

Observation

## HAIR TESTING FOR DRUGS OF ABUSE: A TWO-YEAR EXPERIENCE

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This article gives a brief account of a two-year experience with gas chromatography/mass spectrometry methods developed for the analysis of opiates (morphine, codeine, heroin and 6-acetylmorphine), cocaine, methadone, and amphetamines (amphetamine; methamphetamine; 3,4-methylenedioxyamphetamine – MDA, and 3,4-methylenedioxymethamphetamine – MDMA, Ecstasy) in hair. The methods developed were reproducible (RSD=5.0–16.1%), accurate (85.1–100.6%) and sensitive (LD=0.05–0.30 ng/mg). They were applied in the analysis of 221 hair samples obtained from young subjects aged 15–25 years, who were suspected of drug abuse. Seventy-seven hair samples were found positive for drugs of abuse. Forty-two subjects were found to have consumed amphetamine, of whom 40 were found MDMA (Ecstasy). Heroin consumption, based on positive 6-acetylmorphine, was found in 26 subjects. Cocaine alone was present in three subjects, two were found cocaine and heroin, two cocaine and methadone, and one cocaine and MDMA. These results could indicate the trend in drug abuse among young people in Croatia.

**KEY WORDS:** *amphetamines, cocaine, gas chromatography/mass spectrometry, methadone, opiates, quantitative determination*

The 1990s have seen an increase in illicit drug consumption in Croatia. A number of rapid screening assays and quantitative GC/MS methods have been developed for the determination of drugs in urine (1). However, urine sampling time is critical because all drugs, with the exception of marihuana, can be detected in urine only within 2–3 days after the use (2). Urine testing provides a reliable answer for recent intake. These limitations urged for a biological marker which could yield a cumulative reflection of a long-term drug abuse.

Hair analysis is a reliable tool for confirming or excluding chronic drug abuse (3–6). As hair grows approximately 1 cm a month, the history of drug abuse can be traced months back, depending on the length of the hair.

As a biological matrix, hair has particular

advantages: it can easily be obtained, it is not readily adulterated and, thanks to its stability, it can be stored and transported without particular precaution. Cocaine is the leading subjects in literature on hair analysis, and is followed by opiates and amphetamines (7). This paper describes gas chromatographic/mass spectrometric (GC/MS) methods developed for the determination of opiates (morphine, codeine, heroin and 6-acetylmorphine), cocaine, methadone and amphetamines (amphetamine; methamphetamine; 3,4-methylenedioxyamphetamine, MDA; and 3,4-methylenedioxymethamphetamine, MDMA, “Ecstasy”) in hair (8). The methods had been applied for over two years in the analysis of hair of young subjects suspected for drug abuse. In addition, the paper gives an evaluation of the incidence of drug abuse in young people in Croatia.

## MATERIALS AND METHODS

### *Reagents and standards*

All solvents and chemicals were of the analytical grade. Morphine sulphate, codeine, heroin hydrochloride, methadone hydrochloride, and cocaine hydrochloride were obtained from Sigma Chemical (St. Louis, MO, USA), 6-acetylmorphine, amphetamine, methamphetamine, MDA and MDMA from Radian International LLC (Austin, TX, USA), propionic acid anhydride and heptafluorobutyric 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).

### *Hair samples*

Hair samples were collected from 221 young people aged between 15 and 25 years. As the Institute for Medical Research and Occupational Health is the only institution in Croatia with a laboratory equipped for the analysis of drugs of abuse in hair, the subjects were either sent by the regional drug abuse prevention centres or came on their own, most often with their parents. A tress of hair of about 5 mm in diameter was cut as close to the rear top of the scalp as possible, folded in aluminium foil, and the proximal and the distal ends were marked. The samples were stored under dry conditions at room temperature until analysis. We analysed samples 2–4 cm long, which corresponds to the timeline of approximately 2–4 months.

### *Hair analysis*

GC/MS methods for detecting drug in hair mostly differ between each other in the washing and extraction procedure. We decided to wash hair samples in dichloromethane twice, because our own tests showed that the third wash was always negative, although the two previous washes were positive. After washing, the hair was dried and cut into very small pieces of less than 1 mm. We analysed 50 mg of each sample.

