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Note

Antibody-mediated extraction of the main tetrahydrocannabinol metabolite, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid, from human urine and its identification by gas chromatography—mass spectrometry in the sub-nanogram range

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In forensic chemistry, large numbers of urine samples must be analysed for cannabis content. For this purpose, immunological assays such as enzyme-multiplied immunoassay technique (EMIT) [1, 2] and radioimmunoassay (RIA) [3—5] are widely used. These methods are very rapid, and economical; they can also be automated to a high degree. As they suffer from a lack of substrate specificity, however, a positive EMIT or RIA result must be confirmed by means of a second independent method, in order to comply with standard forensic requirements.

For the detection of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), the main urinary metabolite of Δ^9-tetrahydrocannabinol (THC), several techniques have been described, including high-performance liquid chromatography [6, 7], gas chromatography [8], gas chromatography–mass spectrometry (GC—MS) [9—12], and thin-layer chromatography (TLC) [13, 14]. The detection limits of these methods depend mainly on the clean-up procedure used during extraction. Even after bonded-phase adsorption chromatography, a detection limit no better than 20—50 ng/ml was achieved, using

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TLC [15] and GC—MS [10]. These techniques are sufficient for the confirmation of positive EMIT results. Because RIA has a higher sensitivity (5 ng/ml, Immunalysis, U.S.A.) than EMIT (20 ng/ml), only some of the positive RIA results can be confirmed by the methods cited above.

We applied a new analytical principle for the detection of THC-COOH in urine combining the features of immunoassay and GC—MS [16, 17]. A simple antibody-mediated extraction procedure for cannabinoids yielded a highly pure extract, which led to a considerable improvement of the detection limit with GC—MS.

MATERIALS AND METHODS

Acetone, isooctane and methanol (nanograde) were obtained from Mallinckrodt (St. Louis, MO, U.S.A.) and used without further purification. Dimethyl sulphoxide and methyl iodide were purchased from Merck (Darmstadt, F.R.G.) and tetramethylammonium hydroxide from Fluka (Neu-Ulm, F.R.G.). CNBr-activated Sepharose 4B was obtained from Deutsche Pharmacia (Freiburg, F.R.G.) and the THC antibody from Miles (Munich, F.R.G.). [^{3}H]THC (specific activity 30 mCi/mg) and [^{3}H]flunitrazepam (specific activity 263 mCi/mg) were purchased from Amersham (Buckinghamshire, U.K.). Water was distilled twice before use.

For the coupling procedure, 1 g of Sepharose was washed for 15 min with 500 ml of 1 m*M* hydrochloric acid on a sintered glass filter, and 4.5 mg of THC antibody, dissolved in 0.1 *M* sodium hydrogen carbonate containing 0.5 *M* sodium chloride was then added. After end-over-end rotation for 2 h at room temperature, the excess ligand was washed away with coupling buffer. Inactivation of remaining active groups was performed with Tris—HCl buffer (0.1 *M*, pH 8). The product was washed three times with 0.1 *M* acetate buffer (pH 4) and 0.1 *M* Tris buffer (pH 8), each containing 0.5 *M* sodium chloride. The Sepharose-coupled antibody was stored in 10 ml of 0.1 *M* phosphate buffer (pH 7) at 4°C in the dark.

For extraction, 1 ml of the phosphate buffer (containing 0.45 mg of immobilized antibody) was placed in a Pasteur pipette and held in place by a swab of cotton wool. The cotton wool, as well as the gel, was washed extensively with acetone and water before use. After the extraction procedure, the antibody columns were washed with water and then immersed in 1—2 ml of 0.1 *M* phosphate buffer (pH 7) and stored at 4°C in the dark. The columns were reuseable up to 50 times [16, 17]. The maximum binding capacity was 300 ng of THC-COOH.

For hydrolysis, 1 ml of 10 *M* potassium hydroxide and 1 ml of methanol were added to a 5-ml aliquot of the urine. This mixture was incubated for 15 min at 50°C. After cooling, 2 ml of 0.1 *M* phosphate buffer (pH 7) were added, and the mixture was adjusted to pH 7 with concentrated hydrochloric acid.

A 1—5 ml volume of the hydrolysed and filtered urine was passed through the column, which was then washed with 15 ml of water. Elution of the drug was performed with 10 ml of acetone—water (95:5). The solvent was removed with a dry stream of nitrogen.

For GC—MS analysis, the residue was dissolved in 10 μl of a mixture of 1 g

of tetramethylammonium hydroxide, 1 ml of water and 20 ml of dimethyl sulphoxide [8]. After 2 min, 20 μl of methyl iodide were added. The reaction mixture was incubated at room temperature for 10 min and then extracted with 1 ml of isooctane. The isooctane was removed by a dry stream of nitrogen, and the residue was dissolved in 10 μl of isooctane. A 1-μl aliquot was used for GC—MS analysis.

