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メタデータ	言語: English 出版者: 公開日: 2013-08-27 キーワード (Ja): キーワード (En): 作成者: Hattori, Hideki メールアドレス: 所属:
URL	http://hdl.handle.net/10271/1778

Jpn J Legal Med
38 (3), 294-301, 1984

Detection of Cannabinoids by Gas Chromatography/Mass Spectrometry (GC/MS) Part III. Negative Ion Chemical Ionization GC/MS of Cannabinoids in Human Materials

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(Received Feb. 29, 1984; accepted Mar. 26, 1984)

Abstract. Negative ion chemical ionization (CI) mass spectra of Δ^9-tetrahydrocannabinol, cannabidiol and cannabinol after derivatization with pentafluorobenzoyl (PFB) chloride and trifluoroacetic (TFA) anhydride were presented and compared with those in the positive CI and positive electron impact (EI) modes. The spectra in the negative CI mode showed strong molecular anions in the high mass number region especially after derivatization with PFB chloride. Using the molecular anions and a strong fragment ion, the quantitation of the three cannabinoids, which had been added to human urine and blood, was made by selected ion monitoring (SIM). The detection limit of the cannabinoids was less than 100 pg and 1.0 ng per injected volume for their PFB- and TFA-derivatives, respectively. The isolation procedure with Sephadex LH-20, which had been required for detection of cannabinoids in plasma in the positive EI mode, could be omitted because of the low backgrounds in SIM in the negative CI mode.

Key words : Toxicology, Cannabinoids, Mass fragmentography

Introduction

There is a great need for analyses of cannabinoids or their metabolites in human samples because marihuana or hashish has been widely abused. In the previous papers[1)2)], the author reported detailed procedures of gas chromatography (GC)/mass spectrometry (MS) assays for Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN), the main components of marihuana, in human urine and plasma. Recently, negative ion chemical ionization (CI) MS has been the focus of interest as a new technique in analytical chemistry[3)] because of its advantages over the positive electron impact (EI) or positive CI modes. In the present study, the author has used this new method for detecting the three main cannabinoids from human urine and plasma.

Abbreviations used: GC, Gas chromatography; MS, Mass spectrometry; CI, Chemical ionization; EI, Electron impact; PFB, Pentafluorobenzoyl; TFA, Trifluoroacetic; SIM, Selected ion monitoring; THC, Δ^9-Tetrahydrocannabinol; CBD, Cannabidiol; CBN, Cannabinol.

Materials and Methods

Materials

Human blood was obtained from the Hamamatsu Red Cross Blood Center, and centrifuged at 3,000 rpm for 5 min to separate plasma. Urine was collected from healthy volunteers. THC, CBD and CBN were kindly donated by Prof. I. Yamamoto, Department of Hygienic Chemistry, Hokuriku University School of Pharmacy, Kanazawa. Pentafluorobenzoyl (PFB) chloride was obtained from Aldrich Chemical Company, Inc., Milwaukee, WIS; trifluoroacetic (TFA) anhydride from Wako Pure Chemical Ind. Ltd., Osaka; 3% OV-17 on Gaschrom Q (100/120 mesh) from Nihon Chromato Works Ltd., Tokyo; silicic acid (100 mesh) from Mallinckrodt Chemical Works, St. Louis, MO; and Extrelut powder from E. Merck, Darmstadt and 3 g powder of it was packed in a 20 ml glass syringe before use. Other common chemicals used were of the highest purity commercially available.

GC/MS conditions

A JMS-D300 GC/MS instrument designed for both positive and negative modes equipped with a