Methanol was chosen for extracting opiates and cocaine. The best recoveries for amphetamines were obtained after alkaline hydrolysis of hair.

A variety of derivatisation reagents are used in the analysis of drugs of abuse. We found that a mixture of propionic acid anhydride and pyridine was very convenient and superior to N,O-bis(trimethylsilyl)

trifluoroacetamide for the derivatisation of codeine, 6-acetylmorphine, and morphine. Heptafluorobutyric anhydride is recommended for the derivatisation of amphetamines.

It was not possible to analyse all drugs using the same procedure. They were divided in groups A and B, and each was analysed separately.

### *GROUP A: MORPHINE, CODEINE, HEROIN, 6-ACETYLMORPHINE, METHADONE AND COCAINE*

Methanol was added to 50 mg of hair in a 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 methanol, and both fractions were evaporated to dryness at 40 °C under a stream of nitrogen.

### *Clean-up procedure and derivatisation*

Solid-phase extraction was used to purify hair extracts prior to analysis. Bond Elut Certify columns were conditioned with methanol and 0.1 M phosphate buffer at pH=6.0. After methanol evaporation, the dry hair residue was added 0.1 M phosphate buffer at pH=6.0 and was poured into conditioned columns. Deionised water, 0.1 M acetic acid, and methanol were then added in that order. The cartridges were dried under full vacuum and eluted with 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 microlitres of pyridine and 30 µl of propionic acid anhydride were added to the residues and heated at 60 °C for 30 min. Followed evaporation to dryness, reconstitution in ethyl acetate, and GC/MS analysis.

### *GROUP B: AMPHETAMINES*

Every 50 mg hair sample received 1 M sodium hydroxide. The samples were hydrolysed for 20 min at 70 °C and then cooled. Followed extraction with ethyl acetate and evaporation to dryness in the presence of a mixture of methanol:hydrochloric acid (99:1, v/v). Fifty microlitres of ethyl acetate and 50 µl of HFBA were added to the dry residue and heated at 60 °C for 30 min. Followed evaporation to dryness, reconstitution in ethyl acetate, and GC/MS analysis.

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

### 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, and MDMA were prepared in methanol and stored at -20 °C. Standard calibration curves were obtained through the described method using 20, 50, 100, 200, 400 and 800 ng of the stock solution A or B, and 50 mg of blank control hair, previously washed and cut into very small pieces. Blank control hair samples were obtained from co-workers in our laboratory.

### 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 (5% diphenyl-95% dimethyl polysiloxane, 30 m, 0.25 mm i.d, with a film thick 0.25 µm). For the analysis of the group A of drugs (morphine, codeine, heroin, 6-acetylmorphine, methadone and cocaine), 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 the group B of drugs (amphetamine, methamphetamine, MDA, and MDMA), the initial column temperature of 50 °C was held for 1 min, then programmed to 225 °C at 20 °C/min, and then 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 1 ml/min. Septum-equipped Programmable Injector (SPI) was used; the initial temperature was 40 °C, held for 0.1 min, programmed to 280 °C at 200 °C/min and held for 8 min. The transfer line temperature was 260 °C.

The external standard method of quantitation was used for the analytes in both groups. For each analyte 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, 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, and MDMA-HFBA, m/z 162, 254, 210. The underlined ions were used for quantitation.

## RESULTS AND DISCUSSION

Figure 1 shows the total and selected ion chromatograms of amphetamine-HFBA, methamphetamine-HFBA, MDA-HFBA and MDMA-HFBA in control hair fortified with 4 ng/mg of each analyte (a) and in the hair of amphetamine abusers (amphetamine 2.78; MDA 0.91; MDMA 11.24 ng/mg) (b).

Figure 2 shows the total and selected ion chromatograms of cocaine, codeine-propionyl, heroin, 6-acetylmorphine-propionyl and morphine-dipropionyl in control hair fortified with 4 ng/mg of each analyte(a), and in drug abusers' hair (cocaine 5.99; codeine 2.51; heroin 5.58; 6-acetylmorphine 9.57; morphine 6.52 ng/mg) (b).

