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Determination of drugs in hair using GC/MS/MS

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Abstract

An important task for the forensic toxicologist and expert witness is the detection of the noxa in biological matrices. Because of this, the identification and quantification of residues of illegal drugs in human hair is still of growing interest. Utilizing the advantages of GC/MS/MS testing human hair is performed for most common drugs of abuse like heroin and other opioides, cocaine, cannabis and amphetamine derivatives. Analyzing hair specimens for substances that present a toxicological risk is another challenge. Several quality control parameters must be observed to avoid false positive or false negative results and to gain additional information. Blank sample, blank hair as well as the combined wash extracts are tested for the presence of the relevant compounds within every series. Careful evaluation of the findings can provide an approximate measure of the intensity of drug use in the majority of cases. © 1997 Elsevier Science Ireland Ltd. All rights reserved

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1. Introduction

An important task for the forensic toxicologist and expert witness is the detection of the noxa in biological matrices. The identification and quantification of residues of illegal drugs in human hair is still of growing interest. Currently, analysis of hair samples is often utilized in criminal investigations [1,2], involving drug use, or in cases of chronic intoxication.

Applications of hair analysis for crime laboratories in Germany fall into one of the following categories:

(1) Confirmation or demonstration of chronic abuse in order to attenuate penalties.

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(2) Examination of the reliability of an accused or of a witness.
(3) In cases where the criminal responsibility is questioned. Indication of the intensity of illegal drug use to order a medical and psychological examination.
(4) A confirmation technique when there is a disputed positive urine analysis of police candidates.
(5) Verification of an intoxication over a long period.

Generally, the combination of capillary gas chromatography with mass spectrometry is the method of choice for detection of illicit drugs or of specific toxic substances in hair specimens [3]. In order to overcome the shortcomings of conventional mass spectrometry we have enhanced the range of our capabilities by employing a tandem mass spectrometer [4].

We appreciate the selectivity and sensitivity of this powerful technique while conducting our daily routine detection of the most prevalently abused drugs, i.e. some opioides, cocaine, amphetamine, some amphetamine analogues and cannabis. We also recognize the advantages of a GC/MS/MS [5–7] during the determination of low dose acting drugs and toxic components.

2. Experimental

2.1. Hair samples

The hair samples were taken from persons whose drug background was of interest for the prosecution authorities. Blank hair samples were obtained from drug-free children.

2.2. Chemicals and reagents

Organic solvents and phosphoric acid were analytical grade (Merck, Darmstadt, Germany). The derivatizing agents pentafluoropropionic anhydride and hexafluoroisopropanol were purchased from Aldrich (Steinheim, Germany). The external and internal standard solutions of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid, morphine, codeine, dihydrocodeine, monoacetylmorphine, methadone, benzoylecgonine, D3-benzoylecgonine, cocaine, cocaethylene, amphetamine, D5-amphetamine, methamphetamine, methylenedioxymethamphetamine and methylenedioxyamphetamine were supplied by Sigma (Deisenhofen, Germany). D3-11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid and DL-2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidinium (EDDP) perchlorate were purchased from Radian (Austin, TX). Heroin from seized material was purified by recrystallization (cyclohexane) and its purity was analyzed by HPLC. Methylenedioxyethylamphetamine hydrochloride was isolated from seized tablets by Soxhlet-extraction with methylene chloride, its purity was determined by HPLC.

Solid-phase extraction columns (narc-1 by Bakerbond) were purchased from J.T. Baker Inc. (Phillipsburg, NJ) and chromabond C18 ec from Macherey-Nagel (Dueren, Germany).

2.3. Sample preparation

2.3.1. Hair samples

The hair samples (in the case of opioides, amphetamines, cocaine and methadone) were usually prepared in the following manner. This method is also used for the detection of flunitrazepam, tramadol and azaperone.

The hair samples were cut into segments (2–5 cm) if sectional analysis was requested. They were placed into an Eppendorf micro tube and decontaminated by vortexing in an ultrasonic bath with 1 ml *n*-hexane (30 s) followed by 1 ml acetone (30 s). The rinsing extracts were collected with a Pasteur pipette, combined, and kept for GC/MS/MS analysis.

Remaining residues of solvents were evaporated under a gentle stream of nitrogen. Each hair segment was then cut into 12 mm sections, and a 10.0 mg quantity was weighed into an Eppendorf tube. Then, methanol (0.5 ml) and the internal standard (D3-benzoylecgonine or D5-amphetamine) 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, the samples were allowed to stand overnight. The methanolic phase was decanted (centrifugation for 15 min), transferred into a vial and evaporated (at 40°C under a stream of nitrogen).

