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Detection and quantification of low concentrations of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid from minimal amounts of urine*

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Summary. A simple liquid-liquid extraction and GC/MS-method for detection and quantification of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) from merely 1 ml urine is described. The derivatisation to the methyl ester was carried out using methyl iodide and mefenamic acid was used as internal standard. Experiments with urine spiked with 15 ng THC-COOH/ml resulted in a recovery of 91%. Excellent linearity was obtained over the range 5–100 ng/ml.

Key words: Tetrahydrocannabinol – GC/MS-analysis – Urine

Zusammenfassung. Eine leicht zu handhabende Flüssig-Flüssig-Extraktions- und GC/MS-Methodik zur Identifizierung und Quantifizierung der 11-Nor-Delta-9-Tetrahydrocannabinol-9-Carbonsäure (THC-COOH) aus lediglich 1 ml Urin wird beschrieben. Die Derivatisierung zum Methyl-Ester erfolgt durch den Einsatz von Jodmethan. Mefenaminsäure wird als interner Standard eingesetzt. Versuche mit gespiktem Urin führten zu einer Wiederfindungsrate von 91% bei 15 ng THC-COOH/ml Urin. Eine ausgezeichnete Linearität wurde über den Bereich von 5–100 ng/ml beobachtet.

Schlüsselwörter: Tetrahydrocannabinol – GC/MS-Analyse – Urin

Introduction

In forensic toxicology the problem often exists where many substances must be analysed from minimal amounts of material. With this new method it is possible to confirm the result of a positive immunoassay and to generate quantitative data from merely 1 ml urine. For proof of marihuana abuse a GC/MS-method for detection of the major metabolite 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in urine is normally used. THC-COOH is eliminated in the free form and in its conjugated form. Typically, concentrations in urine may range from nanogrammes to microgrammes per millilitre. However, the American Department of Defense has recommended that concentrations equal to or greater than 15 ng of free THC-COOH/ml, as determined by mass spectral analysis, must be present in urine to be considered a positive indication of marihuana abuse [9].

Current methods of sample preparation using techniques such as solid-phase extraction [1, 5–10] or other liquid-liquid extraction techniques [2, 4, 11, 13] commonly suffer from low drug recovery, incomplete removal of interfering urine components, large volumes of sample material and/or long preparation times.

The following report offers an easy to use liquid-liquid extraction procedure for the isolation of THC-COOH from only 1 ml urine. For this method mefenamic acid was used as the nonisotopic internal standard, instead of meclofenamic acid as introduced by Nakamura [8]. This compound behaves in the same way as THC-COOH in each step of the procedure.

Materials and Methods

Instrumentation. A Model 5890A GC (Hewlett-Packard) with a 5970A Mass Selective Detector (MSD) was used for analysis. Data acquisition and manipulation were performed using standard software supplied by the manufacturer. The instrument was autotuned daily with perfluorotributylamine. For sample analysis the electron multiplier voltage of the detector was set in the range 200–400 V above autotune voltage. A fused silica capillary column OV1 (12 m × 0.2 mm i.d.) was used. The temperature program used with this column consisted of an initial temperature of 100°C, held for 2 min, followed by a linear ramp to 290°C at a rate of

* The results were reported at the 22. Jahrestagung des Nord- und Westdeutschen Arbeitskreises der Deutschen Gesellschaft für Rechtsmedizin, Hannover, April 26–27 1991 (Mußhoff and Daldrup, 48)

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40°C/min. The final temperature was held for 6 min. The split/splitless injector was maintained at a temperature of 260°C.

Chemicals and reagents. 200 ng mefenamic acid/ml methanol (internal standard solution), 0.1 mg THC-COOH/ml methanol (stock solution), 10 *M* potassium hydroxide, maleic acid, hexane/ethyl acetate (9:1), 20% aqueous tetramethylammonium hydroxide (TMAH)/dimethyl sulfoxide (DMSO) (1:20), methyl iodide, 1 *M* hydrochloric acid, ethyl acetate.