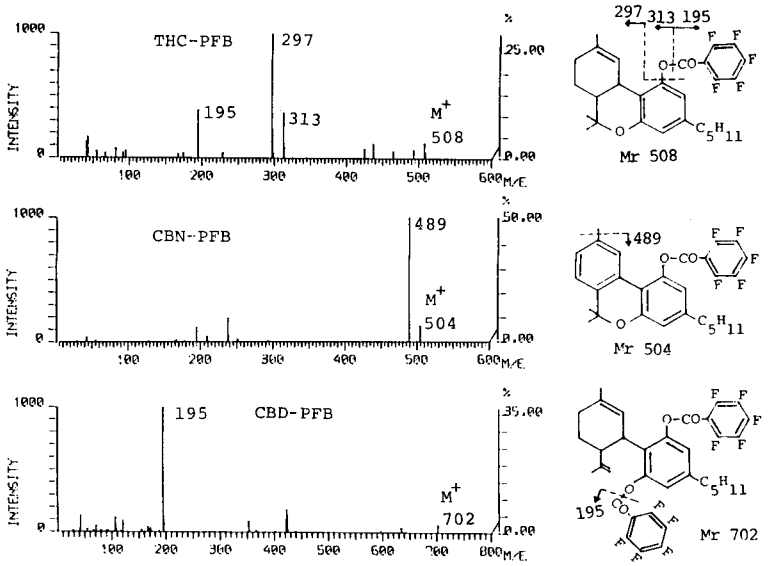

Fig. 1. Positive EI mass spectra of THC-, CBN- and CBD-PFB and their fragmentation mechanisms.

JMA-2000E computer-controlled data analysis system was used. The GC separation was made on a 2.0m × 2mm (internal diameter) glass column packed with 3% OV-17 on Gaschrom Q (100/120 mesh).

GC conditions were: column temperature 290°C for the PFB derivatives and 200-260°C programmed at 10°C/min for the TFA derivatives; injection temperature 320°C and 260°C, respectively; and helium flow rate 25 ml/min.

CI MS conditions were: reagent gas CH_4, electron energy 200 eV, separator temperature 300°C, ion source temperature 200°C, acceleration voltage 3 kV and ionization current 300 μA.

EI spectra were recorded at 70 eV.

Derivatization with PFB chloride

After addition of 200 μl of 1% (v/v) PFB chloride in *n*-hexane and 2 drops of triethylamine to the dried sample, the tube was capped and stood at room temperature for 10 min. The mixture was applied to a small silicic acid gel column (4 × 0.6 cm) with a Pasteur pipett. It was eluted with 4.0ml of ethyl acetate and a yellow band appeared was collected in a small tube and evaporated to dryness under nitrogen gas. The residue was dissolved in 50μl of ethyl acetate; a 2-μl aliquot of it was injected into the GC port.

Derivatization with TFA anhydride

After evaporation of the test sample, 200μl of ethyl acetate and 100μl of TFA anhydride were added to the residue. It was capped, heated at 60°C for 30 min, and evaporated to dryness under nitrogen gas. The residue was dissolved in 50μl of ethyl acetate; 2μl of it was subjected to the GC/MS analysis.

Isolation of cannabinoids from urine and plasma

A mixture of a 1.0ml sample (urine or plasma

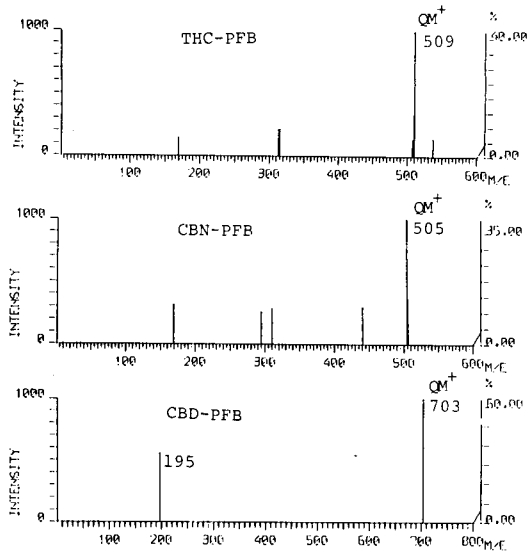

Fig. 2. Positive CI mass spectra of THC-, CBN- and CBD-PFB.

containing cannabinoids) and 3.0 ml of 0.5 M sodium phosphate buffer (pH 7.3) was applied to an Extrelut column. After standing for more than 15 min, 8.0 ml of *n*-hexane was passed through the column to elute cannabinoids. After the eluate was evaporated to dryness *in vacuo*, the residue was subjected to derivatization with PFB chloride or TFA anhydride as described above.

