

Tandem mass spectrometry: a helpful tool in hair analysis for the forensic expert

Michael Uhl

Bayerisches Landeskriminalamt, Maillingerstr. 15, 80636 Munich, Germany

Received 28 June 1999; received in revised form 12 July 1999; accepted 4 August 1999

Abstract

The Bavarian State Bureau of Investigation in Munich has the exclusive responsibility for investigation of criminal acts. One considerable expertise is that of hair analysis. According to the legal system in Germany, there is a special interest when some clients' hair tested positive for illicit drugs. An accused with a lot of drugs in his hair will be treated as a supposed addict and will be guaranteed extenuating circumstances. The instrumentation used for hair analysis is a powerful analytical tool: a Varian 3400 gas chromatograph linked to a Finnigan Tandem-MS (TSQ 700). The methanol extraction method is used for the detection of illegal drugs and metabolites: amphetamine, methamphetamine, MDA, MDMA (ecstasy), MDE, MBDB, methadone, THC, EDDP (metabolite of methadone), cocaine, benzoylecgonine, cocaethylene, opiates (dihydrocodeine, codeine, heroin, 6-monoacetylmorphine, morphine, acetylcodeine). For the detection of 9-carboxy-THC by negative chemical ionization the hair sample is hydrolyzed under alkaline conditions. Solid-phase extraction is used for clean-up. The LOQ for the determination of 11-nor- Δ -9-tetrahydrocannabinol-9-carboxylic-acid is 0.16 pg/mg hair. An unsurpassed combination for rendering an expert opinion based on hair analysis may be: a forensic expert using diligence and experience, coupled with the performance of a sophisticated analytical instrument.

Keywords: Hair analysis; GC/MS/MS; Illicit drugs; 9-Carboxy-THC

1. Introduction

The 1998 International Workshop in Munich 'Cannabinoids in Hair' organized by the 'Society of Hair Testing' was presented in the laboratories of two local institutes. One of

these institutes is the central police agency for Bavaria (Bavarian State Bureau of Investigation). This State Bureau is exclusively responsible for investigating criminal acts, but it is not responsible for investigation of civil law or administration law cases. No service laboratory for workplace testing has been established.

An essential field of a chemistry and toxicology department is hair analysis. Orders for hair testing will be sent from the court, from the prosecutor's office and the police. The forensic expert's skills based on hair analysis will be utilized as corroborative evidence. These skills are mainly used to scrutinize for chronic abuse of illegal drugs or as an adjunct to a medical and physiological examination in order to evaluate criminal responsibility.

In the description of these duties, it is important to emphasize that some clients have a special interest that their hair tests positive for illegal drugs. For example, with regard to the legal system in Germany, a person taken into custody for drug trafficking with no residues of drugs in his hair will probably be sentenced as a dealer. However, an accused with a lot of drugs in his hair could be treated as a supposed addict and will be guaranteed extenuating circumstances. For this reason, some clients are disappointed if the residues of drugs in the hair sample are minimal and not enormous.

Generally, particular information from the mandator about the individual subject can be important for a rendering of an expert opinion. Aspects that could be considered are, for example: when was the person taken into custody? What are the special allegations? Is there a self reported use?

2. Experimental

2.1.1. Chemicals and reagents

Methanol, acetonitrile, ethyl acetate, *n*-hexane, acetone, acetic acid and phosphoric acid were analytical grade (Merck, Darmstadt, Germany). The derivatizing agents penta-fluoropropionic anhydride and hexafluoroisopropanol were purchased from Aldrich (Steinheim, Germany). Reference standard solutions of D5-amphetamine, D5-methylenedioxymethamphetamine, D3-benzoyllecgonine, D3-tetrahydrocannabinol, D3-methadone, D3-cocaine, D3-morphine, D3-dihydrocodeine, D3-codeine, D3-6-mono-acetyl-morphine, D3-11-nor- δ -9-tetrahydrocannabinol-9-carboxylic acid, amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA), *N*-methyl-1-(3,4-methylenedioxy-phenyl)-2-butanamine (MBDB), benzoyllecgonine, DL-2-ethyl-1,5-dimethyl-3,3-diphenyl-pyrrolidinium perchlorate (EDDP), tetrahydrocannabinol, methadone, cocaine, morphine, cocaethylene, dihydrocodeine, codeine, 6-mono-acetylmorphine, acetylcodeine, heroin, and 11-nor- δ -9-tetrahydrocannabinol-9-carboxylic acid were purchased from Promochem (Wesel, Germany).

Solid-phase extraction columns (narc-1 by Bakerbond) were supplied by J.T. Baker (Phillipsburg, NJ).

