Enhanced Selectivity in the Determination of Δ^9-Tetrahydrocannabinol and Two Major Metabolites in Serum Using Ion-Trap GC–MS–MS

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Abstract

Cannabinoids were extracted from serum with C18 SPE cartridges and analyzed as their trimethylsilyl (TMS) derivatives. A benchtop gas chromatography–tandem mass spectrometry (GC–MS–MS) system based on an ion trap with external ionization was used. Quantitation was done in relation to trideuterated internal standards in dual MS–MS mode. Confirmation of the identity for the three compounds of interest, Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH), was achieved by registering the daughter spectra in full scan mode. It was possible to identify the three compounds at concentrations down to 0.25 µg/L for THC, 0.5 µg/L for 11-OH-THC, and < 2.5 µg/L for THCCOOH by comparison with reference spectra. The limits of quantitation are better than 2 µg/L for THC, 5 µg/L for 11-OH-THC, and 8 µg/L for THCCOOH. The within-run and day-to-day precision for the three analytes were very similar and ranged from 4.2 to 10.4%.

Introduction

Methods for the determination of cannabinoids in plasma/serum are numerous. In addition to the immunoassay techniques, which are useful for screening purposes, gas chromatography–mass spectrometry (GC–MS) seems to be the method of choice for confirmation analysis (1–6). Sample preparation is usually done by liquid–liquid or solid-phase extraction (SPE), and the extracts are analyzed after appropriate derivatization by selected-ion monitoring (SIM). The registration of full scan spectra is not routinely performed because of the low concentrations and/or interference with coeluting substances.

Especially for forensic purposes, tandem mass spectrometry (MS–MS) with the possibility of full scan information from a single, selected parent ion seems to be preferable. For a long time it was restricted to expensive triple-quadrupole instruments (tandem in space) (7,8) or to the so-called "research trap" ion-trap MS (ITMS) (9). The introduction of commercial ion-trap GC–MS–MS instruments (tandem in time) in about 1995 made it possible to transmit these techniques to "low-cost" benchtop systems (10).

In this paper, the extraction procedure described by Moeller et al. (1) was used without modification, but the cannabinoids were analyzed after forming TMS derivatives. 11-Hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) is also extracted with good recovery rates. Whole blood can be analyzed by this method after sixfold dilution with water with somewhat lower recovery rates.

Experimental

Materials and methods

Δ^9-Tetrahydrocannabinol (THC), 11-OH-THC, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH), and the trideuterated analogues were purchased from Radian International. MSTFA was from Fluka GmbH. SPE columns (C18, 3 mL, 500 mg, 7020-03) were from Baker, and all solvents or chemicals were of reagent-grade quality.

Sample preparation

One milliliter of serum was spiked with 2.5 ng THC-d_3, 5 ng 11-hydroxy-THC-d_3, and 25 ng Δ^9-carboxy-THC-d_3 (5, 10, and 50 µL solution of 0.5 mg/L standards in methanol) and vortex mixed. The sample was applied within 1 min to the preconditioned Baker C18 SPE column; washed subsequently with water, 0.25 mol/L acetic acid, and water; dried for 10 min by vacuum; and eluted with 3 × 0.5 mL acetone. The eluates are collected in a 2-mL silylated vial and evaporated to dryness in a stream of nitrogen at 60°C. The residue is reconstituted in 40 µL of freshly prepared derivatization

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mixture (isooctane/pyridine/MSTFA, 20:1:2, v/v/v) and heated to 90°C for 30 min. Whole blood was diluted with 5 mL of water and applied to the column after sonification and centrifugation.

Instrumental parameters

The Finnigan GCQ-GC equipped with a standard split/splitless injector, an autosampler (A-200 S, CTC-Analytics, Zwingen, Switzerland), and a DB-1 column (30 m × 0.25-mm i.d., 0.25-µm film thickness, J&W Scientific, Folsom, CA) was used in splitless mode (0.5 min). The injector was held at 275°C. The carrier gas was helium with a constant linear velocity of 40 cm/s. The oven temperature program was 120°C (0.5 min) to 230°C at a rate of 30°C/min and then with 15°C/min up to 310°C (7 min). The injected volume was 1 µL. The MS (Finnigan-GCQ in EI mode) was tuned by autotune, the high-mass adjustment was set to 100%, and the electron multiplier was 200 V above the autotune value.

