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Simultaneous detection of amphetamine-like drugs with headspace solid-phase microextraction and gas chromatography–mass spectrometry

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Abstract

A headspace solid-phase microextraction and gas chromatography–mass spectrometry (HS-SPME–GC–MS) procedure for the simultaneous detection of methylen-dioxyamphetamine (MDA), methylen-dioxymethamphetamine (MDMA), methylen-dioxyethamphetamine (MDE) and *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB) in hair has been developed. This method is suitable for the separation of primary and secondary amines, is reproducible, is not time consuming, requires small quantities of sample and does not require any derivatization. It provides sufficient sensitivity and specificity, with limits of detection (LOD) and limits of quantitation (LOQ) for each substance of <0.7 and 1.90 ng/mg, respectively. Intra- and inter-day precision were within 2 and 10%, respectively. This method is suitable for routine clinical, epidemiological and forensic purposes and can be used for the preliminary screening of many other substances (amphetamine, methamphetamine, ketamine, ephedrine, nicotine, phencyclidine, methadone) in hair and other biological matrices such as saliva, urine and blood. We also describe the first application of this HS-SPME–GC–MS procedure to the analysis of hair and saliva samples from young people attending a disco in the Rome area. All positive hair samples were confirmed by the gas chromatography–mass–mass ($GC–MS^2$) technique in positive chemical ionization (PCI) mode. Some examples of the use of the method in detecting different drugs are reported.

Keywords: Amphetamine-like drugs

1. Introduction

Many countries have observed increasing use of amphetamines and amphetamine-like drugs, especially variations known as ‘‘designer drugs’’ [1–3]. These substances are in great demand, mainly among young people, because they enhance empathy and produce hallucinogenic effects. While the issue of recreational drug use is of great interest, analytical tools are lacking.

Recently, the EMCDDA (European Monitoring Centre for Drugs and Drug Addiction), promoting an Early Warning System in the 15 EU countries,

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suggested paying increased attention to amphetamine-like drugs as well as to new abused substances such as ketamine. The abuse of ketamine has been recorded since 1998 in Italy [4] and recently this substance has been scheduled among the illicit drugs [5].

Hair analysis is frequently used for the long-term monitoring of drug users and, in recent years, headspace solid-phase microextraction (HS-SPME) has assumed an increasing role in hair testing [6–10]. This new extraction technique has a number of advantages, such as simplicity, rapidity and high purity of the extract, producing clear chromatograms. Moreover, it can be used successfully for a variety of matrices. However, some problems still have to be solved, such as recovery and reproducibility for some drugs.

The present paper describes a headspace solid-phase microextraction and gas chromatography–mass spectrometry (HS-SPME–GC–MS) assay for the detection of methylen-dioxyamphetamine (MDA), methylen-dioxymethamphetamine (MDMA), methylen-dioxyethamphetamine (MDE), and *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB) in hair. Furthermore, a preliminary application to the qualitative detection of many other abused substances [amphetamine (A), methamphetamine (MA), ketamine, ephedrine, nicotine, phencyclidine, methadone] in hair and saliva samples is reported.

2. Materials and methods

2.1. Chemicals

MDA, MDMA, MDE, MBDB, A, and MA hydrochloride (1 mg/ml in methanol) standards were purchased from Sigma–Aldrich (Milan, Italy). 3,4-Methylen-dioxypropylamphetamine (MDPA) hydrochloride standard (1 mg/ml in methanol) and ephedrine, phencyclidine, methadone and nicotine were purchased from SALARS (Como, Italy). Ketamine (Ketavet 50) was obtained from Farmaceutici Gellini (Aprilia, Italy). Ultrapure water was obtained from a Milli-Q Unit (Millipore, Bedford, MA, USA). Hydrochloric acid and sodium hydroxide of analytical grade were purchased from Carlo Erba (Milan, Italy).