Mass spectra were run on a Finnigan 4021 GC—MS system: injection port temperature, 280°C; SE 54 chemical-bonded fused-silica capillary column (25 m × 0.23 mm I.D.) directly coupled to the ion source (250°C); column temperature, 75—300°C; rate 15°C/min. The mass spectrometer was run in the electron-impact ionization mode. The main fragments of THC-COOH, *m/z* 313 (100%), *m/z* 357 (50%), and *m/z* 372 (35%), were registered in the multiple-ion detection (MID) mode. The retention time was 15.9 min.

RESULTS AND DISCUSSION

The THC antiserum coupled to Sepharose binds THC as well as THC-COOH [3, 18]. Therefore, the recovery was checked with tritium-labelled THC, as labelled THC-COOH was not available. Unspecific binding was tested with tritium-labelled flunitrazepam. [^{3}H]THC and [^{3}H]flunitrazepam dissolved in urine were submitted to the extraction procedure. The radioactivity in the extracted urine and in the aqueous acetone eluates was measured. The results show high specificity for [^{3}H]THC and low unspecific binding for [^{3}H]-flunitrazepam (Table I).

Extracts of blank urines with the Sepharose-coupled antibody gave no hint

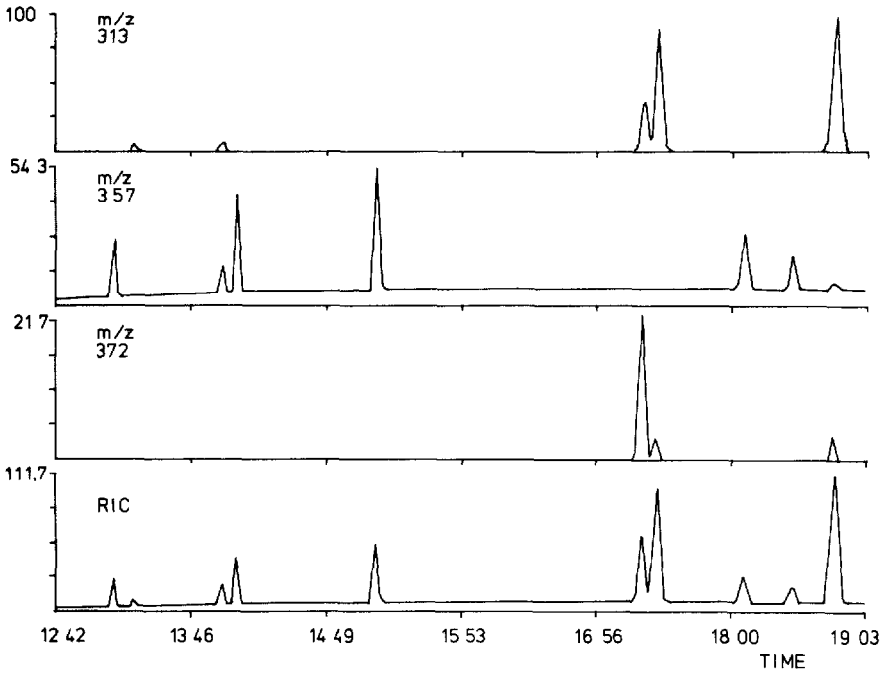

Fig. 1. Mass chromatogram of blank urine extract.

of interfering substances (Fig. 1). For the determination of the detection limit, urine was spiked with THC-COOH. In the MID mode, THC-COOH could be identified at a concentration of 0.5 ng/ml (Fig. 2; only the relevant range of the retention time, 14.49 to 16.56 min, is represented). This is a factor of 10 lower than the detection limit of RIA. A full mass scan could be obtained from samples with 20 ng/ml THC-COOH. The total-ion current chromatogram is shown in Fig. 3, and the mass spectrum of THC-COOH in Fig. 4.

The method presented here permits the routine analysis of a large number of urine samples. It is simple, rapid and cost-effective, because the antibody columns may be used many times. In addition, the high level of purification reduces the contamination of the ion source of the GC—MS. A considerable improvement of sensitivity and specificity is achieved by the strong affinity of THC-COOH for the antibody and the separation of cross-reacting substances by a second analytical step (GC—MS).

