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Short communication

Rapid reversed-phase high-performance liquid chromatographic method for the assay of urinary 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid and confirmation of use of cannabis derivatives

Vincenza Bianchi[a,*], Giovanni Donzelli[b]

[a]*Vincenza Bianchi Laboratory of Clinical Pathology, City Hospital, Via Venezia 18, 15100 Alessandria, Italy*
[b]*Bio-Rad Laboratories, Segrate Milan, Italy*

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Abstract

The main active cannabis (marijuana and hashish) derivative Δ^9-tetrahydrocannabinol is, in vivo, transformed and excreted mainly as 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) and its glucuronide. The method presented here allows the confirmation of the presence of THC-COOH by means of a basic hydrolysis, solid-phase extraction clean-up on reversed-phase (RP) disposable cartridges followed by analysis on a C_8 RP column and UV detection; the mobile phase used was a 55% acetonitrile solution in acid phosphate buffer. Over 600 samples both from drug addicts in therapeutic communities and subjects who were not on any drugs therapy were analysed. This method was precise with a linearity range from 10 to more than 500 ng/ml [the lower limit proposed by the National Institute on Drug Abuse (NIDA) for cannabinoid confirmation method is 15 ng/ml]. The sample preparation is simple and fast, allowing the analysis of large numbers of samples. Perfect correlation was observed between data from the HPLC method and a fluorescence polarization immunoassay screening method. The THC-COOH metabolite was found to constitute 30% of all the cannabinoids excreted in urine of abusers.

1. Introduction

The constant increase in the use of cannabis derivatives, together with stricter legislation on the release of driving and firearms licences, emphasizes the great importance of the availability of chromatographic methods for the confirmation of the presence in urine of drug abuse metabolites when immunoassays are positive [1].

The active constituents of *Cannabis sativa* L., to some of which the pharmacological activity of the plant is connected, are called cannabinoids, a particular group of cyclic C_{21} compounds which are present only in this vegetables species. Their structure contains the benzopyran moiety essential for psychotomimetic activity, whereas analogous molecules with similar structures, but in non-cyclic form, are inactive.

The main cannabis constituents are tetrahydrocannabinol (THC), 11-*nor*-tetrahydrocannabinol-9-carboxylic acid which is present as

* Corresponding author.

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either the Δ^8- or Δ^9-isomer (THC-COOH), cannabinol and cannabidiol; only the first two demonstrate narcotic action. This action is dose dependent; driving performance is seriously compromised because the perception of time and space is disturbed [2]. At high dosages similar effects as with lysergic acid diethylamide (LSD) occur, probably owing to the structural relationship of the active constituents [3]. If inhaled, THC is absorbed faster and in larger amounts than when taken orally, and owing to its strong lipophilic nature it is rapidly spread throughout the system and thereafter metabolized to THC-COOH (the Δ^8-isomer, being less stable, is transformed into the Δ^9-isomer) and as such, or in a glucuronated form, is excreted in the urine where it can be found 12–15 days later after the intake of only a single dose [2].

Numerous HPLC methods have been proposed to confirm the presence in urine of cannabis metabolites and vary in the hydrolysis conditions, type of extraction, elution, mobile phases and column used [4–14]; they are mainly investigative methods, unsuitable for routine workloads.

This paper describes a rapid method to confirm and determine urinary THC-COOH. This method includes a HPLC solid-phase extraction (SPE) to clean up urine samples prior to HPLC analysis. The results obtained with this HPLC method were compared with those obtained with fluorescence polarization immunoassay (FPIA), a class-specific method for cannabinoids.

2. Experimental

2.1. Confirmation method

Instrumentation

The HPLC system (Bio-Rad Labs., Hercules, CA, USA) consisted of a Model 1350 pump, Model AS 100 autosampler, Model 1706 UV–Vis detector and MD 910 diode-array detector. The system was completed with a dry oven for the column and an HP 3393 integrator. The analytical column was reversed-phase RP-8 (250 × 4.6 mm I.D., 5 μm) from Bio-Rad Labs.

