ELSEVIER

Forensic Science International 125 (2002) 120–126

Forensic Science International

www.elsevier.com/locate/forsciint

Quantitative determination of amphetamines, cocaine, and opiates in human hair by gas chromatography/mass spectrometry

L. Skender[a,*], V. Karačić[a], I. Brčić[a], A. Bagarić[b]

[a]*Institute for Medical Research and Occupational Health, Clinical Toxicological Chemistry Unit, Ksaverska c.2, P.O. Box 291, 10000 Zagreb, Croatia*

[b]*Psychiatric Hospital Vrapče, Center for Prevention of Drug Abuse, Bolnička c. 32, 10000 Zagreb, Croatia*

Received 16 March 2001; received in revised form 24 October 2001; accepted 2 November 2001

Abstract

Hair of young subjects ($N = 36$) suspected for drug abuse was analysed for morphine, codeine, heroin, 6-acetylmorphine, cocaine, methadone, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA). The analysis of morphine, codeine, heroin, 6-acetylmorphine, cocaine, and methadone in hair included incubation in methanol, solid-phase extraction, derivatisation by the mixture of propionic acid anhydride and pyridine, and gas chromatography/mass spectrometry (GC/MS). For amphetamine, methamphetamine, MDA, MDMA, and MDEA analysis, hair samples were incubated in 1 M sodium hydroxide, extracted with ethyl acetate, derivatised with heptafluorobutyric acid anhydride (HFBA), and assayed by GC/MS. The methods were reproducible (R.S.D. = 5.0–16.1%), accurate (85.1–100.6%), and sensitive (LoD = 0.05–0.30 ng/mg). The applied methods confirmed consumption of heroin in 18 subjects based on positive 6-acetylmorphine. Among these 18 heroin consumers, methadone was found in four, MDMA in two, and cocaine in two subjects. Cocaine only was present in two, methadone only in two, methamphetamine only in two, and MDMA only in seven of the 36 subjects. In two out of nine coloured and bleached hair samples, no drug was found. Despite the small number of subjects, this study has been able to indicate the trend in drug abuse among young people in Croatia.

Keywords: Quantitative determination; Human hair analysis; Opiates; Cocaine; Amphetamines; Croatian experience

1. Introduction

Hair analysis is a reliable tool for proving or excluding chronic drug abuse [1–4]. As biological matrix, hair gives particular advantages; it can be easily obtained without violating individual privacy, it is not easily adulterated, and it can be stored and transported without specific precautions thanks to its stability. Aware of the disadvantages of urine as a biological specimen for drugs of abuse [5,6] and of the advantages of hair, we extended our routine urine analysis [7] to hair analysis. Most papers on hair analysis deal with cocaine, followed by opiates, and amphetamines [8]. This paper describes how we used gas chromatography/mass spectrometry (GC/MS) to determine morphine, codeine, heroin, 6-acetylmorphine, cocaine, methadone, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy), and 3,4-methylenedioxyethylamphetamine (MDEA) in hair.

2. Materials and methods

2.1. Reagents and standards

All solvents and chemicals were of the analytical grade. Morphine sulphate, codeine, heroin hydrochloride, methadone hydrochloride, cocaine hydrochloride, and methaqualone hydrochloride were obtained from Sigma (St. Louis, MO, USA), 6-acetylmorphine, amphetamine, methamphetamine, MDA, MDMA, and MDEA from Radian International

* Corresponding author. Fax: +385-41-4673303.
E-mail address: ljskender@imi.hr (L. Skender).

0379-0738/02/$ – see front matter
PII: S0379-0738(01)00630-2

LLC (Austin, TX, USA), propionic acid anhydride and heptafluorobutyric acid anhydride (HFBA) from Fluka AG (Buchs SG, Switzerland), pyridine from Kemika (Zagreb, Croatia), and Bond Elut Certify columns (10 ml, 130 mg) from Varian (Harbor City, CA, USA).

