Detection of Cannabinoids by Gas Chromatography/Mass Spectrometry (GC/MS) Part II. Quantitation of Cannabidiol and Cannabinol in Human Urine and Blood Plasma by GC/MS

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Detection of Cannabinoids by Gas Chromatography/Mass Spectrometry (GC/MS) Part II. Quantitation of Cannabidiol and Cannabinol in Human Urine and Blood Plasma by GC/MS

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Abstract. Detailed procedures for gas chromatography/mass spectrometry (GC/MS) assays of cannabidiol and cannabinol in human urine and blood plasma are presented. Both cannabinoids were *t*-butyl dimethylsilylated prior to the GC/MS analysis, because this derivatization gave simple and strong peaks suitable for quantitation. Tetraphenylethylene was satisfactory as an internal standard. The detection limit of the method for both compounds was 200 pg in an injected volume. We can recommend this method for actual forensic examination because of its simplicity, specificity and sensitivity.

Key words : Toxicology, Cannabidiol, Cannabinol, Cannabinoids, Mass fragmentography

Introduction

The main components of hashish is known to be Δ^9-tetrahydrocannabinol, cannabidiol (CBD) and cannabinol (CBN). All these components are general inhaled into the body by smoking, although the latter two have no hallucinogenic activities. In the previous paper[1], the author reported a gas chromatography/mass spectrometry (GC/MS) method for quantitation of Δ^9-tetrahydrocannabinol in human urine and blood plasma. In the present paper, we have extended the study to assay for CBD in CBN in human samples, which are useful for actual forensic examination.

Materials and Methods

Materials

Human blood was obtained from the Hamamatsu Red Cross Blood Center. Urine was collected from healthy volunteers. The authentic CBD and CBN were kindly donated by Prof. I. Yamamoto, Department of Hygienic Chemistry, Hokuriku University School of Pharmacy, Kanazawa. Tetraphenylethylene (TPE), *t*-butyl dimethylsilyl (*t*-BDMS) chloride and imidazole were obtained from Tokyo Kasei Kogyo Co., Ltd., Tokyo; 3% OV-17 on Gaschrom Q (100/120 mesh) from Nihon Chromato Works Ltd., Tokyo; Sephadex LH-20 from Pharmacia, Uppsala, Sweden; and N,N-dimethylformamide from Pierce, Illinois. Other common chemicals used were of the highest purity commercially available.

GC/MS conditions

A JMS-D300 GC/MS instrument with a JMA-2000E computer-controlled data analysis system was used. The GC separation was made on a 2.0 m × 2 mm (internal diameter) glass column packed with 3% OV-17 on Gaschrom Q (100/120 mesh). The MS conditions were: electron energy 20 eV, separater temperature 300°C, ion source temperature 250°C, acceleration voltage 3 kV and ionization current 300 μA.

Derivatization

Samples were evaporated to dryness under the stream of nitrogen in a small glass tube. After adding 50 μl of imidazole solution (2 M in dry N,N-dimethylformamide) and 50 μl of *t*-BDMS chloride solution (2 M in dry N.N-dimethylformamide) to the residue, the tube was capped and heated at 100°C for 1 hr. To the mixture, were added 2.0 ml of *n*-hexane and 1.5 ml of water and shaken with a Vortex-type mixer. After standing for 5 min, the organic layer was evaporated to dryness under nitrogen gas. The residue was dissolved in 60 μl of *n*-hexane and 3 μl of it was injected into the GC port.

Results and Discussion

Basic data

Figure 1 shows mass spectra of the authentic underivatized CBD and CBN. The molecular peaks were small for both compounds. The base peaks, which can be used for quantitation, appeared at m/z 231 for CBD and at m/z 295 for CBN. The peak at m/z 231, however, is not specific for CBD, because Δ^9-tetrahydrocannabinol and many metabolites show peaks at the same mass number[2]. In addition, the use of underivatized compounds with multiple hydroxyl groups, such as CBD, for GC is not desirable because of their loss due to adsorption to the GC column. Therefore, CBD and CBN were derivatized with *t*-BDMS chloride, which gave satisfactory mass spectra for quantitation.

Figure 2 shows mass spectra of the authentic CBD-*t*-BDMS, CBN-*t*-BDMS and TPE. The CBD-*t*-BDMS gave fragment peaks at m/z 417 and 474 and a molecular peak at m/z 542. The CBN-*t*-BDMS showed peaks at m/z 367, 409 and 424 (molecular peak). TPE, which had served as an internal standard for quantitation of Δ^9-tetrahydrocannabinol in our previous paper[1], was also tested in the present study; it shows a very simple spectrum with a molecular and base peak at m/z 332.

The suspected fragmentation mechanisms are shown also in Fig. 2.