The analysis was divided into three segments in time starting at 6.0, 7.8, and 9.15 min to detect THC, 11-OH-THC, THCCOOH, and their trideuterated internal standards using two parent ions (dual MS–MS) in each segment: *m/z* 386/389, 371/374, and 488/491. In contrast, only one parent ion was selected (e.g., 386, 371, 488) in single MS–MS mode. The excitation voltages were 1.00 V for THC and THCCOOH and 1.25 V for 11-OH-THC. The product ions were collected in the mass range from *m/z* 300 to 390, 250 to 375, and 300 to 491. The scan rate was set to 0.5 s/scan.

For quantitation, the GCQ Data System (Rev. 2.2, 1997) was used. The peak-area quotients of the following mass chromatograms were used for quantitation: 371/374 (THC); 265,

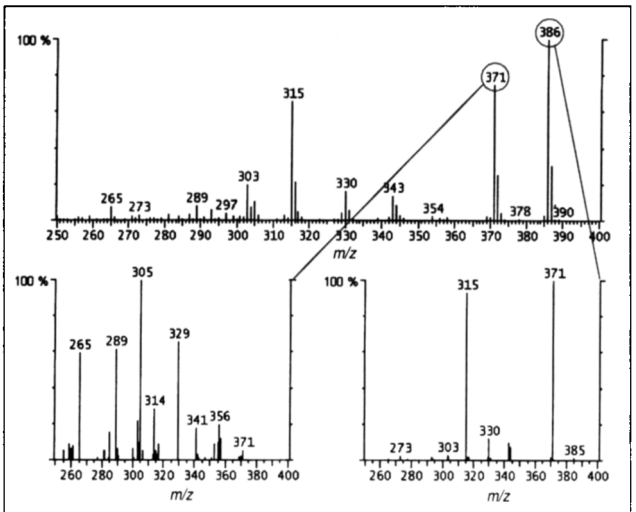


Figure 1. Mass spectra of THC-TMS. Upper, primary spectra; lower left, daughter spectra of *m/z* 371 at 1.5 V; and lower right, daughter spectra of *m/z* 386 at 1 V.

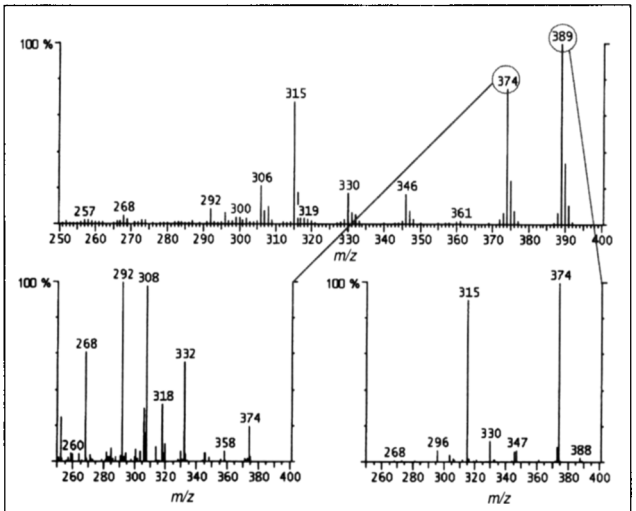


Figure 2. Mass spectra of THC-d_3-TMS. Upper, primary spectrum; lower left, daughter spectrum of *m/z* 374 at 1.5 V; and lower right, daughter spectrum of *m/z* 389 at 1 V.