2.2. Hair samples

Hair samples were collected from 36 young people ranging from 16 to 22 years of age. For most of them, screening tests were positive for drug of abuse. In Croatia, analyses of drug of abuse in hair are performed only at our institute and the subjects were sent from regional centers for prevention of drug abuse. A strand of hair of about 5 mm in diameter was cut from close to the scalp at the vertex posterior area, folded in aluminium foil, and the proximal and distal ends marked. We analysed samples 2–4 cm long.

2.3. Hair analysis

The hair was washed twice in dichloromethane for 2 min at room temperature. Then it was dried, cut into very small pieces of less than 1 mm, and 50 mg was used for analysis. It was not possible to analyse for all drugs simultaneously.

2.3.1. Morphine, codeine, heroin, 6-acetylmorphine, methadone and cocaine

Methanol (2 ml) as an extraction solvent and 200 ng of methaqualone in methanol solution of 2 μg/ml as internal standard (IS) were added to 50 mg of hair in a 10 ml screw-cap tube. The samples were incubated for 18 h in a 40 °C water bath. The methanol was then collected, the remaining hair was rinsed with 0.5 ml methanol, and both fractions were evaporated to dryness at 40 °C under a stream of nitrogen.

2.3.1.1. Clean-up procedure and derivatisation. Solid-phase extraction was used to purify hair extracts prior to analysis. Bond Elut Certify columns were conditioned with 2 ml of methanol and 2 ml of a 0.1 M phosphate buffer at pH 6.0. After methanol evaporation, the dry residue of the hair extract was added to 2 ml of 0.1 M phosphate buffer at pH 6.0 and was poured into the conditioned columns. The sample passed very slowly through the column. Then, 2 ml of deionised water, 1 ml of 0.1 M acetic acid, and 2 ml of methanol were added in that sequence. The cartridges were dried under full vacuum for 5 min and eluted with a 1 ml (3×) mixture of dichloromethane:2-propanol:ammonium hydroxide (80:20:2, v/v/v). The eluents were collected in glass tubes and evaporated to dryness at 40 °C under a stream of nitrogen. One-hundred microliters of pyridine and 30 μl of propionic acid anhydride were added to the residues and the tubes were capped, vortexed, and heated at 60 °C for 30 min. Followed evaporation to dryness, reconstitution in 100 μl of ethyl acetate, and GC/MS analysis.

2.3.2. Amphetamine, methamphetamine, MDA, MDMA, and MDEA

One milliliter of 1 M sodium hydroxide was added to every 50 mg hair sample. The samples were hydrolysed for 20 min at 70 °C and cooled. Followed extraction with 1 ml (2×) of ethyl acetate and evaporation to dryness in the presence of a 100 μl mixture of methanol:hydrochloric acid (99:1, v/v). Fifty microliters of ethyl acetate and 50 μl of HFBA were added to the dry residues and the tubes were capped, vortexed, and heated at 60 °C for 30 min. Followed evaporation to dryness, reconstitution in 100 μl ethyl acetate, and GC/MS analysis.

Each batch of samples (A, B) included standards for drug abuse/metabolites, negative control, and genuine positive sample.

2.4. Standard preparation

Stock solutions containing 2 μg/ml of (A) morphine sulphate, codeine, heroin hydrochloride, 6-acetylmorphine, methadone hydrochloride, and cocaine hydrochloride and (B) amphetamine, methamphetamine, MDA, MDMA, and MDEA, were prepared in methanol and stored at −20 °C. Standard calibration curves were obtained through the described methods using 20, 50, 100, 200, 400, and 800 ng of the stock solution (A) or (B), 200 ng of methaqualone as IS (only for A), and 50 mg of blank control hair, previously washed and cut into very small pieces. Blank control hair samples were obtained from coworkers in our laboratory.

Limit of detection (LoD) for the analyte was determined by decreasing concentrations of spiked samples until the response equalled signal-to-noise ratio (S/N) of 3.