Chemicals

Sodium hydroxide, potassium hydroxide, anhydrous monobasic potassium phosphate, 85% orthophosphoric acid, acetonitrile and methanol were obtained from Merck (Darmstadt, Germany), THC-COOH standard solution (0.1 mg/ml in methanol) from Salars (Como, Italy), quality control urine (Multiconstituent Controls for Abused Drug Assays) from Abbott Labs.(Abbott Park, IL, USA) and disposable C_{18} SPE cartridges (100 mg) from Bio-Rad Labs. Ultrapure water was prepared with a Milli-Q system (Millipore, Milford, MA, USA).

Sample preparation

The basic hydrolysis was performed by adding 300 μl of 10 *M* KOH to 5 ml of urine and, after standing at room temperature for ca. 10 min, the sample was adjusted to pH 4.5 with H_3PO_4 (ca. 1.8 ml) and loaded under vacuum (ca. −1.3 kPa) on a cartridge previously activated with MeOH (2 ml) and water (2 ml). The cartridge was then washed with 5 ml of water, 4 ml of acid solution [0.1 *M* H_3PO_4–CH_3CN (70:30, v/v)], 1 ml of basic solution (0.1 *M* NaOH) and eluted with 0.3 ml of 0.05 *M* NaOH–CH_3CN (20:80, v/v).

Standards, spiked in drug-free urine, were prepared in the same way as the samples.

Only small glass tubes, test-tubes and containers were used.

Mobile phase preparation

The mobile phase was prepared with acetonitrile (550 ml) and 0.125 *M* phosphate buffer (450 ml). The phosphate buffer was prepared with 0.2 *M* KH_2PO_4 (60 ml), 2 *M* H_3PO_4 (1.2 ml) and water to 1 l.

Chromatographic conditions

The column was thermostated at 35°C, the mobile phase flow-rate was 1 ml/min and the detection wavelength was 212 nm. The integrator was set up to measure peak areas.

2.2. Screening method

FPIA was carried out with a completely automated instrument (Abbott AD_X). For the dosage of the cannabinoids, which was performed direct-

ly on urine, the sensitivity declared is 10 ng/ml and the cut-off advised is 50 ng/ml.

2.3. Samples

Six hundred samples of urine from different types of subjects were examined: (i) without any drug intake; (ii) negative to the cannabinoid test, but positive to opiates, methadone, benzoylecgonine, benzodiazepines, tricyclic antidepressants and barbiturates; and (iii) positive to the cannabinoid screening test with values between 50 and 1050 ng/ml.

3. Results

Fig. 1A shows a chromatogram for THC-COOH aqueous standard (250 ng/ml), Fig. 1B for urine from a subject who had not taken any drugs and Fig. 1C and D for urine positive to cannabinoids (screening method) with concentrations of 50 and 300 ng/ml, respectively.

As can be seen, the retention time of THC-COOH is ca. 12 min. In the case of urine from subjects who had not taken any drugs no peak is observed, excluding the solvent front. Furthermore, a concentration of 15 ng/ml can be clearly seen, since the relative signal is higher than the background noise.

Fig. 2 shows a chromatogram obtained with the diode-array detector together with absorbance spectrum relating to the peak of THC-COOH in pooled urine positive to cannabinoids (cannabinoids 300 ng/ml, THC-COOH 102 ng/ml) and of an aqueous THC-COOH standard. The two spectra are almost identical.

In Fig. 3, a comparison is made of the absorption spectra measured at the beginning, centre and end of the peak; they are identical.

The linearity of the proposed method was studied by the analysis of blank urines spiked with different concentrations of THC-COOH. The least-squares regression fit showed good linearity, [concentration = 5 + 0.07 (area), correlation coefficient 0.999]. The detection limit of the assay was 10 ng/ml (signal-to-noise ratio = 2).

