Detection of Cannabinoids by Gas Chromatography/Mass Spectrometry (GC/MS) Part I. Quantitation of ⊿9-Tetrahydrocannabinol in Human Urine and Blood Plasma by GC/MS

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Detection of Cannabinoids by Gas Chromatography/Mass Spectrometry (GC/MS)

Part I. Quantitation of Δ^9-Tetrahydrocannabinol in Human Urine and Blood Plasma by GC/MS

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Introduction

The cannabinoid abuse is now spreading all over the world. Δ^9-Tetrahydrocannabinol (THC), the major active component of marihuana and hashish, is generally enhalated by smoking and absorbed into human body *via* the lung. Therefore, the amounts of THC in the blood and excreted into urine are very low. For detecting the trace amounts of THC, gas chromatography/mass spectrometry (GC/MS) methods provide the most specific and sensitive assays at the present time. Extensive works were carried out on the assays of THC in human body fluids by GC/MS.[1)-9)] In most assays, the deuterated THC was used as an internal standard, but is not suitable for the actual forensic examination. In the present study, the author has found that tetraphenylethylene (TPE) can be an excellent internal standard for the assay of THC, and thus succeeded in establishing a simple GC/MS procedure for the quantitation of THC in human urine and blood plasma.

Materials and Methods

Materials

Blood was obtained from Hamamatsu Red Cross Blood Center. Urine was collected from healthy volunteers. Authentic THC was kindly donated by Prof. I. YAMAMOTO, Department of Hygienic Chemistry, School of Pharmacy, Hokuriku University, Kanazawa. TPE was purchased from Tokyo Kasei Kogyo Co., Ltd., Tokyo; 3% OV-17 on 100/120 mesh Gas Chrom Q from Nihon Chromato Works, Ltd., Tokyo; and Sephadex LH-20 from Pharmacia, Uppsala, Sweden.

GC/MS conditions

The GC separation was made on a 2.0 m×2 mm (internal diameter) glass column packed with 3% OV-17 on 100/120 mesh Gas Chrom Q. The GC conditions were: injection temperature 310°C, column temperature 280°C and helium flow rate 30 ml/min. The MS analyses were carried out on a JEOL D-300 GC/MS instrument equipped with a computer-controlled data analysis system (JMA 2000E). The MS conditions were: electron energy 70 eV, separator temperature 310°C, ion source temperature 200°C, acceleration voltage 3 kV and ionization current 300 μA.

Results and Discussion

Basic data

Fig. 1 shows the mass spectrum of the authentic THC. THC showed characteristic peaks at m/z 314, 299, 271, 258, 246, 243 and 231; the molecular ion at m/z 314 was the base peak. This spec-

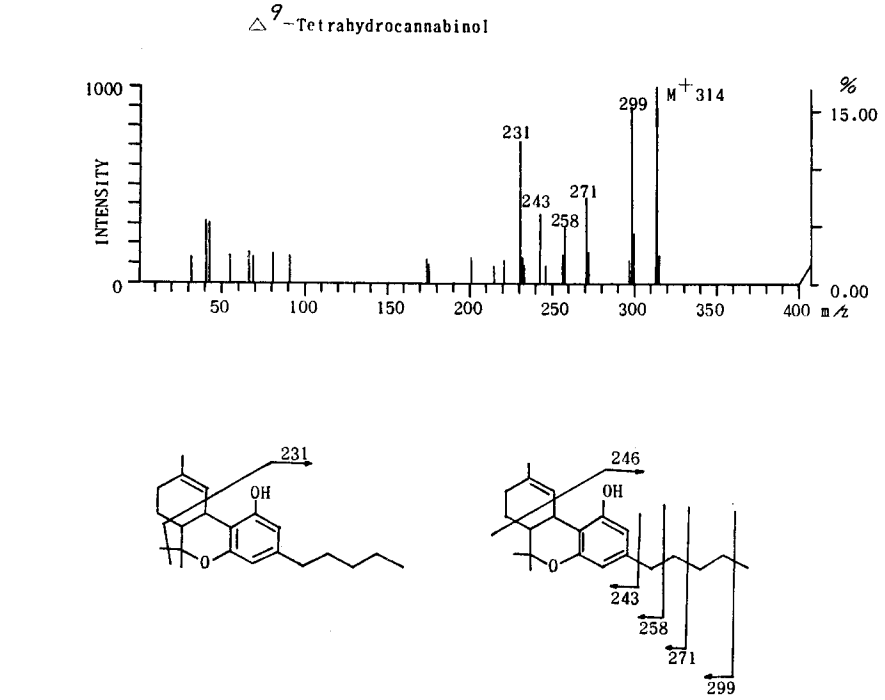

Fig. 1. Mass spectrum of authentic THC and suspected fragmentation mechanisms.

