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Usefulness of multi-parameter opiates–amphetamines–cocainics analysis in hair of drug users for the evaluation of an abuse profile by means of LC–APCI-MS-MS

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Abstract

The report presents a segmental hair analysis for the retrospective multi-parameter evaluation of drugs of abuse including opioids, cocainics and amphetamines. The analysis was carried out with the use of liquid chromatography atmospheric pressure chemical ionization tandem mass spectrometry (LC–APCI-MS-MS). The authors have evaluated the differences in the contents of particular opiates in the hair as related to the origin of a sample taken from Polish drug users taking "Polish heroin", and also from heroin abusers from Western European countries taking "Western heroin". The results indicate distinct differences in the 6-MAM concentration values in the Polish and foreigners, suggesting that the foreigners take products containing high concentrations of heroin and the Polish take the poppy product "compote" characterized by its variable and low heroin content. An additional argument for a different abuse profile in the Polish and Western drug users is found in the presence of cocaine detected in hair samples originating from the latter, while cocaine is much less frequently detected in Polish drug users.

Keywords: Opiates; Hair analysis; LC–APCI-MS-MS

1. Introduction

The statistical data on opiate poisonings in Poland [1,2] point to a continuously large number of cases involving this group of narcotic substances. The profile of opiate abuse in Poland is characterized by the fact that the market does not provide pure morphine and its approximately 10 times stronger semi-synthetic derivative—heroin. Obtaining these narcotics legally is difficult or downright impossible; hence the main source of opiate narcotics is their illegal production from plants, i.e. poppies. The abusers use extracts from poppy straw, the so-called "compote" or "Polish heroin", as well as products derived from poppy juice, mainly *Opium* [3–11].

At present, the opinion prevails that the majority of psychoactive substances introduced into the human body via various routes are incorporated into hair [12,13]. It is additionally believed that the majority of uses for hair analysis is associated with drugs of abuse, which is most likely a consequence of the number of investigated and solved cases of this nature. The advantage of hair as an alternative material is its stability: it is a non-invasive method of collection, does not constitute any breach of privacy and it does not require any particular storage procedures. Drugs of abuse incorporated into hair have a wide "window of detection" when compared with blood and urine [14–19].

The objective of the report is to present investigations confirming the usefulness of hair as evidence of opioid intake by heroin abusers. An opportunity of analyzing hair samples taken from foreign inhabitants of Western European countries taking

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"Western heroin" and Polish abusers taking "Polish heroin" has allowed the comparison of preferential abuse profiles dependent on geographical place of residence.

2. Experimental

2.1. Materials, reagents and solvents

Reference standard solutions of amphetamine, benzoylecgonine, cocaine, cocaethylene, codeine, methamphetamine, 3, 4-methylendioxamphetamine (MDA), 3,4-ethylendioxethylamphetamine (MDEA), 3,4-methylendioxmethamphetamine (MDMA), 6-monoacethylmorphine (6-MAM), morphine (1 mg/ml in methanol or acetonitrile) and the deuterated internal standards (IS) of amphetamine-d_3, benzoylecgonine-d_3, codeine-d_3, methamphetamine-d_5, MDA-d_5, MDEA-d_6, MDMA-d_5, 6-MAM-d_3, and morphine-d_3 (100 μg/ml in methanol or acetonitrile) were purchased from Promochem (Warsaw, Poland).

Acetonitrile was HPLC/GC–MS grade (Merck, Germany), formic acid (100–98%) was ultra pure (Riedel-de Haën, Germany) and methanol, acetone and *n*-hexane were HPLC grade (POCh, Poland).

The hair pulverizer MM 200 series was acquired from Retch (Germany), centrifuge model mini spin from Eppendorf (Germany) and the 24-position N-EVAP analytical evaporator from Organomation Assoc. (Northbourgh, MA).

2.2. Biological materials

Hair samples from seven people were collected from the area at the back of the head with the use of a razor (without bulbs), delivered from Department of Treatment of Abuse from Psychoactive Substances in Chorzów (Poland). Samples were as follows:

(a) Hair samples from three patients with a history of intravenous opiates use—available segment length of 1.3–2 cm;
(b) Hair samples taken from four male-drug abusers from Western European countries—available each segment length of 1 cm;
(c) Control samples, that is, "natural" hair taken for analytical purposes from persons who do not take drugs.