2.2. Sample preparation

2.2.1. Sample preparation for the detection of opiates, cocaine, amphetamines and tetrahydrocannabinol

The hair samples were cut into segments (1–6 cm) if sectional analysis was requested. They were placed into an Eppendorf microtube and decontaminated by vortexing in an ultrasonic bath with 1 ml *n*-hexane (30 s) followed by 1 ml of acetone (30 s). The rinsing extracts were collected with a Pasteur pipette, combined, and kept for GC/MS/MS analysis.

The sample (segment) was transferred into a folded filter, and the remaining residues of solvents were evaporated under a gentle stream of nitrogen. Each hair segment was then cut into 1–2-mm sections, and a 10.0-mg quantity was weighed into an Eppendorf microtube. Then, methanol (0.5 ml) and the internal standards (D3-benzoylecgonine, D5-amphetamine, D5-MDMA, D3-tetrahydrocannabinol, D3-methadone, D3-cocaine, D3-morphine, D3-dihydrocodeine, D3-codeine, D3-6-monoacetylmorphine) were added, and the tube was tightly closed and sealed with a foil of parafilm. After vortexing in an ultrasonic bath (40°C) for 1 h and following centrifugation for 15 min, the samples were allowed to stand overnight. The methanolic phase was decanted, transferred into a vial and evaporated (at 40°C under a stream of nitrogen).

The residue obtained was then derivatized with 50 µl pentafluoropropionic anhydride (PFPA) and 25 µl hexafluoroisopropanol (HFIP) for 30 min at 70°C. The mixture was dried again under nitrogen at 25°C and reconstituted in 100 µl ethyl acetate. A 1-µl quantity of the derivatized sample was then injected into the GC/MS/MS system.

Standard calibration curves (standard solutions to drug-free hair samples) were obtained to provide the concentrations 0.25, 2.0, 4.0, 8.0 and 20 ng/mg (in case of need up to 50 ng/mg) hair, respectively. Quantitation based on the ratio of the peak areas of the analyte to the internal standards.

2.2.2. Sample preparation for the detection of 11-nor- δ -9-tetrahydrocannabinol-9-carboxylic acid

A 15.0-mg quantity of the sample (or segment) was weighed into an Eppendorf tube, the internal standard (50 pg D3-11-nor- δ -9-tetrahydrocannabinol-9-carboxylic acid), methanol (0.5 ml) and 10 N potassium hydroxide (0.5 ml) were added. After vortexing in an ultrasonic bath for 30 min at 70°C and cooling at room temperature, the pH was adjusted to 4–5 with acetic acid.

A narc-1 solid phase cartridge (Bakerbond) was conditioned (twice with methanol, twice with 0.05 N phosphoric acid). At first, 1 ml of 0.05 N phosphoric acid was added to the solid-phase column, then the hair digest and finally, 1 ml of 0.05 N phosphoric acid. After washing the cartridge with 2 ml of acetonitrile/0.1 N hydrochloric acid (2:3, v/v) and aspirating to dryness, the analyte was collected in the elution solvent (1.5 ml of ethyl acetate/*n*-hexane (1:1, v/v)).

Extracts were evaporated to dryness and the residue obtained was then derivatized with 50 µl PFPA and 25 µl HFIP for 30 min at 70°C. The mixture was dried again under

nitrogen at 25°C and reconstituted in 100 µl ethyl acetate. A 2-µl quantity of the derivatized sample was then injected into the GC/MS/MS system.

Standard calibration curves were obtained in the following way: blank hair samples were treated as just described (alkaline hydrolysis, internal standard D3-carboxy-THC, SPE-procedure) and spiked to provide the concentrations 0.16, 0.33, 1.0, 2.0, 3.3 and 8.25 pg/mg of hair, respectively. Quantitation based on the ratio of the peak-areas of the analyte 383 and 492) to the internal standard (D3-carboxy-THC).

2.3. Instrumentation

GC/MS/MS analysis was performed with a Finnigan TSQ 700 triple-stage quadrupole mass spectrometer coupled with a DEC station 2100. The MS/MS is linked to a Varian 3400 gas chromatograph equipped with an A 200 S autosampler.

A fused-silica capillary column (J&W, DB-5, 30 m×0.32 mm i.d., 0.25-µm film thickness) was used for GC separation.

For the detection of drugs and metabolites (except for 9-carboxy-THC) the GC was operated in the following manner: 2 min at 60°C, 60–220°C at 18°C/min, 5.5 min at 220°C, 220–300°C at 25°C/min; 3 min at 300°C.

In the case of 11-nor- δ -9-tetrahydrocannabinol-9-carboxylic acid (9-carboxy-THC) the GC program was: 2 min at 60, 60–230°C at 25°C/min, 5 min at 230, 230–280°C at 15°C/min and finally, within 3 min from 280 to 300°C.