2.5. GC/MS analysis

The analysis was performed using a Varian 3400 CX GC with Saturn ion trap mass spectrometer (mass selective detector, MSD). The chromatographic column was RTX-5

Table 1
Analytical parameters of the applied procedures ($n = 8$)

Analyte	Precision (%)	Accuracy (%)	LoD (ng/mg)
Methadone	6.9	87.5	0.30
Cocaine	16.1	96.3	0.20
Codeine	8.2	100.6	0.10
Heroin	12.6	91.3	0.20
6-Acetylmorphine	6.5	98.8	0.05
Morphine	6.3	99.4	0.10
Amphetamine	7.8	88.0	0.20
Methamphetamine	5.2	97.2	0.05
MDA	8.3	85.1	0.20
MDMA	5.0	90.6	0.10
MDEA	7.0	86.3	0.15

(5% diphenyl-95% dimethyl polysiloxane, 30 m 0.25 mm i.d.). For the analysis of morphine, codeine, heroin, 6-acetylmorphine, methadone, and cocaine (A), the initial column temperature of 50 °C was held for 1 min, then programmed to 300 °C at 50 °C/min and held for 6 min. For the analysis of amphetamine, methamphetamine, MDA, MDMA, and MDEA (B), the initial column temperature of 50 °C was held for 1 min, then programmed to 225 °C at 20 °C/min and held for 1 min, then programmed to 260 °C at 50 °C/min and held for 1 min. Ultra-pure grade helium was used as the carrier gas at a flow rate of about 1ml/min. Septum-equipped programmable injector (SPI) was used;