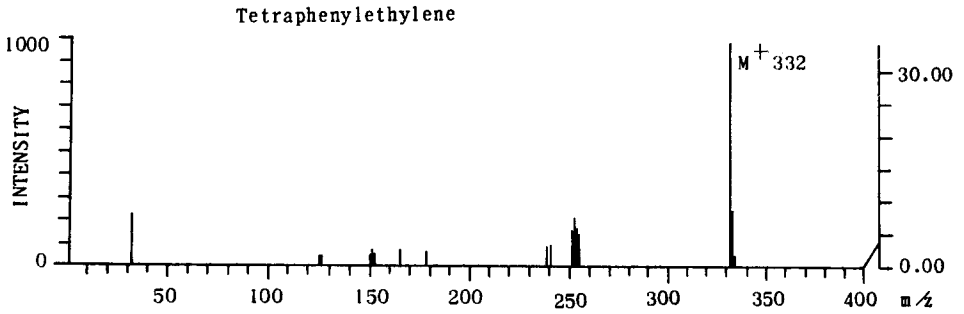

Fig. 2. Mass spectrum of TPE.

trum is in good agreement with those of the previous papers.[10] The suspected fragmentation mechanisms are depicted also in Fig. 1.

The mass spectrum of the authentic TPE, the internal standard used in the present assay, is illustrated in Fig. 2. The molecular ion at m/z 332 was the base peak. Therefore, for the quantitation of THC by selected ion monitoring, the ions at m/z 314 and 332 were used as indicators of THC and the internal standard, respectively.

Fig. 3 shows the mass chromatogram of the authentic THC using its specific ions.

Fig. 4 shows the total ion monitoring for the mixture of the authentic THC and TPE. The retention times of THC and TPE were 7.20 and 8.25 min, respectively.

Both THC and TPE were very lipophilic, suggesting that these compounds are easily extracted

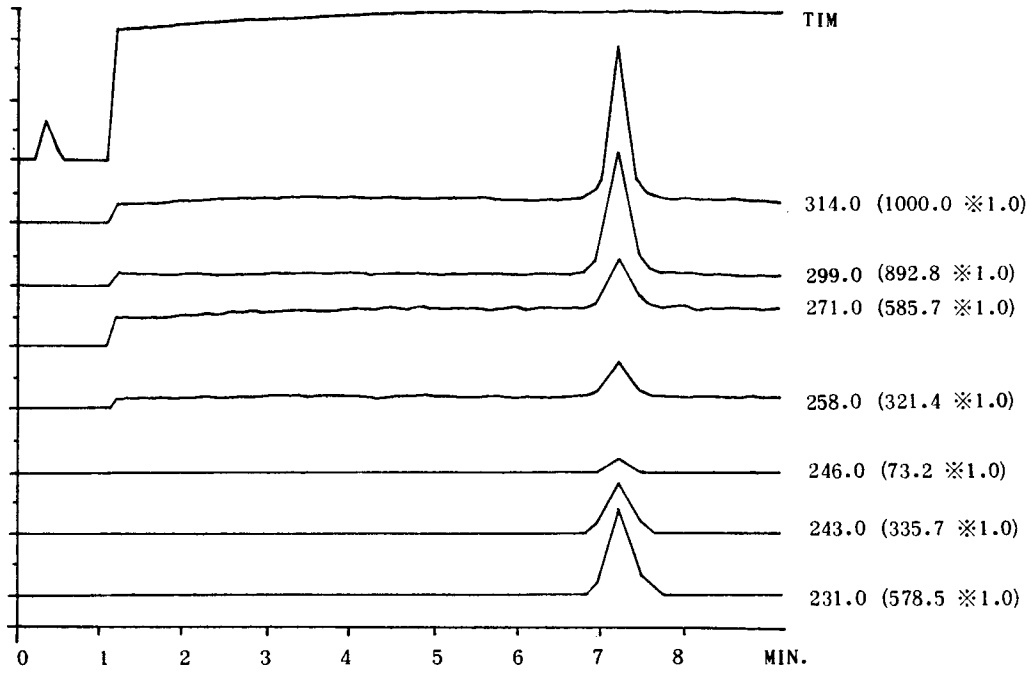

Fig. 3. Mass chromatogram of the authentic THC using its specific ions.