For the quantitation by selected in monitoring (SIM), therefore, the base peaks at m/z 474, 409 and 332 were employed for CBD, CBN and TPE (internal standard) respectively.

The retention times of CBD-*t*-BDMS, CBN-*t*-BDMS and TPE were 3.1, 4.4 and 4.4, respectively.

Analysis with urine

On the basis of the above data, the following procedure was adopted as standard assays for CBD and CBN in human urine.

After adding 30 ng of TPE and 1.0 ml of 1 M phosphate buffer (pH 7.3) to 1.0 ml of urine, the mixture was extracted with 2.0 ml of *n*-hexane three times by repeating shaking and centrifugation (3000 rpm, 3 min). The combined organic layer was evaporated to dryness under nitrogen gas, and the residue was subjected to the derivatization with *t*-BDMS chloride as described before (Materials and Methods). The amounts of CBD and CBN in urine were thus quantitated using the intensities of ions (area) at m/z 474 and 409, respectively, as

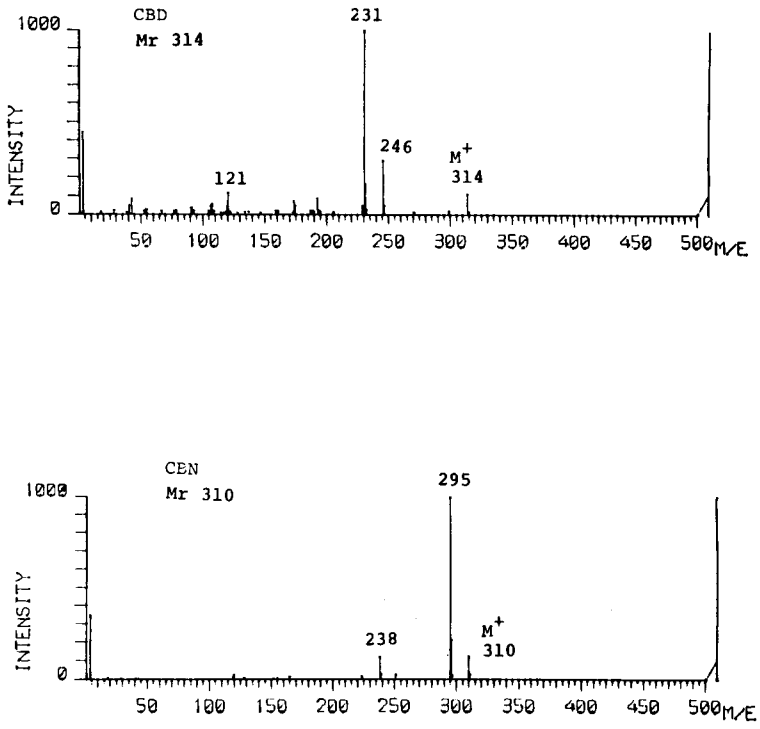

Fig. 1. Mass spectra of underivatized CBD and CBN.

