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Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry Analysis of 11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol in Urine for Monitoring Marijuana Abuse

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ABSTRACT

A specimen pretreatment procedure, combining solid-phase extraction and methylation, was developed for the analysis of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (9-THC-COOH) in urine for monitoring marijuana exposure. d_3-11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol (d_3-9-THC-COOH) was used as an internal standard, while the now universal selected ion monitoring gas chromatography-mass spectrometry approach was used for detection (*m/z* 313, 357, 372 and 316, 360, 375 for 9-THC-COOH and d_3-9-THC-COOH, respectively) and quantitation (*m/z* 372 and 375 for 9-THC-COOH and d_3-9-THC-COOH, respectively). The effectiveness of the overall procedure was evaluated. One of the extraction columns evaluated achieved > 90% recovery of the analyte in 5-mL drug-free urine fortified with 15 ng/mL 9-THC-COOH. Using the one-point calibration approach, the overall protocol achieved the following analytical parameters: detection limit: 2.09 ng/mL; limit of quantitation: 4.18 ng/mL; upper end of linear range: 376 ng/mL (or better); interday precision: 1.49–8.03% CV in the 4.18–376 ng/mL concentration range studied.

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

INTRODUCTION

Analysis of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (9-THC-COOH), a metabolite of tetrahydrocannabinol (THC), in urine has long been established as an effective approach for monitoring marijuana (*Cannabis sativa* L.) exposure[(1)]. Capillary gas chromatography-mass spectrometry (GC/MS) is currently considered the most effective approach and has been widely used to study the effectiveness of various analytical parameters, including sample pretreatment[(2)], derivatization[(3,4)], and internal standards[(4-6)].

Lin *et al*. recently reported a liquid-liquid

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extraction and trimethylsilyl derivatization procedure for the analysis of 9-THC-COOH in urine samples[4]. This study provides an alternative which combines solid-phase extraction and methylation procedures to achieve the same analytical objective.

MATERIALS AND METHODS

I. *Reagents and Chemicals*

Special reagents, tertamethylammonium hydroxide (TMAH) (24% in methanol) and iodomethane were obtained from Sigma (St. Louis, MO). Standard 9-THC-COOH and the internal standard, d_3-9-THC-COOH, were obtained from Radian Corporation (Austin, TX). Extraction columns, Clean Screen^R and ISOLUTE^{TM} are products of United Chemical Technologies (Bristol, PA) and International Sorbent Technology (Ystrad Mynach, Mid Glamorgan, U. K.), respectively.

II. *Extraction Procedure*

A typical analytical process started with the addition of 200 μL 10 N KOH and 75 μL working internal standard solution (1 μg/mL in methanol) into a 5-mL urine sample. The mixture was then incubated at 50–60°C for 20 minutes, cooled, and pH adjusted to 3.5 ± 0.5 with approximately 2 mL glacial acetic acid.