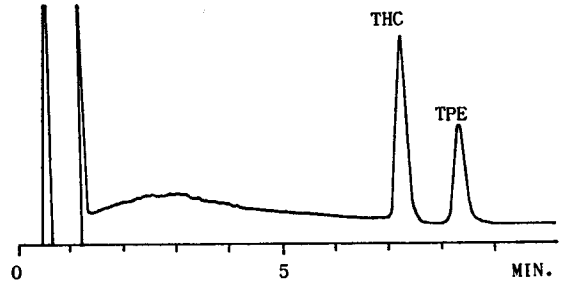

Fig. 4. Total ion monitoring for the mixture of the authentic THC and TPE.

into organic layer. Therefore, TPE is very close to THC in their lipid solubility, retention time and mass number used, showing that this compound is an ideal internal standard for the quantitation of THC.

Recommended procedure

On the basis of the above data, the following procedures are recommended as standard assays for THC in human urine and blood plasma.

After adding 50 ng of TPE and 1.0 ml of 0.1 N HCl to 10 ml of urine, the mixture was extracted with 10 ml of *n*-hexane three times by repeating shaking and centrifugation (3000 rpm, 5 min). The combined organic layer was washed with 10 ml of 0.1 N NaOH and 10 ml of 0.1 N HCl by repeating

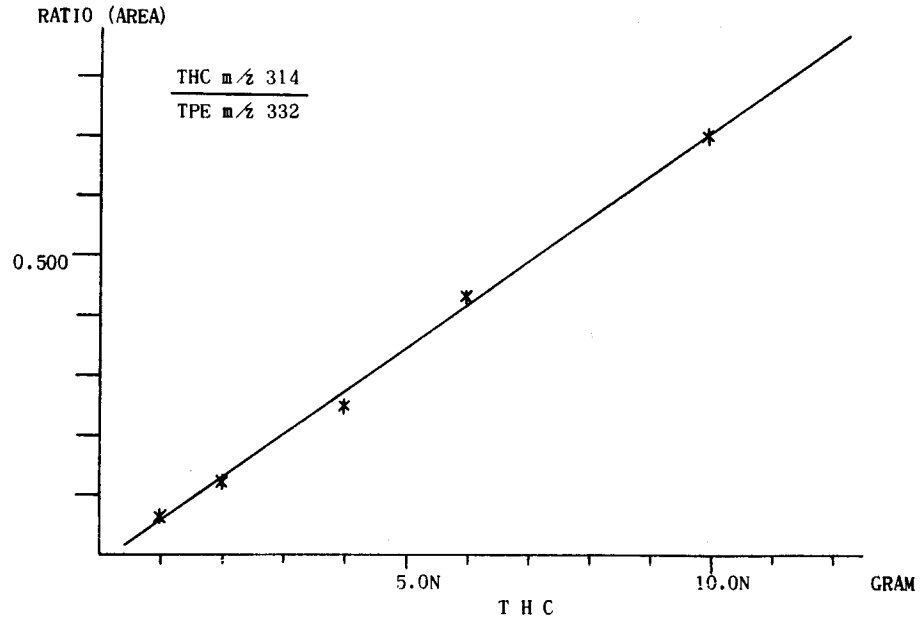

Fig. 5. Calibration curve of THC.

shaking and centrifugation (3000 rpm, 5 min) and then evaporated to dryness. After dissolving the residue in 30 μl of *n*-hexane, 1-3 μl of it was injected to the GC port for the selected ion monitoring with ions at m/z 314 and 332.

For the analysis of THC in blood plasma, 2.0 ml of it was extracted with the same amount of *n*-hexane as described above. The combined organic layer was evaporated to dryness, and the residue was dissolved in 0.2-0.3 ml of chloroform-*n*-hexane-ethanol (10/10/1). The organic solution was subjected to column chromatography with Sephadex LH-20 (40 cm×1.0 cm internal diameter).[1)-3)11)12)] The column was eluted with 50 ml of the same solvent system. The eluate fractions of 25-35 ml were mixed with 10 ng of TPE, evaporated to dryness and dissolved in 30 μl of *n*-hexane. A 1-3 μl aliquot was introduced to the GC port as described above.