2.2. Equipment

Headspace vials (20 ml) and accessories were obtained from Chromacol (London, UK). Hair washing and ultrasonic extraction were performed in a T 310 ultrasonic bath (Carlo Erba). Oral fluid collectors were obtained from Cozart Bioscience (Oxfordshire, UK). A SPME assembly with a replaceable extraction fibre, coated with 100 μm polydimethylsiloxane, a Liner HP-2-6375,05, and a 110 VAC block heater were purchased from Sigma–Aldrich.

2.3. Specimens

Hair and saliva samples were obtained on a voluntary basis and in an anonymous way from 27 young people attending a disco in the Rome area (18 males and nine females, mean age 22±5 years). Hair was cut with scissors from the vertex posterior region of the scalp; saliva was collected by a device consisting of a swab inserted into a tube containing a buffer. Blank hair and saliva samples were obtained in the same manner 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 on each analytical day.

2.4. Sample preparation

Each hair sample was cut into small pieces, washed for 5 min with deionized water and then for 5 min with acetone in an ultrasonic bath. Washed samples were dried under a nitrogen stream at room temperature. The saliva samples did not require any treatment.

2.5. Calibration curve

Stock solutions of the standards (100 μg/ml MDA, MDMA, MDE, MBDB) as well as 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 0.5, 1, 2, 4, 8, 16, 20 and 24 ng/mg. MDPA (10 ng/mg) was used as internal standard.

2.6. Analytical procedure

The washed hair sample (50 mg), 50 μl of the internal standard solution (10 μg/ml, corresponding to 10 ng/mg of hair) and 950 μl of 30% sodium hydroxide were placed in a glass vial (20 ml volume). The vial was rapidly sealed and heated at 70 °C for 20 min. The compounds in the headspace were adsorbed by the extraction fiber in the needle of the SPME device for 5 min at 70 °C.

The saliva sample (50 μl), 50 μl of internal standard solution (10 μg/ml) and 900 μl of 30% sodium hydroxide were placed in a glass vial (20 ml volume). The vial was rapidly sealed and heated at 70 °C for 20 min. The compounds in the headspace were adsorbed by the extraction fiber in the needle of the SPME device for 5 min at 70 °C.

2.7. Gas chromatography–mass spectrometry parameters

A Gas Chromatograph 6890 Plus, and a Mass Selective Detector 5973N (Agilent Technologies, Milan, Italy), equipped with a 30 m×0.25 mm HP-5 trace analysis column (5% PH ME Siloxane, film thickness 0.25 μm), were used. The column temperature was held initially at 60 °C for 2 min, then raised to 250 °C at 20 °C/min 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 detector operated in the selective ion monitoring (SIM) mode. Compounds were identified by their retention times and the relative abundance of three confirming ions with respect to the target. The monitored ions, retention times and relative abundance for each compound are shown in Table 1.

Table 1
Detection of amphetamine-like drugs in spiked hair samples by HS-SPME–GC–MS

Compound	R_t (min)	Ion m/z (relative abundance)
MDA	9.11	44 (100), 136 (44), 135 (44)
MDMA	9.45	58 (100), 77 (27), 135 (29)
MDE	9.74	72 (100), 44 (27), 135 (10)
MBDB	10.00	72 (100), 44 (10), 135 (10)
MDPA (I.S.)	10.26	86 (100), 44 (14), 135 (14)

2.8. Confirmatory analysis (PCI GC–MS^2 technique)

Confirmatory analysis of positive hair samples was carried out using a Thermoquest Trace GC apparatus coupled to a Polaris Q mass spectrometer (ion trap) (Thermoquest Italia, Milan, Italy).

Hair samples were treated as previously described in Analytical procedure and analysed under the following conditions. A capillary column HP-5 trace analysis (12 m×0.2 mm I.D., film thickness 0.33 μm, 5% phenyl methyl silicone) was used. The column temperature was initially held at 40 °C for 1.5 min, raised to 120 °C in linear increments of 40 °C/min, held at 120 °C for 0.5 min, and then raised to 270 °C at 40 °C/min with a final 5-min isotherm. The injector port was set at 240 °C. The splitless injection mode was used. Helium was used as carrier gas at a constant flow-rate of 1 ml/min.

