

Original articles

Determination of dihydrocodeine in hair of opiate addicts by GC/MS

H. Sachs, R. Denk, and I. Raff

Institut für Rechtsmedizin, Universität Ulm/Donau, Prittwitzstrasse 6, W-7900 Ulm, Germany

Received January 28, 1991 / Received in revised form November 7, 1992

Summary. After the examination of more than 300 hair samples of suspected heroin abusers, a large number of which proved positive, we can say that high concentrations of dihydrocodeine can be determined either in addition to, or in the place of, morphine and also frequently in combination with codeine. The opiates were extracted after dissolving the hair samples in NaOH and hydrolysis with HCl. The quantitative determination of dihydrocodeine was achieved by derivatisation with HFBA using GC/MS at $m/u = 497$. Dihydrocodeine is used in antitussive drugs. After the examination of individual hair samples, it was obvious that some heroin consumers had switched to dihydrocodeine. This may lead to the conclusion that dihydrocodeine itself is used either as an intoxicating drug or to reduce withdrawal symptoms. The increasing number of positive samples should be noted by the legal authorities.

Key words: Dihydrocodeine – Hair analysis – GC/MS – Opiate addiction

Zusammenfassung. Im ersten Halbjahr 1990 wurde in einer großen Zahl von Haarproben, die wegen des Verdachts auf Heroinkonsum entnommen worden waren und deshalb auf Morphinderivate untersucht wurden, Dihydrocodein in Konzentrationen über $1 \mu\text{g/g}$ nachgewiesen. Häufig wurde es in Begleitung von Morphin und Codein gefunden, in manchen Fällen aber auch als einziges Morphinderivat. Der Nachweis erfolgt nach Auflösung der Haare in NaOH, Hydrolyse und Extraktion gaschromatographisch-massenspektrometrisch nach Derivatisierung mit Heptafluorbuttersäure. Dihydrocodein wird wie Codein als Antitissivum eingesetzt. Offensichtlich wechseln Heroinkonsumenten in manchen Fällen zu Dihydrocodein oder nehmen beides, so daß man annehmen kann, daß es entweder selbst als berauschendes Mittel oder zur Milderung von Entzugserscheinungen verwendet wird. Die steigende Zahl der Fälle des offensichtlichen Medikamentenmißbrauchs sollte bei den Gesetzgebern zu entsprechenden Konsequenzen führen.

Schlüsselwörter: Dihydrocodein – Haaranalysen – GC/MS – Opiatabhängige

Introduction

Dihydrocodeine like codeine is used as an antitussiva and is sold in Germany under the trade marks Paracodin and Remedacen. It is also increasingly being used as an alternative by heroin addicts. In order to distinguish heroin addicts from those who merely take cough medicine it has been important to look for a specific method to detect dihydrocodeine alone or in combination with codeine and morphine in hair samples. For this purpose radioimmunological determinations are not sufficient as even morphine – specific RIA – kits show higher levels of cross-reaction with dihydrocodeine in hair than those given in the instruction manual for urine analysis. It is therefore necessary to use GC/MS for the detection of dihydrocodeine parallel to morphine and codeine.

All samples which had been sent by the police originated from suspected heroin abusers.

Materials and methods

Sample preparation and GC/MS method. The samples were dissolved in NaOH as described by Klug (1980). The opiates were

Table 1. Scheme of sample preparation for determination of dihydrocodeine in hair

Asservation of the sample, washing with acetone and drying
Dissolving in NaOH and hydrolysis with conc. HCl
Neutralization and pH adjustment
Extraction with Extrelut and toluene/butanol
Re-extraction with H_2SO_4
Neutralization and pH adjustment
Extraction with Extrelut® and dichlormethane/isopropanol
Evaporating and derivatisation with HFBA
Evaporation of HFBA and absorption in ethylacetate