Results

Mass spectra

Figure 1 shows positive EI mass spectra of the authentic THC-, CBN- and CBD-PFB. Small molecular peaks at m/z 508, 504 and 702, respec-

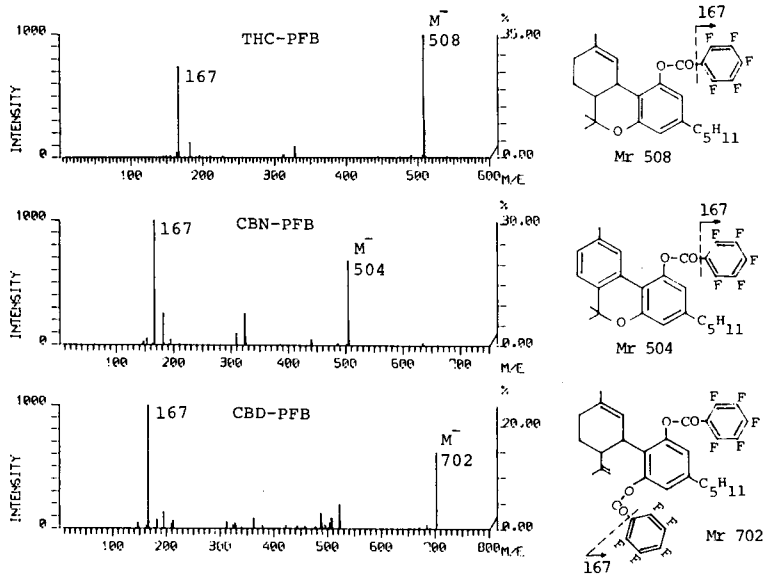

Fig. 3. Negative CI mass spectra of THC-, CBN- and CBD-PFB and their fragmentation mechanisms.

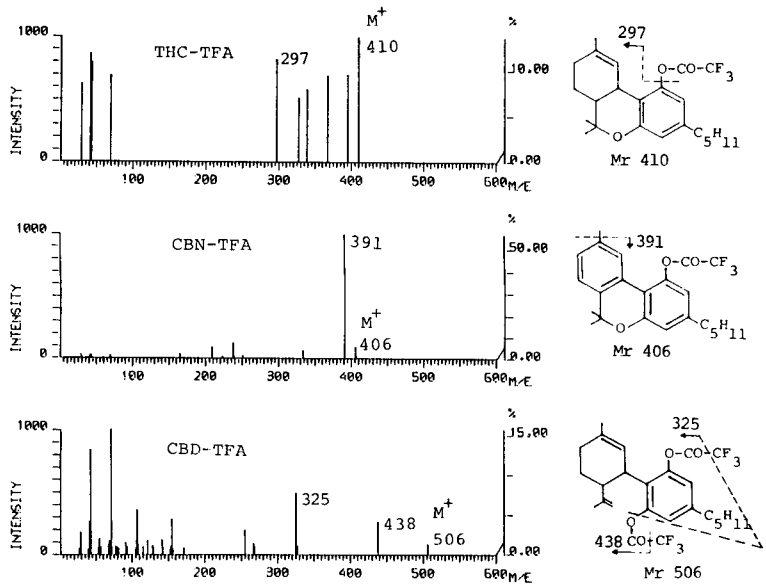

Fig. 4. Positive EI mass spectra of THC-, CBN- and CBD-TFA and their fragmentation mechanisms.

tively, were observed in the spectra. The fragmentation mechanisms are also shown in Fig. 1.

Figure 2 shows positive CI mass spectra of the authentic THC-, CBN- and CBD-PFB. Strong quasi-molecular peaks (M + H) at m/z 509, 505 and 703, respectively, appeared as base peaks.

Figure 3 shows negative CI mass spectra of the PFB derivatives of the cannabinoids. Strong molecular anions appeared at m/z 508, 504 and 702 in the spectra. The peaks equally appearing at m/z 167 in each spectrum corresponded to the pentafluorobenzyl anions. The fragmentation mechanisms are also shown in the figure.