The main parameters for the quantitative validation of the developed methods are shown in Table 1. The precision (N=8), expressed as relative standard deviation (RSD), was <10% for all analytes except cocaine and heroin. The accuracy was >86% for all analytes except MDA. The limit of detection (LD) ranged from 0.05 to 0.30 ng/mg. The correlation coefficients of the calibration curves were >0.997 for all analytes. External quality assessment was verified through participation in the programme Proficiency Test on Drugs of Abuse in Hair, organized by the Society of Hair Testing, Munich, Germany.

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

Analyte	Precision (RSD) %	Accuracy %	Limit of detection 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

The described methods were applied in the analysis of 221 hair samples taken from young subjects (15–25 years of age) suspected of drug abuse. Seventy seven samples (women N=17, men N=60) were found positive for drug of abuse, and 144 were negative.

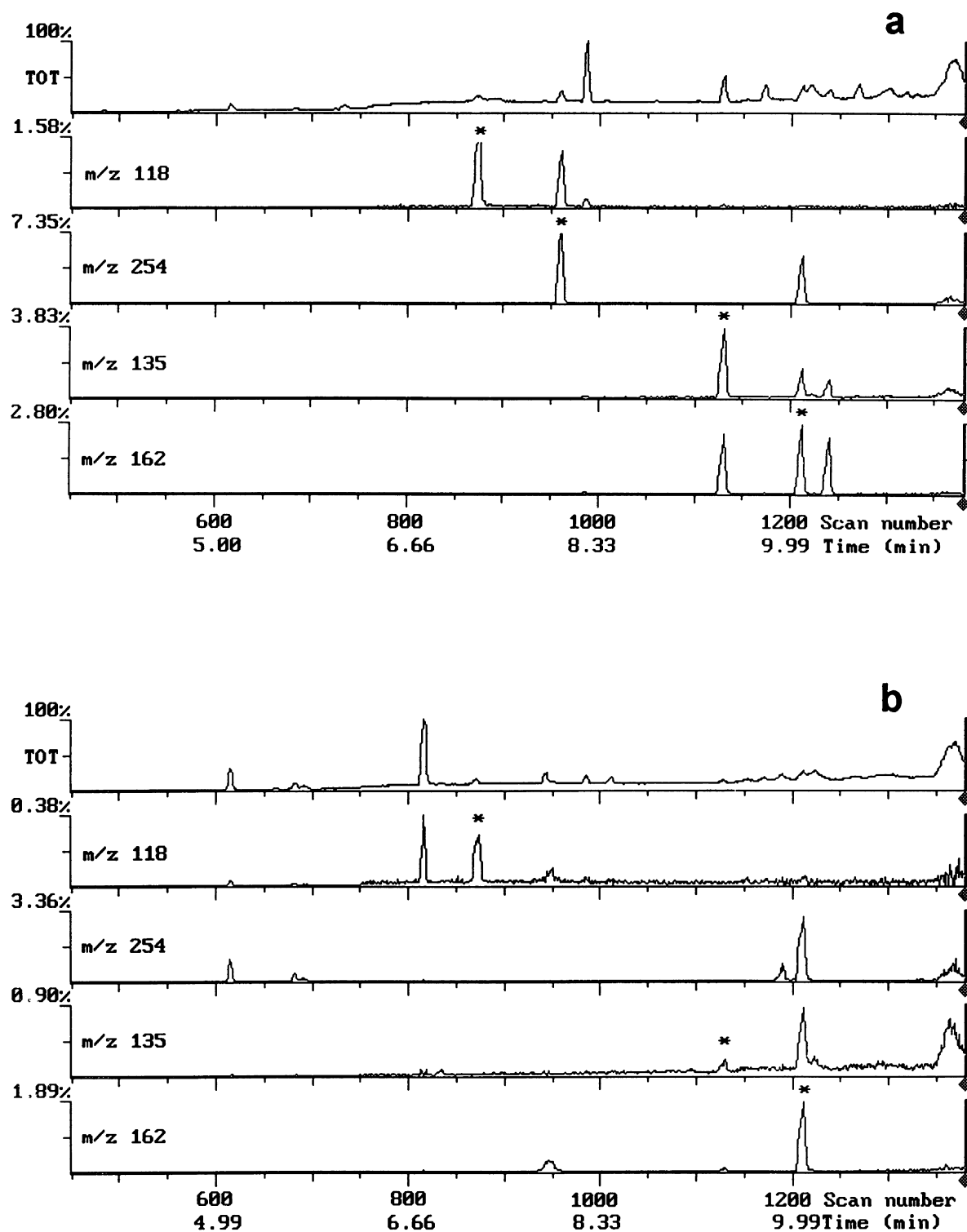


Figure 1

a) Total and selected ion chromatograms of amphetamine-HFBA ( $m/z$  118), methamphetamine-HFBA ( $m/z$  254), MDA-HFBA ( $m/z$  135), and MDMA-HFBA ( $m/z$  162) in control hair fortified with 4 ng/ml of each analyte.

b) Total and selected ion chromatograms of amphetamine abusers' hair: amphetamine 2.78 ; MDA 0.91; MDMA 11.24 ng/mg.

