Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 801 (2004) 289–296
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Rapid screening procedure based on headspace solid-phase microextraction and gas chromatography–mass spectrometry for the detection of many recreational drugs in hair

Stefano Gentili, Maria Cornetta, Teodora Macchia*

Clinical Biochemistry Department, Drug Abuse Section, Istituto Superiore di Sanità, Viale Regina Elena 299, Rome 00161, Italy

Received 10 July 2003; received in revised form 19 November 2003; accepted 19 November 2003

Abstract

An increasing number of synthetic drugs are appearing on the illicit market and on the scene of drug use by youngsters. Official figures are underestimated. In addition, immunochemical tests are blind to many of these drugs and appropriate analytical procedures for routine clinical and epidemiological purposes are lacking. Therefore, the perceived increasing abuse of recreational drugs has not been proved yet. In a previous paper, we proposed a procedure for the preliminary screening of several recreational substances in hair and other biological matrices. Unfortunately, this procedure cannot apply to cocaine. Consequently, we performed a new headspace solid-phase microextraction and gas chromatography–mass spectrometry (HS-SPME–GC–MS) procedure for the simultaneous detection of cocaine, amphetamine (A), methamphetamine (MA), methylen-dioxyamphetamine (MDA), methylen-dioxymethamphetamine (MDMA), methylen-dioxyethamphetamine (MDE), *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), ketamine, and methadone in human hair. Hair was washed with water and acetone in an ultrasonic bath. A short acid extraction with 1 M hydrochloric acid was needed; the fiber was exposed to a 5 min absorption at 90 °C and thermal desorption was performed at 250 °C for 3 min. The procedure was simple, rapid, required small quantities of sample and no derivatization. Good linearity was obtained over the 0.1–20.0 ng/mg range for the target compounds. Sensitivity was good enough: limits of detection (LOD) were 0.7 ng/mg of hair for the majority of substances. The intra-day precision ranged between 7 and 20%. This paper deals with the analytical performance of this procedure and its preliminary application to hair samples obtained on a voluntary basis from 183 young people (138 males and 45 females) in the Rome area.

Keywords: Headspace analysis; Hair analysis; Amphetamine-like drugs; Cocaine

1. Introduction

The abuse of amphetamine-like drugs and other stimulants is one of the most serious problems among Italian youths attending recreational settings [1–4]. In Italy, like in other European countries, data on the spread of illicit drugs, especially synthetic ones, strongly depend on law enforcement seizures, on information from public facilities for treatment of drug addicts, and on self-reported studies. All these sources provide underestimated figures: very few analytical data are produced on substances available on the street market, and very few people abusing recreational drugs refer to public facilities intended for treatment of addicts [5]. Self-reported information suffers from credibility as it relies on the subjective memory for both long-term and recent use. In fact, some studies show that urine tests values and self-reports are poorly correlated [6,7]. The analysis of seized "ecstasy" tablets performed by different laboratories in Italy in 1998 and 1999 revealed quali-quantitatively significant fluctuations in the content of substances per tablet [8]. A number of tablets (approximately 30%) do not contain MDMA, but other substances or active ingredients. The term "ecstasy" has become generic for a wide range of compounds as EMCDDA (European Monitoring Centre for Drugs and Drug Addiction) has claimed since 1997. Since the composition of tablets sold as "ecstasy" varies considerably, unknown types and quantities of drugs are taken by "ecstasy" users.

At present, the Italian legislation on drugs allows testing only of seized pills. Therefore, contrary to what happens in other countries [9,10], the composition of products

* Corresponding author. Fax: +39-0649-903110.
E-mail address: teodora.macchia@iss.it (T. Macchia).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.11.034

currently available on the street market in Italy cannot be assessed.

At present, considerable delay is foreseen in the adoption of a suitable procedure that relies on a compatible legal framework. In view of such difficulties and the growing concerns about the dangers of using these substances, a different analytical approach is advisable in order to evaluate the illicit use of drugs in a defined population such as young people at recreational venues. Unfortunately, the epidemiology of the use of synthetic drugs is hampered by the lack of analytical methodology. In particular, screening procedures for most recreational drugs are still unavailable. Headspace solid-phase microextraction (HS-SPME) coupled with a GC–MS applied to hair has recently been suggested [11]. Hair analysis has recently proved to be a useful technique for long-term control of the intake of many abused substances [12–26]. HS-SPME–GC–MS has had an increasing role in hair testing; several studies on the usefulness of this procedure for analysis of drugs in this biological matrix have been published [27–32]. In a previous paper [11], we reported a simple, HS-SPME–GC–MS, one step procedure suitable for the simultaneous determination of many recreational drugs. This procedure, however, was unable to detect cocaine. The two ester groups of cocaine were easily hydrolysed in 30% sodium hydroxide [30], which could result in a restricted applicability of the procedure in recreational settings. In fact, cocaine is unfortunately becoming increasingly popular among young people in Italy as well as in other countries [5,33]. This substance is now appearing in “ecstasy” tablets and can lead to the increasing abuse of polysubstances. Based on these observations, a screening procedure for the simultaneous detection of cocaine and amphetamine-like drugs has become a critical need. On these grounds, we set up a new HS-SPME–GC–MS procedure which requires an acid extraction, but no additional sample treatment or derivatization, and by which the main recreational drugs can be detected and quantified.