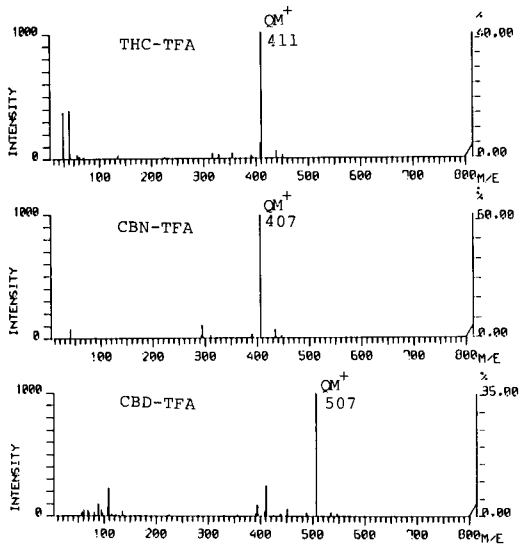

Fig. 5. Positive CI mass spectra of THC-, CBN- and CBD-TFA.

Figure 4 shows positive EI mass spectra of the authentic THC-, CBN- and CBD-TFA. The molecular cation was strong with the THC-TFA, but small with CBN- or CBD-TFA. The suspected fragmentation mechanisms are also shown in the figure.

Figure 5 shows positive CI mass spectra of the TFA derivatives of the cannabinoids. Strong quasi-molecular peak appeared for each spectrum.

Figure 6 shows negative CI mass spectra of the TFA derivatives of the cannabinoids. A strong molecular anion was observed as a base peak for THC-TFA, but in the spectra of CBN- and CBD-TFA, the molecular peak was missing or extremely small. The suspected fragmentation mechanisms are also depicted in the figure.

Selected ion monitoring (SIM)

In an attempt to use the strong negative ions appearing in the spectra (Figs. 3 and 6) for quantitation of cannabinoids, SIM was performed for a mixture of the authentic THC, CBD and CBN and for the extracts of human urine and blood plasma, to which small amounts of cannabinoids had been

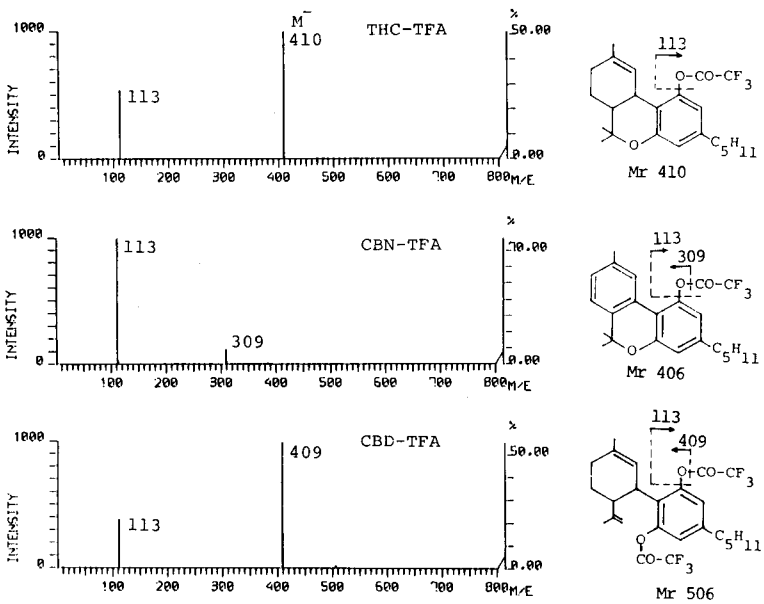

Fig. 6. Negative CI mass spectra of THC-, CBN- and CBD-TFA and their fragmentation mechanisms.