The injector (splitless) was controlled with a cold injection system and with solvent purging (Gerstel, Mülheim, Germany). Injection temperature was 40°C, 40–60°C at 2°C/s, 60 s at 60°C, 60–280°C at 12°C/s and temperature was kept at 280°C for 60 s. The interface was maintained at 280°C. Helium was used as carrier gas with a flowrate of 1.0 ml/min.

The mass spectrometer was operated in selected reaction monitoring (SRM). The MS/MS was operated in the positive chemical ionization mode (PCI) or, in case of the detection of 11-nor- δ -9-tetrahydrocannabinol-9-carboxylic acid in the negative ion chemical ionization mode (NCI) using methane as the reagent gas set at 220 000 mTorr.

The appropriate ion was selected as parent mass in the first quadrupole. The corresponding daughter ion was set in the third quadrupole after collision with argon at a cell pressure between 2.0 and 2.2 mTorr. The collision offset voltage was between –5 and –25 V for PCI and +15 V for NCI.

The electron multiplier was operated at 2100 V. The electrometer gain was 10⁸.

Under NCI, the base peak in the mass spectrum for the detection of 9-carboxy-THC was the molecular ion at m/z 620 (parent ion) while the deuterated analogue D3-9-carboxy-THC exhibited its base peak at m/z 623. The decomposition with argon produced daughter ions at m/z 383/492 and a daughter ion at m/z 386/495 for the deuterated analogue.

3. Results and discussion

The head is an anatomical area where hair samples are collected in the great majority of cases. However, approximately 10% of samples provided for hair analyses in the

laboratories of the State Bureau were obtained from beard, pubic, axillary and chest region [1].

A convenient method to isolate drugs and metabolites that are incorporated in hair is methanol extraction [2]. The methanol extraction procedure is suitable for the determination of amphetamine, methamphetamine, MDA, MDMA (ecstasy), MDE, MBDB, methadone, THC, EDDP (metabolite of methadone), cocaine, benzoylecgonine, cocaethylene, dihydrocodeine, codeine, heroin, morphine, 6-monoacetylmorphine and acetylcodeine. PFPA and HFIP are used for derivatization. The resulting extract is then analyzed using PCI. The impurity of an extract obtained by methanol extraction [3] is of minor importance, if the capabilities of a GC/MS/MS are available.

In cases where cannabis consumption has to be proved [4], confirmation is necessary by determining 9-carboxy-THC using solid-phase extraction for clean-up. The analyte, a derivatized, fluorinated compound, is suitable for NCI [5].

3.1. Case examples

Some case examples should demonstrate that the capability of a good analytical instrument, such as a tandem mass spectrometer, can be helpful for rendering of an expert opinion.

3.1.1. Case 1: testing of pubic hair as adjunctive specimen

A man with short and bleached head hair was seized with a lot of ecstasy (MDMA) tablets and 10 g of cocaine. Only a trace of cocaine (0.35 ng/mg hair), but no benzoylecgonine was detectable in the head hair. The pubic hair of the same person (Fig. 1) was tested positive for MDMA (1.3 ng/mg hair), its potential metabolite MDA (0.35 ng/mg hair), cocaine (4.1 ng/mg hair), benzoylecgonine (0.3 ng/mg hair) and amphetamine (1.1 ng/mg hair).

3.1.2. Case 2: unusual ratio of cocaine/benzoylecgonine

In cases where the time of chronic abuse lies more than several months in the past, the evaluation of the results will be more and more complicated. According to the court it was to be proven that a defendant with 43-cm long hair had only abused cocaine during a time period earlier than 15 months in the past. The sample, a 43-cm long strand of hair was cut into four segments of equal length.

Two proximal segments were tested negative for cocaine and metabolites. As presented in Fig. 2, two distal segments had a concentration of 0.2 ng/mg cocaine and 1.5 and 4.3 ng/mg benzoylecgonine. A cocaine/benzoylecgonine ratio of that kind has been found so far in the hair of ancient Peruvian coca leaf chewers [6].

3.1.3. Case 3: detection of acetylcodeine as additional marker for heroin abuse

A person taken into custody for possession of 100 g of heroin claimed severe addiction to this opiate in order to attenuate penalty. Chronic heroin abuse could be confirmed by the result of a hair analysis (Fig. 3). Concentrations of the parent drug, metabolites and of an impurity due to manufacturing procedure [7] were high: heroin,

CHRO: hf6116 11-MAR-99 Elapse: 00:12:42.6 623
 Samp: L SW Start : 18:25:28 1350
 Comm: IS=200 pg/ul
 Mode: CI +DAU LMR GAS UP LR Study : 18647-99 SW
 Oper: Neumeier Client: da/uhl Inlet : GC Vial 16
 Peak: 1000.00 mmu Label wndw: 1 > 1350 Masses: 91 > 417
 Area: 2, 4.00 Baseline : 0, 3 Label : 2, 40.00