The present paper describes this simple and rapid procedure that can be used for screening purposes. Furthermore, we report the results of a preliminary application to the analysis of 183 hair samples obtained from young people in recreational settings.

2. Material and methods

2.1. Chemicals

Amphetamine (A), methamphetamine (MA), methylen-dioxyamphetamine (MDA), methylen-dioxymethamphetamine (MDMA), methylen-dioxyethamphetamine (MDE), *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), ketamine, methadone and cocaine hydrochloride standards (1 mg/ml in methanol) were purchased from Sigma-Aldrich (Milan, Italy); 3,4 methylen-dioxypropylamphetamine (MDPA) hydrochloride standard (1 mg/ml in methanol) was purchased from SALARS (Como, Italy). Ketamine (Ketavet 50) was obtained from Farmaceutici Gellini (Aprilia, Italy). Ultrapure water was obtained from Milli-Q Unit (Millipore, Bedford, MA, USA). Hydrochloric acid (HCl) and potassium carbonate (K_2CO_3), of analytical grade, were purchased from Carlo Erba (Milan, Italy).

2.2. Equipment

We used a Gas Chromatography 6890 Plus and Mass Selective Detector 5973N (Agilent Technologies, Milan, Italy) equipped with a 19091M–101 HP-5 Trace Analysis capillary column (5% PH ME Siloxane, film thickness 0.33 μm; length 12.5 m; column ID 0.20 mm); an SPME assembly with a replaceable extraction fiber, coated with 100 μm polydimethylsiloxane and a 110 VAC block heater purchased from Sigma–Aldrich were used. Headspace vials (20 and 1.5 ml) and accessories were obtained from Chromacol (London, UK). Hair washing and ultrasonic extraction were performed in a T 310 ultrasonic bath purchased from Carlo Erba.

2.3. Specimens

Hair samples were obtained on a voluntary basis and in an anonymous way from 183 young people in the Rome area (138 males and 45 females, age range 14–40 years, mean 25 years). Hair was cut with round-point scissors from the vertex posterior region of the scalp. All samples were collected over a three-month period in seven different recreational settings such as dancing venues with a mean recorded attendance of 800 (min 200–max 1800) people per night. The youths recruited for the present analytical study were approached by volunteers of non-governmental organizations (NGOs) who were involved in drug abuse information and prevention in the Latium region. For ethical reasons, the subjects labelled there own samples with age, gender and a code known only to themselves. This approach, while respecting privacy, allowed each subject to single out his/her own data from the overall list of analytical results.

Blank hair samples were obtained from the laboratory staff; a considerable amount of blank hair was obtained from a single subject, an aliquot of which was included as a control sample each analytical day.

2.4. Sample preparation

Each hair sample was cut into small pieces, washed for 5 min with deionised water and then for 5 min with acetone in an ultrasonic bath. Washed samples were dried under a nitrogen stream at room temperature.

2.5. Calibration curve

Stock solutions of the A, MA, MDA, MDMA, MDE, MBDB, cocaine, ketamine, methadone standards (100 μg/ml) and of the internal standard (MDPA) were prepared in

methanol and stored at +4 °C until use. An aliquot of each solution was mixed each analytical day, diluted with 0.4 M hydrochloric acid and used to spike hair samples at a final concentration of 0.2, 0.5, 1, 2, 4 and 8 ng/mg. MDPA (10 ng/mg) was used as internal standard.

2.6. Analytical procedure

The washed and dried hair sample (20 mg) was extracted with 200 μl 1 M hydrochloric acid (HCl) in a closed headspace vial (20 ml), containing 10 ng/mg of MDPA as internal standard, at 60 °C for 60 min. After decreasing the temperature to room temperature, the extract was separated and placed into another vial (1.5 ml) containing 80 mg of potassium carbonate (K_2CO_3) and rapidly sealed.

Then the SPME needle was introduced, and the SPME-fiber (100 μm polydimethylsiloxane) was exposed to a 5 min adsorption at 90 °C. A heating block was used for temperature control of hair extraction and HS-SPME adsorption.