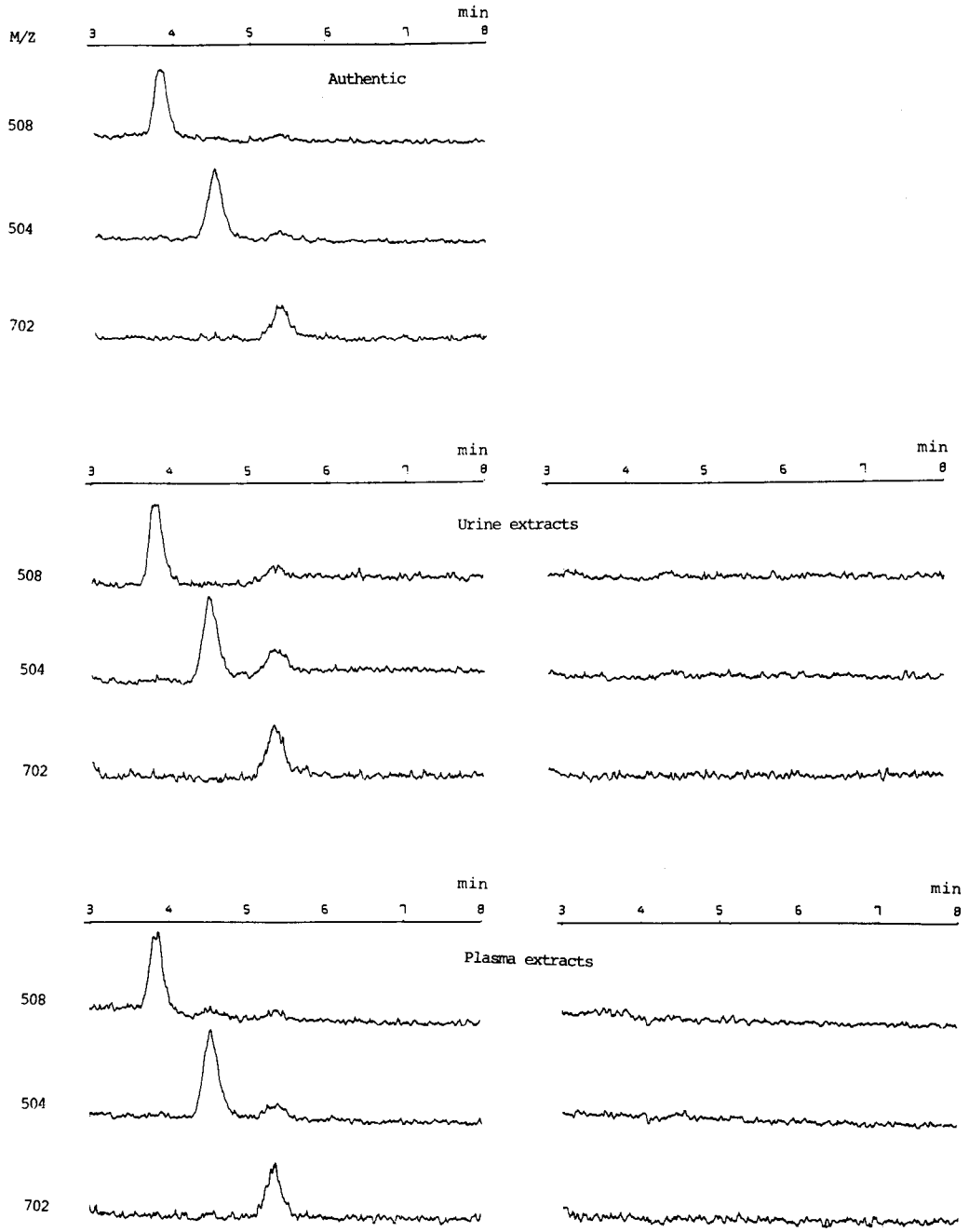

Fig. 7. SIM for the PFB derivatives of the authentic THC, CBN and CBD and the extracts of human plasma and urine. For plasma and urine extracts, the left panels deal with the extracts, to which 500 pg of each cannabinoid had been added at the initial step; the right panels show the extracts with no addition of cannabinoids.

added. Figure 7 shows the SIM in the negative CI mode for the PFB derivatives of the samples. The retention times of THC- (m/z 508), CBN- (m/z 504) and CBD-PFB (m/z 702) on the 3% OV-17 column were 3.8, 4.6 and 5.4 min, respectively.