The residue obtained was then derivatized with 100 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 were obtained to provide the concentrations 0.25 ng/mg, 1.0 ng/mg, 2.5 ng/mg and 7.5 ng/mg of hair, respectively. Quantitation based on the ratio of the peak-areas of the analyte to the internal standard (D3-benzoylecgonine or D5-amphetamine). No derivatization was required for the detection of flunitrazepam, tramadol and azaperone.

2.3.2. Sample preparation for the detection of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THCA)

A 15.0 mg quantity of the sample (or segment) was weighed into an Eppendorf tube, the internal standard (20 pg D3-11-nor-delta-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). The hair digest and 2 ml of acetonitrile/0.1 N hydrochloric acid (2:3, v/v) were added to the solid-phase column. After 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 derivatized as described in the previous paragraph. Two μl of the derivatized sample was then injected into the GC/MS/MS system.

Standard calibration curves were obtained to provide the concentrations 0.20 pg/mg, 1.0 pg/mg, 5.0 pg/mg and 10.0 pg/mg of hair, respectively. Quantitation

based on the ratio of the peak-areas of the analyte to the internal standard (D3-THCA).

2.3.3. Preparation of hair containing residues of fentanyl and sufentanil

A hair tuft 6 cm in length was washed for 1 min each with methanol and acetone. It was then cut into 2 segments which were separately powdered in a ball mill. From each powder 50 mg were incubated in 2 ml Soerensen buffer at pH 7.6 for 2 h at 40°C in a water bath and then centrifuged. The buffer solution was decanted, the residue was shaken with 2 ml fresh buffer and centrifuged again. The combined aqueous layers were extracted with a solid-phase column (Chromabond 18 ec) and eluted three times with 0.5 ml acetone/dichloromethane (3:1, v/v). Evaporation to dryness was carried out under a gentle stream of nitrogen.

No derivatization was required. The dried extract was reconstituted with methanol and injected into the GC/MS/MS system.

2.4. 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 and W, DB-5, 30 m × 0.32 mm i.d., 0.25 μm film thickness) was used for GC separation.

For drugs that are routinely detected (opiates, amphetamines, cocaine and methadone), as well as for THCA and tramadol the GC was programmed in the following manner: 2 min at 60°C, 60–230°C at 25°C/min, 5 min at 230°C, 230–280°C at 15°C/min and, finally, within 3 min from 280–300°C.

In the case of fentanyl and its derivative, the temperature program was: 2 min at 60°C, 60–300°C at 25°C/min, 3 min at 300°C, and for benzodiazepines:2 min at 60°C, 60–180°C at 25°C/min, 180–280°C at 15°C/min and 5 min at 280°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 flow-rate of 1.0 ml/min.

The mass spectrometer was operated in selected reaction monitoring. The appropriate ion (MH^+ or ions derivatized with PFPA/HFIP: MH^+-PFP, MH^+-2 PFP, MH^+-HFIP, HFIP-MH^+-PFP, respectively), usually obtained by chemical ionisation using methane as the reagent gas, 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 at 2.1 or 2.2 mbar for positive chemical ionisation (PCI) and negative chemical ionisation (NCI) at a cell pressure of 2.7 mbar for electron impact ionisation (EI), respectively. The collision offset voltage was between -5 and −20 V for PCI, +15 V and +20 V for NCI and −15 and −20 V for EI. The electron multiplier was operated between 1050 and 2150 V. In case of chemical ionisation (CI) the methane pressure in the ion source was about 1 mbar.

PCI was applied to the detection of tramadol (parent ion at m/z 264, daughter ion at m/z 58), azaperone (parent ion at m/z 328, daughter ion at m/z 165), fentanyl (parent ion at m/z 337, daughter ion at m/z 188), sufentanil (parent ion at m/z 387, daughter ion at m/z 238).

The ionisation by EI (70 eV) was preferred for the detectionof EDDP, morphine, monoacetylmorphine, codeine and heroin.

NCI was used for the detection of THCA (parent ion at m/z 620, daughter ion at m/z 383) and flunitrazepam (parent ion at m/z 313, daughter ions at m/z 278 and 218).

3. Results and discussion

In order to obtain optimum selectivity for different drugs, the selected reaction monitoring (SRM) technique was applied [8,9]. It is desirable to produce an intense ion signal which is characteristic for the target compound. The operating conditions were optimized: argon pressure, collision offset voltage, MSMS factor (a correction factor for increasing the transmission of ions in the MS/MS mode). Only one selected ion was allowed to pass the first quadrupole which is the first mass analyzer. This parent ion on collisionally induced decomposition (CID) with argon produces daughter ions. The third quadrupole (the second mass analyzer) was set to filter a selected daughter ion. A selected reaction monitoring mass spectrometric technique in the daughter ion mode is therefore applied. Two criteria must be fulfilled: the mass-to-charge ratio of the parent and the mass-to-charge ratio of the daughter ion must satisfy the selected values.

