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OBSERVATION

ANALYSIS OF DRUGS OF ABUSE IN URINE BY GAS CHROMATOGRAPHY/ MASS SPECTROMETRY: EXPERIENCE AND APPLICATION

VIŠNJA KARAČIĆ AND LJILJANA SKENDER

Institute for Medical Research and Occupational Health, Zagreb, Croatia

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This paper describes quantitative methods for determination of urinary drugs/metabolites. The analysis included indicators of opiate (morphine, codeine, 6-monoacetylmorphine) and methadone (methadone) consumption, indicator of marihuana/ hashish consumption (11-nor-9-tetrahydrocannabinol-9-carboxylic acid), indicators of cocaine consumption (cocaine, benzoylecgonine, and ecgonine methyl ester) and of amphetamine consumption (amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, and 3,4-methylenedioxethylamphetamine). The methods included solid-phase extraction of urine, concentration of eluent, derivatisation, and quantitative analysis by gas chromatography/ mass spectrometry (GC/MS) on a capillary column in the electron impact and selected ion monitoring (SIM) mode. Sensitivity, reproducibility, and accuracy were determined for all analytes (limit of detection between 3 and 12 ng/ml, precision <10%, accuracy >92%). The accuracy was checked through analysis of standard reference materials and participation in an international quality assessment programme. The methods were used in the analysis of spot urine samples of 60 subjects suspected of drug abuse. Negative findings indicated several disadvantages of urine as a biological sample.

Key words:
amphetamines, cocaine/metabolites, codeine, 6-monoacetylmorphine, methadone, morphine

Urine is generally accepted as the sample of choice for drugs-of-abuse testing. Urine drug testing is reliable, economical, widely utilised, and strictly regulated (1). There are several commercial immunoassay techniques for screening drugs of abuse in urine: radioimmunoassay (RIA), enzyme multiplied immunoassay (EMIT), fluorescence polarization immunoassay (FPIA) (2), and cloned enzyme immunoassay (CE-

DIA) (3). These are used as preliminary screening procedures to identify presumably positive samples. Positive findings are confirmed with specific techniques – usually chromatographic – based on different chemical and physical principles (4–6). This paper describes the development and application of quantitative methods for simultaneous determination of particular drugs/metabolites in urine by gas chromatography/mass spectrometry (GC/MS).

SUBJECTS, MATERIALS, AND METHODS

The work described in this paper presents the results of analysis of spot urine samples taken from 60 subjects, mostly adolescents suspected of drug abuse.

Standards and reagents

(±)Amphetamine, (+)methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy), 3,4-methylenedioxyethylamphetamine (MDEA), 6-monoacetylmorphine (6-MAM), and 11-nor-9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) were purchased from Radian (Austin, TX, USA). Codeine, morphine sulfate, morphine-3-b-D-glucuronide, methadone hydrochloride, cocaine hydrochloride, benzoylecgonine, ecgonine methyl ester and heptafluorobutyric acid anhydride (HFBA), pentafluoropropionic acid anhydride (PFPA), trifluoroacetic acid anhydride (TFA) and N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

All other chemicals (hexane, methanol, ethyl acetate, isopropyl alcohol, ammonium hydroxide, glacial acetic acid, potassium phosphate monobasic, and potassium hydroxide) were analytical-grade-purity products of Merck (Darmstadt, Germany).

Solid-phase extraction was performed using Bond Elut Certify (non-polar C_8 sorbent and a strong cation exchanger) – Certify II (non-polar C_8 sorbent and a strong anion exchanger) extraction columns (10 ml, 130 mg), vacuum manifold Vac Elut SPS24, and small reaction vials Reacti-Vial ™ obtained from Varian (Harbor City, CA, USA).

Standard reference materials

Standard reference materials for morphine and codeine (SRM 2381), cocaine and benzoylecgonine (SRM 1508), and THCCOOH (SRM 15076) in urine were obtained from the National Institute of Standards & Technology, Gaithersburg, MD, USA. Each standard reference material consisted of three different analyte levels (low, middle, and high) and of the blank urine sample. The certified concentrations were 134±14, 295±12, and 580±18 ng/ml for morphine; 130±5, 282±9, and 560±23 ng/ml for codeine; 90±14, 263±22, and 429±20 ng/ml for cocaine; 103±10, 259±20, and 510±29 ng/ml for benzoylecgonine; and 11.7±1.4, 24.1±1.3, and 49.6±4.4 ng/ml for THCCOOH.