Extraction columns were processed on a

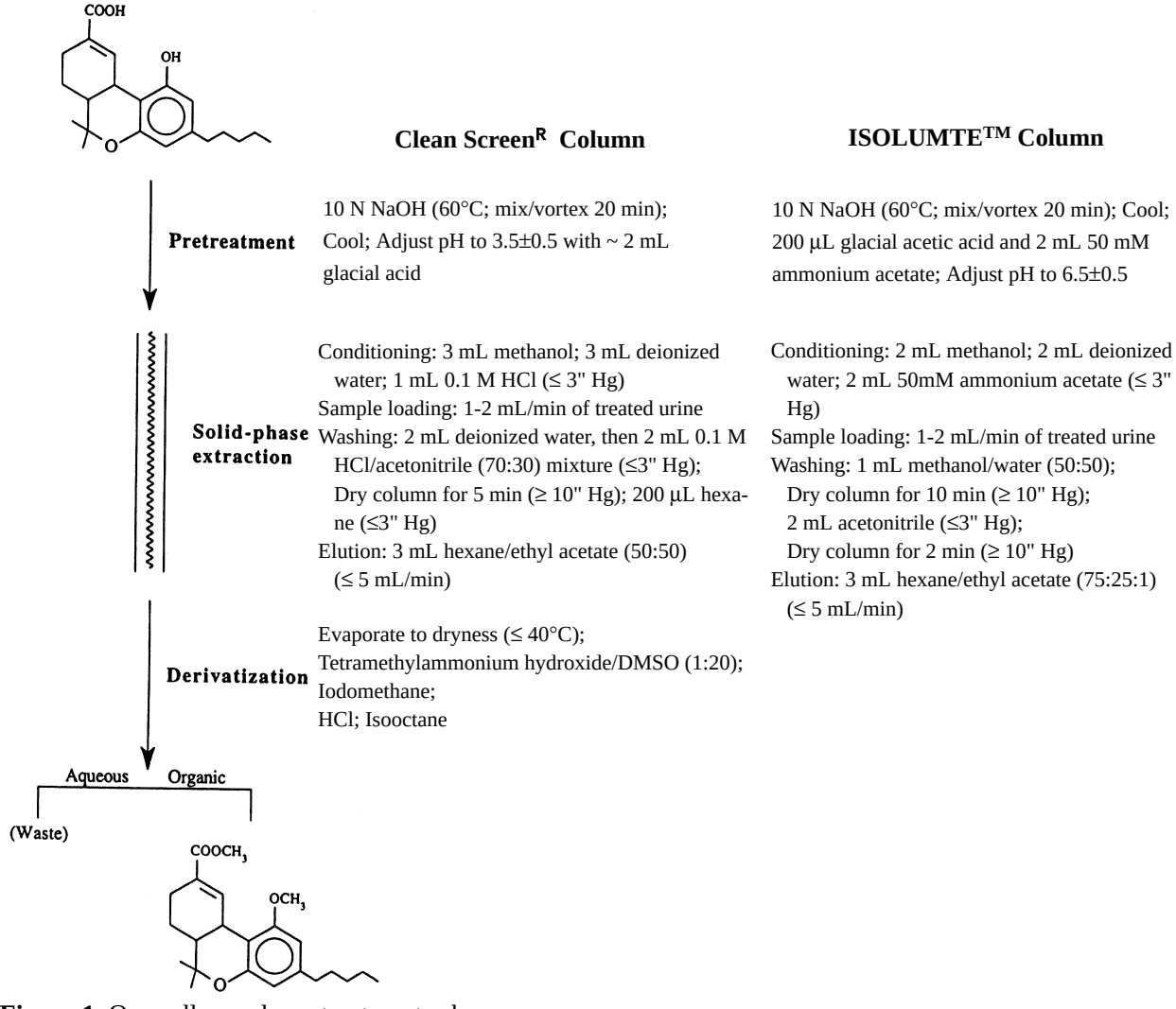

Figure 1. Over-all sample pretreatment scheme.

VISIPREP apparatus (Supelco: Bellefonte, PA). Column conditioning, sample loading, washing, and elution parameters provided by the respective manufacturers were followed. Procedures for Clean Screen and ISOLUTE columns are shown in Figure 1.

III. *Preparation of Methyl Derivatives*

The derivatization procedure developed by the U.S. Department of Navy's drug testing laboratories[7] was adopted for this study. Specifically, 0.1 mL dilute TMAH solution, prepared freshly by mixing one part of 24% TMAH (in methanol) with 20 parts of DMSO, was added to the eluate residue. The mixture was then vortex-mixed for a minimum of 30 seconds. After 2 minutes, 25 μL iodomethane was added to the mixture, vortex-mixed briefly, and after resting for 5 minutes, the pH was adjusted with 0.4 mL 0.1 N HCl. Two mL of isooctane was then added. The tube was capped and vortex-mixed for approximately 2 minutes with frequent stopping and starting of the vortex-mixer (approximately 40 pulses). The tube was centrifuged for 5 minutes at 1500–2000 rpm, followed by transferring the upper organic phase to a clean disposable 5-mL conical tube. The organic solvent was evaporated to dryness at 50–60°C. The tube was capped and stored in a refrigerator until ready for GC/MS analysis.

IV. *Gas Chromatography-Mass Spectrometry Analysis*

A Hewlett-Packard 6890 gas chromatography-5973 mass selective detector (GC-MS) equipped with HP-G1034C Chemstation software was used for this study. The gas chromatograph was equipped with a 15-m RTX-5MS (Restek: Bellefonte, PA) 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 increased (30°C/minute) from 150 to 270°C for 6 minutes. The derivatization product residue was reconstituted with 50 μL of cyclohexane, vortexed, and injected into the column using the following parameters: sample size, 2 μL; injection mode, splitless; injector purge-off duration, 0.75 minute.

The MS is operated under a selected ion monitoring mode using the m/z 313, 357, 372 and 316, 360, 375 for 9-THC-COOH and d_3-9-THC-COOH, respectively. The ions underlined were used for quantitation.