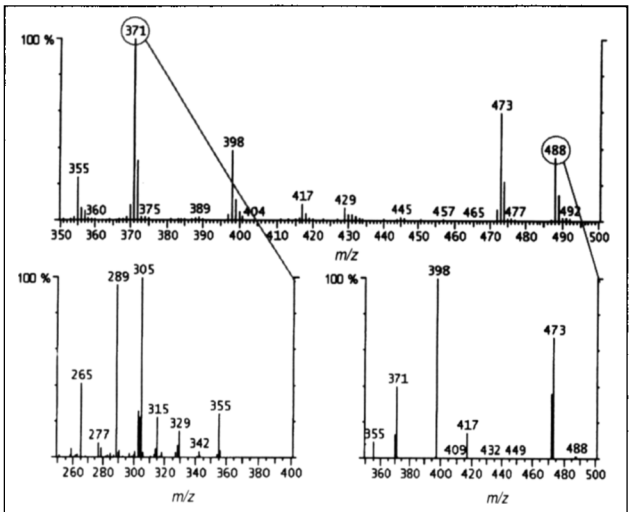


Figure 3. Mass spectra of THCCOOH-TMS. Upper, primary spectrum; lower left, daughter spectrum of *m/z* 371 at 1.5 V; and lower right, daughter spectrum of *m/z* 488 at 1 V.

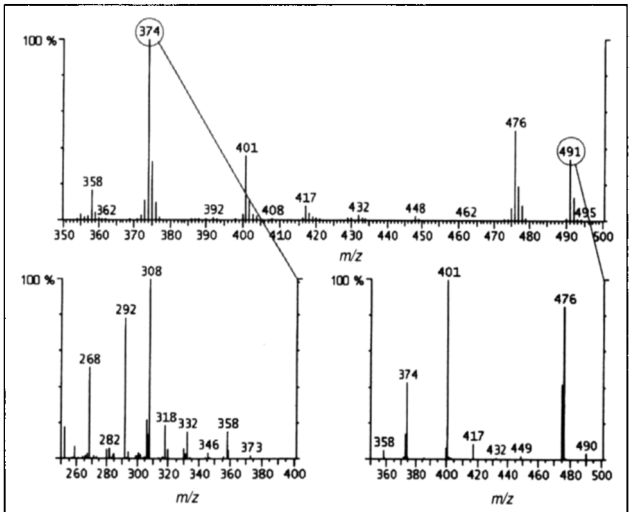


Figure 4. Mass spectra of THCCOOH-d_3-TMS. Upper, primary spectrum; lower left, daughter spectrum of *m/z* 374 at 1.5 V; and lower right, daughter spectrum of *m/z* 491 at 1 V.

289, 305/268, 292, 308 (11-OH-THC); and 371, 398, 473/374, 401, 474 (THCCOOH).

Results and Discussion

The primary EI spectra of THC, 11-OH-THC, and THCCOOH and of the trideuterated internal standards in form of their trimethylsilyl (TMS-) derivatives are shown in Figures 1–5. For the generation of daughter spectra of THC and THCCOOH, two different precursor ions, the molecular ion and the corresponding base peak, were evaluated. Fragmentation of the precursor ion is performed by collision-induced dissociation (CID) with helium molecules in combination with an additional RF voltage (excitation voltage). The necessary energy was optimized by varying the excitation voltage in increments of 0.25 V between 0 V and 2 V for each compound. In each case a voltage of 1 V to 1.5 V was sufficient to minimize the signal of the precursor ion and to maximize the relevant daughter ions. It was decided that the molecular peaks would be used as parent ions for THC and THCCOOH, in account to a better signal-to-noise ratio of the resulting daughter ions. However the other alternative, the use of the base peak (371) (Figures 1 and 2, lower left) as described by Collins et al. (10), also seems to be practical. These authors used rather low excitation amplitudes, resulting in daughter spectra showing relevant intensity of the parent ion. The comparison of the two applied strategies demonstrates the variability of the options given by tandem mass spectrometry. For the determination of 11-OH-THC we preferred the base-peak 371 as parent ion in account of the low intensity molecular ion 474.