Standard preparation

Two-μg/ml stock solutions containing 6-MAM and THCCOOH, and 20-μg/ml stock solutions containing amphetamines, morphine sulfate, codeine, methadone hydrochloride, cocaine hydrochloride, benzoylecgonine, and ecgonine methyl ester were prepared in methanol and stored at -20 °C.

Standard calibration curves – prepared with blank urine samples from the colleagues in the laboratory – were obtained over the range of 100–2000 ng/ml for cocaine, benzoylecgonine, and ecgonine methyl ester, 150–2000 ng/ml for amphetamines and opiates, 10–60 ng/ml for 6-MAM, and 20–200 ng/ml for THCCOOH.

METHODS

Drugs/metabolites were grouped (amphetamines, cocaine/metabolites, THCCOOH, opiates) and each group analysed separately. Preparation of urine samples included conjugate hydrolysis (acid for codeine and morphine glucuronides; alkaline for THCCOOH glucuronide), extraction, concentration, and derivatisation. To achieve high purity of urine, solid-phase extraction of all analytes was performed on a Bond Elut Certify column, except for THCCOOH which was extracted on a Bond Elut Certify II column. Each group of drugs/metabolites was extracted according to the manufacturer's instructions with small modifications. Two millilitres of urine and an appropriate buffer solution were used for all drugs/metabolites. After rinsing and drying under vacuum, the columns were eluted with a mixture of dichloromethane:2-propanol:ammonium hydroxide (80:20:2, v/v/v) for amphetamines, cocaine/metabolites and opiates. THCCOOH was eluted with a mixture of hexane:ethyl acetate (75:25, v/v) and 1% glacial acetic acid. The eluents were collected in glass tubes and evaporated to dryness under a stream of nitrogen at 40 °C for opiates/metabolites and cocaine/metabolites and at room temperature for amphetamines and THCCOOH. Reagents used in derivatisation of amphetamines were HFBA, PFPA, and TFA, whereas all other analytes were derivatised with BSTFA with 1% TMCS. Opiates/metabolites, amphetamines, and cocaine/metabolites were derivatised at 60 °C for 30 minutes and THCCOOH at 90 °C for 15 min. Unlike HFBA, PFPA, and TFA, BSTFA was not further evaporated. After evaporation followed reconstitution in ethyl acetate. For GC/MS analysis, 1–2-μl samples were injected in the gas chromatographic column. Each batch of analysed samples included standards of drug abuse/metabolites, negative control, and a genuine positive sample.

GC/MS analysis

Samples were analysed with a Varian 3400 CX GC with Saturn ion trap mass spectrometer (mass selective detector, MSD) in the electron impact (EI) mode with electron energy of 70 eV. Compounds were separated using a 30 m x 0.25 mm ID DB-5 (5% phenyl-95% methyl polysiloxane) capillary column with a 0.25 μm thick film. Samples were conveyed to the spectrometer with a Septum equipped Programmable Injector (SPI) and ultra-pure grade helium as the carrier gas. The transfer line was

heated at 260 °C. The operating temperature of the column varied according to the group of drugs/metabolites analysed. Identification was based on matching the mass spectrum of the analyte with the one in our own mass-spectrum library of standards created and enhanced during the development of the methods. We used the external standard method for quantitation.

For each analyte three ions were monitored: morphine-2TMS m/z 429,287,324; codeine-TMS m/z 371,234,343; 6-MAM-TMS m/z 399,73,204; methadone m/z 72,309,165; THCCOOH-2TMS m/z 371,488,473; cocaine m/z 182,198,303; benzoylecgonine-TMS m/z 240,256,361; ecgonine methyl ester-TMS m/z 82,96,271; amphetamine-HFBA m/z 118,140,91; 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. The underlined ions were quantified.

RESULTS

Quality assurance of the described methods includes internal quality control and external quality assessment. The internal quality control comprised the following analytical parameters for each drug or metabolite: limit of detection in urine (LD) defined as signal to noise ratio higher than 3, accuracy, and precision (Table 1) according to standardised procedure.