The within-run and between-run precision and

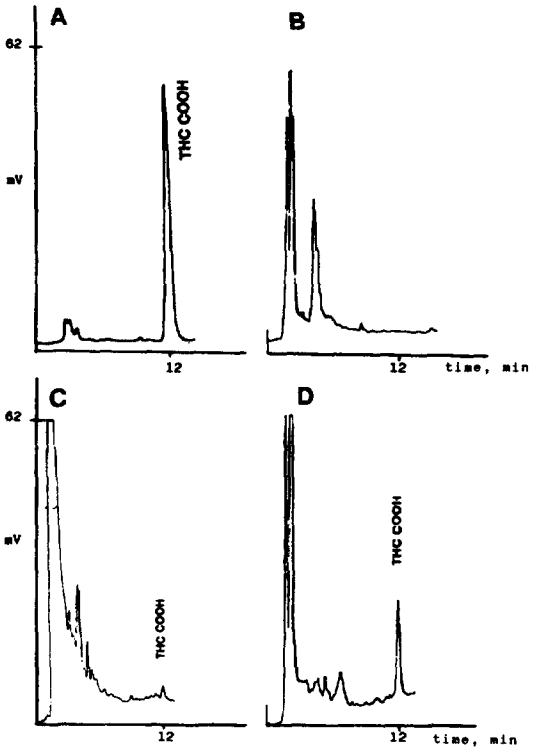

Fig. 1. Chromatograms of (A) THC-COOH aqueous standard (250 ng/ml), (B) Cannabinoid-free urine sample (cannabinoid concentration lower than the FPIA method sensitivity), (C) weakly positive urine sample (cannabinoid concentration = 58 ng/ml, THC-COOH = 15 ng/ml) and (D) positive urine sample (cannabinoid concentration = 300 ng/ml, THC-COOH = 102 ng/ml).

accuracy were evaluated by analysing a commercial quality control urine, as shown in Table 1.

The cannabinoid concentration in spiked samples were determined by the FPIA method and the results were compared with the THC-COOH concentrations obtained with the proposed HPLC method (Fig. 4A), and similarly for urine samples from cannabis abusers (Fig. 4B).

4. Discussion

The HPLC method proposed for the confirmation of the presence of THC-COOH and for its determination appears precise (C.V. = 2.8% within run, 4.7% between run), linear over a wide

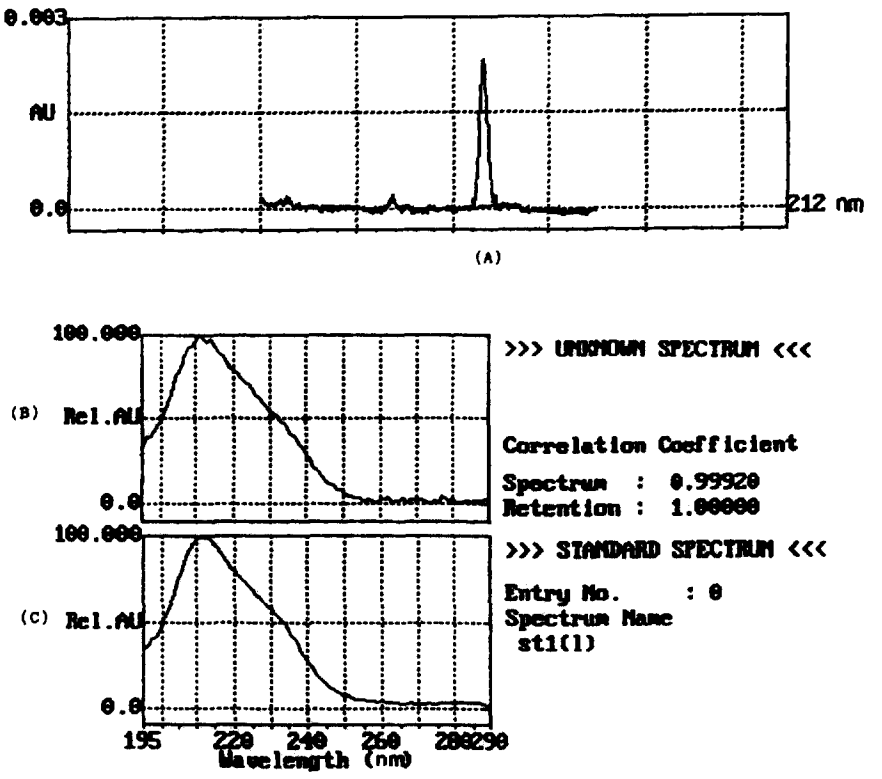

Fig. 2. Comparison of spectra: (A) chromatographic peak (cannabinoids 300 ng/ml, THC-COOH 102 ng/ml); (B) UV spectrum of peak A obtained with the diode-array detector; (C) UV spectrum of the THC-COOH standard.

