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Gas Chromatography-Mass Spectrometry Analysis of 11-Nor-9-carboxy-Δ^9-Tetrahydrocannabinol in Urine

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ABSTRACT

Effective specimen pretreatment and GC-MS protocols were developed for the analysis of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in urine. d_3- and d_9-11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (d_3- and d_9-THC-COOH) were evaluated as internal standards and conclusions were reached that THC-COOH can be effectively analyzed, qualitatively and quantitatively, as trimethylsilyl derivative using selective ion monitoring of the following ions: *m/z* 371, 473 and 488 (for THC-COOH) and *m/z* 380, 479 and 497 (for d_9-THC-COOH). The extraction procedure achieved 90% recovery of the analyte in fortified drug-free urine containing 10-200 ng/ml THC-COOH. Using 2-ml sample size, the overall protocol resulted in the following analytical parameters in the 10-200 ng/ml concentration range studied: interday and intraday precision ranges, 0.35-5.31% and 0.44-5.19%, respectively; linearity, $r^2 > 0.999$.

Key words: **GC-MS, internal standard, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol, urine, drug of abuse.**

INTRODUCTION

Overwhelming literature data resulting from a very significant number of studies have concluded that tetrahydrocannabinol (THC) is the most psychoactive constituent (ranging from trace to 12% in weight) in marijuana (*Cannabis sativa* L.)[1] and the detection of its metabolite, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), in urine can be effectively used for monitoring marijuana exposure.

Several nondeuterated internal standards along with various specimen pretreatment procedures have been adopted for the analysis of THC-COOH in biological media[2,3]. d_3-Analog has been the most popular choice when using GC-MS procedure, especially SIM mode was adopted[4-7]. Use of a d_6-analog has also been reported in a recent study[8].

This study critically evaluated potential interference among ions designated for monitoring THC-COOH and two candidates for internal stan-

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dard, d_3- and d_9-THC-COOH. Selected ions were used along with the adopted pretreatment and other GC-MS parameters to study the effectiveness of the overall analytical procedure, in terms of recoveries, reproducibilities, assay linearity and detection limit.

MATERIALS AND METHODS

I. *Reagents and Chemicals*

All solvents were of HPLC grade and purchased from Baker (Phillipsburg, NJ). All reagents were of analytical grade and purchased from Merck (Darmstadt, Germany). *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was obtained in 10-ml vials from Pierce Chemical (Rockford, IL). THC-COOH, d_3-THC-COOH, and d_9-THC-COOH were obtained from Radian Corporation (Austin, TX).

II. *Extraction Procedure*

Unless indicated otherwise, a typical analytical process utilizes 2 ml urine, which was first spiked with 50 µl d_9-THC-COOH (2 µg/ml in methanol) resulting in the inclusion of 100 ng (or 50 ng/ml) of the internal standard. Hydrolysis was then followed by adding 1 ml 2 N sodium hydroxide and 5 ml hexane containing 0.5% isoamyl alcohol, with the mixture placed on a low-speed shaker for 30 min at room temperature. Following 5 min centrifugation, the top organic layer was aspirated and the aqueous layer was transferred to a clean test tube, which was then added 0.5 ml 6 N HCl and pH checked (pH < 5). Five ml hexane/ethyl acetate (9:1, v/v) mixture was then added, followed by low-speed shaking for 15 min. The mixture was then centrifuged for 5 min and the top organic layer was transferred to a clean screw-top tube and evaporated to dryness at 50°C under a stream of nitrogen.

III. *Preparation of Trimethylsilyl Derivatives*

For derivatization, 75 µl MSTFA was added to the residue in the screw-top tube prepared above. Capped tube was vortexed for 20 sec and then heated at 100°C for 20 min. The reaction mixture was allowed to cool to room temperature prior to GC/MS analysis.