Figure 8 shows SIM for the TFA derivatives of the samples. In this case, anions only at m/z 410 and 409 for THC- and CBD-TFA, respectively, were tested, because no peak suitable for SIM appeared in the spectrum of CBN-TFA (Fig. 6). The retention times for THC- and CBD-TFA were 6.2 and 3.0 min, respectively.

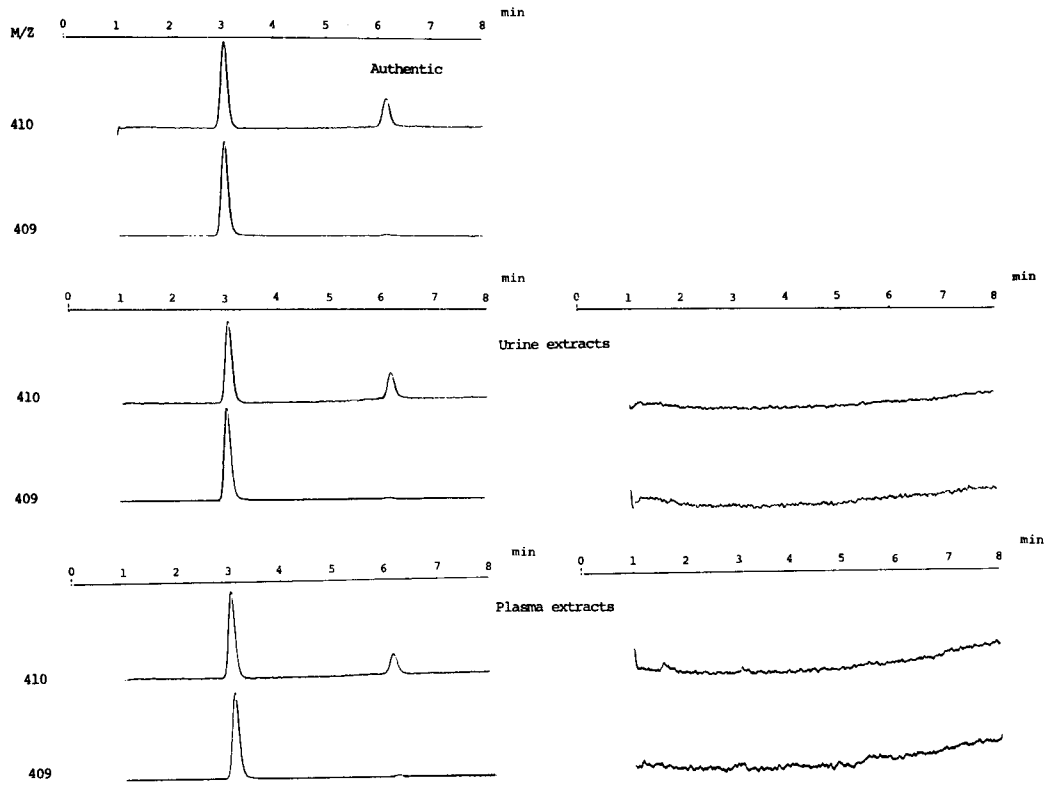

Fig. 8. SIM for the TFA derivatives of the authentic CBD and THC, and the extracts of human plasma and urine. For plasma and urine extracts, the left panels deal with the extracts, to which 5.0 ng of each cannabinoid had been added at the initial step; the right panels show the extracts with no addition of cannabinoids.

In all SIM patterns, the peaks of cannabinoids were not interfered with by impurities and their backgrounds were very low, assuring the high specificity of the SIM determinations.

Calibration curves

For SIM quantitation of cannabinoids in the negative CI mode, various amounts of each cannabinoid were injected into the GC port and the ion intensities (area) were plotted against their amounts. The results are shown in Figs. 9 and 10 for the PFB and TFA derivatives, respectively. It was confirmed that there were linear relationships between them. The detection limit was about 50 pg/2 μl for THC- and CBN-PFB, 100 pg/2 μl for CBD-PFB, 1.0 ng/2 μl for THC-TFA and 500 pg/2 μl for CBD-TFA.