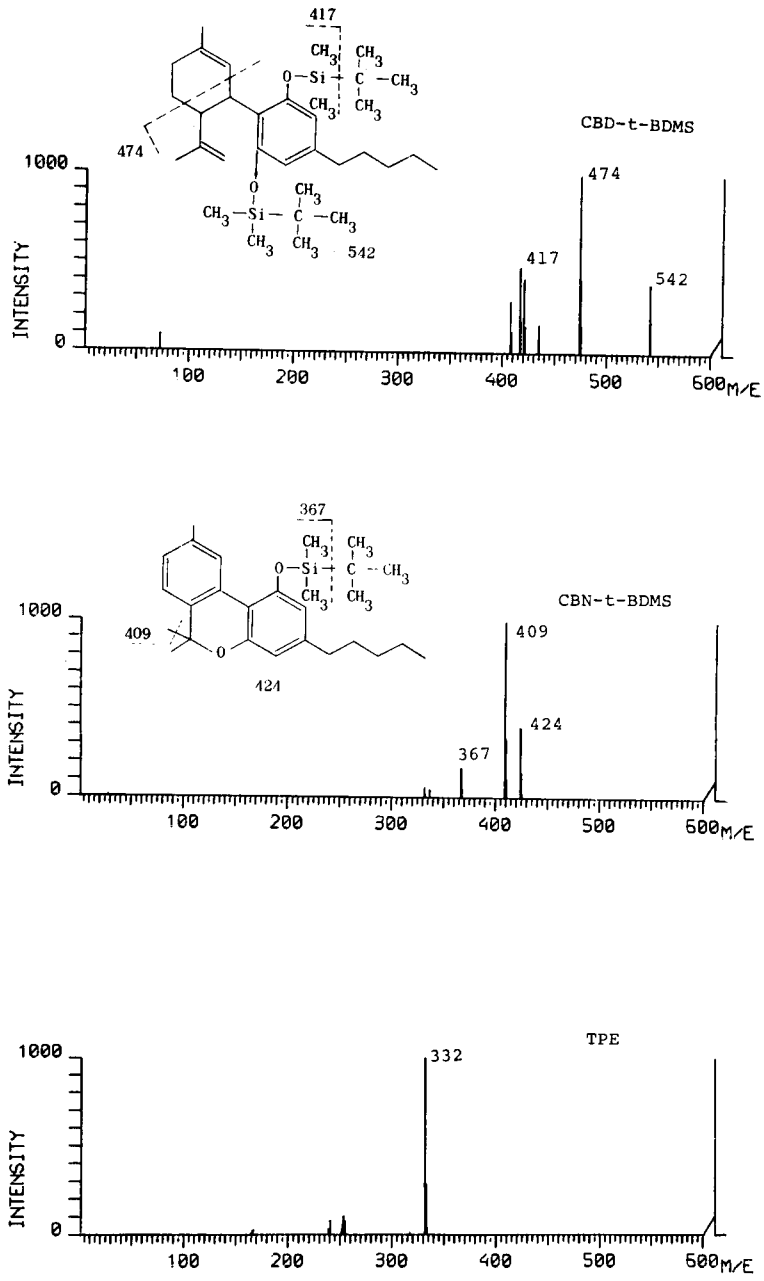

Fig. 2. Mass Spectra of CBD-*t*-BDMS, CNB-*t*-BDMS and TPE.

compared with that at m/z 332 (TPE as an internal standard) by SIM.

Analysis with blood plasma

For the analyses of CBD and CBN in human blood plasma, 1.0 ml of it was extracted with 2.0 ml of *n*-hexane in the presence of 1.0 ml of 1 M phosphate buffer (pH 7.3) as described above. The combined organic layer was evaporated to dryness under the stream of nitrogen and the residue was dissolved in 0.2-0.3 ml of *n*-hexane-chloroform-methanol (10/10/1). The organic solution was subjected to column chromatography with Shephadex LH-20 (16 × 0.8 cm internal diameter). The column was eluted with 30 ml of the same solvent system. The eluate fractions of 9-15 ml were mixed with 20 ng of TPE, and evaporated to dryness under nitrogen gas. The resulting residure was derivatized and subjected to the SIM quantitation as

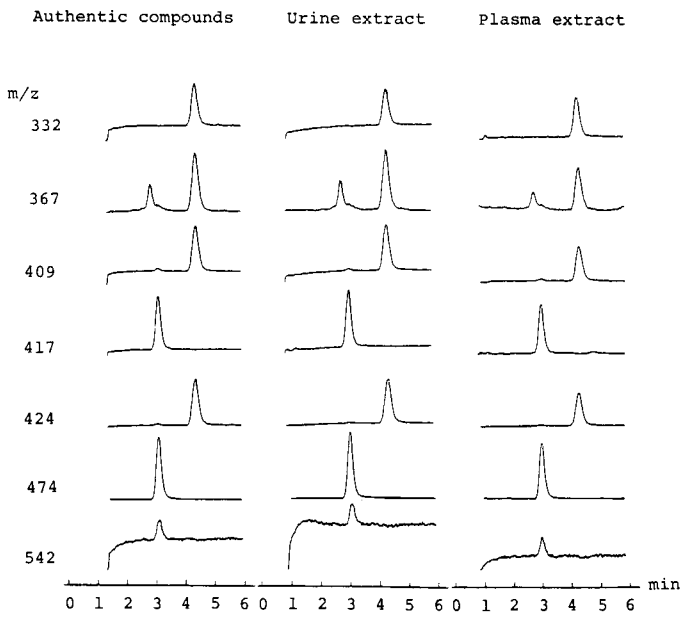

Fig. 3. SIM for a mixture of the authentic CBD-*t*-BDMS, CBN-*t*-BDMS and TPE, and for extracts of human urine and blood plasma (1 ml of each), to which 40, 40 and 20 ng of CBD, CBN and TPE, respectively, had been added.

described above.

It should be noted that, for the analyses of CBD and CBN in urine, TPE was added to urine at the initial step of extraction, but, for those 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

SIM was performed with ions at m/z 332, 367, 409, 417, 424, 474 and 542 for a mixture of the authentic CBD, CBN and TPE and for the extracts of human urine and blood plasma, to which trace amounts of CBD and CBN had been added, as shown in Fig. 3. All peaks were not interfered with impurities, showing the high specificity of the present method.

For the SIM quantitation of CBD, the ion intensity ratios (area) at m/z 474 to at m/z 332 (internal standard) were plotted against the concentrations of CBD; satisfactory linearity was obtained up to 5 ng of CBD in an injected volume (3 μl) (Fig. 4). The calibration curve for CBN with ions at m/z 409 and 332 was also satisfactory (Fig. 4). The detection limit was 200 pg/3 μl for both compounds.