2.7. GC–MS parameters

The column temperature was held initially at 60 °C for 2 min, then raised by 20 °C/min to reach 250 °C and finally held at 250 °C for 5 min. The temperatures of the injection port, ion source and transfer line were set at 250, 230 and 280 °C, respectively. Thermal desorption was performed at 250 °C for 3 min inside the gas chromatograph. Helium was used as carrier gas at a flow rate of 0.7 ml/min. The splitless injection mode was used. The mass spectrometer uses electron impact ionisation. The mass spectra were collected by the total ion chromatography. Compounds were identified by using the retention time and the relative abundance of three confirming ions with respect to the target. Quantitative data were obtained by selected ion monitoring (SIM) for each compound and I.S. Monitored ions, retention times and relative abundance for each compound are shown in Table 1.

Table 1
Detection of amphetamine-like drugs, ketamine, methadone and cocaine in spiked hair samples by HS-SPME–GC–MS

Compound	Rt (min)	Ion $m/z_{(\text{relative abundance})}$
A	5.13	$*44_{(100)}$, $91_{(20)}$, $65_{(6)}$
MA	5.60	$*58_{(100)}$, $91_{(11)}$, $77_{(2)}$
MDA	7.74	$*44_{(100)}$, $136_{(60)}$, $135_{(40)}$
MDMA	8.08	$*58_{(100)}$, $77_{(6)}$, $135_{(8)}$
MDE	8.36	$*72_{(100)}$, $135_{(19)}$, $44_{(16)}$
MBDB	8.62	$*72_{(100)}$, $135_{(7)}$, $44_{(2)}$,
MDPA (I.S.)	8.88	$*86_{(100)}$, $44_{(35)}$, $135_{(13)}$
Ketamine	10.01	$*180_{(100)}$, $182_{(34)}$, $209_{(27)}$
Methadone	11.39	$*72_{(100)}$, $294_{(4)}$, $91_{(3)}$
Cocaine	11.73	$*182_{(100)}$, $82_{(80)}$, $303_{(30)}$

* Quantifier ion.

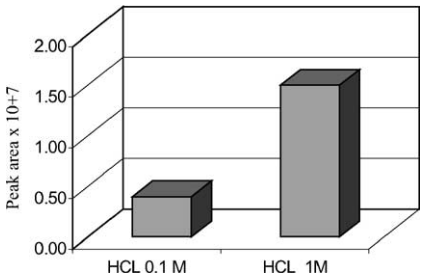

Fig. 1. Effect of 0.1 and 1 M HCl concentration for real hair sample extraction and HS-SPME on the GC–MS abundance of the cocaine.

3. Results and discussion

Major issues, such as analysis procedure, type of fiber used, extraction method, treatment of the matrix, adsorption and desorption conditions, were addressed by meeting most of the criteria reported in the literature on the development of HS-SPME methods [15]. However, for the detection of cocaine in the compounds mixture a compromise had to be reached.

3.1. Acid extraction of drugs from hair

By acid extraction we could simultaneously analyse cocaine, amphetamine-like drugs and other commonly used drugs [34,35]. In order to optimize the extraction of all the substances, especially cocaine, the effects of different concentrations of HCl were investigated. The results are shown in Fig. 1. When 80 mg of K_2CO_3 were added to the extract, we measured pH = 11 in the extracts with 0.1 M HCl, and pH = 10.3 in those with 1 M HCl. On the same way, slight changes of the pH were recorded in unspiked real hair samples, spiked hair samples and aqueous standard solution indifferently.

The increase in HCl concentration produced, on the one hand, a slight change of the pH regardless of the matrix and on the other, an increase in cocaine yield always greater than 60%, in real hair samples. In spiked samples as well as in aqueous solution the difference of yield was negligible, whereas 1 M HCl led to an increase in cocaine extraction yield from the hair matrix. Although 1 M HCl did not cause the hair matrix to dissolve, as 30% NaOH did in our previous procedure [11], it provided a good means of extraction for cocaine as well as for amphetamine-like drugs, methadone and ketamine (data not shown). Consequently, 1 M HCl was used throughout the present study.

3.2. Fiber adsorption

A good fiber adsorption efficiency and a fast process were obtained by PDMS at 90 °C; most of the substances reached equilibrium conditions in only 5 min by adding K_2CO_3. The addition of salt increases the ionic strength of the solution making the target compounds less soluble and allowing a

better response in SPME. Several kinds of salts, such as sodium sulfate, potassium carbonate and sodium chloride were used by other authors [31]. In particular, potassium carbonate was found suitable [28]. In our experience, potassium carbonate proved to be the one and only salt suitable for detection of cocaine as well. Adsorption times of 5, 10 and 20 min were checked. Unexpectedly, times longer than 5 min led to progressively less satisfactory and reproducible results mainly for cocaine. Therefore, 5 min was set as the most suitable adsorption time. In headspace adsorption we used a 1.5 ml vial in order to minimize the gaseous phase volume and increase the yield. In this step the specimen was neither stirred nor sonicated.