RESULTS AND DISCUSSION

Full-scan mass spectra of 9-THC-COOH and d_3-9-THC-COOH (Figure 2) were obtained from aqueous solutions prepared from respective standard compounds following the analytical scheme shown in Figure 1. These spectra provided quali-

Table 1. Within-run and interday precision for analysis of urine fortified with THC-COOH

Spiked concn	Within-run			Interday		
(ng/mL)	Mean[a]	Std dev	%CV	Mean[a]	Std dev	%CV
2.09	_	_	_	1.55	0.03	2.23
4.18	_	_	_	4.16	0.17	4.20
7.84	7.33	0.14	4.97	7.82	0.63	8.03
15.6	15.6	0.36	0.88	15.6	_	_
31.3	29.5	1.55	5.26	32.5	2.42	7.44
62.7	60.5	0.72	1.19	61.0	0.91	1.49
105	_	_	_	103	3.49	3.38
188	_	_	_	183	3.37	1.84
272	_	_	_	260	6.88	2.65
376	_	_	_	367	8.03	2.19

[a] Sample size: triplicates.

tative indications that the "extraction-derivatization-GC/MS analysis" scheme is a viable approach for the intended purpose. Extraction efficiency, precision and accuracy, and linearity and detection limit were further studied to evaluate the effectiveness of the entire analytical protocol.

I. *Extraction Efficiency*

Since the development of a solid-phase extraction procedure is the main objective of this study, extraction columns from two different

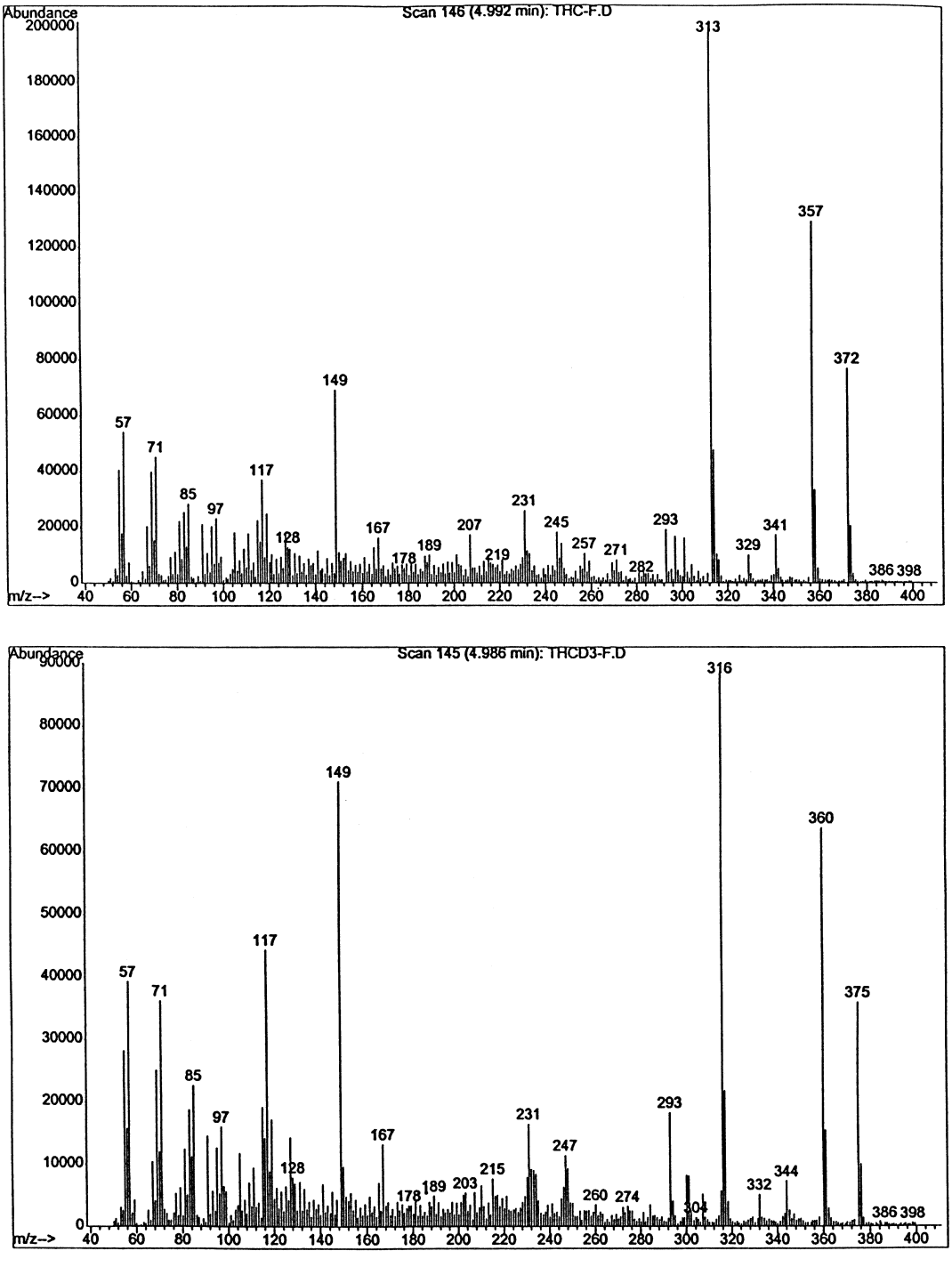

Figure 2. Full-scan mass spectra of methyl-derivatized 9-THC-COOH (upper) and d_3-9-THC-COOH