2.3. Calibrators and controls

For the calibrator samples and quality control (QC), three working solution mixtures of amphetamine, benzoylecgonine, cocaine, cocaethylene, codeine, methamphetamine, MDA, MDEA, MDMA, 6-MAM and morphine were prepared in methanol at the following concentrations: 10 μg/ml, 1 μg/ml and 0.1 μg/ml. For the deuterated internal standards (IS), a working solution mixture of 1 μg/ml of amphetamine-d_3, benzoylecgonine-d_3, codeine-d_3, methamphetamine-d_5, MDA-d_5, MDEA-d_6 MDMA-d_5, 6-MAM-d_3, morphine-d_3 was prepared. All working solutions were stored at –20°C when not in use. Daily calibration samples were prepared by fortifying 20 mg of blank hair with known amounts of analytes at concentrations ranging from 0.2 to 20 ng/mg for opioids and amphetamines and 0.05 to 20 ng/mg for cocainics. Low- and high-QC specimens were also prepared daily at concentrations of 0.6 and 15 ng/mg. Twenty microliters of working solution mixtures was added to each sample prior to extraction, giving a final deuterated IS concentration of 1 ng/mg.

2.4. Sample preparation and extraction

Hair samples were cut into segments (1–2 cm). 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 sample (segment) was pulverized in a ball mill and 20 mg quantity was weighed into eppendorf microtube. Then 1 ml of methanol and 1 ng/mg of each deuterated IS was added and the tube was tightly closed and sealed with a foil of parafilm. After vortexing in an ultrasonic bath (50 °C) for 1 h and following centrifugation, the samples were allowed to stand overnight. The methanolic phase was decanted, transferred into an eppendorf microtube and evaporated at 40 °C under a stream of nitrogen.

2.5. LC–APCI-MS-MS analysis

LC–MS system consisted of liquid chromatograph, degasser T, gradient pump TSP 4000 and autosampler TSP 3000 (Finnigan MAT, San Jose, USA) and mass spectrometer–ion trap LCQ series with atmospheric pressure chemical ionization inlet (APCI) (Finnigan MAT, San Jose, USA). A LiChroCART column 125 mm × 3 mm I.D., 5 μm particle size, filled with Purospher RP 18, and a LiChroCART precolumn 4 mm × 4 mm I.D., particle size 5 μm, filled with LiChrospher 60 RP-select B, was used for separation (Merck, Germany).

2.5.1. Chromatographic parameters

The liquid chromatograph was operated in the gradient composition mode of 0.1% formic acid in water [A] and acetonitrile [B] phases. The gradient program was as follows: 95% [A] + 5% [B] for 2 min, followed by a linear change to 30% [A] and 70% [B] in 30 min, 30% [A] and 70% [B] were held for 2 min, then changed to 95% [A] and 5% [B] for 8 min. The constant flow rate was 400 μl/min and the injection volume was 10 μl. The total run time for one injection was 40 min.

2.5.2. Mass spectrometry parameters

The mass spectrometer was operated in the MS-MS mode. The transitions $[M+H]^+ \rightarrow$ product ions were monitored as follows: 286 → 268 for morphine, 289 → 271 for morphine-d_3, 300 → 282 for codeine, 303 → 285 for codeine-d_3, 328 → 268 for 6-MAM, 331 → 271 for 6-MAM-d_3, 304 → 182 for cocaine, 307 → 185 for cocaine-d_3, 290 → 168 for benzoylecgonine, 293 → 171 for benzoylecgonine-d_3, 343 → 195 for cocaethylene, 136 → 119 for amphetamine, 139 → 122 for amphetamine-d_3, 150 → 119 for methamphetamine, 155 → 121 for methamphetamine-d_5, 180 → 163 for MDA, 185 → 168 for

MDA-d_5, 194 → 163 for MDMA, 199 → 165 for MDMA-d_5, 208 → 163 for MDEA and 214 → 166 for MDEA-d_6. The isolation window for each analyte and deuterated internal standard was 1.5 a.m.u. Collision-induced dissociation (CID) of each opioid and cocainic was 37% while the expected CID for ion of amphetamines was 30%. The dwell-time for each ion was determined to be 200 ms. The ion products were collected in full scan mode. The APCI inlet parameters were as follows: sheath gas (nitrogen) pressure 60 p.s.i., vaporizer temperature 400 °C, capillary temperature 150 °C and discharge current 5 μA. The solvent delay was 4 min. The detector was turned off after 20 min.