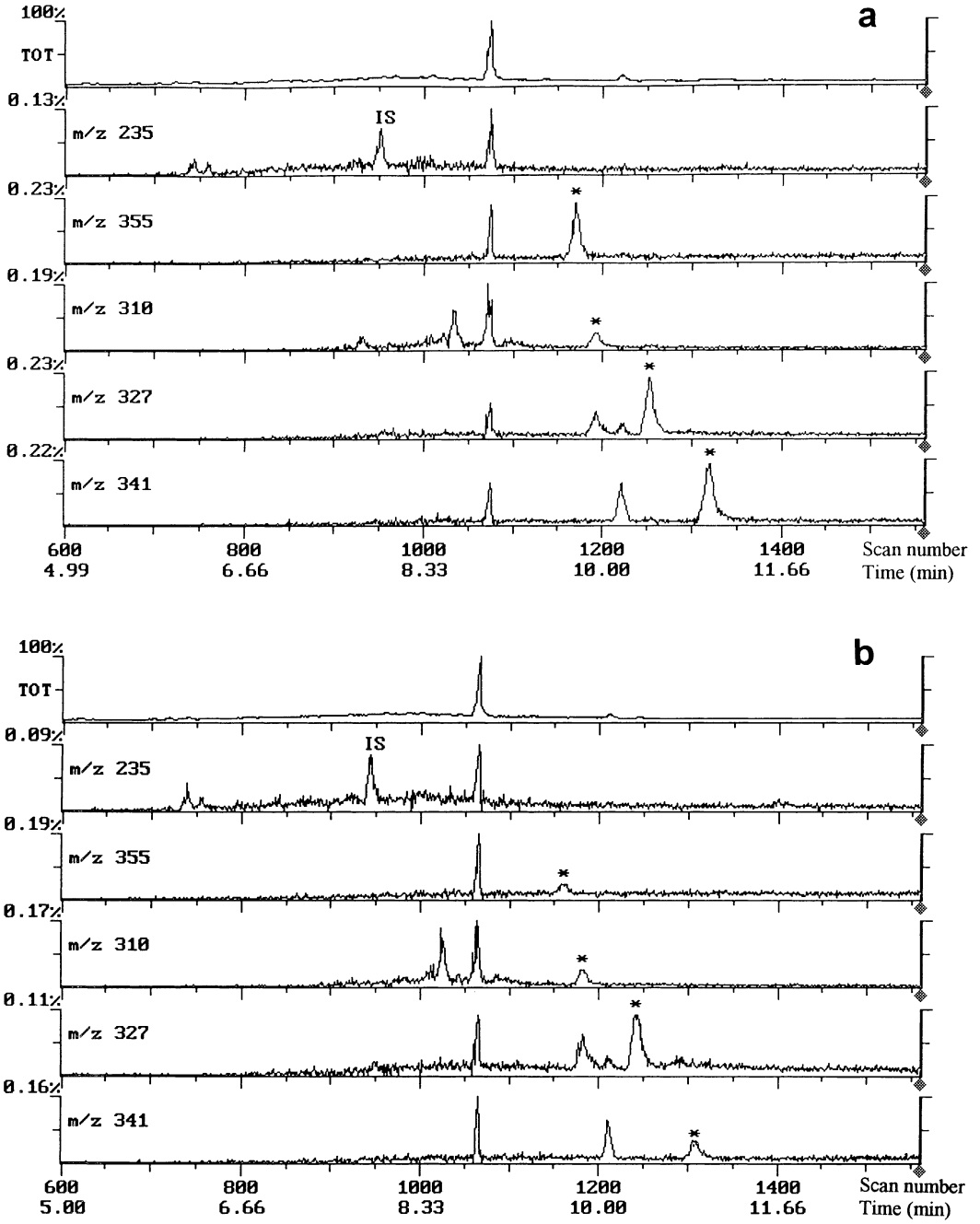

Fig. 1. (a) Selected ion chromatograms of methaqualone (IS, *m/z* 235), codeine propionyl (*m/z* 355), heroin (*m/z* 310), 6-acetylmorphine propionyl (*m/z* 327), and morphine dipropionyl (*m/z* 341) in control hair fortified with 4 ng/mg of each analyte; (b) selected ion chromatograms of heroin abuser's hair: codeine, 0.56 ng/mg; heroin, 1.85 ng/mg; 6-acetylmorphine, 2.52 ng/mg; morphine, 1.10 ng/mg.

the initial temperature of 40 °C was held for 0.1 min, then programmed to 280 °C at 200 °C/min and held for 8 min. The transfer line temperature was 260 °C.

The MSD was operated in the selected ion-monitoring mode. Analytes (A) were identified and quantitated using a comparison with the retention times and relative abundance of three confirming ions to methaqualone (IS). The external standard method of quantitation was used for amphetamines (B). Quantitative calculations were automated by Saturn® software. For each drug the following ions were used: methadone, *m/z* 72, 309, 165; cocaine, *m/z* 182, 82, 303; codeine propionyl, *m/z* 355, 282, 341; heroin, *m/z* 310, 327,