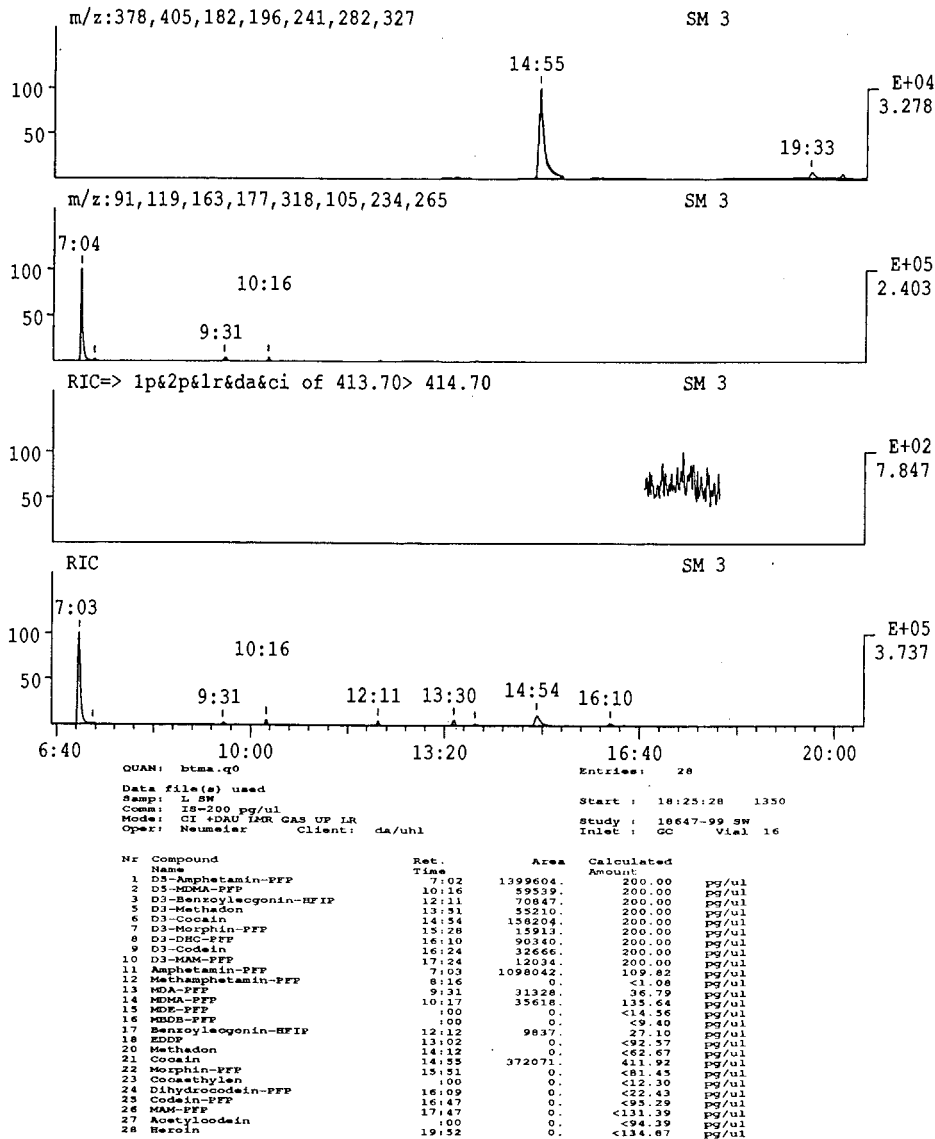
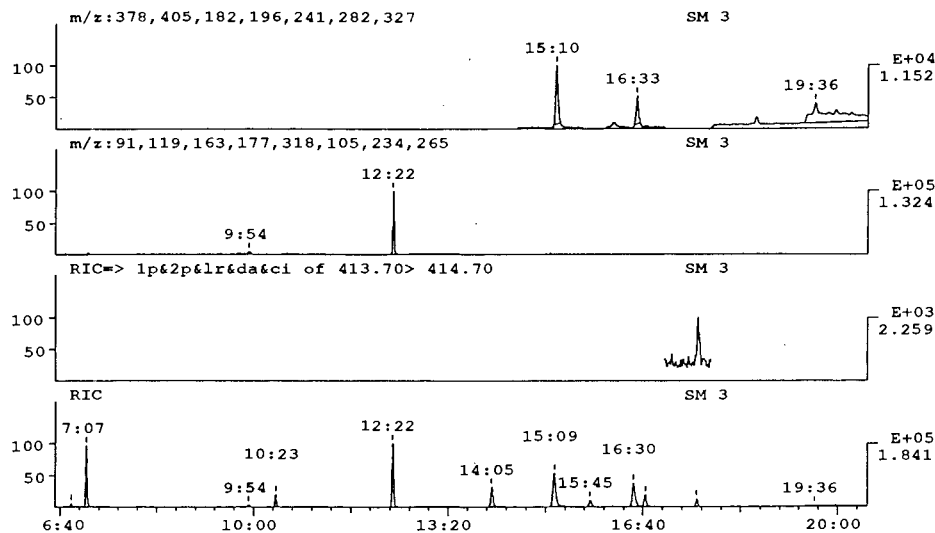