The recoveries of CBD added to urine and blood plasma were 96.5 and 85.0%, and those of CBN were 98.5 and 82.0%, respectively.

In the present paper, the auther has presented detailed procedures for the assays of CBD andCBN in human urine and blood plasma by GC/MS. Although many papers have been published on the detection of Δ^9-tetrahydrocannabinol in biological samples by GC/MS[1)3)~6)], to our knowledge, comparable information is lacking for CBD and CBN. Only Ohlsson's group[3)7)] dealt with GC/MS assays for CBD and CBN in human blood plasma; they synthesized the deuterated compounds as internal standards, which are not suitable for actual forensic practice. We have employed TPE as an internal standard in the present study. TPE seems excellent also for the assays of CBD and CBN, because its lipophilicity, retention time and the mass number employed are close to those of CBD and CBN. Our present method seems useful for actual forensic analyses of the cannabinoids in human samples and also in other materials such as *Cannabis* plants, because of its simplicity, high specificity and sensitivity.

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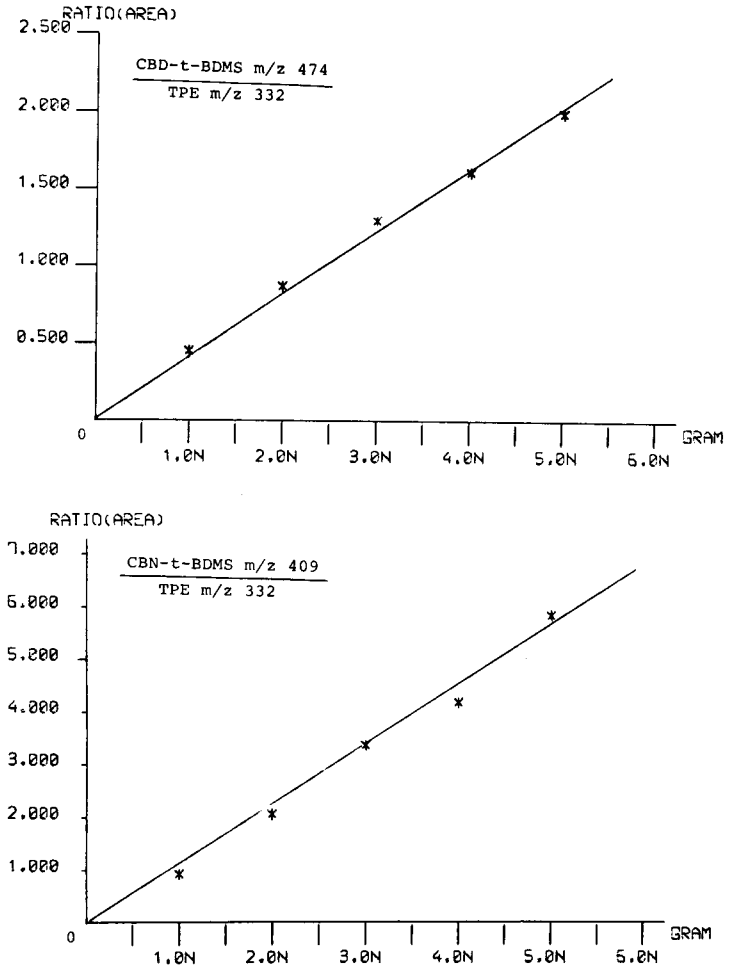

Fig. 4. Calibration curves for CBD (upper panel), and CBN (lower panel). Twenty nanograms of TPE were added to each vial.

Prof. I. Yamamoto for his providing the author with the authentic cannabinoids.

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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

2. GC/MSによるヒト尿ならびに血漿中のカンナビジオールとカンナビノールの定量

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（受付：昭和58年5月30日，掲載決定：昭和58年6月7日）

抄録 大麻の主成分はΔ^9-テトラヒドロカンナビノール，カンナビジオール（CBD）ならびにカンナビノール（CBN）である．前回の報告ではヒト尿と血漿中のΔ^9-テトラヒドロカンナビノールのGC/MSによる定量の詳細を報告したが，今回の研究ではCBDとCBNについて検討を加えたので報告する．CBDとCBNをt-butyl dimethylsilyl化してそのスペクトルを測定したところ，比較的高質量領域に単純でしかも強いピークを認め，定量に適していることを知つた．CBDではm/z 474，CBNではm/z 409の各ピークを用い，テトラフェニルエチレンを内部標準として用いて，selected ion monitoringによる尿と血漿中のCBDとCBNの定量法の詳細を設定した．検出限界は共に200pgであつた．本法は操作が簡便で，感度も高く，ヒト試料の検索のみならず，大麻植物の同定にも有用であると思われる．