Selectivity and sensitivity are extraordinarily increased by almost completely suppressing the noise level.

As a consequence, sample preparation can be considerably reduced: dividing into segments (if required), prewashing with *n*-hexane and acetone, drying, cutting into 1–2 mm sections, extracting with methanol by vortexing in a warm ultrasonic bath and finally, centrifugating and derivatizating.

Several quality control measures must be observed to avoid false positive or false negative results:

- Negative control samples (blank sample and blank hair sample) must be analyzed within every series;
- Test mixtures of relevant compounds in low concentrations must be checked;
- A brief rinse of hair samples to remove potential contamination, analyzing the combined extracts;
- In particular cases hair examination by SEM (scanning electron microscopy).

3.1. Case examples

The use of GC/MS/MS for the detection of codeine, dihydrocodeine, methadone, heroin (and its metabolites 6-acetylmorphine and morphine), cocaine (and its metabolites benzoylecgonine, cocaethylene), amphetamine and some of its

most commonly abused analogs (methylene dioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDE or MDEA), methamphetamine) in hair is conducted on a routine basis. A few examples should demonstrate that this technique is advantageous both for routine work and special forensic problems:

3.1.1. MDMA

A young man was seized with nearly one thousand pills of Ecstasy. His ingestion of MDMA was confirmed by hair analysis. The presence of MDA in hair can be interpreted as additional marker for Ecstasy use (Fig. 1).

3.1.2. Fentanyl and sufentanil

The detection of fentanyl in hair from a tumour patient is presented in Fig. 2 [10,11]. The patient had taken this potent synthetic narcotic for a period of 25 days percutaneously, the total dose was 15 mg.

The hair analysis of the extract from a patient who had intravenously injected sufentanil within a period of 5 days is presented in Fig. 3. The overall uptake of this opioid was 7.75 mg.

The concentration of fentanyl was 100 pg/mg hair, while the concentration of the sulfur containing compound sufentanil was about 10 pg/mg. These concentra-

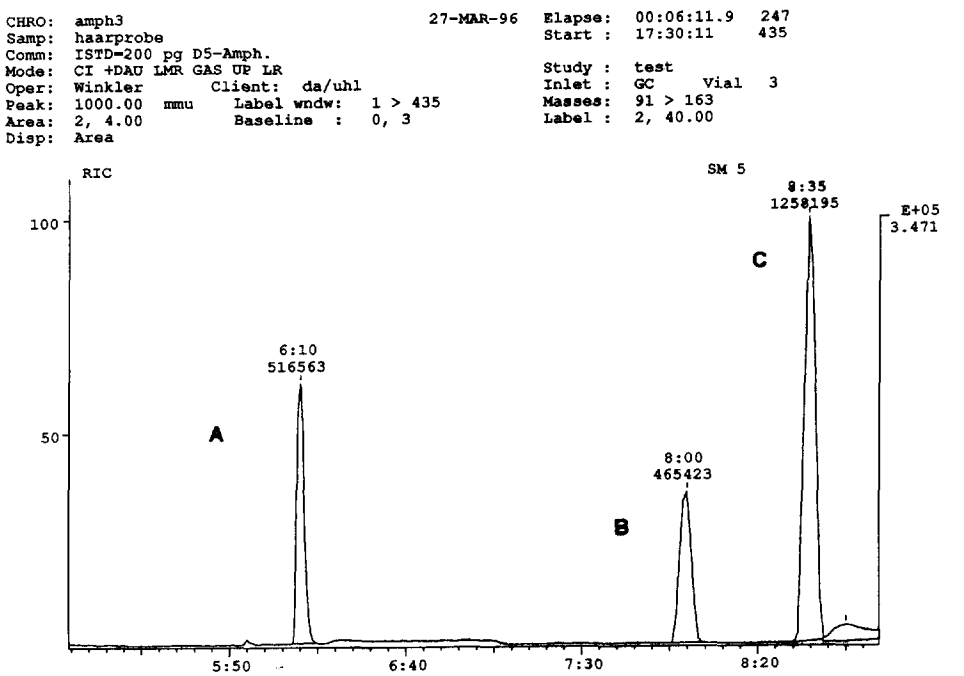

Fig. 1. Daughter ion chromatogram (PCI) of a hair sample from a confirmed MDMA user; A = D5-amphetamine (internal standard), B = MDA, C = MDMA.