Fig. 1. Testing of pubic hair as adjunctive specimen: MDMA (1.3 ng/mg hair), MDA (0.35 ng/mg hair), cocaine (4.1 ng/mg hair), benzoylcegonine (0.3 ng/mg hair) and amphetamine (1.1 ng/mg hair).

CHRO: he8132 30-JUN-98 Elapse: 00:12:54.9 662
 Samp: S 3 RS Start: 05:00:46 1456
 Comm: IS=200 pg/ul
 Mode: CI +DAU LMR GAS UP LR Study: 11961-97 RS
 Oper: Neumeier Client: si/uhl Inlet: GC Vial 32
 Peak: 1000.00 mmu Label wndw: 1 > 1456 Masses: 91 > 417
 Area: 2, 4.00 Baseline: 0, 3 Label: 2, 40.00



QUAN: btma.q0

Entries: 28

Data file(s) used

Samp: S 3 RS

Start: 05:00:46 1456

Comm: IS=200 pg/ul

Mode: CI +DAU LMR GAS UP LR

Study: 11961-97 RS

Oper: Neumeier Client: si/uhl

Inlet: GC Vial 32

Nr	Compound Name	Ret. Time	Area	Calculated Amount	
1	D5-Amphetamin-PFP	7:07	525185.	200.00	pg/ul
2	D5-MDMA-PFP	10:22	123373.	200.00	pg/ul
3	D3-Benzoyllecgonin-HFIP	12:22	235400.	200.00	pg/ul
5	D3-Methadon	14:05	161093.	200.00	pg/ul
6	D3-Cocain	15:09	597550.	200.00	pg/ul
7	D3-Morphin-PFP	15:46	115300.	200.00	pg/ul
8	D3-DHC-PFP	16:31	408628.	200.00	pg/ul
9	D3-Codein	16:42	190968.	200.00	pg/ul
10	D3-MAM-PFP	17:34	95897.	200.00	pg/ul
11	Amphetamin-PFP	7:08	9978.	3.80	pg/ul
12	Methamphetamin-PFP	8:22	0.	<1.56	pg/ul
13	MDA-PFP	9:37	0.	<3.00	pg/ul
14	MDMA-PFP	10:44	0.	<8.32	pg/ul
15	MDE-PFP	10:57	0.	<8.05	pg/ul
17	Benzoyllecgonin-HFIP	12:23	504336.	428.49	pg/ul
18	EDDP	13:14	0.	<12.95	pg/ul
20	Methadon	14:27	0.	<7.37	pg/ul
21	Cocain	15:10	68903.	23.06	pg/ul
22	Morphin-PFP	16:08	0.	<10.18	pg/ul
23	Cocaethylen	16:09	2612.	0.87	pg/ul
24	Dihydrocodein-PFP	16:32	34327.	16.80	pg/ul
25	Codein-PFP	17:04	0.	<9.28	pg/ul
26	MAM-PFP	17:58	0.	<21.47	pg/ul
27	Acetylcodein	19:28	0.	<24.80	pg/ul
28	Heroin	20:04	0.	<40.91	pg/ul

Fig. 2. Unusual cocaine/benzoyllecgonine ratio determined in a distal segment: cocaine, 0.2 ng/mg hair; benzoyllecgonine, 4.3 ng/mg hair.

CHRO: hf7524 26-APR-99 Elapse: 00:09:49.6 335
 Samp: N 2 AS Start : 20:25:20 1393
 Comm: IS=200 pg/ul
 Mode: CI +DAU LMR GAS UP LR Study : 30389-99 AS
 Oper: Neumeier Client: jr/uhl Inlet : GC Vial 24
 Peak: 1000.00 mmu Label wndw: 1 > 1393 Masses: 91 > 408
 Area: 2, 4.00 Baseline : 0, 3 Label : 2, 40.00