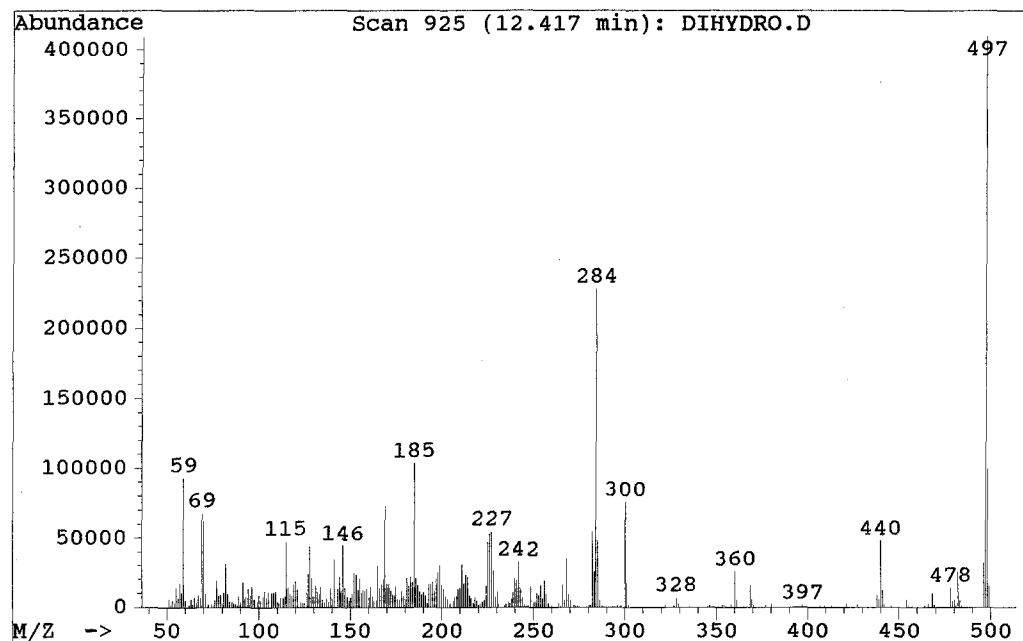


Fig. 1. Mass spectrum of dihydrocodeine-HFBA

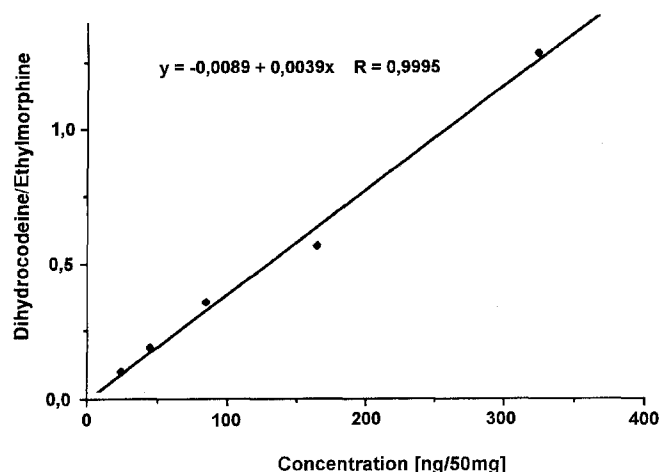


Fig. 2. Calibration curve for hair samples impregnated with dihydrocodeine

determined by GC/MS after derivatization with HFBA, an improved method similar to that already described by Sachs and Brunner (1986), and Sachs and Arnold (1989). The individual hair fragments were heated in an evaporator with 3 ml NaOH (30 g/l) and 500 ng ethylmorphine as an internal standard until the material disintegrated. Then the samples were hydrolyzed for 30 mins together with 2 ml 25% HCl at 100°C. After cooling, solid Na₂CO₃/NaHCO₃ (1:4 w/w) was added until a pH value of 8.5–9 was attained. The volume was adjusted to 20 ml with glycylglycine buffer pH 8.5 (56 g glycine in 500 ml H₂O and 2 ml 25% NH₃) and extracted with a mixture of toluene/n-butanol (7:3 v/v) in an Extrelut-column (Merck, Darmstadt) and re-extracted in 10 ml H₂SO₄ (0.1 mol/l) by shaking for 5 minutes. The aqueous phase was re-adjusted to pH 8.5–9 with Na₂CO₃/NaHCO₃. The solution was again adjusted to 20 ml with glycylglycine buffer and extracted with dichloromethane/propanol (9:1 v/v) by Extrelut®. The organic phase was evaporated under a nitrogen stream and the residue dried for approximately 12 h over silica gel. After adding 100 µl heptafluorobutyric acid anhydride, the samples were derivatized at

65°C. The samples were evaporated in a stream of nitrogen, until odourless, then dissolved in 20 µl ethyl acetate (Table 1).

For GC/MS analysis, 1 µl was injected without splitting into a GC/MS Hewlett Packard 5995 A at 100°C on an Ultra 2 column (cross-linked 5% phenylmethylsilicone, 12 m, 0.2 mm I.D., 0.33 µm film) and analysed from 250 to 280°C programmed at 5°C/min. Performing this method retention times were between 12 and 12.5 min. Because of low concentrations, quantitative determinations were only possible in the selected-ion-monitoring (SIM) mode. Morphine-HFBA was identified by m/u = 464, codeine-HFBA by m/u = 282 and 495(M⁺), dihydrocodeine-HFBA by m/u = 284 and 497(M⁺) (Fig. 1), and ethylmorphine-HFBA by m/u = 509.