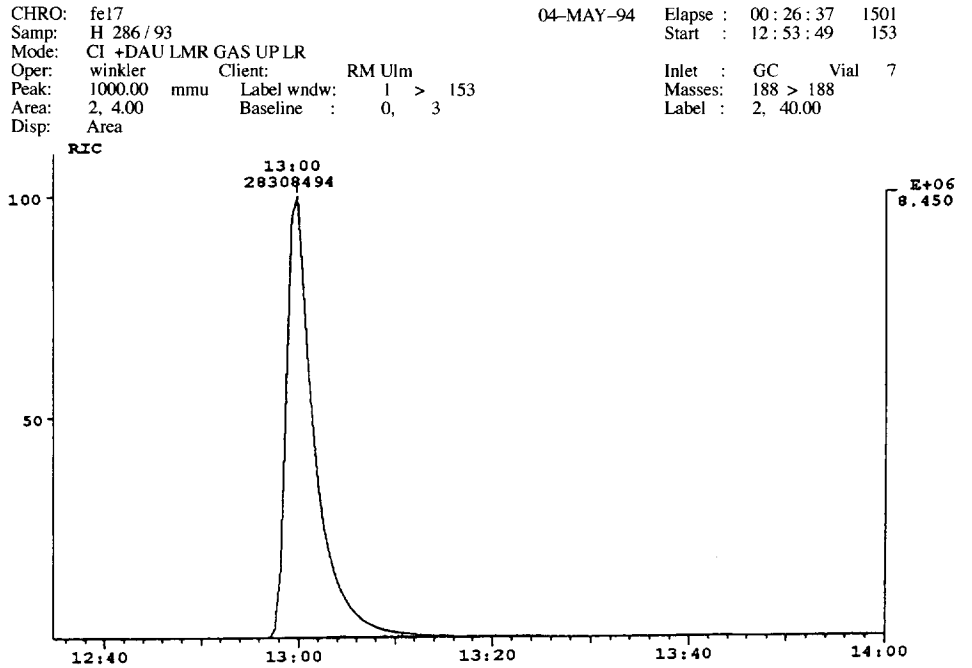

Fig. 2. Daughter ion chromatogram (PCI) of a fentanyl positive hair extract taken from a tumour patient; parent ion at m/z 337,daughter ion at m/z 188.

tions were estimated by comparing the peak-area of the analyte with the peak-area of the external standard.

3.1.3. Tramadol

A nurse working in a hospital was suspected of diluting, stealing and consuming the narcotic analgesic tramadol. The corresponding hair sample was cut into three 2-cm segments. Residues of tramadol were found in each segment (Fig. 4).

3.1.4. Flunitrazepam

Some benzodiazepines are abused in combination with drugs like heroin, cocaine or alcohol. Despite the low dosage of flunitrazepam [12] the detection in hair is performed easily by GC/MS/MS using NCI (Fig. 5).

3.1.5. THCA

The detection and quantification of THCA in hair is problematic due to its low concentration [13,14]. In our laboratory the procedure for the THCA detection involves a basic digestion of the hair samp' ' followed by acidification and solid-phase extraction. After elution and derivatization with PFPA/HFIP the resulting derivatized extract is analyzed using NCI. The parent ions selected for the collision with argon have the masses 620 and 623 representing the THCA derivative and the