IV. *Gas Chromatography-Mass Spectrometry Analysis*

A Hewlett-Packard 5890 gas chromatography-5972 mass selective detector (GC-MS) equipped with an HP-G1034C Chemstation software was used for this study. The gas chromatograph was equipped with a 12-m Hewlett-Packard (Andover, MA) HP Ultra-1 (100% dimethyl polysiloxane phase) fused silica capillary column (0.20-mm ID; 0.33-µm film thickness). The injector and interface temperature were maintained at 260°C and 280°C, respectively. Column oven temperature was held at 150°C for 1 min, then programmed to 300°C at 20°C/min, and held at the final temperature for 6.5 min. The following parameters were used for injecting samples into the GC-MS system: sample size, 3 µl; injection mode, splitless; injector purge-off duration, 0.75 min.

V. *Selection of Ions for Selective Ion Monitoring*

THC-COOH and the two perspective internal standards were derivatized and analyzed using the procedure and parameters described above. Full-scan spectra of the derivatization products were first collected and examined for preliminary selection of candidate ions for collecting SIM data for qualitative and quantitative analysis purposes. SIM data of these ions were then collected to provide more definite information on cross-contributing the intensity of ions designated for the analyte by the perspective internal standard and vice versa.

RESULTS AND DISCUSSION

I. *Selection of Deuterated Internal Standards and Ions*

The full-scan spectra of THC-COOH (analyte) and its two deuterated analogs (d_3-THC-COOH and d_9-THC-COOH) were shown in Figure 1. The mass spectrum of d_9-THC-COOH