Standard reference materials were also tested using the routine analytical procedure. The accuracy of determination of standard reference materials of morphine, codeine, cocaine, benzoylecgonine, and THCCOOH in urine ranged from 96% to 104%.

Table 1 *Precision, accuracy, and limit of detection of each analyte in a random sample (N=9)*

Analyte	Concentration (ng/ml)	RSD (%) (%)	Accuracy (%)	Limit of detection (ng/ml)
Amphetamine	300	4.5	98.2	10
Methamphetamine	300	3.8	97.5	3
MDA	300	4.1	99.0	4
MDMA	300	3.7	99.1	8
MDEA	300	5.0	98.4	5
Cocaine	150	7.5	97.1	5
Benzoylecgonine	200	5.5	98.3	12
Ecgonine methyl ester	200	8.8	95.5	7
6-MAM	30	6.8	92.4	5
Morphine	300	7.0	98.3	3
Codeine	300	6.6	96.5	5
THCCOOH	30	8.5	97.7	7
Methadone	300	6.6	98.1	5

N – number of determinations

The quality of the methods was externally assessed through participation in the UK National External Quality Assessment Scheme, a programme for drugs-of-abuse testing organised by the Cardiff Bioanalytical Service. Our laboratory was receiving lyophilised urine samples in sets of 3 at three-month intervals. The samples contained a mixture of drugs and metabolites, but some were blank. The concentrations of drugs and metabolites ranged as follows: morphine (N=17) 180–8000 ng/ml; 6-MAM (N=4) 23–42 ng/ml; codeine (N=2) 1310–3080 ng/ml; methadone (N=11) 470–3462 ng/ml; THCCOOH (N=6) 27–58 ng/ml; cocaine (N=1) 1060 ng/ml; benzoylecgonine (N=7) 220–18620 ng/ml; amphetamine (N=13) 302–2120 ng/ml; methamphetamine (N=3) 360–1196 ng/ml and MDMA (N=2) 3100–3176 ng/ml. The score was calculated for each analyte and for each of the twelve samples. So far, our score has been 100%.

Each analytical procedure has a cut-off or threshold concentration; a level that determines whether a result is positive or not. It is usually significantly greater than the limit of detection. The quality control specimens are positive if the concentration of a drug of abuse or a metabolite exceeds the threshold proposed by the UK National External Quality Assessment Scheme (Table 2).

Table 2 *Cut-off (threshold) concentrations proposed by the UK National External Quality Assessment Scheme*

Analyte	Cut-off concentration (ng/ml)
Amphetamine	200
Methamphetamine	200
MDA / MDMA / MDEA	200
THCCOOH	15
Benzoylecgonine	150
Morphine	200 (total after hydrolysis)

We applied our validated methods to the analysis of spot urine samples of 60 subjects suspected of drug abuse. Fifty samples were positive for drugs of abuse and 10 were negative. In four urine samples more than one drug of abuse was detected.

Table 3 shows the concentrations (range and median) of morphine, codeine, 6-MAM, methadone, THCCOOH, cocaine, benzoylecgonine, ecgonine methyl ester, and 5 amphetamines in urine of subjects suspected of drug abuse. Most subjects (N=35) were found THCCOOH which confirmed marihuana and/or hashish consumption. Ecstasy consumption was confirmed in one subject. Figure 1 shows selected ion chromatograms of THCCOOH-2TMS in blank urine fortified with 20 ng/ml THCCOOH (Figure 1-A) and in a marihuana consumer's urine in the concentration of 337 ng/ml THCCOOH (Figure 1-B).

Opiate consumption was confirmed by positive findings of morphine, codeine, and 6-MAM. The specific heroin metabolite, 6-MAM, was detected in one urine sample. Morphine was found in 11 and codeine in seven urine samples. Methadone was found in four urine samples.