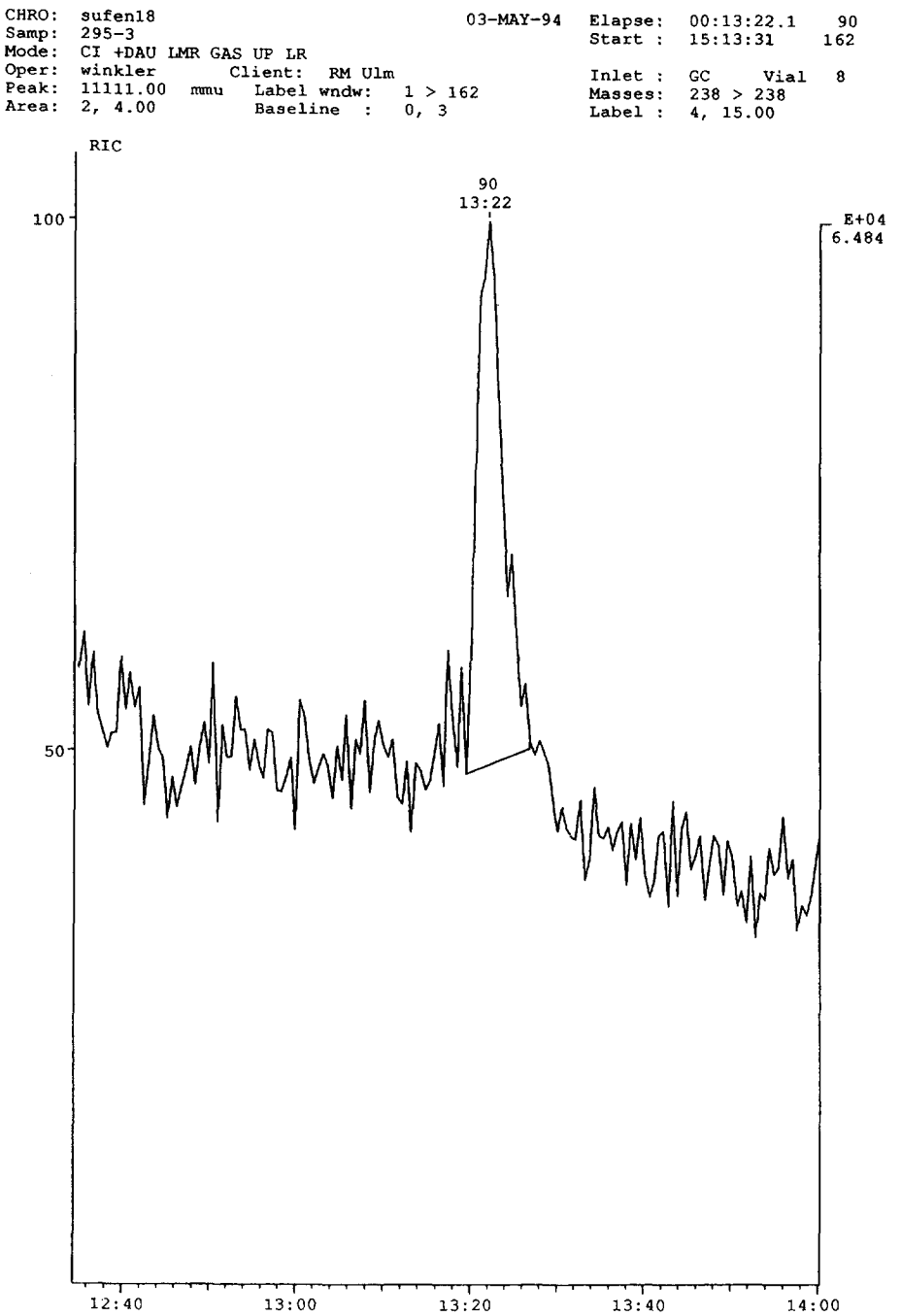

Fig. 3. Daughter ion chromatogram (PCI) of a sufentanil positive hair extract taken from a tumour patient; parent ion at m/z 387, daughter ion at m/z 238.