Recovery

For the samples with PFB derivatization, the recoveries of THC (100 ng), which had been added to urine (1.0 ml) and plasma (1.0 ml), were 98.5 and 94.0%, respectively; those of CBN (100 ng) were 98.7 and 94.3%; and those of CBD (100 ng) were 98.8 and 94.2%. With the TFA derivatization, the recoveries of THC, which had been added to urine and plasma, were 86.5 and 83.2% and those of CBD were 95.4 and 92.4%, respectively.

Discussion

In the present paper, the author has presented negative CI mass spectra of the three cannabinoids after derivatization with PFB chloride and TFA anhydride (Figs. 3 and 6). Although negative CI MS has recently been studied on THC[4)5)] and its metabolites[5)6)], the data on CBN and CBD in the present paper are new observations. It has been widely demonstrated that negative CI mass spectra generally give different information from that of positive EI and positive CI mass spectra[3)]; thus they are usuful for discrimination of closely related analogs. The spectral pattern was different in different modes also in the spectra of cannabinoids (Figs. 1-6).

The author has tried to use the strong anions appearing in the negative spectra (Figs. 3 and 6) for

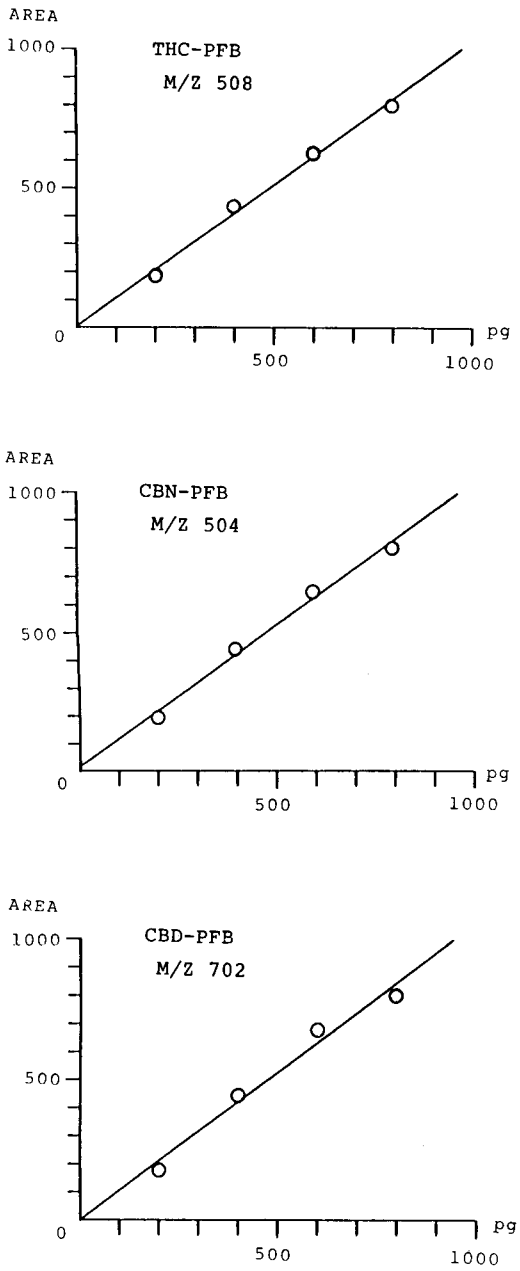

Fig. 9. Calibration curves for the PFB derivatives of THC, CBN and CBD.