TABLE I

RECOVERY (n = 4) OF [^{3}H]THC AND [^{3}H]FLUNITRAZEPAM IN URINE AND AQUEOUS ACETONE ELUATES AFTER PASSAGE THROUGH SEPHAROSE-COUPLED ANTIBODY COLUMNS

Substance added	Percentage recovery in urine after extraction (mean ± S.D.)	Percentage recovery in aqueous acetone fraction (mean ± S.D.)
[^{3}H]THC (100 ng)	6.6 ± 0.8	92.3 ± 3.9
[^{3}H]Flunitrazepam (100 ng)	99.5 ± 3.5	0.9 ± 0.3

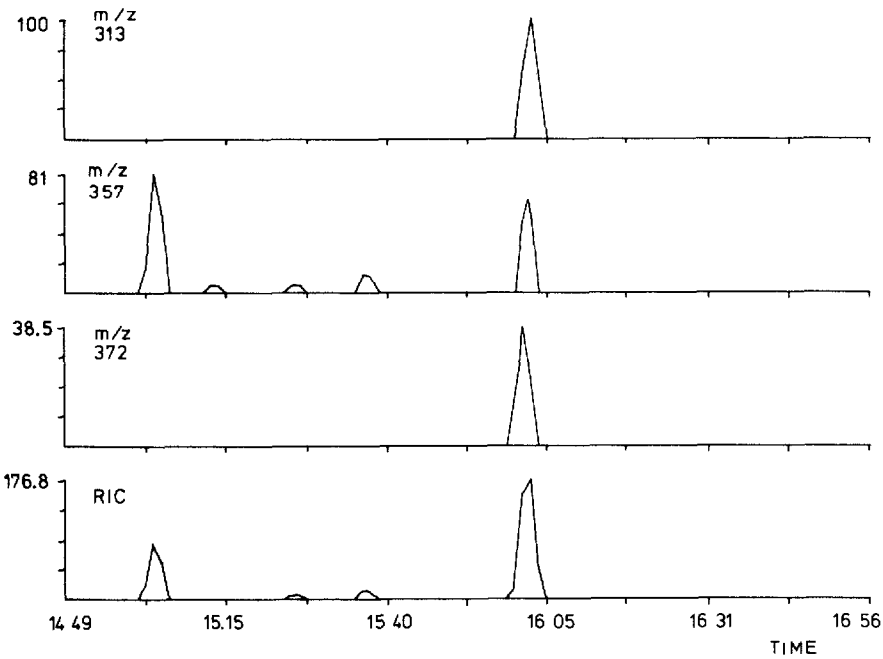

Fig. 2. Mass chromatogram of urine spiked with 0.5 ng/ml THC-COOH.

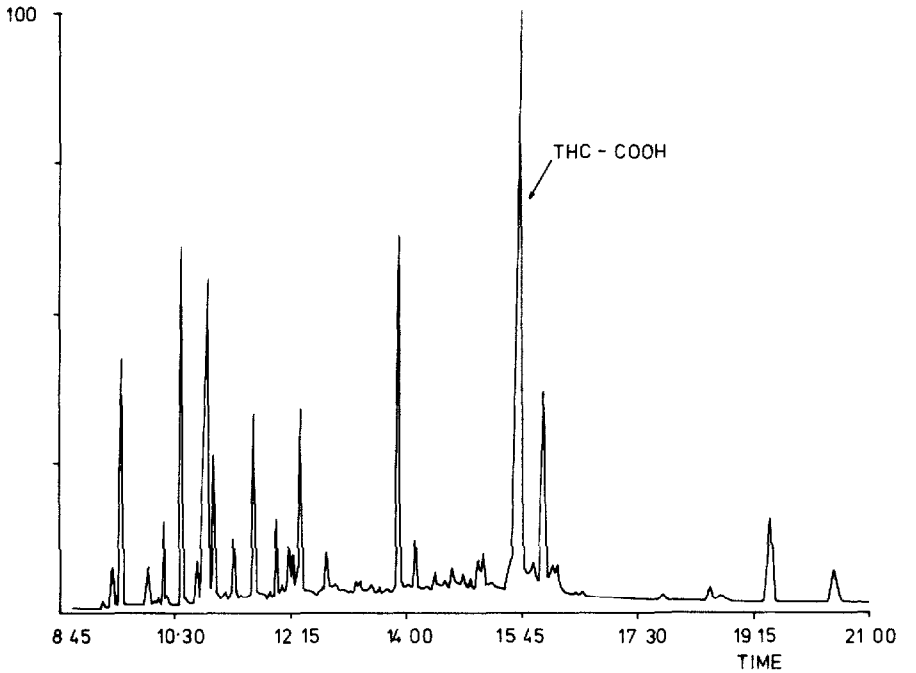

Fig. 3. Total-ion current chromatogram of urine with 20 ng/ml THC-COOH.