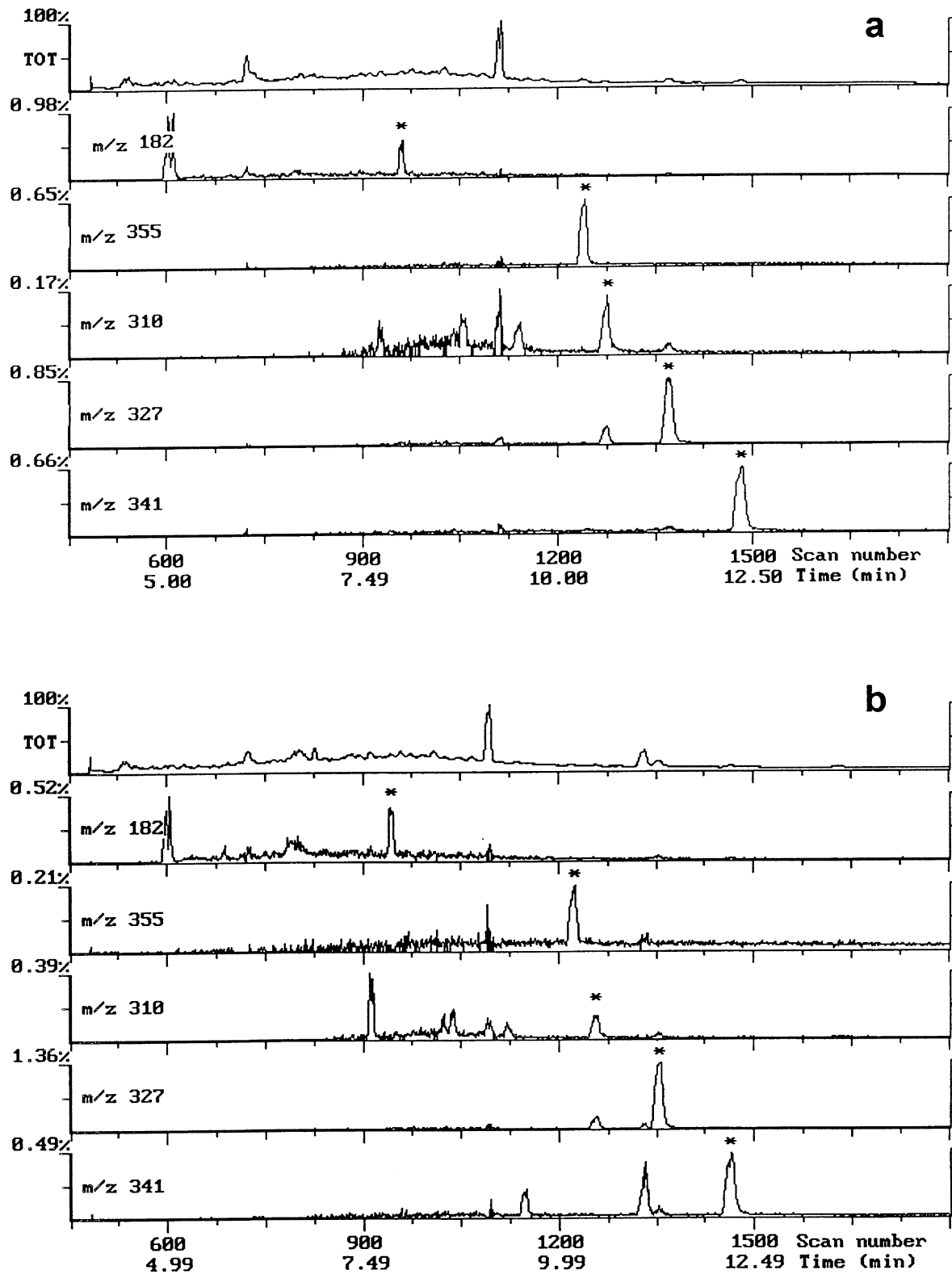


Figure 2  
a) Total and selected ion chromatograms of cocaine ( $m/z$  182), codeine-propionyl ( $m/z$  355), heroin ( $m/z$  310), 6-acetylmorphine-propionyl ( $m/z$  327), and morphine-dipropionyl ( $m/z$  341) in control hair fortified with 4 ng/mg of each analyte.  
b) Total and selected ion chromatograms of heroin and cocaine abusers' hair: cocaine 5.99; codeine 2.51; heroin 5.58; 6-acetylmorphine 9.57; morphine 6.52 ng/mg.