High concentrations of cocaine and metabolites were determined in three urine samples. Codeine and morphine were found in two samples. In the cocaine consum-

Figure 1 *Selected ion chromatograms of THCCOOH-2TMS in blank urine fortified with 20 ng/ml THCCOOH (A) and in marihuana consumer's urine in concentration of 337 ng/ml THCCOOH (B)*

Figure 2 *Selected ion chromatograms of blank urine fortified with 250 ng/ml amphetamine, methamphetamine, MDA, MDMA and MDEA (A) and of amphetamine consumer's urine containing MDA (1373 ng/ml), MDMA (11747 ng/ml), and MDEA (4409 ng/ml) (B)*

Table 3 *Concentrations of morphine, codeine, 6-MAM, methadone, THCCOOH, cocaine, benzoylecgonine, ecgonine methyl ester, MDA, MDMA, and MDEA found in urine samples of drug consumers*

Analyte	Concentration (ng/ml)		
	Number of positive samples	Range	Median
Morphine	11	259–27954	1657
Codeine	7	18–475	40
6-MAM	1	479	–
Methadone	4	235–1421	874
THCCOOH	35	15–669	46
Cocaine	3	56–552	76
Benzoylecgonine	3	1880–19438	12595
Ecgonine methyl ester	3	177–8375	418
MDA	1	1373	–
MDMA	2	286–11748	–
MDEA	1	4409	–

ers' urine benzoylecgonine concentrations were the highest, followed by ecgonine methyl ester and cocaine. The results indicate that all three urine samples were from severe abusers.

Amphetamines were found in two urine samples. The first sample of the two samples showed high concentrations of MDA, MDMA, and MDEA (1373 ng/ml, 11748 ng/ml and 4409 ng/ml, respectively) and the second showed MDMA alone in the concentration of 286 ng/ml. The cut-off value for amphetamines is 200 ng/ml. Figure 2 shows selected ion chromatograms of HFBA derivates of MDA, MDMA, and MDEA found in the urine of amphetamine consumers (Figure 2-B) and compared to the blank urine sample fortified with 250 ng/ml amphetamine, methamphetamine, MDA, MDMA, MDEA (Figure 2-A). Table 4 gives the percentages of positive findings by

Table 4 *Percentage of positive urine samples by particular drugs and combinations of drugs*

Group	Number of positive samples	Percentage (%)
Morphine	4	8
Morphine/codeine	4	8
Morphine/codeine/methadone	1	2
Morphine/codeine/6-MAM/methadone/cocaine and metabolites	1	2
Morphine/codeine/cocaine and metabolites	1	2
Cocaine and metabolites	1	2
THCCOOH	34	68
MDA, MDMA, MDEA	1	2
MDMA/THCCOOH	1	2
Methadone	2	4

selected groups of drugs and metabolites. THCCOOH was found in the majority of urine samples (68%), which suggests a widespread marihuana consumption in adolescents.

DISCUSSION

Quantitative methods employing solid-phase extraction combined with GC/MS analysis for determination of morphine, codeine, 6-MAM, THCCOOH, amphetamines, cocaine/metabolites and methadone in urine showed good precision and accuracy. Although GC/MS is recognized as the technique of choice for confirming positive immunoassay screening results for drugs of abuse in urine, GC/MS analysis does have limitations. *Wilson and Smith* (7) demonstrated that GC/MS often missed morphine and benzoylecgonine at concentrations above 1.3 mg/l and 0.5 mg/l, respectively. We had no such experience. Literature also reports cases of false positive results due to similar retention time and mass spectrum of different compounds (8). Interference of over-the-counter medications ephedrine and pseudoephedrine with methamphetamine in urine has often been reported (9, 10). The results should be interpreted with utmost care. When using HFBA derivatisation, it is imperative to monitor 91 and 118 ions for positive methamphetamine, because these ions are not present in ephedrine or pseudoephedrine. By contrast, the 344 ion is present in ephedrine and pseudoephedrine, but not in methamphetamine. Having paid attention to these facts, we had no false positive results in the quality assessment program, although both ephedrine and pseudoephedrine were sometimes present in high concentrations in urine samples.