All solvents and reagents were of analytical grade (Merck). The THC-COOH was purchased from Sigma (Deisenhofen), the internal standard mefenamic acid was purchased from Parke & Davis (München).

Extraction and derivatization. The extraction was carried out according to the TOXI-LAB procedure of Analytical Systems [12]. Fifty µl of the internal standard solution and 0.1 ml 10 *M* potassium hydroxide were added to 1 ml urine samples (standards) spiked with 5, 15, 30, 50, 75 or 100 ng THC-COOH. The alkalized urine was hydrolyzed at room temperature for 30 min, acidified with maleic acid (0.1 g) and 5 ml hexane/ethyl acetate (9:1) was added. The mixture was shaken for 10 min at room temperature and centrifuged for 5 min at 2000 rpm. The organic layer was transferred to a conical tip glass tube and evaporated to dryness in a heating block at 50°C under a stream of nitrogen.

The derivatisation procedure was similar to that described by Whiting and Manders [13]. The extracted residue was treated at room temperature with 0.2 ml TMAH/DMSO for 2 min, followed by 50 µl of methyl iodide for 15 min, with agitation on a vortex mixer after the addition of each reagent. The mixture was acidified with 0.2 ml hydrochloric acid, extracted with 1 ml ethyl acetate and centrifuged at 2000 rpm for 5 min. The supernatant was transferred to another conical tip glass tube and evaporated to dryness at 50°C under a stream of nitrogen. Ten µl ethyl acetate was used to dissolve the residue and a 2 µl aliquot was injected into the GC/MSD.

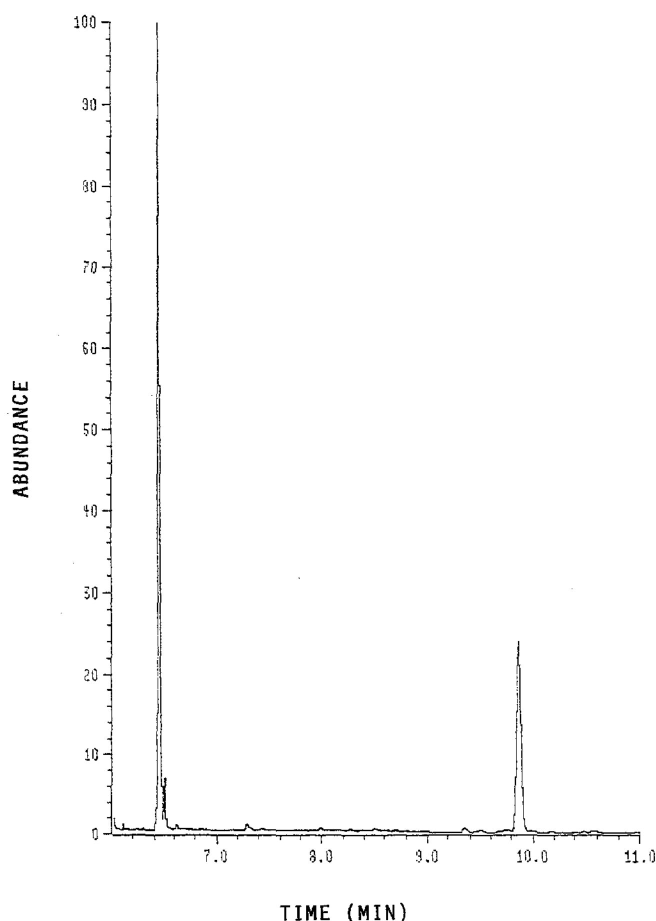

Fig. 1. Gas chromatogram of methylated mefenamic acid (retention time: 6.45 min) and methylated 11-nor-delta-9-THC-9-COOH (retention time: 9.95 min)

