TECHNICAL NOTE

George R. Nakamura,[1] *Ph.D.; Walter J. Stall,*[1] *B.S.; and Raymond D. Meeks,*[1] *M.S.*

Analysis of Dihydrocodeine in Urine Using Sep-Pak® C_{18} Cartridges for Sample Cleanup

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ABSTRACT: A rapid and precise method for the isolation and identification of dihydrocodeine from urine is reported. The narcotic is isolated from urine using Sep-Pak® C_{18} cartridges for cleanup, requiring less than 30 min for preparation. Identification is performed by gas chromatography/mass spectrometry.

KEYWORDS: toxicology, dihydrocodeine, drug identification, urine, chemical analysis

Dihydrocodeine is generally dispensed as a mild analgesia in solid dosage forms and is listed as a controlled substance under Schedules III and V of the United States Code. However, in the Far East, it is present as an antitussive agent in cough syrups as well as in solid dosage forms, and widely sold over the counter. While the amount of dihydrocodeine is small in these syrup preparations, commonly 0.1% or less, it is sometimes abused.

The analysis of urine for the identification of dihydrocodeine is facilitated by cleanup of samples using Sep-Pak® C_{18} cartridges. We have previously found that urine samples can be rapidly and efficaciously analyzed for 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid using Sep-Pak C_{18} cartridges for cleanup [*1*]. This solid phase extraction procedure is also applicable for extracting dihydrocodeine. A review of the literature indicates that methods used for the analysis of urine for dihydrocodeine involve liquid-liquid extraction and identification by gas chromatography [*2,3*]. Our procedure incorporates a rapid solid phase extraction of urine samples with an absolute identification of dihydrocodeine by gas chromatography/mass spectrometry (GC/MS).

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[1]Chief chemist, forensic chemist, and forensic chemist, respectively, U.S. Army Criminal Investigation Laboratory Pacific, APO San Francisco 96343-0086.

Experimental Procedures

Materials and Reagents

The extraction cartridges used were Sep-Pak C_{18} type, Waters Associates, Milford, MA. Disposable syringes, 20 mL, were Plastipak® with Luer-Lok® tips, from Becton-Dickenson, Rutherford, NJ. The drug standard used was dihydrocodeine bitartrate, Alltech-Applied Science, State College, PA. All other chemicals and solvents were analytical grade.

Equipment

A Finnigan Model 1020 gas chromatograph/mass spectrometer was used. The glass column (6 ft by 1/8 in. [2 m by 3 mm]) was packed with OV-1 on 100–200 mesh Gas Chrom Q (Applied Science). The column temperature was programmed from 200 to 270°C at a rate of 20°C/min. The temperatures of the injector port, jet separator, and ion source were 265, 240, and 80°C, respectively. Helium was used as the carrier gas at 30 mL/min. Electron impact ionization mode was used with ionization at an electron energy of 70 eV. The spectra were scanned from m/z 40 to 400 with a scan time of 2 s. The retention time for dihydrocodeine was in the range of 5 to 7 min under these conditions.

Method

The extraction method for dihydrocodeine was essentially the same as for the marijuana metabolite described in an earlier report [*1*]. A Sep-Pak cartridge was activated by passing 5 mL of methanol followed by 5 mL of water, using a 20-mL hypodermic syringe. Ten millilitres of urine were adjusted to approximately pH 8.0 with a 10% mixture of sodium bicarbonate:sodium carbonate ($NaHCO_3$:Na_2CO_3), (8:3 w/w), and introduced into the cartridge. The cartridge was washed with 5 mL of water followed by 5 mL of acetonitrile:water (25:75 v/v). Two millilitres of methanol were used to elute the dihydrocodeine. The eluant was collected in a 5-mL conical tube and evaporated in a heating block at 60°C under a flow of nitrogen or air. The residue was dissolved in 20 μL of methanol and a 2 to 3-μL aliquot was injected into the GC/MS.

Recovery

The efficiency of extraction was tested by determining the recovery of various amounts of dihydrocodeine added to the urine. Three different concentrations were used: 10, 20, and 50 μg in 10 mL of urine. Each of these was extracted under standard conditions. The final residue was dissolved in methanol containing 0.5 mg/mL of methadone internal standard, and was subjected to gas chromatography under standard conditions.

The results, shown in Table 1, indicated a recovery of 86.2 to 90.5% of dihydrocodeine added to urine. The precision was shown by a standard deviation of 3.3 to 4.1 and a coeffi-

TABLE 1—*Recovery and reproducibility of dihydrocodeine from urine using Sep-Pak extraction.*

N	Concentration, μg/mL	Recovery, %, Mean	SD	CV, %
4	2.5	86.2	3.4	3.9
4	5.0	87.1	3.3	3.8
4	7.5	90.5	4.1	4.5

cient of variation of 3.9 to 4.5%. These values compared closely with those obtained by extracting the same concentrations of dihydrocodeine in urine by the liquid-liquid extraction method of Peat and Sengupta [*2*]. Our method, however, provided a simple, rapid procedure in which absorption, cleanup, extraction, and filtration are performed rapidly and efficiently in a single 1-cm cartridge. In comparison, we found that the liquid-liquid extraction method, which entailed a double extraction of the basic urine sample and the acidic, aqueous back-extract, required three to four times the analytical time. Unless care was exercised, recovery values were difficult to reproduce.