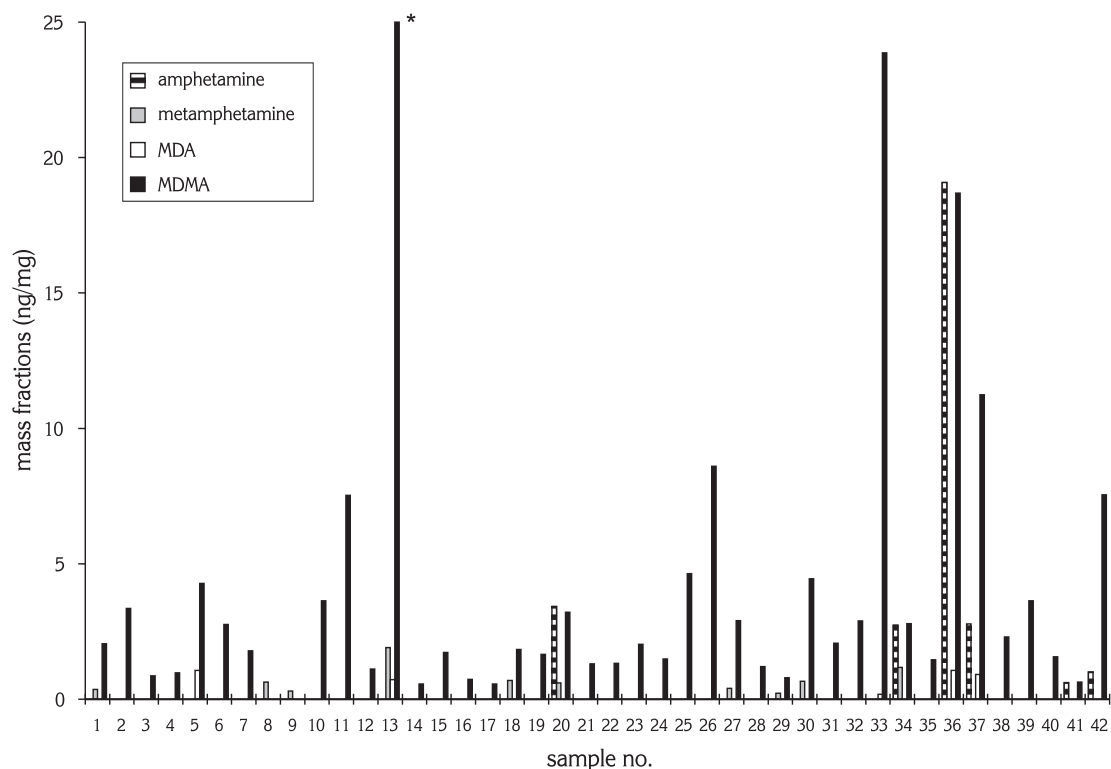
Table 2 summarises positive results of drugs of abuse (N=77) in which one or more than one kind of drug were found. In most subjects, amphetamines (N=42) were found alone (N=39) or with other drugs (N=3). The leading amphetamine was MDMA, which confirmed dominant “Ecstasy” consumption. “Ecstasy” is believed to be popular as a “dance drug” among the young, particularly those who attend “rave” parties.