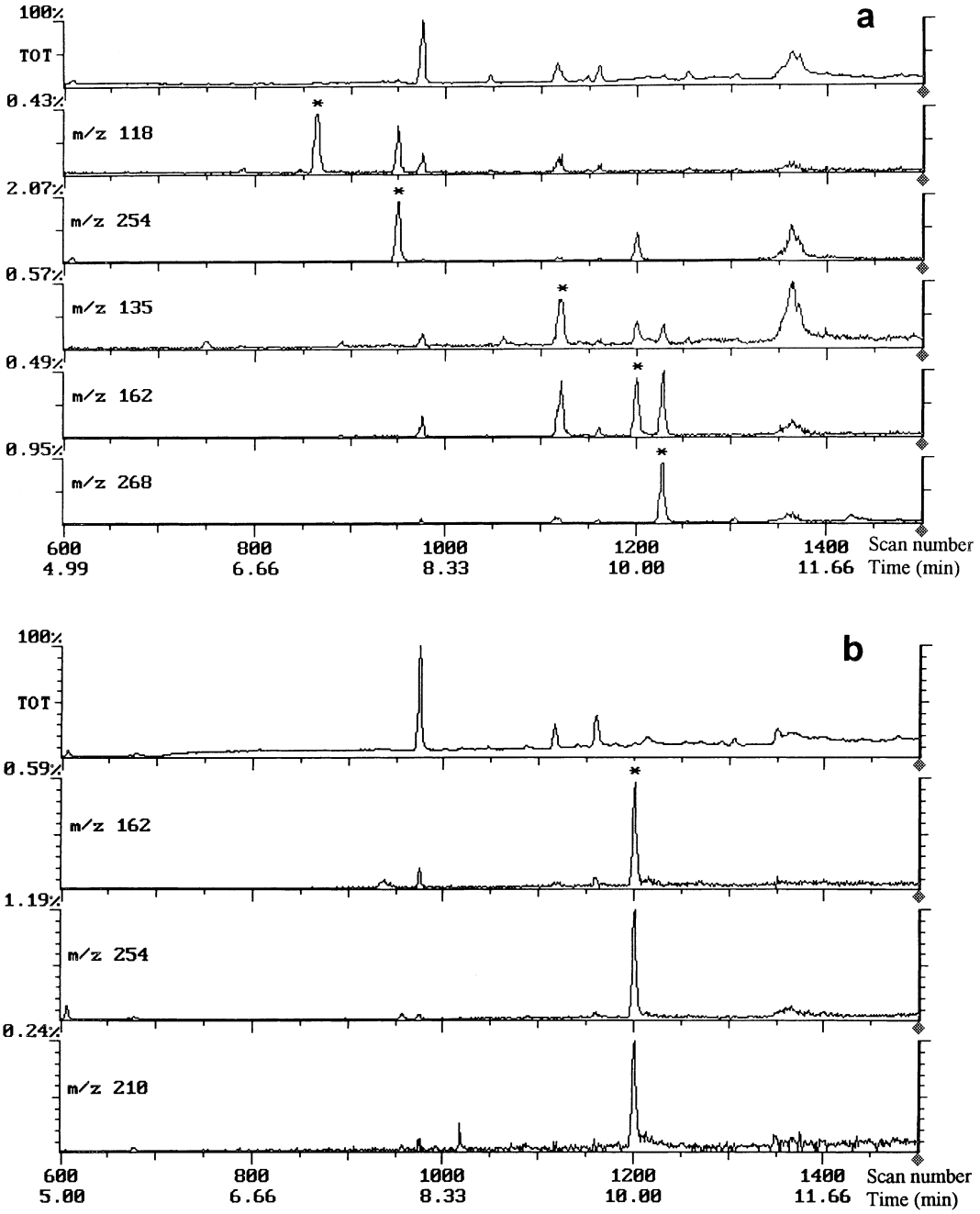

Fig. 2. (a) Selected ion chromatograms of amphetamine HFBA (*m/z* 118), methamphetamine HFBA (*m/z* 254), MDA HFBA (*m/z* 135), MDMA HFBA (*m/z* 162), and MDEA HFBA (*m/z* 268) in control hair fortified with 4 ng/mg of each analyte; (b) selected ion chromatograms of MDMA abuser's hair (MDMA 7.53 ng/mg).

369; 6-acetylmorphine propionyl, *m/z* 327, 268, 383; morphine dipropionyl, *m/z* 341, 268, 397; and amphetamine HFBA, *m/z* 118, 240, 91; methamphetamine HFBA, *m/z* 254, 210, 118; MDA HFBA, *m/z* 135, 162, 240; MDMA HFBA, *m/z* 162, 254, 210; MDEA HFBA, *m/z* 268, 135, 162; and methaqualone, *m/z* 235, 250, 91. The underlined ions were used for quantitation.

3. Results and discussion

Table 1 shows the main parameters for quantitative validation of the methods. The precision ($n = 8$), expressed as relative standard deviation (R.S.D.), was <10% for all analytes except cocaine and heroin. The accuracy was >86% for all analytes except MDA. LoD ranged from 0.05 to 0.30 ng/mg. The correlation coefficients of the calibration curves were >0.997 for all analytes.

Possible conversions of heroin to 6-acetylmorphine and morphine, and 6-acetylmorphine to morphine during the extraction were checked by adding 200 ng of heroin or 6-acetylmorphine (three times each) to 50 mg of the blank sample and analyses were performed as described. Heroin hydrolysed up to 18% to 6-acetylmorphine and up to 1% to morphine, and 6-acetylmorphine up to 6% to morphine. Our results agree with the findings of Kintz et al. [9].