2.6. Selectivity

To evaluate peak-purity and selectivity, blank hair samples (no analytes or IS added) were analyzed with each batch to check for peaks that might interfere with detection of analytes or IS. Also, unextracted methanolic IS samples were analyzed by MS-MS mode to verify negligible amounts (<1%) of non-deuterated analyte ions, and negative samples (blank hair + IS) were analyzed to verify the absence of native analyte in the IS solution.

2.7. Linearity, limit of quantitation and detection

Quantitation of opioids, amphetamines, and cocaines was performed by the internal standard method. A six-point calibration curve was made for opioids, and amphetamines and an eight-point calibration curve for cocaines was prepared by linear least-square regression analysis of the ratio of the peak area of analyte to the peak area of its deuterated IS.

Calibration curves were prepared daily by spiking blank hair with corresponding analytical working solution mixtures to obtain calibration concentrations of 0.2, 0.5, 1, 5, 10 and 20 ng/mg opioids and amphetamines and 0.05, 0.1, 0.2, 0.5, 1, 5, 10 and 20 ng/mg cocainics. Validation samples were prepared in triplicate at the following concentrations: 0.05, 0.1, 30, 50 ng/mg opioids and amphetamines and 0.01, 0.02, 30, 50 ng/mg cocainics. Negative QC samples were analyzed after each linearity sample to evaluate potential carry over.

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by analyzing validation samples ($n = 5$) to determine if acceptance criteria were met for each analyte.

2.8. Precision and accuracy

Intra-assay data were assessed by comparing data from within one run ($n = 10$) and inter-assay data were determined between five separate runs ($n = 34$). Accuracy, expressed as a percentage, was calculated by taking the difference between the mean calculated concentrations and target concentrations, dividing by the calculated mean and multiplying by 100. Precision, expressed as percent mean and standard deviation (% R.S.D.), was determined by calculating the percent ratio of the standard deviation divided by the calculated mean concentration times 100.

3. Results

3.1. Analytical and validation study

Blank hair samples were analyzed with each validation run ($n = 6$). All samples were free of co-eluting peaks at the retention times of morphine, codeine, 6-MAM, amphetamine, methamphetamine, MDA, MDMA, MDEA, cocaine, benzoylecgonine, cocaethylene and their respective deuterated IS. The analysis of negative hair samples in each assay also demonstrated that the IS did not contain relevant amounts of native opioids, amphetamines and cocainics.

An overview of characteristic calibration data over a dynamic range from the LOD/LOQ to 20 ng/mg each of opioids, amphetamines and cocainics is given in Table 1.

Additional quality control samples ($n = 3$) were analyzed to evaluate the upper limit of linearity and potential carry over. The 30 ng/mg opioids, amphetamines and cocainics samples were

Table 1
Characteristics of opiates, amphetamines and cocainics calibration curves[a]

Analyte	Range (ng/mg)	Regression equation of calibrators[b]	Correlation coefficient (r^2)	LOD[c] and LOQ[d]
Morphine	0.2–20	$y = 1.3088x - 0.1163$	0.9998	0.2
Codeine	0.2–20	$y = 1.0065x + 0.1734$	0.9982	0.2
6-MAM	0.2–20	$y = 1.0679x + 0.0185$	0.999	0.2
Amphetamine	0.2–20	$y = 1.408x - 0.2593$	0.9997	0.2
Methamphetamine	0.2–20	$y = 1.6324x - 1.309$	0.9991	0.2
MDA	0.2–20	$y = 1.8763x - 1.693$	0.9993	0.2
MDMA	0.2–20	$y = 1.488x + 0.0554$	0.9991	0.2
MDEA	0.2–20	$y = 1.5589x - 0.7794$	0.9989	0.2
Cocaine	0.05–20	$y = 0.8402x + 0.0198$	0.9992	0.05
Benzoylecgonine	0.05–20	$y = 0.7022x + 0.0229$	0.9984	0.05
Cocaethylene	0.05–20	$y = 1.9509x + 0.0209$	0.9998	0.05

[a] $N = 5$.
[b] Mean value.
[c] Limit of detection.
[d] Limit of quantitation.