Interpretation of positive findings of morphine and codeine is not easy. Heroin (diacetyl morphine), morphine, and codeine (methyl morphine) are related metabolically. Heroin is rapidly (half-life is about 3 min) deacetylated to 6-MAM (11). The only specific heroin metabolite 6-MAM can only be detected 2–8 hours after consumption of heroin, as it is metabolised rapidly (half-life 0.6 hours) to morphine (11). Codeine is metabolised by O- demethylation to form morphine and N- demethylation to form norcodeine (1). Thus morphine is a metabolite of both heroin and codeine. The main morphine metabolite found in urine is morphine-3-glucuronide. Street heroin may also contain acetylcodeine which is metabolised to codeine (12). When a urine sample contains relatively low amounts of codeine and morphine, it is impossible to distinguish between heroin and morphine or codeine intake. If the codeine concentration exceeds that of morphine, that points to the codeine use. A sample of urine collected 2–3 days after codeine ingestion may contain only morphine, rendering the interpretation difficult. An additional problem is that codeine and morphine are present in poppy seed and its consumption may lead to further misinterpretation of positive opiate urine test results (13).

Despite all those problems in interpretation, it is most likely that the positive finding of morphine in 11 urine samples was the result of heroin consumption, although it was not possible to confirm it because 6-MAM was found in only one urine sample. The combined presence of morphine, codeine, and cocaine metabolites indicates heroin and cocaine consumption.

Urine is accepted as the sample of choice for drugs-of-abuse testing; it is easily obtainable, testing is reliable, economical, widely utilised, and strictly regulated. However, urine also has disadvantages; it can be adulterated, diluted, and substituted. Since the concentration of a drug in urine depends on many factors such as dose, frequency, route of administration, purity, and individual metabolism, it is not possible to infer the amount or the time of drug consumption from the level found in the urine (14). Common use of urine is only to indicate presence or absence of drugs of abuse. Sampling time is also critical because most drugs can be detected in urine not later than 2–3 days after use (15). An exception is THCCOOH which can be detected up to two months after chronic use of marihuana/hashish. Fast urinary excretion of most drugs could explain the negative finding of drugs of abuse in 10 urine samples; a few days of abstinence before testing are enough to evade detection of most drugs. Positive findings of amphetamines in only three urine samples can also be explained by fast urinary excretion of amphetamines. By contrast, the positive finding of THCCOOH in 35 subjects could be explained both by frequent consumption of marihuana and by its prolonged excretion. Generally, the success of urine drugs-of-abuse testing depends on the testing frequency.

CONCLUSION

Quality assurance in the analysis of drugs of abuse in urine by GC/MS is essential to confidence in the result, since the consequences of a positive test can be quite severe. It should be kept in mind that urine tests only reveal that an individual has used a drug of abuse i.e. they cannot establish the length of time and the quantity of drug taken. Furthermore, drug abuse may not be followed by positive results, as these depend on the time between drug intake and urine collection, as well as on the time needed for a drug to get eliminated from the body.

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REFERENCES

1. Kwong TC, Chamberlain RT, Frederik DL, Kapur B, Sunshine I. Critical issues in urinalysis of abused substances: Report of the substance-abuse testing committee. Clin Chem 1988;34:605–32.
2. George S, Braithwaite RA. A preliminary evaluation of five rapid detection kits for on site drugs of abuse screening. Addiction 1995;90:227–32.
3. Wu AH, Forte E, Casella G, et al. CEDIA for screening drugs of abuse in urine and the effect of adulterants. J Forensic Sci 1995;40:614–8.