Table 2
Concentrations (ng/mg) of methadone, cocaine, codeine, heroin, 6-acetylmorphine, morphine, methamphetamine, MDA, and MDMA in the hair of drug abusers

Subject		Methadone	Cocaine	Codeine	Heroin	6-Acetylmorphine	Morphine	Methamphetamine	MDA	MDMA
No.	Sex									
1	M	ND[a]	ND	ND	ND	0.95	0.42	ND	ND	ND
2	M	1.64	ND	ND	ND	1.09	0.56	ND	ND	ND
3	M	ND	ND	ND	ND	ND	ND	0.36	ND	2.05
4	M	ND	ND	ND	ND	2.32	2.00	ND	ND	3.35
5	M	8.00	ND	ND	ND	ND	ND	ND	ND	ND
6	F[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	M	4.53	ND	ND	3.19	2.87	0.78	ND	ND	ND
8	M	ND	ND	0.56	1.85	2.52	1.10	ND	ND	ND
9	M	ND	ND	ND	ND	ND	ND	ND	ND	0.86
10	M	ND	ND	ND	ND	3.11	1.73	ND	ND	ND
11	F[b]	3.95	ND	ND	ND	1.13	1.33	ND	ND	ND
12	F[b]	ND	ND	ND	ND	ND	ND	ND	ND	0.97
13	M	ND	1.42	1.89	63.57	33.21	4.02	ND	ND	ND
14	M	ND	ND	ND	ND	2.13	0.72	ND	ND	ND
15	M	ND	ND	ND	ND	ND	ND	ND	1.06	4.28
16	F[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND
17	M[b]	ND	3.29	ND	ND	ND	ND	ND	ND	ND
18	M	10.59	ND	ND	ND	ND	ND	ND	ND	ND
19	M	ND	ND	ND	4.43	4.80	1.10	ND	ND	ND
20	M	ND	ND	ND	ND	ND	ND	ND	ND	2.76
21	F	ND	ND	ND	ND	ND	ND	ND	ND	1.79
22	F[b]	1.26	ND	ND	ND	3.41	ND	ND	ND	ND
23	M	ND	ND	ND	ND	ND	ND	0.63	ND	ND
24	M	ND	ND	ND	ND	ND	ND	0.30	ND	ND
25	M	ND	ND	ND	3.52	5.05	3.30	ND	ND	ND
26	M	ND	2.84	ND	ND	ND	ND	ND	ND	ND
27	M	ND	ND	ND	0.83	2.94	0.77	ND	ND	3.63
28	F[b]	ND	ND	1.10	ND	1.39	ND	ND	ND	ND
29	M	ND	ND	ND	ND	ND	ND	ND	ND	7.53
30	M	ND	1.74	0.52	4.96	4.59	2.02	ND	ND	ND
31	M	ND	ND	ND	ND	ND	ND	ND	ND	1.11
32	M	ND	ND	ND	ND	0.99	0.94	ND	ND	ND
33	M[b]	ND	ND	ND	ND	ND	ND	1.91	0.72	64.42
34	F[b]	ND	ND	0.68	ND	0.60	0.87	ND	ND	ND
35	M	ND	ND	ND	ND	ND	ND	ND	ND	0.56
36	M	ND	ND	ND	6.22	4.25	0.65	ND	ND	ND

[a] ND: not detected.
[b] Coloured and bleached hair.

There are many similar GC/MS methods, although most differ in the washing and extraction procedure. We chose to wash hair samples twice in dichloromethane because our own tests and findings of Kintz and Mangin [1] showed that the third wash was always negative, although the two previous washes were positive.

Methanol turns to be the solvent of choice for extracting heroin and 6-acetylmorphine [10,11], although acidic extraction [1,12] and enzymatic digestion [3,13] are also used for the extraction of the same substances from hair. Kintz and Cirimele [14] found that the best recoveries for amphetamine, MDA, and MDMA were observed after alkaline hydrolysis of hair.