The pH of Sep-Pak extraction was not critical over a wide range. No significant differences could be discerned in the recovery between pH 7.5 to 9.5.

Results and Discussion

The mass spectrum shown in Fig. 1 is that of dihydrocodeine extracted from a urine sample submitted to this laboratory for analysis. The principal peaks were well-delineated and the spectrum was virtually indistinguishable from spectra of standard dihydrocodeine. Since the separation of codeine, norcodeine, and dihydrocodeine was not discrete under our standard GC conditions, full spectrum scanning was used to prevent the possible misidentification of dihydrocodeine as codeine or norcodeine.

In our analysis of urine samples collected from a large number of dihydrocodeine users, we detected no codeine or norcodeine in samples containing significant quantities of dihydrocodeine. This tends to support the proposal that the pathways of dihydrocodeine metabolism do not favor the conversion of dihydrocodeine to codeine [*4*]. While this observation was based on samples analyzed in the past by a liquid-liquid extraction method [*2*], codeine can be also extracted using Sep-Pak C_{18} cartridges under standard conditions. The percent recovery of codeine was found to be similar to that of dihydrocodeine.

Although most of the brands of over-the-counter cold remedies sold in the Far East containing dihydrocodeine vary widely in their ingredients, we have experienced no interference

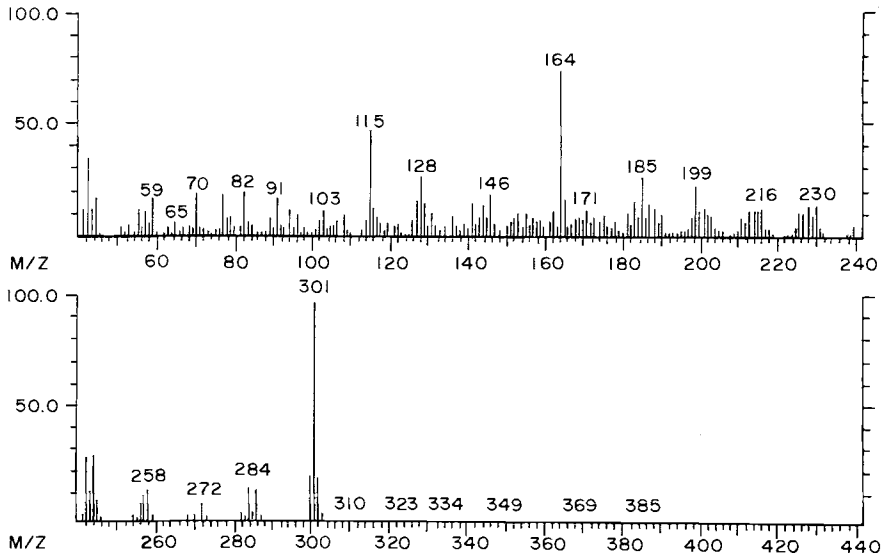

FIG. 1—*Mass spectrum of dihydrocodeine extract from a urine specimen taken from an individual using cough syrup containing dihydrocodeine.*

problems in the analysis of urine from individuals using these products. These remedies commonly include antihistamines such as chlorpheniramine; analgesics such as acetaminophen, ethanzamide, and aspirin; analeptics such as caffeine and methylephedrine; vitamins such as thiamine, riboflavin, and ascorbic acid; expectorants such as guaiacol; and flavoring substances. The analysis of cough syrups themselves was also simplified by using Sep-Pak cartridges for sample cleanup. The product most frequently examined in this laboratory is sold under the brand name, "Bron," which contains senega syrup, 1.5 mL; dihydrocodeine, 30 mg; *dl*-methylephedrine HCl, 40 mg; chlorpheniramine maleate, 6.3 mg; and caffeine, 62 mg in 30 mL of the syrup. One millilitre of this syrup was diluted 1:10 with water and extracted in the Sep-Pak cartridges and a mass spectrum for dihydrocodeine was obtained under standard conditions.

Our ongoing studies show that our method can be used also for extracting methamphetamine and benzoylecognine from urine. The extraction procedure is the same as that for dihydrocodeine. We find that our solid phase system lends itself admirably for extracting highly polar metabolities like benzoylecognine from urine in view of the difficulty by which these compounds are extracted in liquid-liquid procedures. Data obtained from our study conducted on urine samples collected from users of methamphetamines and cocaine are being appraised for accuracy and reproducibility. They will be correlated with results obtained from enzyme multiple immunoassay technique (EMIT®) and the findings will be included in a future report.

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Address requests for reprint or additional information to
George R. Nakamura
USACIL-Pacific
APO San Francisco 96343-0086