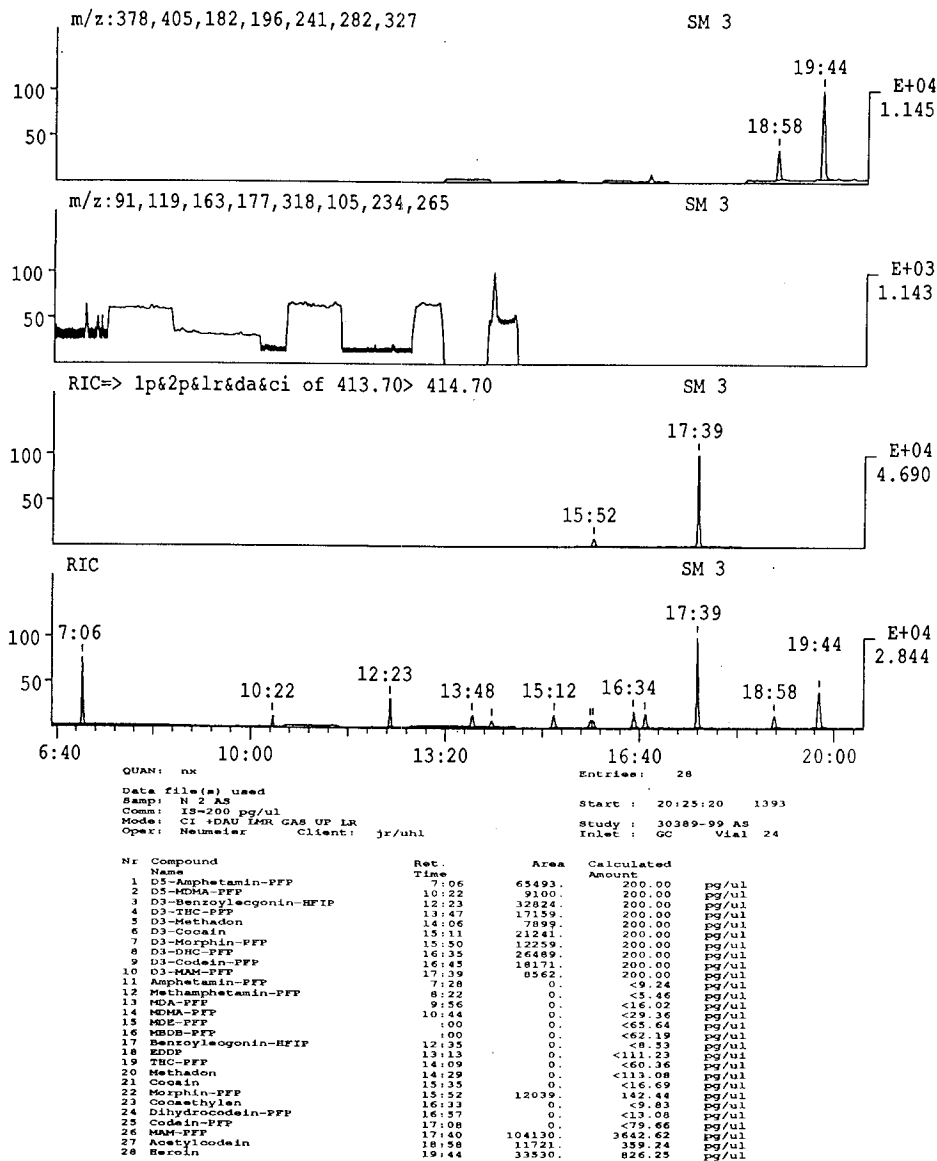


Fig. 3. Confirmation of a claimed addiction to heroin: 6-monoacetylmorphine, 36 ng/mg; morphine, 1.4 ng/mg; heroin, 8.2 ng/mg; and acetylcodeine, 3.6 ng/mg hair.

8.2 ng/mg; 6-monoacetylmorphine, 36 ng/mg; morphine, 1.4 ng/mg; and acetylcodeine, 3.6 ng/mg hair.

3.1.4. Case 4: detection of 9-carboxy-THC

Two examples should demonstrate capabilities and limits of GC/MS/MS. The scalp hair of a supposed hashish dealer was to be tested for cannabinoids. According to the dealer's self-admitted use he characterizes himself as a moderate cannabis smoker. The sample was cut into a 3-cm root and a 3-cm tip segment: the concentration of 11-nor- Δ -9-THC-9-carboxylic-acid in the root segment was very close to the LOQ of 0.16 pg (Fig. 4).

However, the result for this metabolite was not positive in the tip segment (Fig. 5).