A variety of derivatisation reagents are used in the analysis of drugs of abuse [15]. After examination of several derivatisation reagents, we found that a mixture of propionic acid anhydride and pyridine was very convenient and superior to *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) for derivatisation of codeine, 6-acetylmorphine and morphine. Namely, propionylation formed very stable derivatives (tested for more than 2 weeks without change) with good GC properties. Another advantage of propionylation over BSTFA is that it does not decrease the resolution power of the capillary column, as it evaporates before analysis. HFBA is often recommended for derivatisation of amphetamines [16].

Fig. 1 compares ion chromatograms of codeine propionyl, heroin, 6-acetylmorphine propionyl, and morphine dipropionyl in the subject's hair with the control hair. Fig. 2 compares ion chromatograms of MDMA HFBA in the subject's hair with the control hair.

Table 2 shows the results of hair analysis of drug abusers. Predominant analytes were 6-acetylmorphine (50%) and morphine (44.4%). The presence of 6-acetylmorphine, the only specific heroin metabolite, confirmed heroin consumption in most subjects, which is consistent with their statements. In 16 subjects, we found both 6-acetylmorphine and morphine. The 6-acetylmorphine/morphine ratio (mean: 2.84; median: 2.11; range: 0.69–8.26) was similar to that found by Moeller et al. [17] although individual concentrations were very different and the latter study involved far more subjects. Higher concentrations of 6-acetylmorphine than those of morphine are usual for consumers of heroin. Only rarely does the morphine concentration exceed the concentration of 6-acetylmorphine [8,9,17]; we recorded only two such cases among our subjects (No. 11 and 34) and they concerned coloured hairs. The morphine/6-acetylmorphine ratio (mean: 0.55; median:0.44; range: 0.15–1.45) is in accordance with results of Gaillard and Pepin [2]. Heroin was found in only eight hair samples, which is consistent with literature [10,18]. Methadone was present in six hair samples. Finding of codeine in only five hair samples is a surprisingly low rate having in mind the notorious impurity (associated with acetylcodeine which eventually metabolises to codeine) of heroin sold in the streets. However, the purity and amount of consumed heroin are always very questionable. Kintz et al. [9] found no codeine in subjects to whom heroin was administered in the controlled study and explained that result with high heroin purity. Cocaine was present in four hair samples and in two together with heroin. Of amphetamines predominant drug was MDMA, mostly alone. All women but one had coloured and bleached hair. In two samples no drug was found (subjects 6 and 16) and in two only 6-acetylmorphine was present without morphine (subjects 22 and 28). Several authors recently published results about a decreasing tendency of the hair drug content because of cosmetic treatment [19–22]. Bleaching, colouring, or permanent waving were found to affect the stability of incorporated drugs and to cause alterations of the fibres at an ultra-structural level. This may result in partial or complete loss of drug substances, depending on particular drug molecule and on its concentration prior to cosmetic treatment.

4. Conclusion

The methods we have been using in our laboratory for over 2 years have been validated and found acceptable for the analysis of drugs of abuse in hair. Despite uncertainties related to the analysis of died or otherwise treated hair, the methods were able to establish drug abuse. Furthermore, despite the small number of subjects, we believe that this study has been able to indicate the trend in drug abuse among young people in Croatia.

Acknowledgements

This study was supported by Ministry of Science and Technology of the Republic of Croatia through Project 00220303. The authors wish to thank Ms. Vesna Triva for the most skilful technical assistance.

References

[1] P. Kintz, P. Mangin, Simultaneous determination of opiates, cocaine and major metabolites of cocaine in human hair by gas chromatography/mass spectrometry (GC/MS), Forensic Sci. Int. 73 (1995) 93–100.

[2] Y. Gaillard, G. Pepin, Simultaneous solid-phase extraction on C_{18} cartridges of opiates and cocinics for an improved quantitation in human hair by GC–MS: one year of forensic applications, Forensic Sci. Int. 86 (1997) 49–59.

[3] K.M. Hold, D.G. Wilkins, D.E. Rollins, R.E. Joseph, E.J. Cone, Simultaneous quantitation of cocaine, opiates, and their metabolites in human hair by positive ion chemical ionization gas chromatography–mass spectrometry, J. Chromatogr. Sci. 36 (1998) 125–130.