3.3. GC analysis

Desorption of analytes from the fiber occurred in splitless mode introducing the main desorbed amount of analytes in the GC column. Desorption for 3 min at 250 °C was as efficient as adsorption for less than 5 min at 90 °C. Our procedure was set up for cocaine, A, MA, MDA, MDMA, MDE, MBDB, ketamine and methadone in hair. An example of a SIM chromatogram of a 20 mg hair sample spiked with A, MA, MDA, MDMA, MDE, MBDB, cocaine, ketamine and methadone to a final concentration of 4 ng/mg is shown in Fig. 2a. Fig. 2b shows the HS-SPME–GC–MS total ion chromatogram and relative mass spectrum of a hair sample positive for MDMA and cocaine.

Peaks of the individual substances present in the mixture appeared well resolved, and chromatographic conditions proved to be appropriate for the separation of all these substances. Good linearity was obtained over the 0.1–20 ng/mg range for all the considered drugs, with little deviation observed for A and MDA. When we explored concentrations lower than 1 ng/mg, no linearity was observed for these substances in the lowest parts of their calibration curves. The main parameters of the linear regression analysis along with sensitivity (LOD) and specificity (LOQ) are shown in Table 2.

Table 3 shows the accuracy, expressed as the relative recovery according to Kintz [24] and Jurado [25], and the precision, evaluated for five replicates of each concentration.

LOD and LOQ were calculated according to Miller et al. [36], and were in agreement with suggestions from US Pharmacopoeial Convention [37] and Alvencar et al. [38].

For the analysis of both hair and urine a statistical approach would be preferable to an experimental one when passive intake must be controlled [7]. The analysis of hair for the presence of cocaine also reveals passive exposure to external contamination. This could account for the higher percentage of positive subjects in comparison with urinalysis [7] and in comparison with the positive use rate in the population. In fact, Kidwell [34] reported a 6% of hair samples to be positive for cocaine vs. the 2% expected in the reference population. The same author showed that by applying a cut-off level of 1.0 ng/mg, rather than the LOD, the positive rate declined from 6 to 1.3%, which is more consistent with the rate obtained from survey data. A cut-off level of 1.0 ng/mg is considered suitable in the literature [35] especially when hair is the only biological specimen available. In the case of cocaine detection, our HS-SPME–GC–MS procedure showed a LOD = 0.35 ng/mg and a LOQ = 1.05 ng/mg. We used a statistical approach to determine the LOD (footnote of Table 2). This approach produced higher LODs and consequently higher LOQs, though more conservative measurements were obtained.

To check the procedure we analysed the hair specimens obtained from 183 young people in the Rome area. Table 4