According to our available data, we do not observe a significant correlation between the concentration of THC and 9-carboxy-THC. It is a preliminary observation that, in

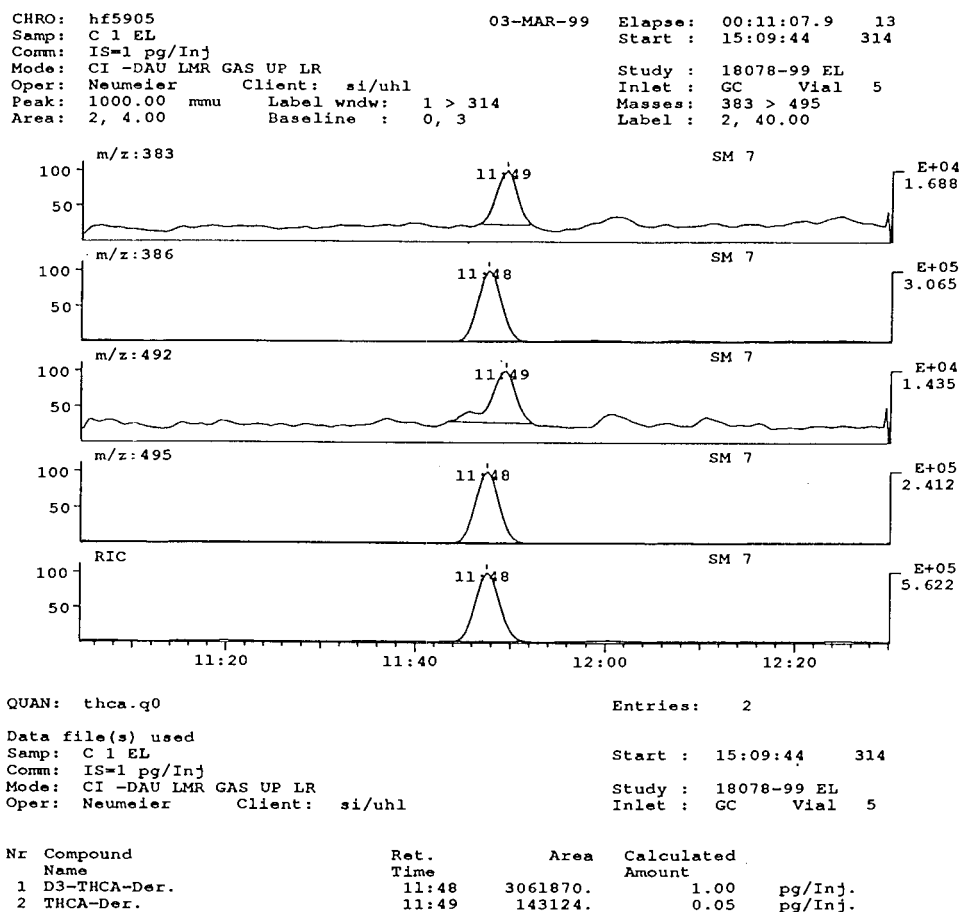


Fig. 4. Detection of 9-carboxy-THC in low concentration: 0.16 pg/mg hair.