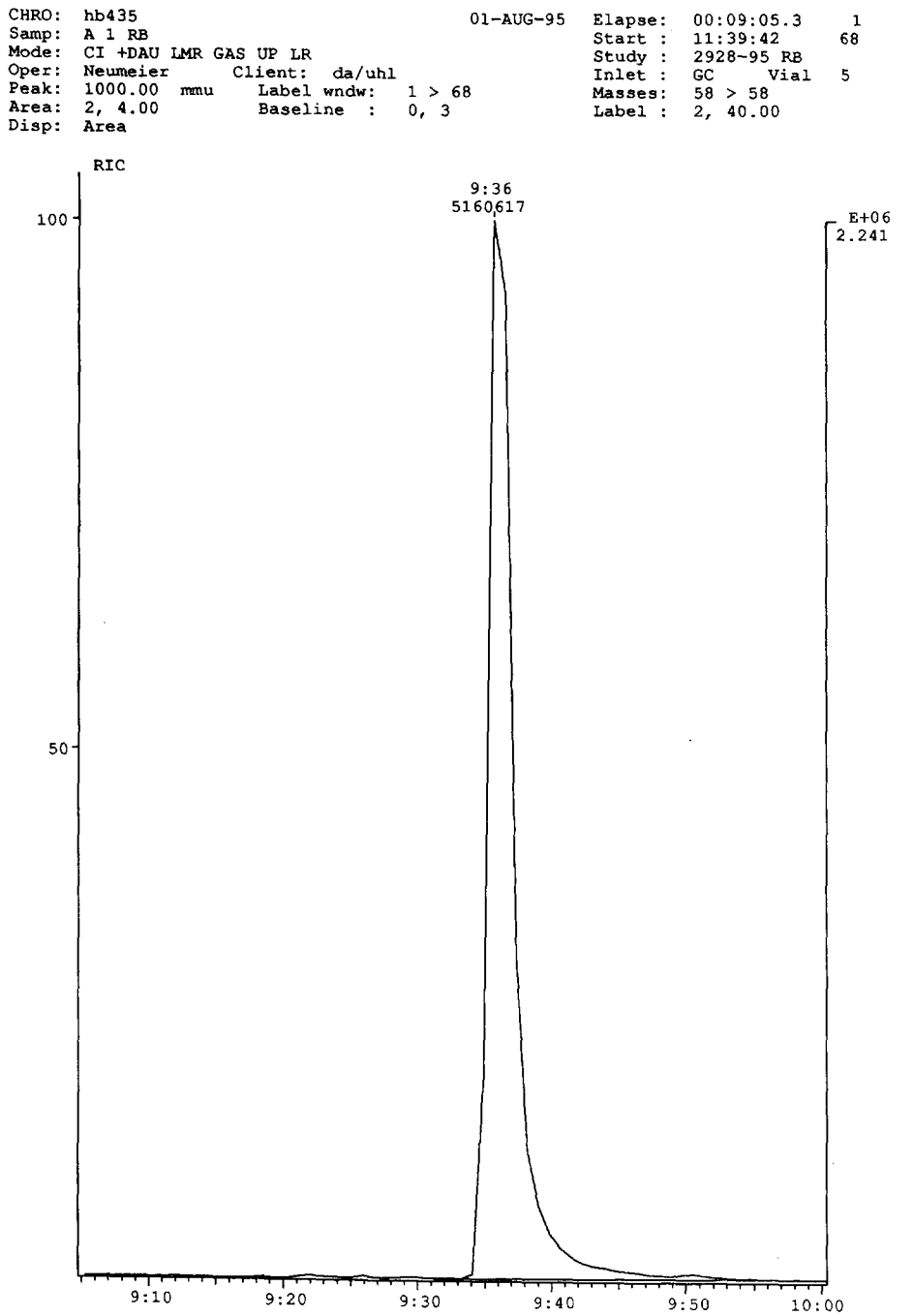

Fig. 4. Daughter ion chromatogram (PCI) of a hair sample from a confirmed tramadol user; parent ion at m/z 264, daughter ion at m/z 58.