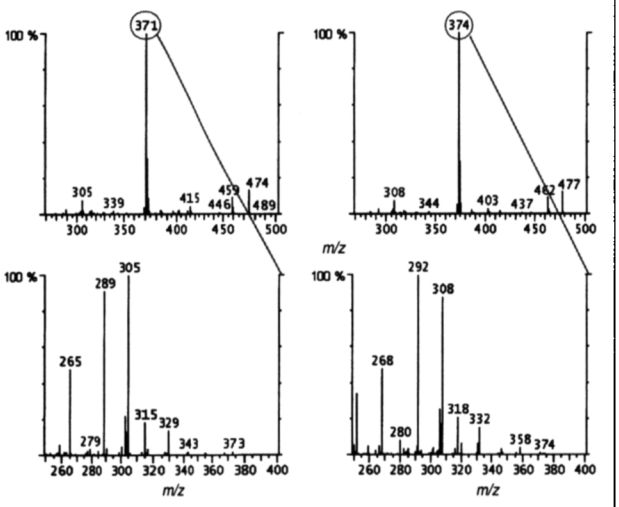


Figure 5. Mass spectra of 11-OH-THC-TMS. Upper, the two primary spectra and lower, the daughter spectra of *m/z* 371 and *m/z* 374 at 1.25 V.

Recovery rates were determined using a pooled serum spiked with the deuterated internal standards and calculated in the relation to the analytes that were added after sample preparation. The recovery rates of THC at a level of 5 µg/L and of THCCOOH (25 µg/L) which were determined to 78% (± 4) and to 93% (± 3) are in accordance to the findings of Moeller et al. (1). For 11-OH-THC (10 µg/L) the recovery was determined to 85% (± 3) (n = 24).

The benefit of the described technique is demonstrated in Figure 6. Mass chromatograms resulting from a serum sample containing 2.5 µg/L THC are shown: *m/z* 300–500 in full-scan (Figure 6A), *m/z* 371 and 386 in SIM (Figure 6B), and *m/z* 371 in MS–MS mode (Figure 6C), where the last shows the best selectivity and specificity with the lowest noise.

From our experience a disadvantage of the MS–MS technique seems to be the moderate reproducibility in the determination of the ion ratios of the analytes as well as the internal standards. In contrast to SIM, where the system reproducibility averaged to a relative standard deviation (RSD) of 3.8% for THC, 2.7% for 11-OH-THC, and 2.9% for THCCOOH (n = 10), in dual MS–MS mode, the average RSD is about 2–3 times higher (e.g., 7.6% for THC) (Table I). On the other hand, the instrumental precision in the presence of matrix is impaired only insignificantly. Based on these results, the SIM technique seems to be preferable for quantitation, but there were great