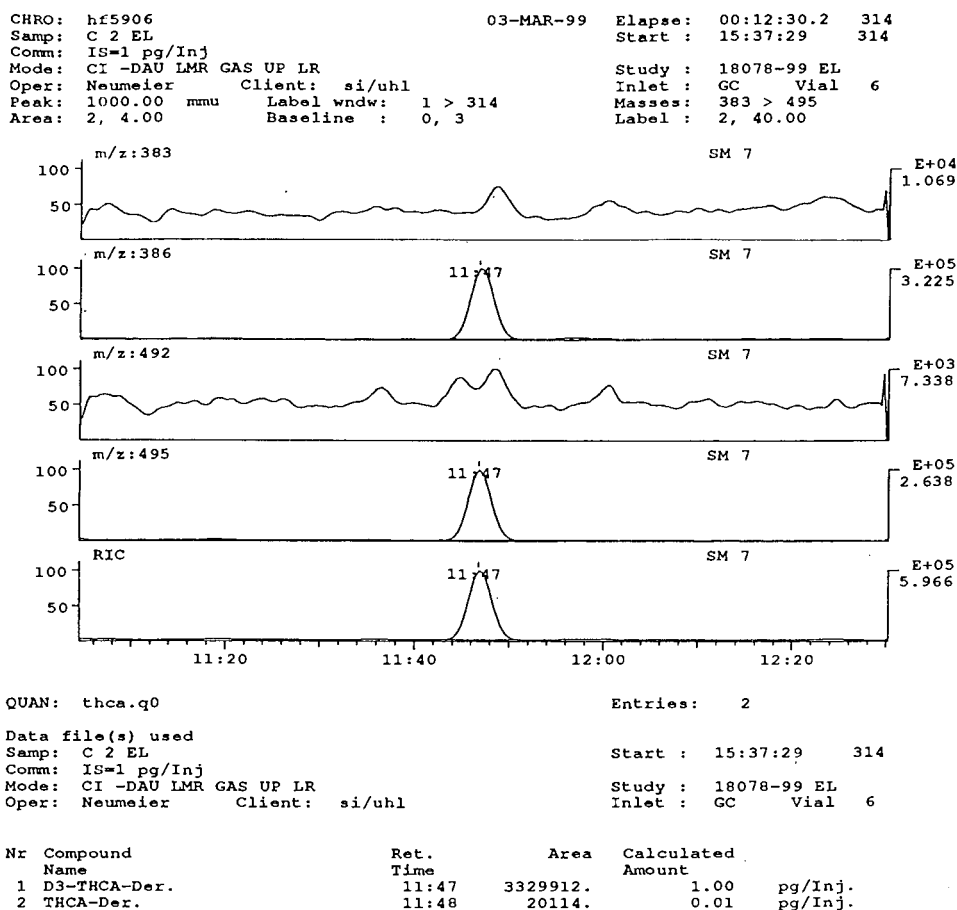


Fig. 5. Futile detection of 9-carboxy-THC: an attempt exceeding the capabilities of the procedure.

segmental hair analysis, the highest concentration of 9-carboxy-THC is detected in the root segment, a lower concentration in the middle segment (if available), the minimum concentration in the tip segment.

4. Conclusion

Each forensic expert will appreciate the solid basis of reliable data. He will probably prefer two, three or even more data in order to evaluate the drug history of a certain person. A helpful tool for reaching this goal can be a gas chromatograph/tandem-mass spectrometer in order to detect components and metabolites in very low concentration.

Possible criteria for obtaining a positive hair result as used in the State Bureau are the following.

- Cocaine positive: only if cocaine exceeds 0.5 ng/mg hair and benzoylecgonine is detectable, cocaethylene occasionally.
- Heroin positive: if 6-monoacetylmorphine is more than 0.5 ng/mg, and morphine and/or heroin, and occasionally acetylcodeine, are detectable.
- Cannabis positive: if THC is detectable and optional 9-carboxy-THC (if more than 0.16 pg/mg).
- MDEA, MDMA positive: if MDEA, MDMA is more than 0.5 ng/mg and MDA is detectable.
- Methadone positive: if methadone is more than 0.5 ng/mg and optional EDDP.

The concentrations of drugs that were quantified in hair samples range from 0.25 to more than 50 ng/mg. The concentrations of metabolites range from 0.25 ng/mg to (e.g., benzoylecgonine) 20 ng/mg and even (monoacetylmorphine) 40 ng/mg. Lower concentrations (0.05–0.10 ng/mg) can be detected.

The whole pattern of metabolites should be evaluated in order to diagnose drug consumption. It is essential that all these analytical results are in good harmony.

An unsurpassed combination for rendering an expert opinion based on hair analysis may be: a forensic expert using diligence and experience coupled with the capabilities of a sophisticated analytical instrument.

Acknowledgements

The author would like to thank, for their excellent support and engagement, Mrs. S. David and Mrs. G. Sichelstiel for sample preparation and Mr. X. Neumeier for performing GC/MS/MS analyses.

References

- [1] P. Mangin, Drug analysis in nonhead hair, in: P. Kintz (Ed.), *Drug Testing in Hair*, CRC Press, Boca Raton, FL, 1996, pp. 279–287.
- [2] J. Röhrich, G. Kauert, Determination of amphetamines and methylenedioxy-amphetamine-derivatives in hair, *Forensic Sci. Int.* 84 (1997) 179–188.
- [3] V. Cirimele, P. Kintz, P. Mangin, Comparison of different extraction procedures for drugs in hair of drug addicts, *Biomed. Chromatogr.* 10 (1996) 179–182.
- [4] Society of Hair Testing, *Forensic Sci. Int.* 84 (1997) 3–6.
- [5] T. Cairns, D. Kippenberger, H. Scholtz, W. Baumgartner, Determination of carboxy-THC in hair by mass spectrometry/mass spectrometry, in: R.A. de Zeeuw, I. Al Hosani, S. Al Munthiri, A. Maqbool (Eds.), *Proceedings of the 1995 International Conference and Workshop for Hair Analysis in Forensic Toxicology*, Abu Dhabi, Nov. 19–23, 1995, pp. 185–193.
- [6] A.C. Springfield, L.W. Cartnell, A.C. Aufderheide, J. Buikstra, J. Ho, Cocaine and metabolites in the hair of ancient Peruvian coca leaf chewers, *Forensic Sci. Int.* 63 (1993) 269–275.
- [7] P. Kintz, C. Jamey, V. Cirimele, R. Brenneisen, B. Ludes, Evaluation of acetylcodeine as a specific marker of illicit heroin in human hair, *J. Anal. Toxicol.* 22 (1998) 425–429.