4. Tai SSC, Christensen RG, Paule RC, Sander LC, Welch MJ. The certification of morphine and codeine in a human urine standard reference material. J Anal Toxicol 1994;18:7–12.
5. Wang WL, Darwin WD, Cone EJ. Simultaneous assay of cocaine, heroin and metabolites in hair, plasma, saliva and urine by gas chromatography-mass spectrometry. J Chromatogr B Biomed Appl 1994;660:279–90.
6. Bermejo Barrera AM, Strano Rossi S. Hair and urine analysis: relative distribution of drugs and their metabolites. Forensic Sci Int 1995;70:203–10.
7. Wilson JF, Smith BL. Evaluation of detection technique and laboratory proficiency in testing for drugs of abuse in urine: an external quality assessment scheme using clinically realistic urine samples. Ann Clin Biochem 1999;36:592–600.
8. Wu AH. Mechanism of interferences for gas chromatography/mass spectrometry analysis of urine for drugs of abuse. Ann Clin Lab Sci 1995;25:319–29.
9. Thurman EM, Pedersen MJ, Stout RL, Martin T. Distinguishing sympathomimetic amines from amphetamine and methamphetamine in urine by gas chromatography/mass spectrometry. J Anal Toxicol 1992;16:19–27.
10. Kraemer T, Maurer HH. Determination of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments in blood and urine. J Chromatogr B Biomed Appl 1998;713:163–87.
11. Inturrisi CE, Max MB, Foley KM, Schutz M, Shin SJJ, Houde RW. The pharmacokinetics of heroin in patients with chronic pain. N Engl J Med 1984;310:1213–7.
12. Braithwaite RA, Jarvie DR, Mintry PSB, Simpson D, Widdop B. Screening for drugs of abuse. I: Opiates, amphetamines and cocaine. Ann Clin Biochem 1995;32:123–53.
13. Hayes LW, Krasselt WG, Mueggler PA. Concentrations of morphine and codeine in serum and urine after ingestion of poppy seeds. Clin Chem 1987;33:806–8.
14. Bosomworth MP. Drugs of abuse in urine: some pitfalls in testing. Annotation 1993;50:150–5.
15. James WH, Moore DD. Adolescents and drug abuse: Clinical use of urine drug screening. J Child Adolesc Subst Abuse 1997;7:19–36.

Sažetak

ANALIZA DROGA U URINU GC/MS TEHNIKOM: STEČENA ISKUSTVA I PRIMJENA

Za provjeru uzimanja droga razvijen je niz brzih, relativno jeftinih testova za analizu droga u urinu, kojima se razlikuju negativni od vjerojatno pozitivnih nalaza. Preporučeni analitički protokol zahtijeva potvrdu svih pozitivnih nalaza specifičnom i osjetljivom metodom. S tim ciljem opisane su kvantitativne metode za simultano određivanje pojedinih droga i njihovih metabolita u urinu: 1) morfina, kodeina i 6-acetilmorfina (6-MAM) pokazatelja uzimanja morfina, kodeina i heroina; 2) 11-nor-tetrahidrokanabinol-9-karboksilne kiseline (THCCOOH) pokazatelja uzimanja marihuane i hašiša; 3) kokaina, benzoilekgonina i metilekgonina pokazatelja uzimanja kokaina i 4) amfetamina, metamfetamina, 3,4-metilendioksiamfetamina (MDA), 3,4-metilendioksimetamfetamina (MDMA, Ecstasy) i 3,4-metilendioksietilamfetamina (MDEA) pokazatelja uzimanja tih istih spojeva, tehnikom plinske kromatografije sa spektrometrijom masa (GC/MS). Metode uključuju ekstrakciju urina na kolonama punjenim sorbensom, koncentriranje eluata, derivatizaciju te kvantitativnu analizu GC/MS sustavom s kapilarnom kolonom uz ionizaciju elektronskim snopom i detekciju karakterističnih iona. Osjetljivost, preciznost i točnost postupka određene su za sve analite. Točnost određivanja provjerena je analizom standardnih referentnih uzoraka. Sudjelovanjem u međunarodnom programu provjere kvalitete analiza droga u urinu potvrđena je točnost razvijenih metoda za sve analite. Opisane metode primijenjene su pri identifikaciji zloporabe droga u 60 osoba za koje se sumnjalo da uzimaju droge. U 35 uzoraka urina nađena je THCCOOH, amfetamini u dva, metadon u četiri, morfin/kodein/6-MAM u 11 i kokain i metaboliti u tri uzorka urina. U četiri uzorka urina određeno je više droga/metabolita. Negativni nalazi droga u 10 uzoraka urina upućuju na nedostatke urina kao biološkog uzorka: može biti zamijenjen drugim urinom, izmijenjen dodacima raznih sredstava i razrijeđen vodom. Kako se većina droga/metabolita izlučuje urinom 2–3 dana nakon uzimanja droge (s izuzetkom THCCOOH), kritično je vrijeme uzimanja urina za analizu.

Ključne riječi:
amfetamin, kokain/metaboliti, kodein, 6-monoacetilmorfin, metadon, morfin

Requests for reprints:

Višnja Karačić, Ph. D.
Institute for Medical Research and Occupational Health
P. O. Box 291, HR–10001 Zagreb, Croatia
E-mail: *Visnja.Karacic@imi.hr*