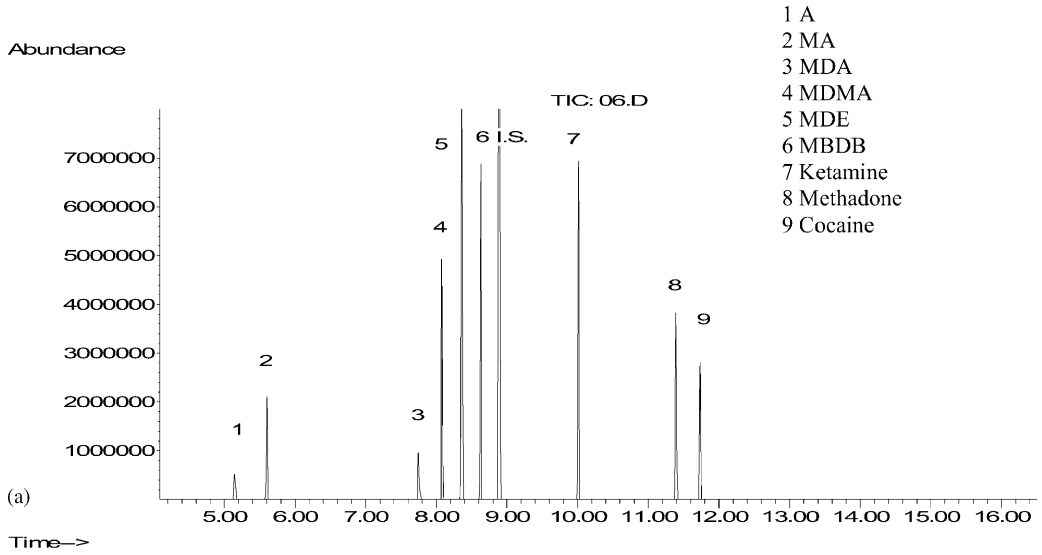

Fig. 2. (a) HS-SPME–GC–MS–SIM chromatogram of a 20 mg hair sample spiked with 4 ng/mg A, MA, MDA, MDMA, MDE, MBDB, ketamine, methadone, cocaine and 10 ng/mg MDPA as IS. For SIM measurement, masses given in Table 1 were used. (b) HS-SPME–GC–MS total ion chromatogram and relative mass spectrum of a blank hair sample and of a hair sample positive for MDMA (8.23 ng/mg) and cocaine (4.51 ng/mg).

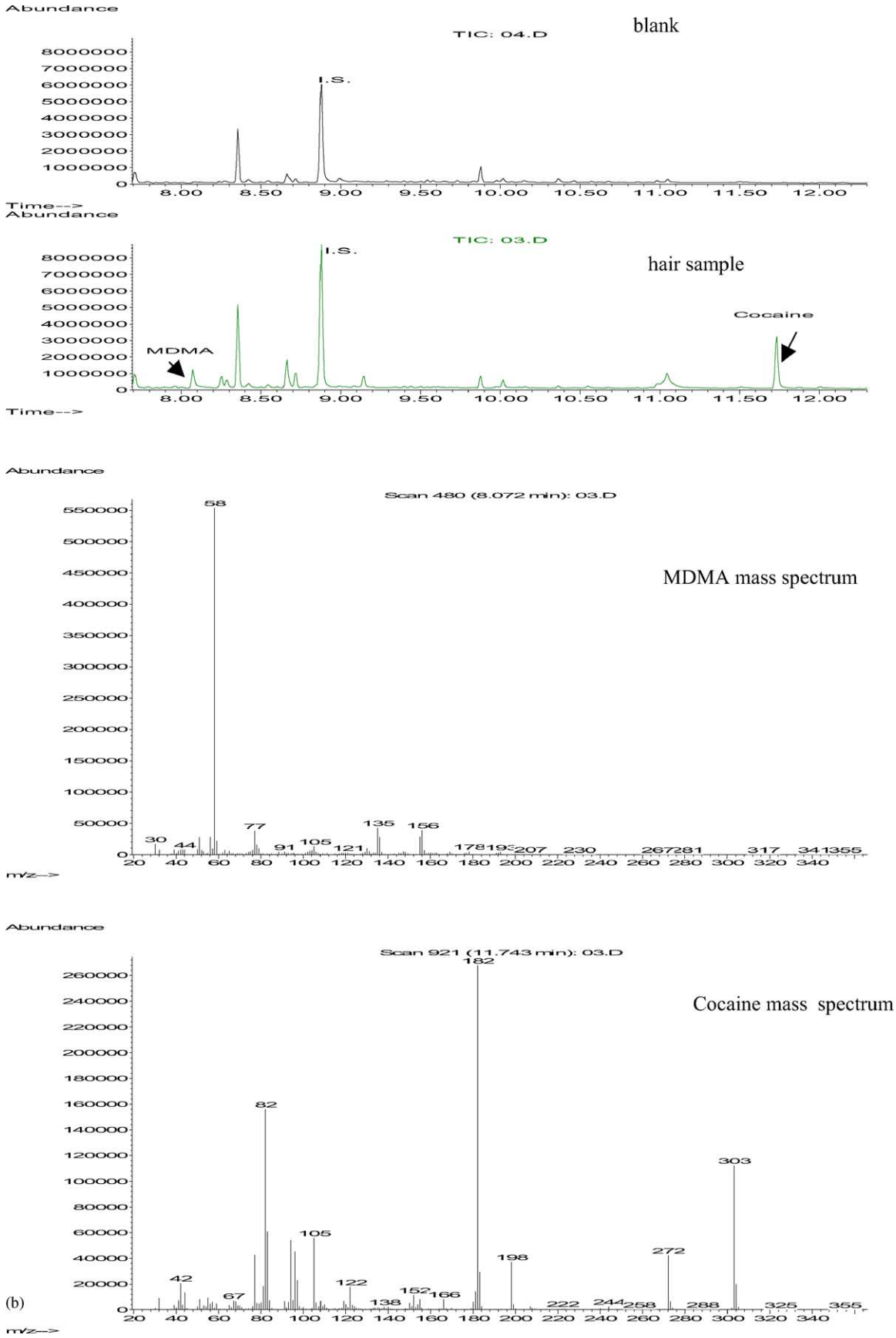

Fig. 2. (*Continued*).