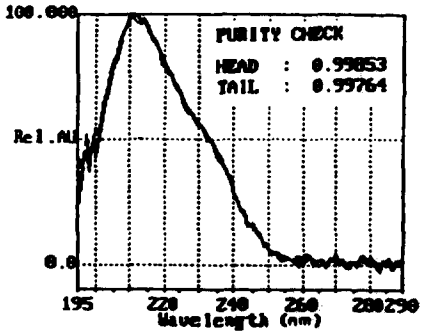

Fig. 3. Superimposed spectra obtained at different times during peak elution of THC-COOH.

concentration range (10–500 ng/ml) and sensitive enough to identify and measure down to 10 ng/ml 15 ng/ml being the lower limit proposed by NIDA for a confirmation method suitable for ascertaining the usage of cannabis derivatives. Further, it is a simple and rapid method and does not require more than 30 min for hydrolysis (10 min), extraction (5 min) and chromatography (ca. 15 min), and is therefore suitable for a large routine testing programmes.

By means of the diode-array detector it has also been possible to confirm that the peak considered is due mainly to the THC-COOH and owing to the selective extraction by SPE and to the analytical conditions used, no interferences from endogenous or exogenous substances have been observed. With spiked urine samples, FPIA

Table 1
Precision and accuracy of the method

Parameter	Within run	Between run	Within run	Between run
Precision				
Number of samples	10	10	10	10
Mean values (ng/ml)	104.4	97.4	14.3	14.6
S.D. (ng/ml)	2.9	4.6	0.5	0.6
C.V. (%)	2.8	4.7	3.5	4.2
Accuracy				
HPLC mean value (ng/ml)	104.4	14.3		
Acceptable values (ng/ml)	98.6–110.2	13.3–15.3		
Theoretical mean values (ng/ml)	100	15		
Theoretical acceptable values (ng/ml)	80–120	12–18		
Experimental vs. theoretical values (%)	4.4	4.7		

and HPLC give identical results ($y = x + 0.1$, $r = 0.999$); in fact, the FPIA monoclonal antibody has been chosen to give 100% reactivity against THC-COOH.

In urine from cannabis abusers the comparison between FPIA and the HPLC methods showed a good correlation ($y = 0.30x + 4.3$, $r = 0.99$), indicating that, if the cross-reactivity of the single species is not taken into account, the levels of molecules with a cannabinoid-like structure found in urine after the intake of cannabis are approximately three times higher than the free and bound THC-COOH.

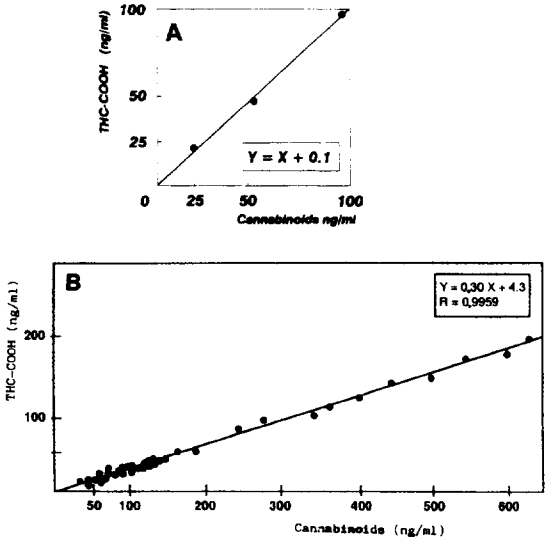

Fig. 4. Comparison between FPIA screening method and HPLC confirmation method: (A) in spiked urine samples; (B) in cannabis abusers' urine samples.

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