sources were evaluated for their efficiencies and reproducibilities. Recovery efficiencies of these extraction columns 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. A set amount of 9-THC-COOH (in methanol concentrated stock) was spiked into a drug-free urine blank, while the same amount of stock was spiked into an empty tube at the same time. Samples in the former set were first proceeded through the solid-phase extraction process to the step ready

Table 2. Theoretical versus observed values (in ng/mL) of 9-THC-COOH controls derived from one-point calibration methodology

Theoretical	Set 1			Set 2			Set 3		
Concn	Ion ratio	Cal concn	Dev. (%)	Ion ratio	Cal concn	Dev. (%)	Ion ratio	Cal concn	Dev. (%)
2.09	0.087	1.58	-24.4	0.083	1.51	-27.8	0.088	1.55	-25.8
4.18	0.221	4.02	-3.83	0.240	4.35	-4.07	0.231	4.09	-0.09
7.84	0.393	7.13	-0.06	0.438	7.95	+1.40	0.473	8.37	+6.76
15.62	0.860	Calibrator		Calibrator			Calibrator		
31.34	1.637	29.73	-5.13	1.888	34.26	+9.32	1.890	33.47	+6.76
62.68	3.302	59.97	-4.32	3.383	61.37	-1.56	3.483	61.67	-1.61
104.5	5.891	107.0	+2.39	5.515	100.1	-4.21	5.806	102.8	-1.60
188.0	9.886	179.6	-4.47	10.24	185.8	-1.17	10.45	185.0	-1.60
271.6	14.44	262.3	-3.42	14.59	264.6	-2.58	14.22	251.7	-7.33
376.1	19.78	359.3	-4.47	20.69	375.3	-0.21	20.72	366.9	-2.45