Table 2
Linearity of the HS-SPME–GC–MS procedure in the concentration range 0.12–8 ng/mg

	Slope, *b* (S.E.)	Intercept, *a* (ng/mg)	S.D.$_{yx}$	*n*	r^2	LOD[a] (ng/mg)	LOQ[b] (ng/mg)
A[c]	1.86 (008)	−0.55	0.80	20	0.97	1.29	3.87
MA	8.73 (0.31)	0.19	1.07	25	0.97	0.37	1.11
MDA[c]	1.34 (0.12)	0.64	0.72	20	0.85	1.61	4.83
MDMA	14.36 (1.04)	2.80	3.62	25	0.88	0.76	2.28
MDE	23.50 (1.48)	2.79	5.15	25	0.91	0.66	1.98
MBDB	20.29 (1.17)	2.00	4.09	25	0.92	0.60	1.80
Ketamine	19.73 (1.13)	1.86	3.94	25	0.92	0.59	1.77
Methadone	19.09 (0.63)	0.78	2.20	25	0.97	0.35	1.05
Cocaine	4.48 (0.15)	0.00	0.52	25	0.97	0.35	1.05

Five replicates for each concentration point: 0.12, 0.25, 0.5, 1 and 2 ng/mg in spiked hair samples.
[a] LOD = limit-of-detection = (3 S.D.$_{yx}$/slope).
[b] LOQ = limit-of-quantification = 3 LOD.
[c] Five replicates for each concentration point: 1, 2, 4 and 8 ng/mg. S.D.$_{yx}$ = standard deviation of the regression line; S.E. = standard error of the slope.

Table 3
Precision and accuracy of the HS-SPME–GC–MS procedure

Compound	Expected concentration (ng/mg)	Response ratio mean	Measured concentration (ng/mg)	CV% intra-day ($n = 5$)	Relative recovery (%)
A	4	7.31	4.22	19.15	105.50
MA	2	17.60	1.99	16.68	99.50
MDA	4	5.87	3.90	15.47	97.50
MDMA	2	30.34	1.92	16.65	96.00
MDE	2	48.54	1.95	16.97	97.50
MBDB	2	41.32	1.94	15.33	97.00
Ketamine	2	40.10	1.94	17.52	97.00
Methadone	2	38.20	1.96	7.37	98.00
Cocaine	2	9.12	2.03	7.97	101.50

shows the results obtained. When the amounts of compounds were below the LOQs, but greater than the LODs, the sample was considered detectable, but not quantifiable. We set the cut-off level = LOQ for the evaluation of the results; this value is consistent with the threshold considered suitable enough to rule out a false positive exposure or an accidental contamination [39].

The distribution of results of hair analysis is shown in Table 4. In 84/183 hair samples (46%), one or more substances were detected; 43 samples were drug-free. Fifty six samples (31%) were quantified for one or more substances: twenty tree specimens (41%) for one substance and 33 (59%) for two or more drugs. In the "one-substance" group ($n = 78$) cocaine was present in 94% of the cases versus 4% MDMA, 1% MA and 1% ketamine. In the "two or more-substances" group ($n = 62$) cocaine was present in 93% of specimens versus 87% MDMA, 26% ketamine and 18% MA. A, MA, MDE, MBDB and methadone were found in a minority of the samples and always with other substances. Cocaine proved to be the most popular recreational drug. Fig. 3 shows the distribution of 131 hair samples, either quantified for cocaine (≥LOQ) or in which cocaine was detected (≥LOD). The first column (0.35–1 ng/mg) groups samples in which cocaine was detected (≥LOD), but not quantified because below the LOQ (1.05 ng/mg). In 50 hair samples quantified for cocaine (concentration range: 1.35–100.00 ng/mg), a variable amount of lidocaine was identified. Lidocaine, a synthetic local anaesthetic, is often an adulterant in the preparation of substances such as cocaine and heroin. In Italy, the Group of Forensic Toxicologists reported lidocaine with increasing frequency in biological samples of people that died from drug use. The

Table 4
Results of hair analysis (total subjects: 183 (100%); drug-free: 43 (23%))

	Detected (≥LOD) 84 (46%)	Quantified (≥LOQ) 56 (31%)	Compounds detected ≥ LOD								
			A	MA	MDA	MDMA	MDE	MBDB	Ketamine	Methadone	Cocaine
One substance group	55 (65%)	23 (41%)	–	1 (1%)	–	3 (4%)	–	–	1 (1%)	–	73 (94%)
Two or more substances[a] group	29 (35%)	33 (59%)	1	11 (18%)	1	54 (87%)	1	3	16 (26%)	3	58 (93%)

[a] Many subjects were positive for two or more substances, the number of positive samples for each substance exceeding the total number of tested subjects.