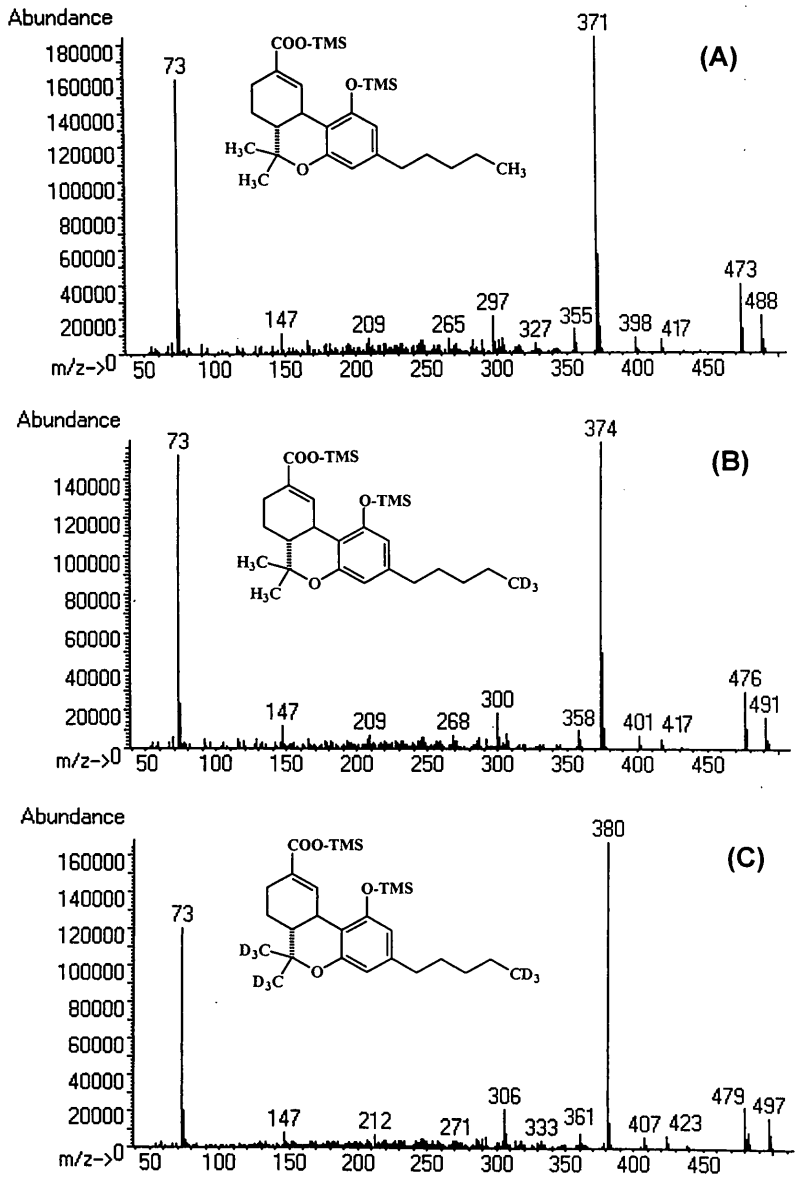

Figure 1. Full-scan mass spectra of TMS-derivatized THC-COOH (A), d_3-THC-COOH (B) and d_9-THC-COOH (C).

revealed relative intensities of 100%, 14.2%, and 10.5% at *m/z* 380, 479, and 497, respectively (Table 1). Since the full-scan mass spectrum of THC-COOH showed no peaks designated for these three ions (Table 1), indicated that it is free from interference by THC-COOH and can be designated for d_9-THC-COOH in SIM data acquisition. The full-scan mass spectrum of THC-COOH showed relative intensities of 100%, 21.7%, and 12.0% at *m/z* 371, 473, and 488, respectively (Table 1). The absence of these ions in the full-scan mass spectrum of d_9-THC-COOH (Table 1) indicated no cross-contribution and being suitable for SIM data acquisition of THC-COOH. The data of SIM peak area integration (Table 2) indicated that all the cross-contributions to peaks at *m/z* 371, 473, 488 (designated for THC-COOH), 380, 479, and 497 (designated for d_9-THC-COOH) were 0%. These six ions appeared to be free from interference and being suitable for quantitation

Table 1. Relative intensity (in %) of selected ions from the full-scan mass spectra of TMS-derivatized THC-COOH, d_3- and d_9-THC-COOH

Ion (*m/z*)	THC-COOH	d_3-THC-COOH	d_9-THC-COOH
Ions designated for THC-COOH			
488	12.0	0.44	0
473	21.7	0.54	0
398	4.8	0.87	0
371	100	1.8	0
355	8.2	0.56	0.30
297	12.4	1.45	0.70
Ions designated for d_3-THC-COOH			
491	0	10.8	—[a]
476	0	19.3	—
401	0	4.74	—
374	0.46	100	—
358	0.48	6.74	—
300	1.00	11.9	—
Ions designated for d_9-THC-COOH			
497	0	—[a]	10.5
479	0	—	14.2
407	0	—	3.8
380	0	—	100
361	0	—	5.0
306	0.6	—	13.0

[a]Since d_3- and d_9-THC-COOH will not be used together, the intensity data of these ions are irrelevant.

Table 2. SIM cross-contribution data of ion designated for the analyte and perspective internal standards

Deuterated analog	Analyte			Deuterated analog		
d_3-THC-COOH	488 (4.0%)	473 (3.3%)	398 (18%)	491 (0%)	476 (0%)	401 (0%)
	371 (1.6%)	355 (6.4%)	297 (13%)	374 (0.63%)	358 (9.5%)	300 (9.2%)
d_9-THC-COOH	488 (0%)	473 (0%)	398 (0%)	497 (0%)	479 (0%)	407 (0%)
	371 (0%)	355 (7.2%)	297 (6.3%)	380 (0%)	361 (11%)	306 (4.9%)

analysis.

The full-scan mass spectrum and SIM peak area integration data of d_3-THC-COOH/THC-COOH were shown in Table 1 and 2. Data in Table 2 indicated that cross-contribution to *m/z* 371, 473, 488 (designated for THC-COOH) and 374 (designated for d_3-THC-COOH) were 1.56%, 3.27%, 3.96% and 0.63%, respectively. Therefore, d_9-THC-COOH is a preferable internal standard than d_3-THC-COOH for the quantitation of THC-COOH by GC/MS.