Table 2
Accuracy and precision for simultaneous determination of opioids in human hair by LC–APCI-MS-MS

Analyte (ng/mg)	Intra-assay ($n = 10$)			Inter-assay ($n = 34$)		
	Mean (ng/mg)	Accuracy[a]	Precision[b] (%)	Mean (ng/mg)	Accuracy[a]	Precision[b] (%)
Morphine						
0.6	0.57 (±0.04)	−5.00	7.02	0.58 (±0.03)	−3.33	5.17
15	14.10 (±1.15)	−6.00	8.16	14.23 (±0.98)	−5.13	6.87
Codeine						
0.6	0.57 (±0.08)	−5.00	14.03	0.58 (±0.05)	−3.33	8.62
15	14.67 (±1.15)	−2.22	7.84	15.23 (±0.98)	1.53	6.43
6-MAM						
0.6	0.52 (±0.08)	−13.33	15.38	0.55 (±0.06)	−8.33	10.91
15	14.11 (±1.21)	−5.93	8.57	14.23 (±1.08)	−5.13	7.59

[a] Percent difference between mean and target concentration.
[b] Percent relative standard deviation.

Table 3
Accuracy and precision for simultaneous determination of amphetamines in human hair by LC–APCI-MS-MS

Analyte (ng/mg)	Intra-assay ($n = 10$)			Inter-assay ($n = 34$)		
	Mean (ng/mg)	Accuracy[a]	Precision[b] (%)	Mean (ng/mg)	Accuracy[a]	Precision[b] (%)
Amphetamine						
0.6	0.55 (±0.04)	−8.33	7.27	0.58 (±0.03)	−3.33	5.17
15	14.55 (±1.01)	−3.00	6.94	15.33 (±0.97)	2.20	6.33
Methamphetamine						
0.6	0.52 (±0.06)	−13.33	11.54	0.54 (±0.05)	−10.00	9.26
15	14.67 (±1.25)	−2.20	8.52	15.84 (±0.98)	5.60	6.19
MDA						
0.6	0.51 (±0.08)	−15.00	15.67	0.55 (±0.05)	−8.33	9.09
15	14.11 (±1.25)	−5.93	8.86	14.41 (±0.92)	−3.93	6.38
MDMA						
0.6	0.56 (±0.05)	−6.67	8.93	0.59 (±0.04)	−1.67	6.78
15	14.55 (±1.02)	−3.00	7.01	14.43 (±0.95)	−3.80	6.58
MDEA						
0.6	0.52 (±0.06)	−13.33	11.54	0.57 (±0.05)	−5.00	8.77
15	15.55 (±1.31)	−3.67	8.42	15.23 (±0.94)	1.53	6.17

[a] Percent difference between mean and target concentration.
[b] Percent relative standard deviation.

Table 4
Accuracy and precision for simultaneous determination of cocainics in human hair by LC–APCI-MS-MS

Analyte (ng/mg)	Intra-assay ($n = 10$)			Inter-assay ($n = 34$)		
	Mean (ng/mg)	Accuracy[a]	Precision[b] (%)	Mean (ng/mg)	Accuracy[a]	Precision[b] (%)
Cocaine						
0.6	0.53 (±0.05)	−11.67	9.43	0.58 (±0.04)	−3.33	6.90
15	15.67 (±1.11)	4.47	7.08	15.23 (±0.94)	1.53	6.17
Benzoyloecgonie						
0.6	0.55 (±0.05)	−8.33	9.09	0.58 (±0.04)	−3.33	6.90
15	14.67 (±1.15)	−2.20	7.84	15.23 (±1.04)	1.53	6.83
Cocaethylene						
0.6	0.57 (±0.06)	−5.00	10.54	0.58 (±0.04)	−3.33	6.90
15	14.67 (±1.15)	−2.20	7.84	15.23 (±0.94)	1.53	6.17

[a] Percent difference between mean and target concentration.
[b] Percent relative standard deviation.