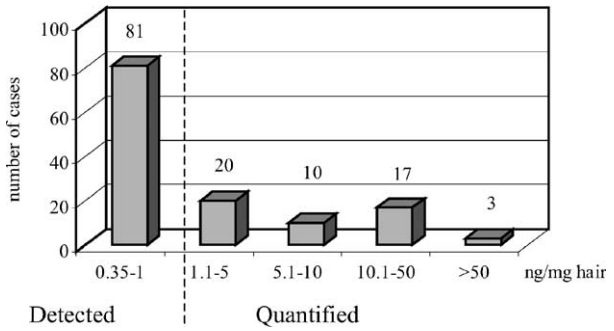

Fig. 3. Distribution of 131 hair samples, either quantified for cocaine (≥LOQ) or in which cocaine was detected (≥LOD). The number of subjects in each group is reported. Concentrations are not uniformly grouped due to the wide range of values considered.

interest in lidocaine analysis is growing and recently an HS-SPME–GC–MS method for the detection of lidocaine in hair has been developed [32]. Cocaethylene was singled out in our four positive real hair samples where cocaine concentration was greater than 10 ng/mg.

The performance of the method proposed hereby is a reasonable compromise between practicability and the capacity to simultaneously detect a variety of substances, cocaine included. This renders the method highly satisfactory for the intended field of application. The performance could be further improved by adopting the solid-phase dynamic extraction technique (SPDE), recently validated in some studies [40–42]. A new method combination, headspace solid-phase dynamic extraction coupled with gas chromatography–tandem mass spectrometry (HS-SPDE–GC–MS–MS) [40] sounds particularity interesting. This solventless extraction technique represents an advancement with respect to SPME, and allows repeated aspirate/dispense cycles. A better performance in terms of reliability, feasibility and total analysis time is obtained. This procedure uses deuterate standards and derivatization even in an automated manner, and was applied to hair analysis of many recreational drugs, not yet cocaine. The authors referred the main advantage of the SPDE technique, on respect of SPME, is the robustness of the capillary allowing more than twice the sampling possible with SPME. In our experience, yet, the fiber we used in HS-SPME had a very long life owing to the settled procedure did not need derivatives or fiber immersion. The same fiber was used throughout the study. The procedure we propose is simple, rapid and detects cocaine and many other abused drugs in a reliable, reproducible and simultaneous fashion, while most of the available tests cannot. Furthermore, some abused drugs, like ketamine, are not detectable by available immunological assays. Good linearity and precision were obtained for the compounds we considered. The proposed method showed a high benefit/cost ratio: minimising sample treatment and the number of analytical steps, it was able to detect most of the drugs used in recreational settings. A satisfactory compromise with respect to analytical parameters also allowed quali-quantitative detection of cocaine.

A preliminary application to the analysis of hair samples obtained from young people suggested that the procedure is suitable and feasible. It meets the requirements for quali-quantitative analysis of several compounds, and showed good potentiality in screening hair samples as well as other biological matrices. The proposed method could fill the gap between the lack of appropriate screening analytical tools and knowledge on the actual use of recreational drugs. Last but not least, it allows cocaine analysis by HS-SPME.

Acknowledgements

We gratefully acknowledge the Social Agencies “PARSEC”, “IL CAMMINO”, “LA TENDA”, “MAGLIANA 80”, for specimen collection.