**Table 2** Percentage of hair positive samples (N=77) for amphetamines, opiates, cocaine, methadone, and their combination

Group	Number of cases	Percentage
Amphetamines only	39	50.6
Opiates only	15	19.5
Cocaine only	3	3.9
Methadone only	6	7.8
Amphetamines / cocaine	1	1.3
Opiates / cocaine	1	1.3
Opiates / amphetamines	2	2.6
Opiates / methadone	7	9.1
Opiates / cocaine / methadone	1	1.3
Cocaine / methadone	2	2.6

Figure 3 shows positive amphetamine findings. The leading MDMA ranged from 0.56 to 64.42 ng/mg. In most hair samples (78.6%), MDMA was found in mass fractions of up to 5.0 ng/mg, which reflects the occasional and the most common, “party” abuse of 1–2 tablets in average. Only three hair samples stand out with high MDMA mass fractions, that is, 64.42; 23.87, and 18.62 ng/mg. It was not even possible to represent the real MDMA mass fraction of 64.42 ng/mg in Figure 3.

Opiates alone were detected in 15 hair samples, with cocaine in one sample, amphetamines in two, methadone in seven, and methadone plus cocaine in one sample. The marker of heroin consumption, 6-acetylmorphine, was found in all these samples. Both 6-acetylmorphine and morphine were found in 23 subjects. The 6-acetylmorphine/morphine ratio (mean: 1.98; median: 2.28; range: 0.69–6.54) was similar to that found by Moeller and co-workers (9). Higher concentrations of 6-acetylmorphine than those of morphine are usual for heroin consumers. Only rarely does the morphine concentration exceed the concentration of 6-acetylmorphine (7, 9, 10). We recorded only three such cases among our subjects. The morphine/6-acetylmorphine ratio (mean: 0.61; median: 0.58; range: 0.15–1.45) is in accordance with the results of Gaillard and Pepin (4). Heroin was



**Figure 3** Mass fractions of amphetamines (amphetamine, methamphetamine, MDA, and MDMA) in hair. \*The real MDMA mass fraction is 64.42 ng/mg.

found in only 12 hair samples, which is consistent with literature (11, 12). The finding of codeine in only seven hair samples is a surprisingly low rate, having in mind the notorious impurity of heroin sold in the streets.

Figure 4 shows 26 positive cases for opiates in hair. The confirmation marker for heroin consumption, 6-acetylmorphine (6-MAM), ranged from 0.57 to 6.82 ng/mg. Cocaine was present in eight hair samples; in three of them together with heroin, and in one with MDMA. Methadone was found in 16 hair samples.

It is rather unexpected that we found only 77 positive results in the total of 221 analysed hair samples. However, there is a plausible explanation for that. Most hair samples were taken from young adolescents (15–17 years of age) whose parents were upset by their change in behaviour and poor results in school, which led them to suspect that their children were taking drugs. This suspicion was not altogether unjustified as most of them smoked marihuana (1). However, we have not yet developed a method for the analysis of marihuana's psychoactive constituent tetrahydrocannabinol in hair. One reason is that, in contrast to other drugs whose excretion through urine is relatively quick, the marihuana metabolite 11-nor-9-tetrahydrocannabinol-9-carboxylic acid can be detected for up to two months after the chronic use of marihuana or hashish (2).

All but five women and three men had coloured and bleached hair. Only three women with treated hair were found 6-acetylmorphine without morphine. There are several reports about a lower hair drug content which is associated with the cosmetic treatment of hair (13–16).

## CONCLUSION

The described GC/MS methods used in our laboratory for over two years have been validated and found acceptable for the routine analysis of drugs of abuse in hair. Despite uncertainties related to the analysis of coloured and bleached hair, the methods were able to confirm drug abuse with high reliability. This study also indicates the trend in drug abuse among young people in Croatia.