Results and discussion

The retention times of methylated mefenamic acid and THC-COOH were 6.45 and 9.95 min, respectively (Fig. 1). In Figures 2 and 3 the mass spectra of the methylated derivatives, extracted from urine samples, are shown with a scan range 40–500 m/z. Twenty ng/ml of THC-COOH in urine appears to be the lowest concentration level at which the metabolite can be clearly identified by its full mass spectrum. For quantification the metabolites were detected by selective ion monitoring (SIM) of 3 fragments (Table 1). A plot of the peak area ratios for the base peak m/z 313 [THC-COOH] and molecular peak m/z 255 [mefenamic acid] was linear over the concentration range 5–100 ng/ml (Fig. 4). The relative recovery of THC-COOH was determined to be 98% (± 2.9% SD) at a concentration of 100 ng/ml, 95% (± 3.1% SD) at 50 ng/ml and 91% (± 3.1% SD) at 15 ng/ml, respectively. The limit of determination was 2 ng/ml and the coefficient of correlation for the calibration curve was 0.997. The reproducibility of the method was measured by analysing 5 replicates of 2 urine samples assayed to contain 75 ng/ml and 30 ng/ml. The coefficient of variation of the high-concentration sample was 5.5% and that of the low-concentration sample was 10.1%.

The procedure offers a rapid and reproducible method for the analysis of the major metabolite of THC in urine samples. Even at low concentrations, 1 ml of urine appears to be sufficient. This is a great advantage for forensic toxicology, particularly if only minimal amounts of material are available.

The gas chromatographic properties of the silyl derivatives of THC-COOH showed no advantage compared to those of the methylated compounds, but the silylated compounds were relatively unstable and chromatography had to carried out within minutes of preparation, whereas the methylated THC-COOH was stable at 4°C for at least one week.

Mefenamic acid is a suitable internal standard. The trideuterated THC-COOH as internal standard is expensive and is not easily available. El Sohly [3] discussed the problem when using the base peak in the mass spectrometry to determine the THC-COOH with the deuterated internal standard, because the natural and deuterated metabolites are not separated by chromatography and the coincidence of 2 fragments with the same mass, one produced by the deuterated, the other by the natural compound, produced a 4–5% error in the results. Mefenamic acid is discretely separated from THC-COOH chromatographically and shows a clear difference in the spectral configuration. It is not a controlled drug and behaves in the same way as THC-COOH in each step of sample preparation. It is not affected by alkaline hydrolysis and can be extracted in acid milieu and derivatized by methylation.

Fig. 2. Structure of mefenamic acid and mass spectrum of its derivative

Fig. 3. Structure of 11-nor-delta-9-THC-9-COOH and mass spectrum of its derivative

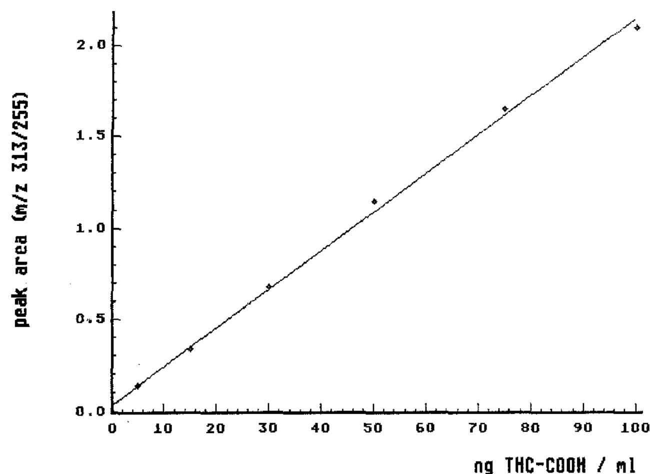

Fig. 4. Calibration curve for the peak area ratios (m/z 313/255) versus concentration of 11-nor-delta-9-THC-9-COOH in urine

Table 1. Detected fragments by selected ion monitoring (SIM)

Mefenamic acid (m/z)	11-nor-delta-9-THC-9-COOH (m/z)
208	313 (base peak)
223 (base peak)	357
255 (molecular peak)	372 (molecular peak)

In summary, this method can easily be used in forensic toxicology for the analysis of the major metabolite of THC in urine samples, specially to confirm presumptive positive immunoassay results.

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