Mass–Mass detection of MA, MDA, MDMA and ketamine was performed in positive chemical ionization mode (using methane as reagent gas at a flow-rate of 1.2 ml/min), isolating the parent ion (corresponding to the protonated molecular ion [M+1] for each target compound) and monitoring the products ions.

The main parameters related to the mass spectrometer settings can be summarized as follows:

- source temperature: 200 °C;
- scan event 1 (MS fragmentation): microscans, 3; maximum ion time, 25; scan mode, full scan;
- scan event 2 (MS–MS fragmentation): microscans, 3; maximum ion time, 25; scan mode, MS–MS; isolation time, 8; collision energy, 1.00; collision time, 15.

Table 2
Selected ions and retention times for PCI GC–MS^2

Compound	R_t (min)	Precursor ion m/z	Product ion m/z (relative abundance)
MA	4.51	150	91 (100), 119 (40)
MDA	6.86	180	163 (100), 135 (18), 133 (12)
MDMA	7.52	194	163 (100), 133 (22), 135 (18)
MDPA (I.S.)	8.54	222	163 (100), 135 (33), 133 (28)
Ketamine	10.04	238	220 (100), 207 (10)

The above scanning events were repeated over five analytical segments during the analysis based on the retention times of the target compounds, as summarized in Table 2.

With our conditions, a limit of quantitation (LOQ) of 1 ng/mg was achieved. The limit of quantitation was estimated from the signal-to-noise ratio of about 10:1 in the analysis of spiked samples.

3. Results and discussion

HS-SPME coupled with digestion of hair with 30% sodium hydroxide is a very advantageous procedure because dissolution of the hair matrix can be combined with a satisfactory extraction in the same vial without additional treatment or derivatization.

Table 3
Linearity of the HS-SPME–GC–MS procedure in the concentration range 2–24 ng/mg

	Slope, b (SE)	Intercept, a (ng/mg)	$S_{y\cdot x}$	n^a	r^2	LOD (ng/mg)	LOQ (ng/mg)
MDA	1.92 (0.04)	5.40	1.83	30	0.99	3.14	9.43
MDMA	7.18 (0.06)	−2.43	2.61	30	0.99	1.19	3.59
MDE	12.59 (0.09)	−11.02	4.49	30	0.99	1.17	3.53
MBDB	10.79 (0.14)	−15.73	6.27	30	0.99	1.92	5.75

$S_{y\cdot x}$, standard deviation of the regression line; SE, standard error of the slope; LOD, limit of detection ($3.3\cdot S_{y\cdot x}$/slope); LOQ, limit of quantification (3·LOD).

[a] Five replicates for each concentration point: 2, 4, 8, 16, 20 and 24 ng/mg in spiked hair samples.

Table 4
Linearity of the HS-SPME–GC–MS procedure in the concentration range 0.5–4 ng/mg

	Slope, b (SE)	Intercept, a (ng/mg)	$S_{y\cdot x}$	n^a	r^2	LOD (ng/mg)	LOQ (ng/mg)
MDA	2.81 (0.41)	1.08	0.53	20	0.90	0.62	1.86
MDMA	12.48 (1.32)	−1.67	1.72	20	0.94	0.45	1.35
MDE	20.21 (2.27)	0.78	2.98	20	0.93	0.48	1.44
MBDB	18.98 (1.93)	−3.43	2.53	20	0.94	0.43	1.29

$S_{y\cdot x}$, standard deviation of the regression line; SE, standard error of the slope; LOD, limit of detection ($3.3\cdot S_{y\cdot x}$/slope); LOQ, limit of quantification (3·LOD).

[a] Five replicates for each concentration point: 0.5, 1, 2 and 4 ng/mg in spiked hair samples.

Our procedure was set up for MDA, MDMA, MDE, and MBDB in hair. Good linearity throughout the explored range characterized the procedure for all drugs considered. The main parameters of the linear regression analysis, together with the sensitivity and specificity, are shown in Tables 3 and 4. The procedure is also suitable for analyzing biological samples such as saliva.