Table 5
Toxicological findings in hair of Polish opiate users

Information	Length of hair segment (cm)	Opioids (ng/mg)	Amphetamines (ng/mg)
(1) Patient: M.P., 21-year-old woman, "Polish heroin" abuser	S_1 (2)	6-MAM-0.20, M-1.54, C-0.20	–
	S_2 (2)	6-MAM-0.20, M-2.53, C-0.32	–
	S_3 (2)	M-1.66, C-0.22	–
	S_4 (2)	M-1.53, C-0.29	–
	S_5 (2)	M-1.50, C-0.20	–
	S_6 (2)	M-1.31, C-0.20	–
	S_7 (2)	M-1.62, C-0.20	–
	S_8 (2)	M-1.65, C-0.20	–
	S_9 (2)	M-0.97	–
	S_{10} (2)	M-0.96, C-0.20	–
	S_{11} (2)	M-1.14	–
	S_{12} (2)	M-1.12	–
	S_{13} (2)	M-1.24	–
	S_{14} (2)	M-1.28	–
	S_{15} (2)	M-1.14	–
	S_{16} (2)	M-1.30	–
(2) Patient: P.B.. 25-year-old man, "Polish heroin" and amphetamine abuser; carrier of HIV	S_1 (1.3)	M-0.68, C-0.20	A-1.86
	S_2 (1.3)	6-MAM-0.20; M-0.50; C-0.20	A-1.87
	S_3 (1.3)	M-0.47, C-0.20	A-2.65
(3) Patient: P.P., 21-year-old man, "Polish heroin" and amphetamine abuser	S_1 (1.5)	6-MAM-0.20, M-1.80,C-0.20	A-27.76
	S_2 (1.5)	6-MAM-0.20, M-1.07, C-0.20	A-18.49
	S_3 (1.5)	M-0.59, C-0.20	A-17.73
	S_4 (1.5)	6-MAM-0.20, M-0.60, C-0.20	A-15.87

S: segment; M: morphine; 6-MAM: 6-monoacethylmorphine; A: amphetamine; C: codeine.

quantified within the acceptable criteria of ±20% of target concentration. Negative samples were analyzed between samples of increasing analyte concentration. No detectable carry over occurred for 50 ng/mg opioids, amphetamines and cocainics samples.

The precision and accuracy of the method were evaluated at two concentrations (low and high). Data for both intra-assay ($n = 10$) and inter-assay ($n = 34$) are presented in Tables 2–4. The intra-assay accuracy and precision (%R.S.D.) ranged from −15.00 to 6.80 and from 6.94 to 15.67, respectively. The inter-assay accuracy and precision ranged from −11.66 to 6.67 and from 5.17 to 10.91, respectively.

3.2. Application for segmental hair analysis of drug abusers

Using the analytical procedure, determinations were made of the level of particular opioids and concomitantly taken phar-

Table 6
Toxicological findings in hair of opiate users from Western European countries (foreigners)

Information	Hair segment 1 cm, concentration (ng/mg)		
	Opioids	Amphetamines	Cocainics
(1) T.C., 25-year-old woman; opioids, amphetamines and cocainics abuser	6-MAM-1.45 M-0.20 C-0.20 6-MAM/M-7.2	A-0.40 MA-4.39 MDA-0.20 MDMA-14.07	Coc-8.15 B-5.15 CE-0.05
(2) B.C., 30-year-old man, cocainics abuser	6-MAM-0.20	–	Coc-111.5 B-37.61 CE-0.06
(3) D.F., 34-year-old man, opioids and cocainics abuser	6-MAM-6.09 M-0.82 C-0.24 6-MAM/M-7.4	–	Coc-4.80 B-4.98
(4) P.P., 33-year-old man, opioids and cocainics abuser	6-MAM-8.00 M-1.61 C-0.32 6-MAM/M-4.9	–	Coc-2.91 B-5.83

6-MAM: 6-monoacethylmorphine; M: morphine; C: codeine; A: amphetamine; MA: methamphetamine: MDA: 3,4-methylendioxyamphetamine; MDMA: 3,4-methylendioxymethamphetamine; Coc: cocaine; B: Benzoylecgonine; CE: cocaethylene.