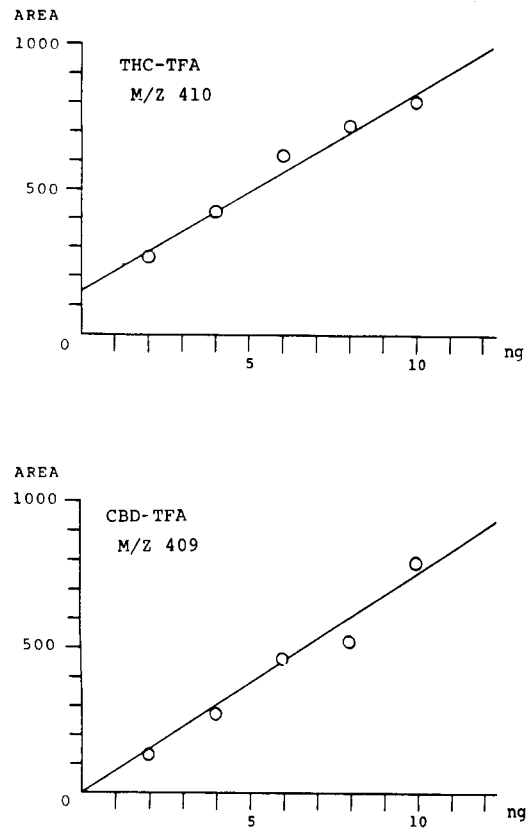

Fig. 10. Calibration curves for the TFA derivatives of CBD and THC.

quantitation of cannabinoids in human samples by SIM (Figs. 7-10). It is well documented that the background in SIM in the negative mode is generally low; thus specific analysis can be achieved even if some of the purification steps are omitted[3]. In fact, the author could omit a tedious chromatographic procedure with Sephadex LH-20 in the present study, which had been required for the assays of cannabinoids in plasma in the positive EI mode[1)2)]. Thus the present assay method in the negative CI mode is employable for actual measurements of cannabinoids in human samples because of its high specificity, excellent recovery and simplicity.

The sensitivity of cannabinoid assays by SIM in the negative mode with the PFB derivative (Fig. 9) was comparable to or a little higher than that in the positive EI mode[1)2)]. Hunt and Crow[4] reported that the negative CI mass spectrometric determination of THC gave an enormously low detection limit of as little as 10 fg. However, they did not try actual determination of THC in biological samples, but only the authentic compound in solutions. In addition, it should be pointed out that the sensitivity greatly depends upon the capability of the apparatus used.

In this paper, the author has tested the derivatization of cannabinoids with both PFB chloride and TFA anhydride. In view of sensitivity, the PFB

derivatization can be recommended rather than the TFA derivatization, although its procedure for the PFB derivatization is a little more complicated than the latter.

Negative ion CI MS has many advantages over the conventional positive EI and CI MS; it gives different spectral information (Figs. 1-6), low backgrounds (Figs. 8 and 9) and sometimes high sensitivity. It seems correct that negative CI MS will be a powerful tool also in the field of forensic toxicology.

Acknowledgements

The author is very greteful to Prof. M. Asano and Assoc. Prof. O. Suzuki for their kind guidance throughout this study, and to 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

3. ヒト試料中のCannabinoid類の負イオン化学イオン化GC/MS

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（受付：昭和59年2月29日，掲載決定：昭和59年3月26日）

摘要 著者は第1，2編において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(THC)，カンナビジオール(CBD)ならびにカンナビノール(CBN)の，正イオン電子衝撃（EI）法によるGC/MS分析を報告したが，今回の研究では最近注目を集めている負イオン化学イオン化（CI）GC/MSによつて三種のCannabinoid類の分析を試みたので報告する．試料をペンタフルオロベンゾイル（PFB）クロリドもしくは無水トリフルオロ酢酸（TFAA）で反応させたのちに負イオンCIマススペクトルを測定し，正イオンEI法と正イオンCI法マススペクトルと比較検討を行つた．Cannabinoid類のPFB誘導体の負イオンCIスペクトルでは強い分子負イオンが三者ともに認められた．TFA誘導体では，THCのみで強い分子負イオンを生じ，CBDでは強いフラグメントイオンが高質量領域に出現したがCBNでは定量に適当な負イオンは得られなかつた．以上の定量に適する各負イオンを使用し，selected ion monitoringによる生体試料中のCannabinodid類の定量法を設定したところ，検出限界はPFB誘導体では注入量で100pg以下，TFA誘導体では1.0ng以下であつた．負イオンCI法ではバックグラウンドが低く，したがつて正イオンEI法で血漿中のCannadinoidの検出に必要とされていたSephadex LH-20による分離を省略することができ，操作を一段と簡略にすることができた．