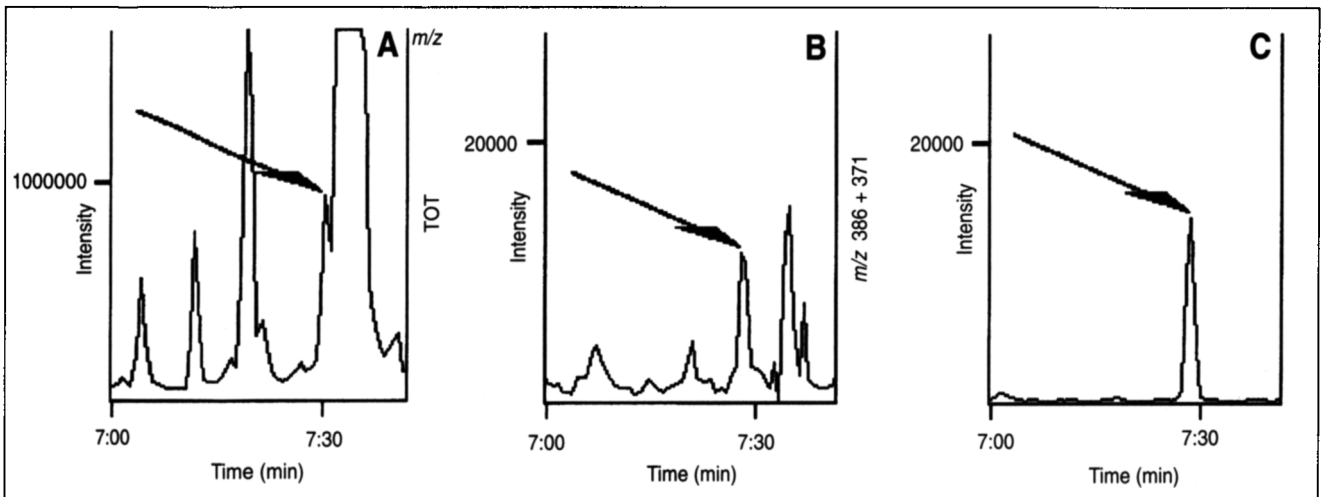


Figure 6. Mass chromatograms resulting from a serum sample containing 2.5 µg/L THC: A, *m/z* 300–500 in full-scan; B, *m/z* 371 and 386 in SIM; and C, *m/z* 371 in MS–MS mode.

drawbacks in specificity when an ion trap was used on real samples, especially in samples with degraded matrix leading to interfering peaks and loss of sensitivity. The comparison of Figure 6B (SIM) and Figure 6C (MS–MS) may demonstrate these problems.

Using model compounds, the combination of methaqualone and its homologue ethaqualone, for example, the same differences in reproducibility comparing SIM and dual MS–MS mode were observed, whereas in SIM by internal standardization, a typical RSD of about 3.3% could be achieved (variability of the internal standard area counts about 6–9%), and in MS–MS mode, an RSD of only 6.6% (variability of the internal standard area counts similar to SIM) was possible. In this technique, in contrast to common analytical experience, the precision with reference to the internal standard was not improved to the usually expected degree.

Table I. System Reproducibility with Pure Substance Mixtures in Dual MS–MS Mode

THC (386/389)		11-OH-THC (371/374)		THCCOOH (488/491)		
Conc. (µg/L)	RSD	Conc. (µg/L)	RSD	Conc. (µg/L)	RSD	
1.25	13.1%	2.5	5.1%	5	9.7%	(n = 8)
2.5	6.1%	5	5.9%	10	8.4%	(n = 10)
3.75	4.2%	7.5	10.8%	15	12.9%	(n = 10)
5.0	7.1%	10.0	12.5%	20	7.2%	(n = 10)
6.25	7.4%	12.5	9.5%	25	6.5%	(n = 10)
average RSD	7.6%		8.8%		8.9%	

Table II. Linearity of Analysis (Dual MS–MS)*

Analyte	Range (µg/L)		SD	r	LOQ†	LOD‡
THC	1.25–6.25	y = 0.389x + 0.063	0.075	0.9947	1.76	0.56
11-Hydroxy-THC	2.5–12.5	y = 0.232x – 0.117	0.106	0.9926	4.13	1.32
THCCOOH	5–25	y = 0.0383x – 0.026	0.033	0.9935	7.77	2.47

* Five equidistant calibration points were analyzed in tetraplicate.
† Limit of quantitation.
‡ Limit of detection calculated according to DIN 32 645 (Perkin-Elmer SQS Rev. 3.3 statistical package).

Table III. Interday Precision and Accuracy using Certified Control Serum (Dual MS–MS)*

Assigned value (µg/L)	Substance	Amount found (µg/L) Mean	% RSD
9.2	THC	10.6	9.24
13.6	11-OH-THC	14.6	9.93
20.1	THCCOOH	15.4	5.43

* n = 10.

This lack of precision in measuring ion ratios on ion-trap MS in contrast to quadrupole instruments was already described by Fitzgerald et al. (11) using diazepam as a model compound. Their results can be summarized in the statement that ion-trap MS generally provides greater signal-to-noise ratios and equivalent precision in full scan mode but is 5–10 times less precise in measuring ion ratios compared with quadrupole instruments in the SIM mode. The cause of this obvious lack in precision is uncertain. Perhaps the rather complicated time-sharing processes of the scan function (tandem in time) may be responsible for this effect because the chromatographic signal was incompletely depicted. It may be hoped that further improvements of hard- or software can overcome these disadvantages.