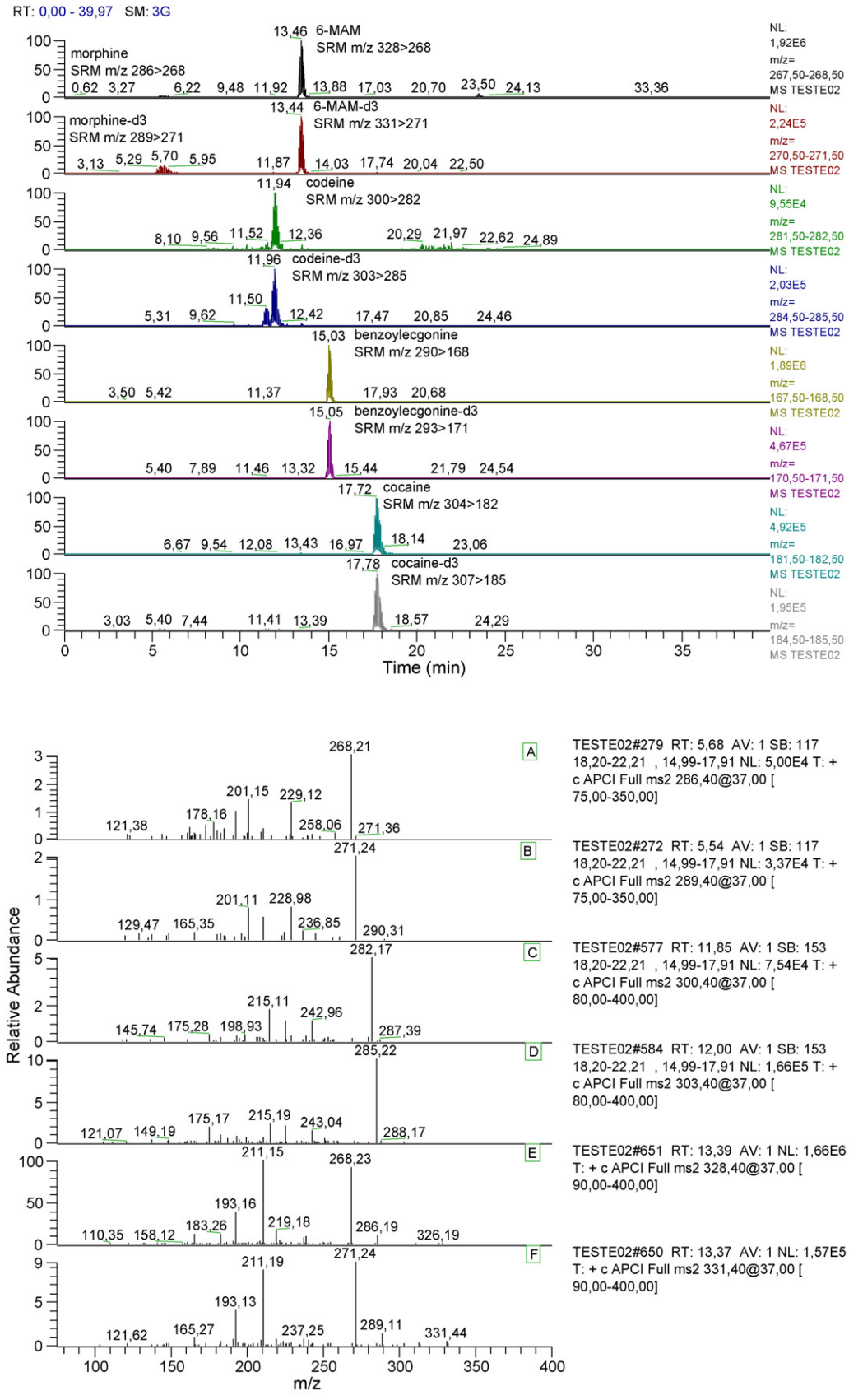

Fig. 1. Mass chromatograms and mass spectra of hair sample taken from Western European drug abuser (no. 4 in Table 6) contained morphine and 6-MAM, morphine-d_3 and 6-MAM-d_3, codeine, codeine-d_3, benzoylecgonine, benzoylecgonine-d_3, cocaine, cocaine-d_3, respectively.