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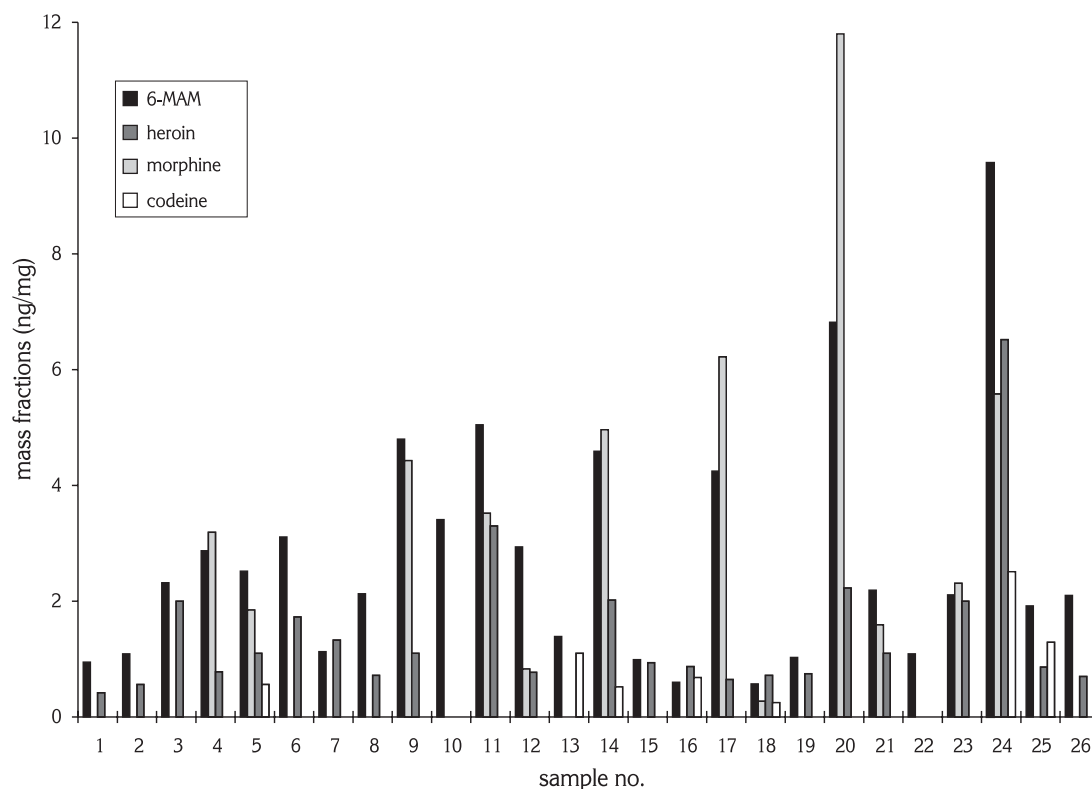


Figure 4 Mass fractions of 6-acetylmorphine (6-MAM), heroin, morphine, and codeine in hair.

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**Sažetak**

## PROCJENA ZLOPORABE DROGA ANALIZOM KOSE: DVOGODIŠNJE ISKUSTVO

Razvijene su GC/MS metode za analizu opijata (morfin, kodein, heroin i 6–acetilmorfin), kokaina, metadona i amfetamina (amfetamin; metamfetamin; 3,4–metilendioksiamfetamin –MDA i 3,4–metilendioksimetamfetamin – MDMA) u kosi. Osjetljivost, preciznost i točnost metode određene su za sve analite. Opisane metode primijenjene su pri identifikaciji zlorabe droga u kosi 221 mlade osobe (15–25 god.) za koje se sumnjalo da uzimaju drogu. U 77 osoba dokazano je uzimanje droga, dok su u uzorcima kose 144–ju ispitanika rezultati negativni. U 54,5% (N=42) osoba za koje je utvrđeno da uzimaju droge nađeni su amfetamini, pretežno MDMA (N=40), što upućuje na uzimanje tableta “Ecstasy”. Konzumiranje heroina dokazano je u 33,8% (N=26) osoba na temelju prisutnosti 6–acetilmorfina, jedinog specifičnog metabolita heroina. Uzimanje kokaina dokazano je u osam ispitanika.

Razrađenim metodama moguće je procijeniti učestalost i vrstu droga koju uzima mlada populacija u Hrvatskoj.

**KLJUČNE RIJEČI:** *amfetamini, kokain, kvantitativno određivanje, metadon, opijati, plinska kromatografija/spektrometrija masa*

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