For quantitative determinations m/u = 464, 495, and 497 were used compared to m/u = 509. Calibration curves were obtained by adding 20–320 ng dihydrocodeine to 50 mg of drug-free hair. Regression coefficients were always greater than 0.99. As the example of a calibration curve demonstrates (Fig. 2), ethylmorphine is suitable as an internal standard, in spite of the disadvantage that it must be initially excluded that the drug addict used ethylmorphine. Deuterated standards, therefore, should be used as soon as they are available.

Results

The smallest amount detected was 30 pg (Fig. 3), which can be considered to be the detection limit. In Fig. 4 the results of a person with a high dihydrocodeine consumption over a long period are shown. High levels of dihydrocodeine were found in all segments of a hair sample over a total length of 13 cm and only traces of morphine and codeine, corresponding to an intake of antitussive drugs over a period of about 13 months. The case shown in Fig. 5 involves a heroin-addict who subsequently consumed only dihydrocodeine. Morphine/codeine concentrations typical for heroin users were found in the hair sections from 3–10 cm, corresponding to an approximate growth of 3–10 months before cutting the hair. The last

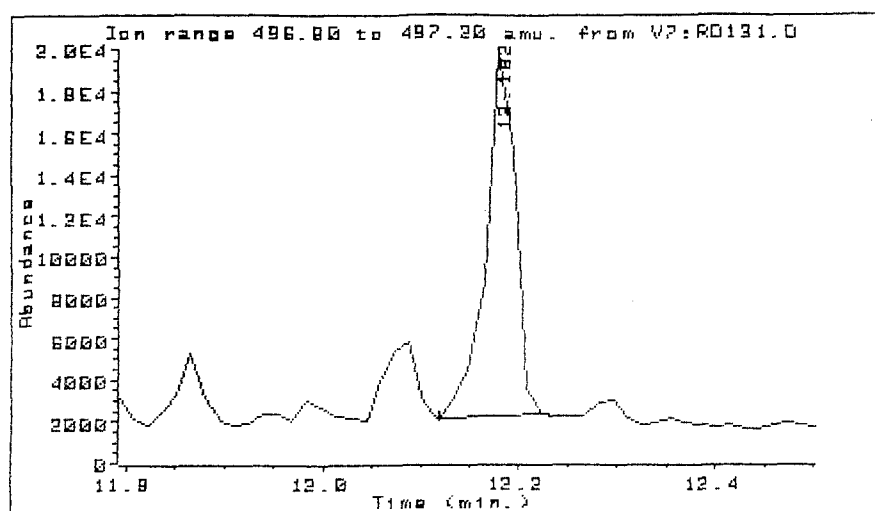


Fig. 3. Selected ion-chromatogram at $m/u = 497$ of 30 pg dihydrocodeine extracted from hair sample

Ion range 496.80 to 497.20 amu. from V7:RD131.D
 A5H 593 I

Peak#	Ret Time	Type	Width	Area	Start Time
1	12.182	PBA	0.035	367365	12.118

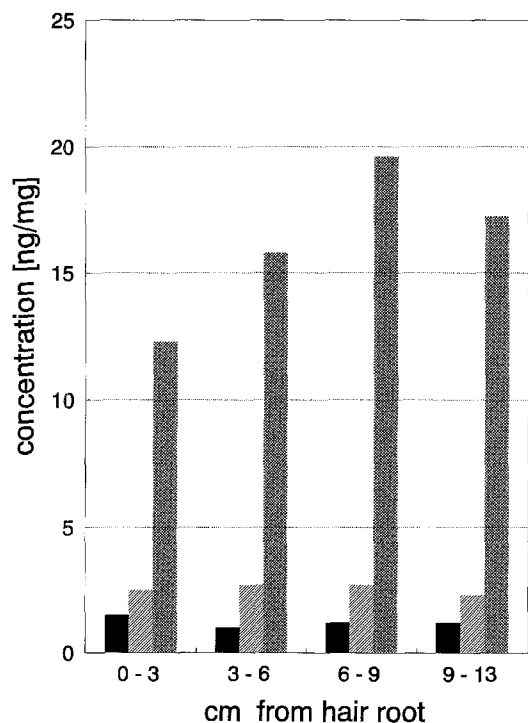


Fig. 4. Opiate concentrations in hair segments after long periods of dihydrocodeine intake