[4] J. Segura, C. Stramesi, A. Redon, M. Ventura, C.J. Sanchez, G. Gonzales, L. San, M. Montagna, Immunological screening of drugs of abuse and gas chromatographic–mass spectrometric confirmation of opiates and cocaine in hair, J. Chromatogr. B 724 (1999) 9–21.

[5] M.P. Bosomworth, Drugs of abuse in urine: some pitfalls in testing, Annotation 50 (1993) 150–155.
[6] W.H. James, D.D. Moore, Adolescents and drug abuse: clinical use of urine drug screening, J. Child Adolesc. Abuse 7 (1997) 19–36.
[7] V. Karačić, L. Skender, Analysis of drugs in urine by gas chromatography/mass spectrometry: experience and application, Arh. Hig. Rada Toksikol. 51 (2000) 389–400.
[8] Y. Nakahara, Hair analysis for abused and therapeutic drugs, J. Chromatogr. B 733 (1999) 161–180.
[9] P. Kintz, P. Bundeli, R. Brenneisen, B. Ludes, Dose–concentration relationships in hair from subjects in a controlled heroin-maintenance program, J. Anal. Toxicol. 22 (1998) 231–236.
[10] E.J. Cone, W.D. Darwin, W.L. Wang, The occurrence of cocaine, heroin and metabolites in hair of drug abusers, Forensic Sci. Int. 63 (1993) 55–68.
[11] M. Rothe, F. Pragst, Solvent optimization for the direct extraction of opiates from hair samples, J. Anal. Toxicol. 19 (1995) 236–240.
[12] C. Jurado, M.P. Gimenez, M. Menendez, M. Repetto, Simultaneous quantitation of opiates, cocaine and cannabinoids in hair, Forensic Sci. Int. 70 (1995) 165–174.
[13] M.R. Moeller, P. Fey, R. Wennig, Simultaneous determination of drugs of abuse (opiates, cocaine and amphetamine) in human hair by GC/MS and its application to a methadone treatment program, Forensic Sci. Int. 63 (1993) 185–206.
[14] P. Kintz, V. Cirimele, Interlaboratory comparison of quantitative determination of amphetamine and related compounds in hair samples, Forensic Sci. Int. 84 (1997) 151–156.
[15] J. Segura, R. Ventura, C. Jurado, Derivatization procedures for gas chromatographic–mass spectrometric determination of xenobiotics in biological samples, with special attention to drugs of abuse and doping agents, J. Chromatogr. B 713 (1998) 61–90.
[16] E.M. Thurman, M.J. Pedersen, R.L. Stout, T. Martin, Distinguishing sympathomimetic amines from amphetamine and methamphetamine in urine by gas chromatography/mass spectrometry, J. Anal. Toxicol. 16 (1992) 19–27.
[17] M.R. Moeller, P. Fey, H. Sachs, Hair analysis as evidence in forensic cases, Forensic Sci. Int. 63 (1993) 43–53.
[18] B.A. Goldberger, Y.H. Caplan, T. Maguire, E.J. Cone, Testing human hair for drugs of abuse. III. Identification of heroin and 6-acetylmorphine as indicators of heroin use, J. Anal. Toxicol. 15 (1991) 226–231.
[19] V. Cirimele, P. Kintz, P. Mangin, Drug concentration in human hair after bleaching, J. Anal. Toxicol. 19 (1995) 331–332.
[20] L. Potsch, G. Skopp, Stability of opiates in hair fibers after exposure to cosmetic treatment, Forensic Sci. Int. 81 (1996) 95–102.
[21] C. Jurado, P. Kintz, M. Menendez, M. Repetto, Influence of cosmetic treatment on hair in drug testing, Int. J. Legal Med. 110 (1997) 159–163.
[22] G. Skopp, L. Potsch, M.R. Moeller, On cosmetically treated hair-aspects and pitfalls of interpretation, Forensic Sci. Int. 84 (1997) 43–52.