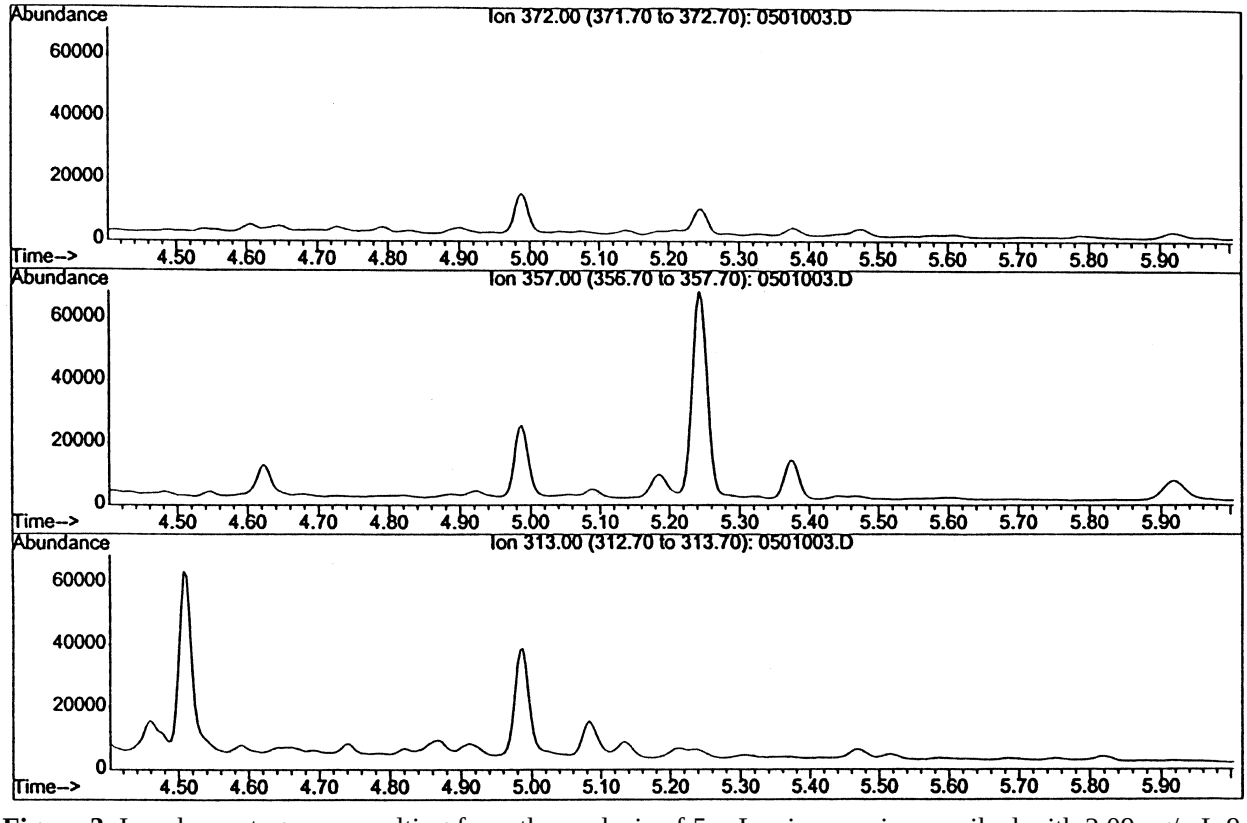

Figure 3. Ion chromatograms resulting from the analysis of 5-mL urine specimen spiked with 2.09 ng/mL 9-THC-COOH (retention time approximately 4.985 min): m/z 372 (upper); m/z 357 (middle); m/z 313 (lower).

for derivatization (Figure 1). Samples in the latter set were evaporated to dryness. The internal standard was added to samples in both sets at this time and processed in parallel for the derivatization and GC/MS analysis procedures.

With the amount of 9-THC-COOH spiked into all tubes equivalent to 15 ng/mL (sample size, 5 mL), duplicate results derived from the Clean Screen columns were 14.25 and 13.39 ng/mL, representing an average recovery of 92.1%. The corresponding results derived from the ISOLUTE columns were 11.02 and 11.84 ng/mL, representing an average recovery of 76.2%. Thus, Clean Screen columns were adopted for the remaining studies.

II. *Assay Precision*

To evaluate the precision of the overall analytical protocol, standard solutions of 9-THC-COOH at various concentrations, each in triplicates, were prepared and analyzed three times in one day (intraday study) and on three consecutive days (interday study). Retention time and ion ratio data all met compound identification criteria. Data shown in Table 1 indicate all precision data are acceptable.

III. *Assay Linearity and Limit of Detection*

Three sets of standard solutions were analyzed on three different dates. Ion ratios (*m/z* 372 and 375 for 9-THC-COOH and d_3-9-THC-COOH, respectively), calculated concentrations, and deviations between the respective theoretical and calculated values are shown in Table 2. These results show excellent linearity within the 4–376 ng/mL concentration range studied.

Data shown in Table 2 also indicate that the quantitative results of the 2.09 ng/mL standard deviate more than 20% from its theoretical value and are thus considered outside the linear range. However, the ion ratios monitored are still acceptable and the three single-ion signal displays shown in Figure 3 indicate this concentration is within the protocol's detection limit.

Urine samples collected from known marijuana users (provided by the Substance Abuse Programs of the University of Alabama at Birmingham, Birmingham, Alabama, U.S.A.) have also been analyzed along with the certification of standards and controls and found to generate reliable results.

In summary, the protocol developed in this study, in terms of the method of "solid-phase extraction, methylation, and using d_3-9-THC-COOH as internal standard", provides a viable approach for the analysis of 9-THC-COOH in urine for monitoring marijuana exposure.

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以固相萃取法及氣相層析質譜儀分析尿中大麻代謝物監測大麻濫用者

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摘 要

本報告以固相萃取及甲基衍生化方法來分析尿中大麻代謝物11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (9-THC-COOH)，用以判定受檢者是否使用大麻。d_3-9-THC-COOH為內標準品，並以氣相層析質譜儀中之離子監測法（selected ion monitoring）做定性及定量分析。監測9-THC-COOH離子為m/z 313, 357, 372; d_3-9-THC-COOH離子為m/z 316, 360, 375。5 mL空白尿液配製濃度15 ng/mL之9-THC-COOH時，其固相萃取之回收率大於90%。以單點校正方式定量分析，最低可檢濃度為2.09 ng/mL；線性範圍位於4.18 ng/mL–376 ng/mL間；而線性範圍內之日間精密度試驗其變異係數（Coefficient of variation）為1.49%-8.03%。

關鍵詞：氣相層析質譜分析，氘內標準品，尿中大麻代謝物，藥物濫用。