Relative intensity and cross-contribution data shown in Tables 1 and 2 indicated that selections of *m/z* 488, 473 and 371 (for THC-COOH) and *m/z* 497, 479 and 380 (for d_9-THC-COOH) provides ions that have significant intensities (> 10%)

with no cross-contribution interference. The most intense ions *m/z* 371 and 380 were used for quantification purposes. These ions were then used for the rest of this study.

II. *Extraction Efficiency*

Recoveries of the extraction procedure were studied by comparing the amount of the analyte (observed at the final GC-MS measuring step) in two sets of samples containing the same amount of the analyte. One set of these samples were proceeded with the extraction process, while the another set did not. Specifically, one set (Set I) of drug-free urine were spiked with THC-COOH stock (2 μg/ml in methanol) to arrive at the following concentrations: 10, 25, 50, 100 and 200 ng/ml. Another set (Set II) of samples was prepared by spiking, into empty tubes, the corresponding amounts of THC-COOH stock (2 μg/ml in methanol) used to prepare samples in Set I. Samples in set II were evaporated to dryness, while those in Set I were proceeded with the extraction process. The same amount of the internal standard was added to each sample in Set I and Set II immediately prior to the deviratization step.

Table 3. Extraction recoveries (%) of THC-COOH at different concentration (ng/ml) from fortified urine samples

Spiked conc.	Replicates	Mean ± S.D. (%)
10	3	89.33 ± 0.89
25	3	89.44 ± 0.88
50	3	90.60 ± 0.93
100	3	87.66 ± 0.39
200	3	88.36 ± 1.45

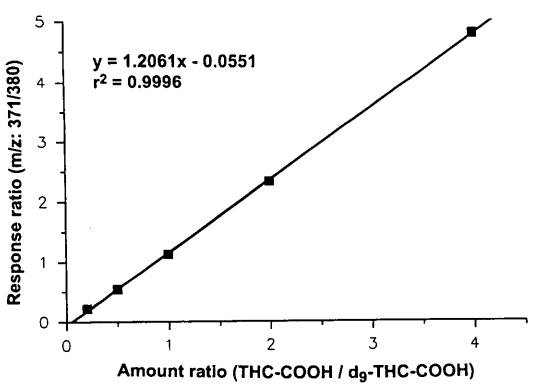

Figure 2. A typical calibration curve (10-200 ng/ml) for the analysis of THC-COOH using d_9-THC-COOH as internal standard.

Samples in both sets were then proceeded with identical derivatization and GC-MS analysis protocols. Amounts of the analyte in Set I was compared to that found in the corresponding samples in Set II. Resulting data are shown in Table 3. Approximately 90% recoveries were achieved.

III. *Precision and Accuracy*

To evaluate the precision of the overall analytical protocol, standard solutions of THC-COOH at 10, 25 ,50, 100 and 200 ng/ml, each in triplicates, were prepared and analyzed for three times in one day (intraday study) and three times on three consecutive days (interday study). Results are shown in Table 4.

IV. *Assay Linearity and Limit of Detection*

A calibration curve based on the response

Table 4. Intraday and interday precision for analysis of urine fortified with THC-COOH

Spiked conc. (ng/ml)	Intraday Mean[a]	Intraday Std dev	Intraday %CV	Interday Mean[a]	Interday Std dev	Interday %CV
10	11.13	0.58	5.19	11.40	0.41	3.61
25	24.65	0.60	2.42	24.76	1.31	5.31
50	49.23	0.22	0.44	49.47	0.59	1.19
100	100.6	1.94	1.93	100.9	0.36	0.35
200	203.2	3.40	1.67	200.6	4.37	2.18

[a]Sample size: triplicates.

