9-tetrahydrocannabinol-9-Carboxylic Acid., J. Chromatogr., 414, 187-193 (1987).

9. Vinson, J.A., and Lopatofsky, D.J., A Semi-Automated Extraction and Spotting System for Drug Analysis by TLC I. Procedure for Analysis of

the Major Metabolite of Δ-9-Tetrahydrocannabinol in Urine., J. Anal. Toxicol., 9, 6-9 (1985).

10. Kanter, S.L., Hollister, L.E., and Musumeci, M., Marijuana Metabolites in Urine of Man X. Identification of Marijuana Use by Detection of Δ-9-Tetrahydrcannabinol-11-oic Acid Using TLC. J. Chromatogr., 234, 201-207 (1982).

11. Meatherall, R.C., and Garriott, J.C., A Sensitive TLC Procedure for the Detection of Urinary 11-Nor-Δ-9-Tetrahydrocannabinol-9-Carboxylic Acid., J. Anal. Toxicol., 12, 136-140 (1988).

12. McCurdy, H.H., Lewellen, L.J., Callahan, L.S., and Childs, P.S., A Simple Enzymic Digestion Procedure of Intact Samples in Pharmokinetic Drug Analysis. J. Anal. Toxicol., 11, 175-178 (1986).

13. Joern, W.A., Detection of Past and Recurrent Marijuana Use by Modified GC/MS Procedure., J. Anal. Toxicol., 11, 49-52 (1987).

14. Paul, B.D., Mell, L.D., Mitchell, J.M., Jr., Mckinley, R.M., and Irving, J., Detection and Quantitation of Urinary 11-Nor-Δ-9-Tetrahydrocannabinol-9-Carboxylic Acid, A Metabolite of Tetrahydrocannabinol, by Gas Chromatography and Electron Impact Mass Fragmentography., J. Anal. Toxicol., 11, 1-5 (1987).

15. Elshohly, M.A., Arafat, E.S., and Jones, A.B., Analysis of the Major Metabolite of Δ-9-Tetrahydrocannabinol in Urine III. A GC/ECD Procedure., J. Anal. Toxicol., 8, 7-9 (1984).

16. Peat, M.A., Deyman, M.E., and Johnson, J.R., HPLC-Immunoassay of Δ-9-Tetrahydrocannabinol and its Metabolites in Urine., J. Forensic Sci., 1, 110-114 (1984).

17. Parry, R.C., and Gisch, D.J., Confirmation of 11-nor-Δ-9-tetrahydrocannabinol-9-carboxylic acid in urine using solid phase extraction pretreatment. LC-GC International, 3, 29-35 (1990).