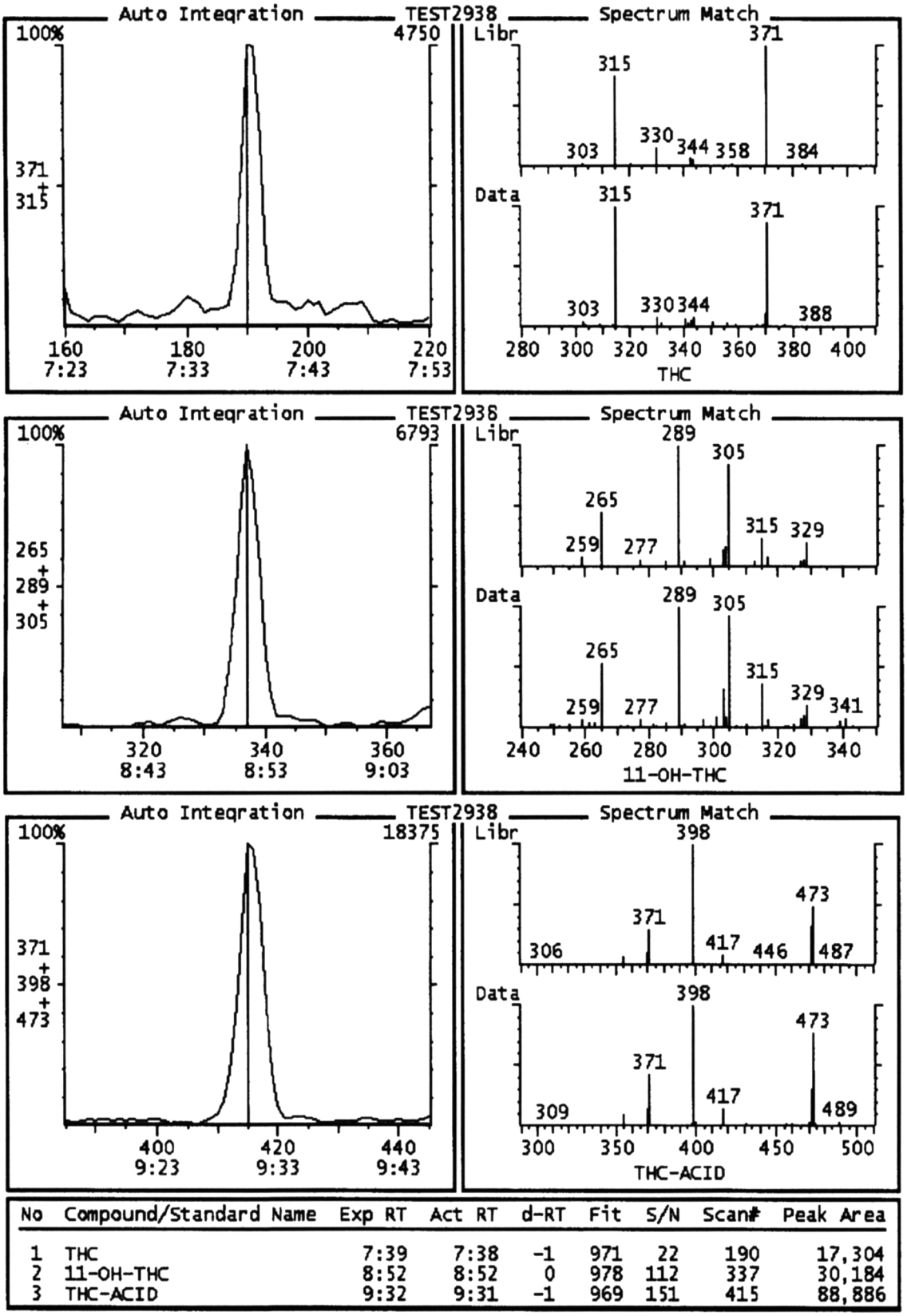
Calibration mixtures were analyzed in tetraplicate with five equidistant levels. Linear regression analysis was used to construct calibration curves for the three analytes as shown in Table II. The limits of detection (LOD) and quantitation (LOQ) are calculated using a statistical package (Perkin-Elmer SQS Rev. 3.3, Ueberlinger, Germany) from the data of the calibration graph (linear regression) according to the German regulation DIN 32654 (12). Briefly, the LOD means that at this limit the analyte may be present with a probability of 50% at a level below the detection limit. Above the LOD, the analyte may be present with a certainty of 99%. For the determination of the LOQ, the presence of the analyte is pre-condition. At this limit the uncertainty of the result is equal to 33%.