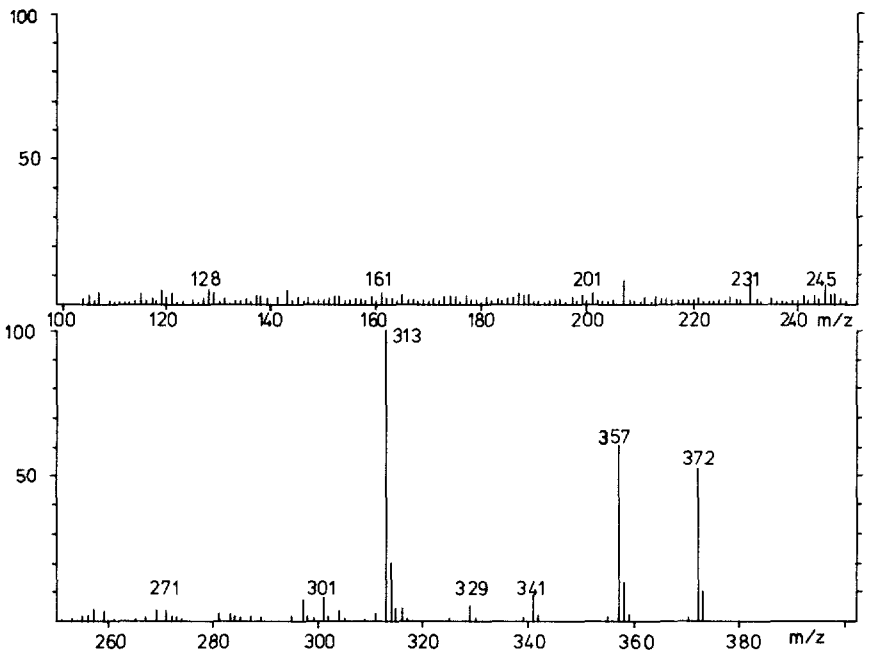

Fig. 4. Mass spectrum of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid from urine with 20 ng/ml THC-COOH.

The proposed procedure could also be used in the near future for the specific extraction of other drugs from biological fluids, thus opening a broad field of application in forensic and toxicological chemistry.

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REFERENCES

1 EMIT Drug Abuse Urine Assays, Cannabinoid Urine Assay, Syva, Palo Alto, CA, 1982.
2 B. Riesselmann, Deut. Apoth. Ztg., 121 (1981) 2078–2082.
3 J.R. Soares, J.D. Grant and S.J. Gross, NIDA Research Monograph, 42 (1982) 44–55.
4 S.M. Owens, A.J. McBay, H.M. Reisner and M. Perez-Reyes, Clin. Chem., 27 (1981) 619–624.
5 C.E. Cook, H.H. Seltzmann, V.H. Schindler, C.R. Tallent, K.M. Chin and C.G. Pitt, NIDA Research Monograph, 42 (1982) 19–32.
6 M.A. ElSohly, H.N. ElSohly and A.B. Jones, J. Anal. Toxicol., 7 (1983) 262–264.
7 A.C. Moffat, P.L. Williams and L.J. King, NIDA Research Monograph, 42 (1982) 56–68.
8 J.D Whiting and W.W. Manders, J. Anal. Toxicol., 6 (1982) 49–52.
9 L. Karlsson, J. Jonsson, K. Aberg and C. Ross, J. Anal. Toxicol., 7 (1983) 198–202.
10 M. Hanke and G. Megges, Z. Rechtsmed., 90 (1983) 105–108.
11 J.E. O'Connor and T.A. Rejent, J. Anal. Toxicol., 5 (1981) 168–173.
12 K. Harzer and M. Kächele, J. Chromatogr., 278 (1983) 63–70.
13 S.L. Kanter, L.E. Hollister and M. Musumeci, J. Chromatogr., 234 (1982) 201–208.
14 S.L. Kanter, L.E. Hollister and J.U. Zamora, J. Chromatogr., 235 (1982) 507–512.
15 M.J. Kogan, E. Newman and N.J. Willson, J. Chromatogr., 306 (1984) 441–443.
16 W. Krause, U. Jakobs, P.E. Schulze, B. Nieuweboer and M. Hümpel, Prostagland. Leukotr. Med., 17 (1985) 167–182.
17 R.G. Glencross, S.A. Abeywardene, S.J. Corney and H S. Morris, J. Chromatogr., 223 (1981) 193–197.
18 M. Cais, S. Dani, Y. Josephy, A. Modiano, H. Gershon and R. Mechoulam, FEBS Lett., 55 (1975) 257–260.