References

[1] P. Griffiths, L. Vingoe, K. Jensen, J. Lewis, R. Harnoll, M. Nelson, Insights, EMCDDA (European Monitoring Centre for Drugs and Drug Addiction), Series, No. 1, November 1997.
[2] Ministry of Interior, Italian Focal Point for “Reitox” Project, Relazione Annuale per l’Osservatorio Europeo sulle Droghe e Tossicodipendenze, EMCDDA, 1995–1996.
[3] T. Macchia, A.M. Cioce, R. Mancinelli, Bollettino Farmacodipendenze ed Alcolismo XVII (1995) 16.
[4] EMCDDA (European Monitoring Centre for Drugs and Drug Addiction). Risk Assessment of Ketamine in the Framework of the Joint Action on Synthetic Drugs, 2001.
[5] EMCDDA. (European Monitoring Centre for Drugs Addiction). Annual on the State of the Drugs Problems in the European Union, 2002.
[6] R.N. Ehrman, S.J. Robbins, J.W. Cornish, Exp. Clin. Psychopharmacol. 5 (2) (1997) 150.
[7] D.L. Blank, D.A. Kidwell, Forensic Sci. Int. 63 (1993) 145.
[8] Ministry of Welfare-Social and Providential Policy Department, Annual Report to the Parliament on the State of the Addictions in Italy, 2002.
[9] J.D. Ramsey, M.A. Butcher, M.F. Murphy, T. Lee, A. Johnston, D.W. Holt, Br. Med. J. 323 (2001) 603.
[10] J.D. Ramsey, A. Johnson, D.W. Holt, Lancet 354 (1999) 2166.
[11] S. Gentili, A. Torresi, R. Marsili, M. Chiarotti, T. Macchia, J. Chromatogr. B 780 (2002) 183.
[12] Y. Nakahara, Forensic Sci. Int. 70 (1995) 135.
[13] S. Strano Rossi, M. Chiarotti, J. Anal. Toxicol. 23 (1999) 7.
[14] A.M. Bermejo, R. Seara, A.C. Dos Santos Lucas, M.J. Tabernero, P. Fernandez, R. Marsili, J. Anal. Toxicol. 24 (2000) 66.
[15] N.H. Snow, J. Chromatogr. A 885 (2000) 445.
[16] G. Theodoridis, E.H.M. Koster, G.J. De Jong, J. Chromatogr. B 745 (2000) 49.
[17] P. Kintz, V. Cirimele, A. Tracqui, P. Mangin, J. Chromatogr. B 670 (1995) 162.
[18] M.R. Moeller, Ther. Drug. Monit. 18 (1996) 444.
[19] R. Kikura, Y. Nakahara, T. Mieczkowski, F. Tagliaro, Forensic Sci. Int. 84 (1997) 165.
[20] M. Rothe, F. Pragst, K. Spiegel, T. Harrach, K. Fischer, J. Kunkel, Forensic Sci. Int. 89 (1997) 111.
[21] J. Röhrich, G. Kourt, Forensic Sci. Int. 84 (1997) 179.
[22] P. Kintz, V. Cirimele, Forensic Sci. Int. 84 (1997) 151.
[23] H. Sachs, P. Kintz, J. Chromatogr. B 713 (1998) 147.
[24] P. Kintz, N. Samyn, J. Chrmatogr. B 733 (1999) 137.

[25] C. Jurado, M.P. Gimenez, T. Soriano, M. Menendez, M. Repetto, J. Anal. Toxicol. 24 (2000) 11.
[26] O. Quintela, A.M. Bermejo, M.S. Tebernero, S. Strano-Rossi, M. Chiarotti, A.C.S. Lucas, Forensic Sci. Int. 107 (2000) 273.
[27] R. Wennig, Forensic Sci. Int. 107 (2000) 5.
[28] M. Yashiki, T. Kojima, T. Miyazaki, N. Nagasawa, Y. Iwasaki, K. Hara, Forensic Sci. Int. 76 (1995) 169.
[29] I. Koide, O. Noguchi, K. Okada, A. Yokoyama, H. Oda, S. Yamamoto, J. Chromatogr. B 707 (1998) 99.
[30] F. Sporkert, F. Pragst, Forensic Sci. Int. 107 (2000) 129.
[31] L. Junting, C. Peng, Forensic Sci. Int. 97 (1998) 93.
[32] F. Sporkert, F. Pragst, J. Anal. Toxicol. 24 (2000) 316.
[33] L. Williams, H. Parker, Int. J. Drug Policy 12 (2001) 397.
[34] D.A. Kidwell, M.A. Blanco, F.P. Smith, Forensic Sci. Int. 84 (1997) 75.
[35] P. Kintz, P. Mangin, Forensic Sci. Int. 70 (1995) 3.
[36] J.C. Miller, J.N. Miller, in: E. Horwood (Ed.), Statistics for Analytical Chemistry, second ed., 1988, Ellis Horwood Ltd. Publishers, Chichester, UK, p. 115 (Chapter 5).
[37] US Pharmacopoeial Convention (1995), United States Pharmacopoeia 23/National Formulary 18, Rockville, MD, 1982, p. 1225.
[38] R.V. Alvencar, S. Sidhom, T.U. Okarter, Drug Dev. Ind. Pharm. 25 (1999) 929.
[39] W. Baumgartner, in: P. Kintz (Ed.), Drug Testing in Hair, CRC Press, Boca Raton, Florida, 1995, p. 223.
[40] D.W. Lachenmeir, L. Kroener, F. Musshoff, B. Medea, Rapid Common Mass Spectrum 17 (2003) 472.
[41] F. Musshoff, D.W. Lachenmeier, L. Kroener, B. Madea, J. Chromatogr. A 958 (2002) 231.
[42] A. Namera, M. Yashiki, T. Kojima, M. Ueki, J. Chromatogr. Sci. 40 (2002) 19.