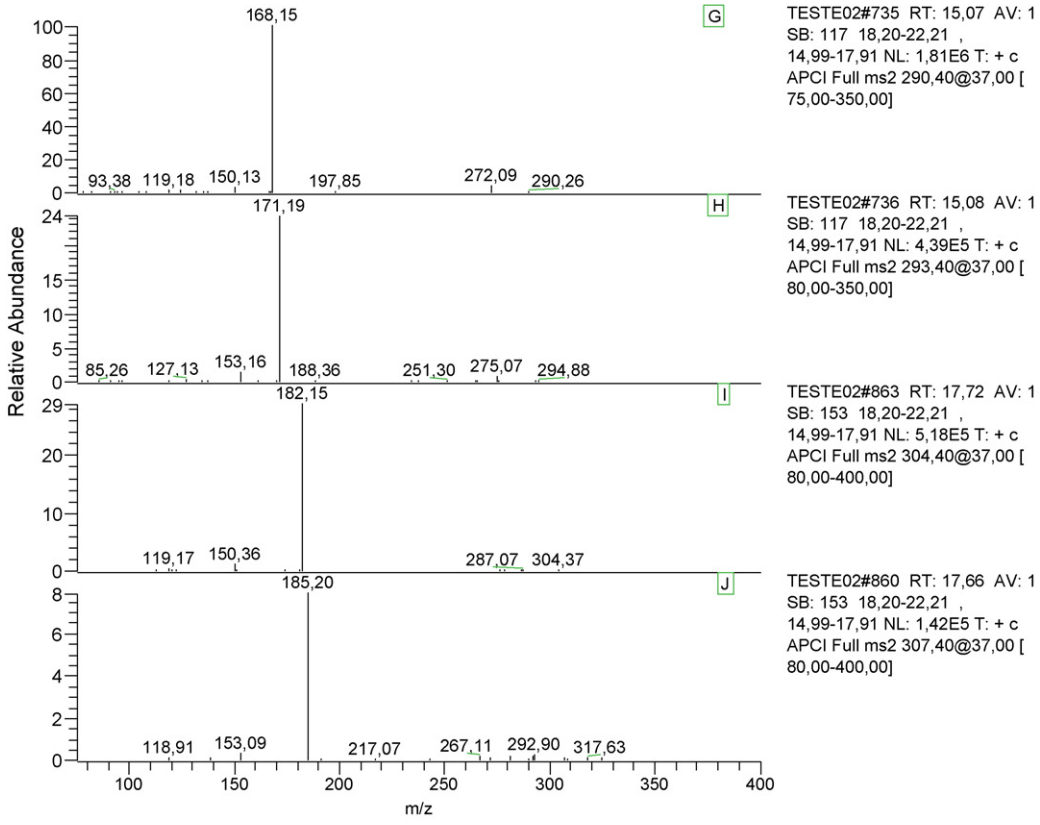

Fig. 1. (*Continued*).

maceuticals in hair samples originating from seven opiate users divided into two groups.

Group 1 consisted of three selected Polish patients who had been opiates addicts for 3–12 years. The data are presented in Table 5. The analysis indicated that the investigated subjects had been taking opium preparations characterized by their low heroin content, that is, the "Polish heroin", often in combination with amphetamine.

Group 2 consisted of four Western European drug users taking "Western heroin". The results obtained from these subjects are presented in Table 6.

In all the four cases, opiates were accompanied by cocaine and its main metabolite, benzoyloecgonine. In two subjects, cocaethylene was also detected, which pointed to a simultaneous partaking of cocaine and alcohol. Only in one instance was the presence of amphetamine and its derivatives (MA, MDMA, MDA) determined.

The graphical documentation of the analytical procedure is presented in Fig. 1 which illustrates mass chromatograms and mass spectra of drugs revealed in the hair of a Western European drug abuser (no. 4 in Table 6).

4. Discussion

Opiates are very dangerous in view of their high potential of leading to dependence. In this context, determinations of mind-altering substance levels in body fluids of drug users is fully justified in view of both clinical issues related to emergency services in cases of poisoning as well as in legal problems associated with various crimes, including traffic accidents caused by drivers under the influence of narcotic substances.

Regular urinalysis is the main method of monitoring detoxification and therapeutic programs. The point is, however, that within such systems there is a potential possibility of falsifying samples or using narcotic substances only in a specified period before the test. Hair analysis basically eliminates such attempts [16–20].