For the analysis of THC in urine, TPE was added to urine on the initial step of extraction, but, for that in blood plasma, it was added after the column chromatography. The latter is because TPE was found to bind to the Sephadex tightly and thus not eluted efficiently.

Reliability of the method

Fig. 5 shows the calibration curve for the quantitation of THC using the peak ratio of the ion at m/z 314 to that at m/z 332. The linearity was obtained up to 10 ng of THC. The detection limit of THC was found to be 400 pg.

Fig. 6 shows the results of the selected ion monitoring with the extracts of urine and blood plasma using m/z 314 after adding trace amounts of THC. As can be seen in the figure, there were no interfering peaks due to impurities around the peaks of THC for both urine and blood plasma, showing that THC can be measured specifically using the ion at m/z 314. The selected ion monitoring on the extracts of urine and blood plasma using the ion at m/z 332 (TPE) was also performed; it was confirmed that the peak of TPE was not interfered with by impurities.

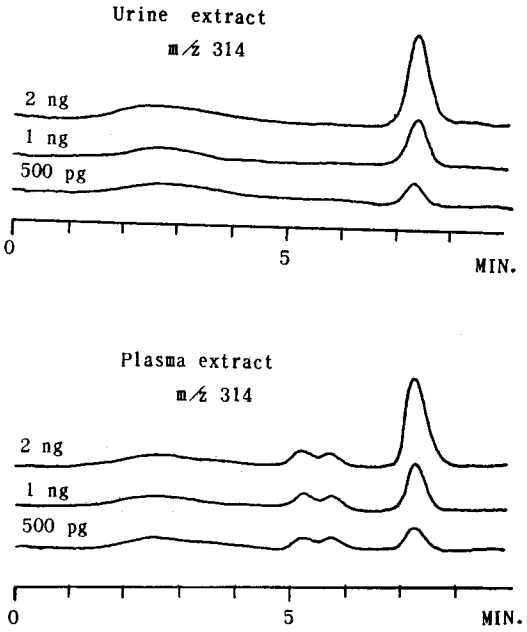

Fig. 6. Selected ion monitoring on the extracts of human urine and blood plasma, to which various amounts of THC had been added.

The recoveries of THC added to urine and blood plasma were 72% and 68%, respectively.

In the present paper, the author has presented detailed procedures for the assay of THC in human urine and blood plasma by GC/MS. Our method employs TPE as an internal standard; this compound seems very excellent as an internal standard, because its lipid solubility, retention time and the mass number used are very close to those of THC. Since in actual forensic examination, the use of the deuterated isotope of THC is not recommendable owing to its expensiveness or labor to synthesize it, out present method seems of great use in forensic practice. In addition, our method is relatively simple, very specific and sensitive.

Summary

Detailed procedures for the assay of Δ^9-tetrahydrocannabinol (THC) in human urine and blood plasma by selected ion monitoring of gas chromatography/mass spectrometry are presented. This method employs tetraphenylethylene as an internal standard; this compound was very close to THC in its lipid solubility, retention time and mass number used for the assay, and thus ideal as an internal standard. The detection limit of the method was 400 pg. Because of its simplicity and sensitivity, our method seems suitable for medicolegal examination.

Acknowledgement

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ガスクロマトグラフィー/質量分析法 (GC/MS) による Cannabinoids の検出

1. GC/MS によるヒト尿ならびに血漿中の Δ^9-テトラヒドロカンナビノールの定量

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大麻中毒の証明には，大麻の主成分である Δ^9-テトラヒドロカンナビノール (THC) をガスクロマトグラフィー/質量分析法 (GC/MS) によつて検出するのが最も有力と思われる．今回ヒト尿ならびに血液からの GC/MS による THC の検出法を検討し，その詳細を設定したので報告する．本法の特色は内部標準としてテトラフェニルエチレン (TPE) を用いることであり，保持時間ならびに抽出性からも THC にきわめて類似し，すぐれた内部標準であると思われる．TPE を用いることにより高価な安定同位体を用いないですみ，法医学的実用に適しているものと考えられる．しかも本法は特異性が高く，操作も比較的簡便で，感度の点でもすぐれ，selected ion monitoring における検出限界は 400pg であつた．