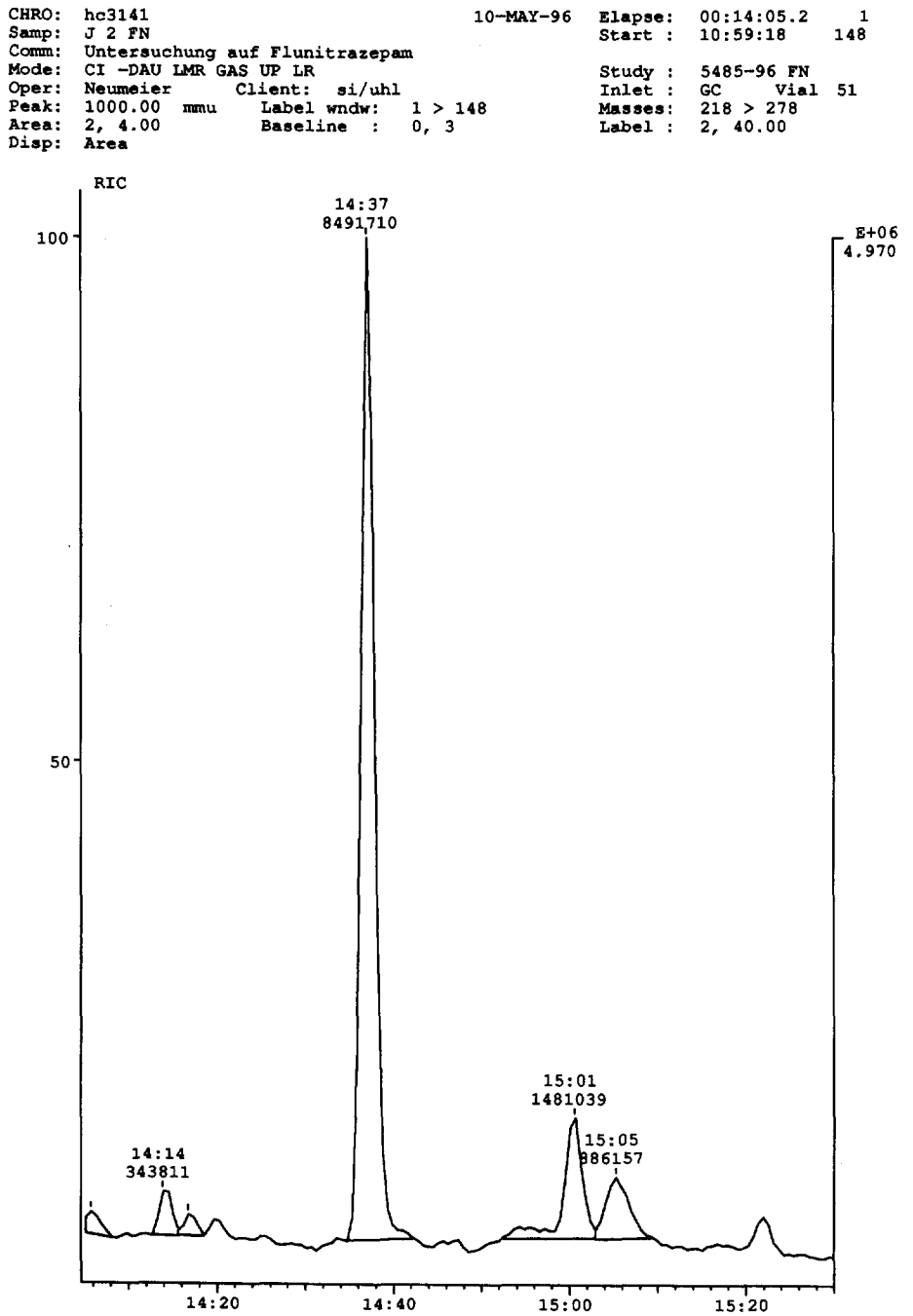

Fig. 5. Daughter ion chromatogram (NCI) of a hair sample from a confirmed flunitrazepam user; parent ion at m/z 313, daughter ions at m/z 278 and 218.

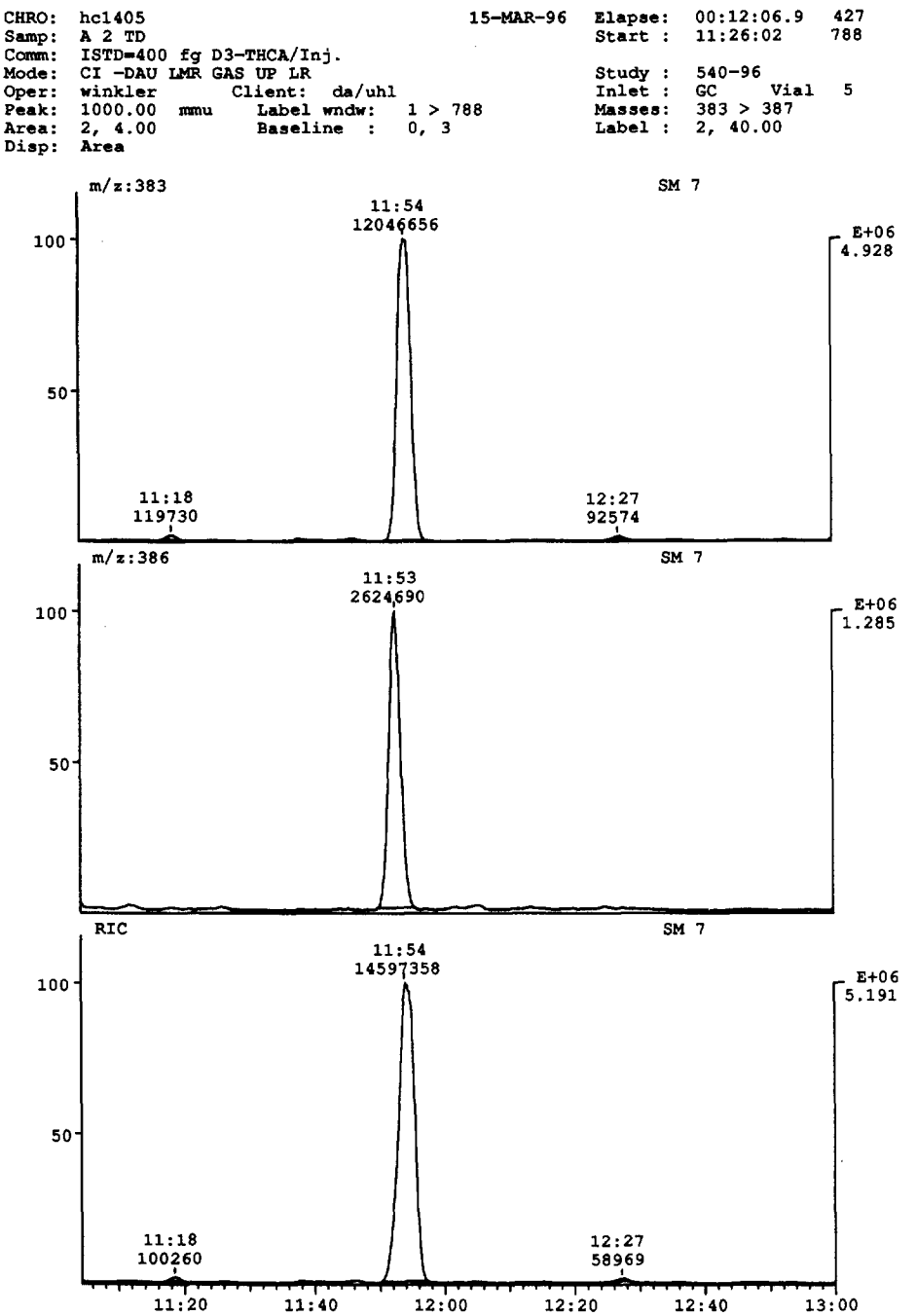

Fig. 6. Total ion current (RIC) and daughter ion chromatograms (NCI) of a hair sample from a confirmed cannabis user. Sample: parent ion at m/z 620, daughter ion at m/z 383; internal standard: parent ion at m/z 623, daughter ion at m/z 386.