A certified control serum (BTMF 2/98 S-plus, Medichem,

Table IV. Interday Precision and Accuracy at Low Level*

Concentration (µg/L)	Substance	Dual MS–MS Amount found (µg/L) Mean	% RSD	Single MS–MS S/N-ratio Mean	% RSD	Spectral-FIT Mean	% RSD
0.25	THC	0.24	18.9	23	28.9	971	2.9
0.5	OH-THC	0.82	14.2	117	42.8	939	9.3
2.5	THCCOOH	2.29	41.1	122	64.9	960	6.8

* n = 10.



No	Compound/Standard Name	Exp RT	Act RT	d-RT	Fit	S/N	Scan#	Peak Area
1	THC	7:39	7:38	-1	971	22	190	17,304
2	11-OH-THC	8:52	8:52	0	978	112	337	30,184
3	THC-ACID	9:32	9:31	-1	969	151	415	88,886

Figure 7. Mass chromatograms and resulting daughter spectra, signal-to-noise ratios, and spectra fit to library spectra resulting from a serum sample containing 0.25 µg/L THC (upper), 0.5 µg/L 11-OH-THC (middle), and 2.5 µg/L THCCOOH (lower) in single MS–MS mode.

Steinenbronn, Germany) was extracted on 10 consecutive days and analyzed in tetraplicate. The instrumental precision in the determination of THC (four injections) averaged to 6.21% (SD = 1.98), for 11-OH-THC to 8.71% (SD = 1.66), and for THCCOOH to 7.48% (SD = 2.45). The interday precision and accuracy of the total procedure are listed in Table III.

For the determination of the absolute detection limits with a special view to qualitative aspects, serum spiked with 0.25 µg/L THC, 0.5 µg/L 11-OH-THC, and 2.5 µg/L THCCOOH was analyzed on 10 consecutive days in dual and single MS–MS modes. The following parameters were evaluated: 1. Dual MS–MS mode. Nominal concentration of the three analytes, disregarding that the levels are below the LOQ as computed following DIN 32654 and their RSD and 2. Single MS–MS mode. The signal-to-noise ratios of the resulting peaks based on the mass chromatograms of *m/z* 371 and 315 (THC); *m/z* 265, 289, and 305 (11-OH-THC); and *m/z* 371, 398, and 473 (THC-COOH) and the spectral fit of the resulting daughter spectra in comparison with reference spectra. The results are summarized in Table IV. Typical mass chromatograms and mass spectra (Figure 7) for the three analytes using the autoquantitation software demonstrates high signal-to-noise ratios and a good agreement with the reference spectra. The injected amount of THC in this example corresponds to less than 6.25 pg (0.25 ng/40 µL). It is shown that at concentrations lower than the mathematical limit of quantitation (e.g., half of the concentration of the internal standards or lower, by disregarding the internal standards in single MS–MS mode) forensic proof of the presence of the analyte in a qualitative manner can be given in form of the (quantitative) spectral-fit value. In this kind of view the detection level can be assumed to be about 0.25 µg/L for THC at a signal-to-noise ratio greater than 10:1 in combination with a spectral-fit value better than 900 and for better than 0.5 or 2.5 µg/L for 11-OH-THC and THCCOOH at signal-to-noise ratios better than 50:1 and similar fit values. The high variability of the signal-to-noise ratio (RSD = 28.9–64.9%) is due to the very low noise of the typical MS–MS experiments, which makes it difficult to calculate this parameter because some scans of the analytical run may contain no counts at all. So an additional visual inspection of chromatograms and spectra should be done.

At very low levels confirmation of the spectral identity may be done in single MS–MS mode by trapping only one parent ion of the analytes (386, 374, and 488). In this way, pure daughter spectra that are not disturbed by the daughter spectra of the trideuterated internal standards are acquired. Using the actual revision of the GCQ data system (Xcalibur, 1999) a separate analytical run is not necessary because the data of the two simultaneous MS–MS experiments are collected separately.