Applying LC–APCI-MS-MS method and using an ion trap allowed the present investigators to screen 11 illegal and therapeutic drugs, including opioids (morphine, codeine, 6-MAM,), amphetamine derivatives (amphetamine, methamphetamine, MDA, MDMA, MDEA) and cocainics (cocaine, benzoyloecgonine and cocaethylene) in human hair samples. A satisfactory chromatographic separation of the investigated drugs of abuse was achieved, which made it possible to monitor identical fragmented ions- 268 produced in the course of disintegration of a morphine and 6-MAM, while for MDA, MDMA and MDEA product ion 163 was observed. However, the extracts obtained using the above isolation method were characterized by a rich biological matrix, the effect of which was attenuated using the MS-MS option in the quadruple ion trap detector LCQ. The use of such a mode of mass spectrometer operations allowed for achieving concentration levels of the analyzed drugs that are recommended by the Society of Hair Testing LOQ [21].

Numerous authors have suggested that proof of an individual's using the "compote" or extract from poppy straw could be

found in the presence of other opium alkaloids, such as tebaine, noscapine or papaverine, as well as the ratio of morphine and codeine concentration levels in body fluid samples collected from a drug user. In turn, in heroin detection, numerous investigators [22–25] pointed to an acetylated morphine derivative, i.e. 6-MAM, as an indicator of heroin exposure, also in view of the fact that the above derivative was not isolated from poppies [24,25]. However, the disadvantage of employing 6-MAM as a biomarker of exposure to heroin may be found in the low content of this metabolite in biological materials originating from drug users, as well as a relatively short half-life, which is 5 min for heroin and 30–45 min for 6-MAM. These particular properties of heroin and 6-MAM impose the conditions under which the analysis must be carried out and the results interpreted.

Heroin may be used for both smoking and injections. The term "heroin" should only be used as a name for the final product mixed with any adulterants, which may be present. Two samples of heroin may have an identical appearance, since heroin is manufactured in a batch process. In any event, depending on its geographical origin (Eastern Asia, Western Asia) and the employed chemical procedure while manufacturing the final product, it may contain numerous other xenobiotics as additives (quinine, methaqualone, barbital, caffeine, diacetylmorphine hydrochloride, acetyl codeine, 6-MAM [26].

Morphine and 6-MAM, as compounds of lower polarity, may easily penetrate hair and when detected in hair samples, prove the usage of opium alkaloids. The presence of 6-MAM in hair confirms heroin usage, since – as a substance that is not present in poppy products – it cannot be taken this way.

To demonstrate or rule out heroin usage, the main detected substance in hair analysis is 6-MAM, followed by morphine and heroin. The ratio of 6-MAM to morphine concentration levels amounting to >1.3 [26,27] indicates the use of pure heroin. When we relate the above information to investigations presented in our report, we may demonstrate differences in the abuse profile in the investigated drug users.

6-MAM has been detected in numerous hair samples originating from Polish subjects. The metabolite has been also confirmed in all the four individuals from Western European countries. What is interesting here is the fact that in the latter samples, the level of 6-MAM was significantly higher and the 6-MAM/morphine ratio exceeded 7. Although it is difficult to pass any judgement on the correlation between the dose and hair level; nevertheless, the clear differences in 6-MAM concentration values in the Polish and foreign drug users suggest that the latter take pure heroin ("Western heroin") and the Polish take the poppy compote ("Polish heroin") characterized by its variable and low heroin content.

An additional argument for a different abuse profile in the Polish and foreign drug users is found in the presence of cocaine detected in hair samples originating from the latter. Cocaine is much less frequently detected in Polish drug users. Such a model of drug dependence is observed in countries situated at various latitudes, including the United States [28] or Western Europe [4,6,18,19].

Cocaine, as a mother substance, is rarely detected in urine, but it is the main component found in hair, following the use of the narcotic. On the other hand, the highest concentrations of its metabolite, benzoyloecgonine, is present in blood and urine, and – as a result of cocaine hydrolysis – can be detected in small amounts in hair matrix [16,17]. Similarly, as in the case of cocaethylene that is formed in consequence of a simultaneous use of cocaine and alcohol and whose trace amounts have been detected in Western drug users, the presence of benzoyloecgonine confirms that alcohol was taken together with cocaine.

In Polish users of mind-altering drugs, opiates are very often taken with amphetamine and/or its derivatives. This conclusion confirms an observation taken from clinical and medico- legal practice.

To conclude, it should be stressed that widespread opinions holding that the "Polish heroin" as a narcotic is slowly becoming a thing of the past to be replaced by a new-generation drugs of abuse, such as amphetamines and hallucinogens are not warranted [4,5,6]. The problem is still pertinent and it has to be taken into consideration.

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