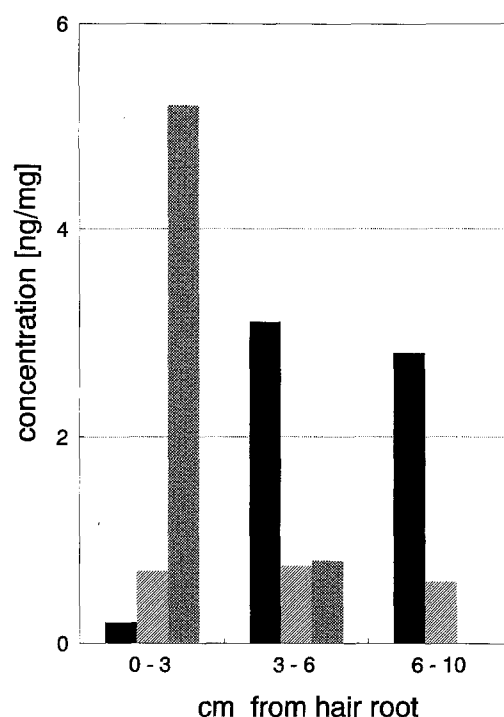


Fig. 5. Opiate concentrations in hair segments after changing from heroin to dihydrocodeine uptake

hair section contained only very little morphine in spite of a high concentration of dihydrocodeine.

During the first 6 months of 1990 a high proportion of case samples contained dihydrocodeine (Table 2). Out of a total of 68 cases examined for heroin, dihydro-

codeine could be demonstrated in 14. During these examinations only those cases were recorded where the concentration was over $1 \mu\text{g/g}$ (Table 3). From experience with codeine it is known that therapeutic doses result in low concentrations in hair.

Table 2. Number of investigations in Ulm in the first half of 1990

Total number of investigations	104
Question of opiate uptake	68
Positive in at least 1 segment	52
Certain heroin uptake	25
Dihydrocodeine uptake	13

Table 3. Results of 13 cases in the first half of 1990. Segments 3 cm in length are numbered from root to tip. Morphine and codeine concentrations are given for comparison

Case no.	Segment no.	Dihydrocodeine [ng/mg]	Codeine [ng/mg]	Morphine [ng/mg]
176	1	2.4	1.2	2.5
	2	1.2	1.4	3.2
167	1	3.4	0.1	0.6
	2	1.7	0.1	1.0
	3	1.9	0.1	1.2
	4	2.4	0.4	1.8
166	1	1.2	1.9	12.3
93	1	5.2	0.6	2.0
186	1	24.6	0.1	0.6
	2	4.6	—	0.4
31	1	13.7	0.4	—
	2	4.8	—	—
	3	1.2	—	—
232	1	11.0	1.7	1.9
	2	7.2	0.3	0.5
	3	2.8	0.5	2.8
244	1	8.4	0.9	—
	2	13.8	0.8	—
	3	11.6	1.3	0.1
	4	12.7	1.5	0.1
272	1	21.4	2.8	10.8
295	1	2.7	—	0.5
322	1	31.2	2.3	9.2
348	1	2.3	—	—
	2	3.0	—	—
515	1	20.8	4.3	4.6
	2	6.6	5.3	11.0
	3	6.1	3.0	4.2

Discussion

Dihydrocodeine, like morphine and codeine, can be determined even in very small concentrations. The analysis of individual opiates in hair samples helps to describe a drug career. The cases examined lead to the conclusion that dihydrocodeine like codeine is not only used therapeutically. The danger of codeine abuse and the possibility of its detection by hair analysis has already been described by Reinhardt and Sachs (1986), and Scheller and Sachs (1989). However this substance is frequently used by heroin addicts as an alternative in the absence of the regular drug. It has not yet been determined whether dihydrocodeine itself has a habit-forming potential comparable to heroin or whether it is only used to alleviate withdrawal symptoms. The case shown in Fig. 5 involved a heroin addict who subsequently consumed only dihydrocodeine, either because he had no heroin at his disposal or because he was trying to cure his heroin dependency.

In some cases, although high levels of dihydrocodeine have been found in all segments of hair, no other opiate or only traces of morphine and codeine were detected (e.g. case no. 31 with 13.7 µg/g dihydrocodeine). The highest level of dihydrocodeine found was 86 µg/g. This concentration cannot be explained by a therapeutic dose of the drug. The increasing number of highly positive samples should be noted by the legal authorities, since drug abuse is to be suspected in such cases.

References

- Klug E (1980) Zur Morphinbestimmung in Kopfharen. *Z Rechts-med* 84:189–193
- Reinhardt G, Sachs H (1986) Codein und das Betäubungsmittelgesetz. In: Heberer G, Opderbecke HW, Spann W (eds) *Ärztliches Handeln (Festschrift für Walther Weißbauer)*. Springer Verlag, Berlin Heidelberg New York, pp 128–131
- Sachs H, Brunner H (1986) Opiatuntersuchungen in Haaren mit GC/MS. *Beitr Gerichtl Med* 44:281–288
- Sachs H, Arnold W (1989) Results of comparative determination of morphine in human hair using RIA and GC/MS. *Clin Chem Clin Biochem* 27:873–877
- Scheller M, Sachs M (1989) Nachweis von Codeinmißbrauch durch Haaranalyse. *Dtsch Med Wochenschr* 115:1313–1315