Conclusions

By the presented GC–MS–MS assay THC and the main metabolites 11-OH-THC and THCCOOH may be analyzed as sensitive as in other published GC–MS assays. On account of the precision, which is somewhat lower when compared to quadrupole-SIM techniques, the mathematical limits of quantitation may thus result somewhat higher. On our opinion this disadvantage seems to be of only theoretical importance for qualitative specificity must be emphasized in legal cases even and especially at low concentrations of analytes. The high selectivity of the assay with the absence of interfering peaks in the resulting ion current profiles and the gathered daughter spectra from defined parent ions allows the unequivocal proof of presence of these cannabinoids even below the mathematical detection limits.

References

1. M.R. Moeller, G. Doerr, and S. Warth. Simultaneous quantitation of delta-9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) in serum by GC/MS using deuterated internal standards and its application to a smoking study and forensic cases. *J. Forensic Sci.* **37:** 969–983 (1992).
2. L.M. Shaw, J. Edling-Owens, and R. Mattes. Ultrasensitive measurement of delta-9-tetrahydrocannabinol with a high energy dynode detector and electron-capture negative chemical-ionization mass spectrometry. *Clin. Chem.* **37(12):** 2062–2069 (1991).
3. P.M. Kemp, I.A. Abukhalaf, J.E. Manno, B.R. Manno, D.D. Alford, and G.A. Abusada. Cannabinoids in humans. I. Analysis of Δ^9-tetrahydrocannabinol and six metabolites in plasma and urine using GC–MS. *J. Anal. Toxicol.* **19:** 285–291 (1995).
4. L.J. McBurney, B.A. Bobbie, and L.A. Sepp. GC/MS and EMIT analysis for delta-9-tetrahydrocannabinol metabolites in plasma and urine of human subjects. *J. Anal. Toxicol.* **10:** 56–64 (1986).
5. R.L. Foltz, K.M. McGinnis, and D.M. Chinn. Quantitative measurement of delta-9-tetrahydrocannabinol and two major metabolites in physiological specimens using capillary column gas chromatography negative ion chemical ionization mass spectrometry. *Biomed. Mass Spectrom.* **10:** 316–323 (1983).
6. K. Harzer and M. Kaechele. Detection of intake of hashish in biological material. *J. Chromatogr.* **278(1):** 63–70 (1983).
7. C.C. Nelson, M.D. Fraser, J.K. Wilfarth, and R.L. Foltz. Gas chromatography/tandem mass spectrometry measurement of delta-9-tetrahydrocannabinol, naltrexone and their active metabolites in plasma. *Ther. Drug Monit.* **15:** 557–562 (1993).
8. T. Mieczkowski. A research note: the outcome of GC/MS/MS confirmation of hair assays on cannabinoid (+) cases. *J. Forensic Sci.* **79**: 83–91 (1995).
9. C.S. Phinney and M.J. Welch. Analysis by a combination of gas chromatography and tandem mass spectrometry: development of quantitative tandem-in-time ion trap mass spectrometry: isotope dilution quantitation of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid. *Rapid Commun. Mass Spectrom.* **9**: 1056–1060 (1995).
10. M. Collins, J. Easson, G. Hansen, A. Hodda, and K. Lewis. GC–MS–MS confirmation of unusually high Δ^9-tetrahydrocannabinol levels in two postmortem blood samples. *J. Anal. Toxicol.* **21:** 538–552 (1997).
11. R.L. Fitzgerald, C.L. O'Neal, B.J. Hart, A. Poklis, and D.A. Herold. Comparison of an ion-trap and a quadrupole mass spectrometer using diazepam as a model compound. *J. Anal. Toxicol.* **21:** 445–450 (1997).
12. DIN 32645. Arbeitsausschuss Chemische Terminologie (AchT) im Deutschen Institut für Normung e.V., Beuth-Verlag Berlin, Berlin, Germany, 1994.

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