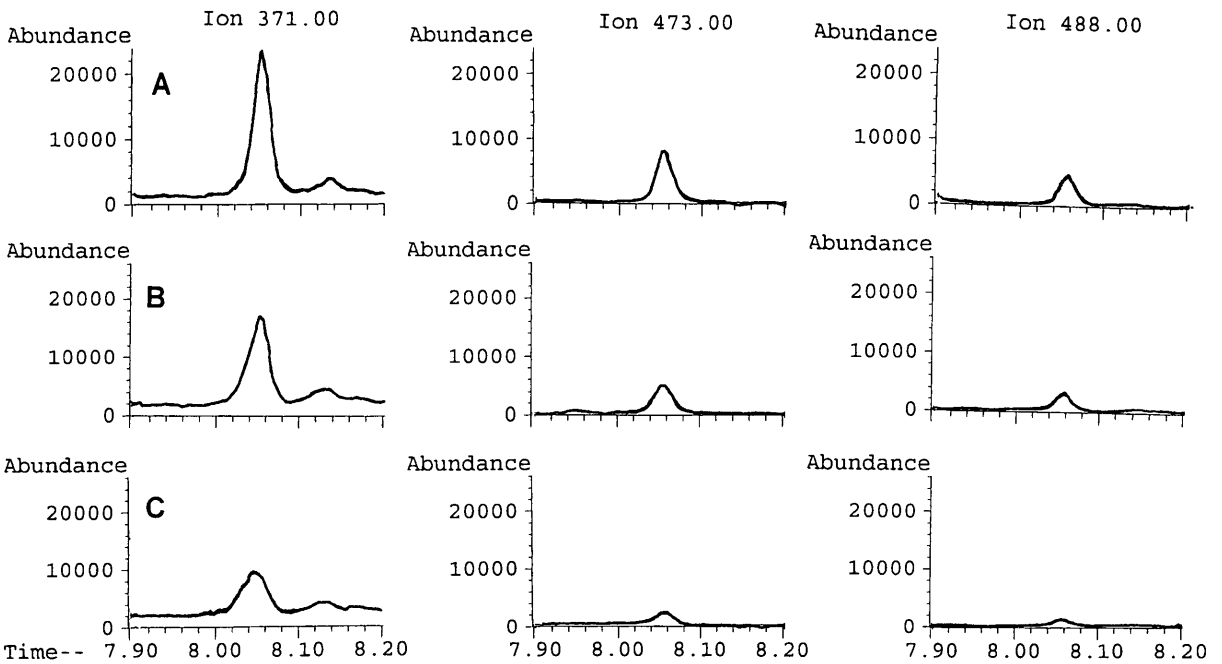

Figure 3. Ion chromatograms resulting from the analysis of 2-ml urine specimen spiked with 2.5 (A), 1.0 (B) and 0.5 (C) ng/ml THC-COOH.

ratios of quantification ions selected for THC-COOH and d_9-THC-COOH (*m/z* 371 and 380, respectively) is shown in Figure 2. Assay protocol achieved excellent linearity within the 10-200 ng/ml concentration range studied: $r^2 > 0.999$. Ion intensity data shown in Figure 3 indicate the overall procedure can at least achieve 1 ng/ml detection limit.

In summary, d_9-THC-COOH is an excellent internal standard, in terms of providing ions free of cross-contribution among ions designated for the analyte and the internal standard. Recovery, reproducibility, assay linearity, and detection limit data fully demonstrated the reliability of the protocol hereby reported.

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以氣相層析質譜儀分析尿中大麻代謝物

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摘 要

本研究係以氣相層析質譜分析法來定量尿中大麻代謝物11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH)。經評估d_3-與d_9-THC-COOH二種內標準品後，發現d_9-THC-COOH對尿中大麻代謝物並無任何干擾定量分析之離子，適合GC/MS做定量分析。本實驗採三甲基矽化(trimethylsilyl)衍生物，以離子監測法(SIM, Selected Ion Monitoring)來定量，監測離子THC-COOH爲 *m/z* 371, 473, 488，內標準d_9-THC-COOH爲 *m/z* 380, 479, 497。以2 ml空白尿液配製濃度在10至200 ng/ml範圍內，其抽取回收率平均約爲90%，日間與日內精密度試驗，其變異係數分別爲0.35～5.31%與0.44～5.19%，並具有良好的線性關係$r^2 > 0.999$。

關鍵詞：氣相層析質譜分析法，氘內標準品，尿中大麻代謝物，定量分析。