D3-THCA derivative (Fig. 6). The following daughter ions for each parent ion are detected: m/z 383 and m/z 386. The concentrations were calculated by comparing the ratio of *mz* 383 and *mz* 386.

3.1.6. Azaperone

The butyrophenone derivative azaperone is a neuroleptic and is used in animals to prevent population stress and aggressive behaviour. This active substance is commonly used in veterinary medicine as a sedative for pigs. No therapeutic applications in humans are known so far [15]. But there are inhuman applications of azaperone: the blood and urine samples of a victim of crime tested positive for azaperone. When confronted with these results, the wife suspected of attempted poisoning of her husband admitted a single use but denied repeated applications of azaperone. In order to prove exposure over a long period the victim's hair sample was cut into two 3-cm segments (Fig. 7). Both segments tested positive for residues of the unchanged compound, evidence that there was a long-term intoxication and not only a recent accidental ingestion.

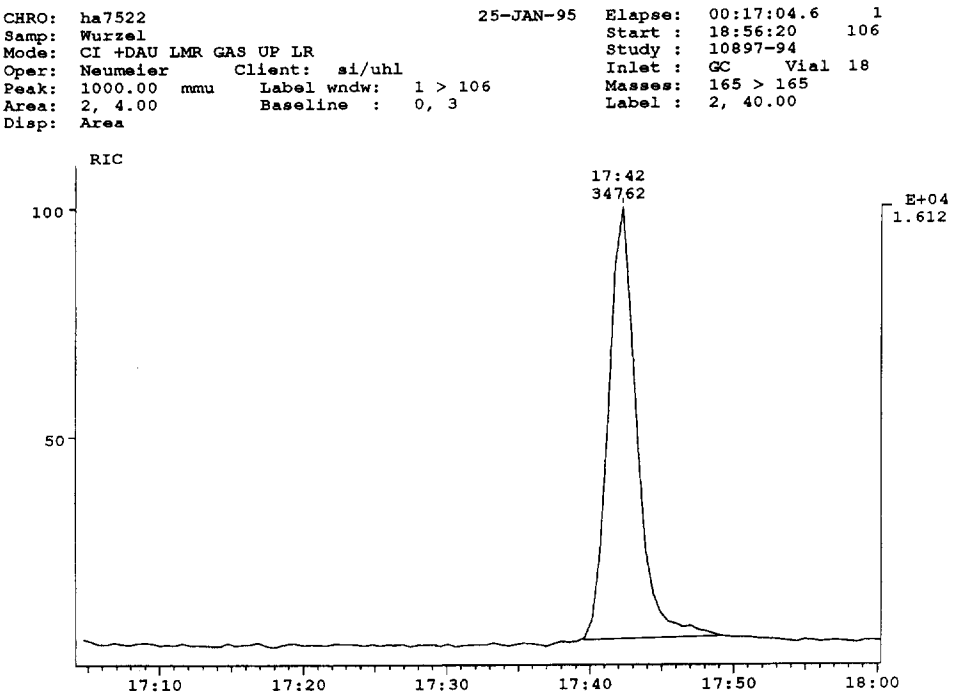

Fig. 7. Daughter ion chromatogram (PCI) of a hair sample (root segment) from a victim of an azaperone intoxication; parent ion at m/z 328, daughter ion at m/z 165.

4. Conclusions

The expert serves as a technical consultant in criminal court proceedings. The testing of hair for drugs is an invaluable aid and a necessary tool for law enforcement. Hair analysis gives the forensic investigator historical information on drug consumption or on cases of chronic intoxication.

In case of need it may be possible in the majority of cases to estimate roughly the extent and time of drug use by measuring drug concentrations in sequential segments of hair. In cases of the detection of rare illegal drugs or in special cases of intoxication, both the dose–response relationship and the incorporation rate are uncertain. A reliable statement could only be: generally, there was use or an administration of the substance.

Excellent GC/MS/MS methods with its extreme selectivity and high sensitivity could facilitate the expert's job. Nevertheless interpretation of the findings will continue to be complex and difficult.

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