The first test over a wide concentration (2–24 ng/mg) range showed limits of detection (LOD) and limits of quantitation (LOQ) for each substance ranging between 1 and 4 ng/mg and between 3 and 10 ng/mg, respectively (Table 3). However, these values are not suitable for the routine analyses of hair, since they are higher than those reported in the literature for a more complex procedure [11–26]. Therefore, we decided to explore lower concentrations (0.5–4 ng/mg) and obtained a more acceptable sensitivity, with a LOD and LOQ of <0.7 and 1.9 ng/mg, respectively (Table 4). Our LOD and LOQ were calculated according to Miller et al. [27] as reported in Tables 3 and 4. Furthermore, they are in agreement with suggestions of the U.S. Pharmacopoeial Convention [28].

An example of a SIM chromatogram of a 50 mg hair sample spiked with MDA, MDMA, MDE and MBDB at a final concentration of 4 ng/mg is shown in Fig. 1. The peaks of the compounds appear well resolved, confirming that the chromatographic conditions used are appropriate for the separation of all these amines without derivatization.

The parameters described in the literature [7,10] as being relevant for the HS-SPME of hair are alkaline digestion, the extraction temperature, the adsorption time in the headspace by the extraction fiber and thermal desorption inside the gas chromatograph, and, according to Jurado et al. [24], these factors account for the reproducibility, sensitivity and recovery in biological samples.

Under our experimental conditions we obtained a satisfactory extraction of analytes by performing the hydrolysis with 30% sodium hydroxide solution. Fifty milligrams of hair were used. The viscosity of the hydrolysate was greater when compared with the 10 mg suggested by many authors as being more suitable in terms of methodological efficacy. In spite of this, the use of 30% sodium hydroxide, an incubation time of 20 min and a larger volume reaction vial (20 ml) in our procedure gave a satisfactory accuracy and a better reproducibility than the use of the experimental conditions referred to above. In any case, when applied to smaller samples, the procedure was sufficiently robust and sensitive to identify all of the drugs examined (data not shown).

Table 5 shows the accuracy expressed as the

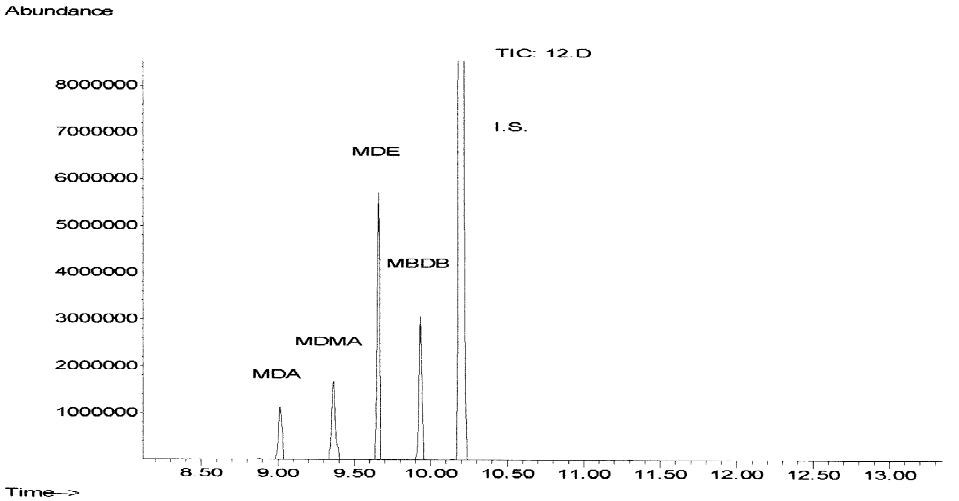

Fig. 1. GC–MS-SIM chromatogram of a 50 mg hair sample spiked with 4 ng/mg MDA, MDMA, MDE and MBDB and 10 ng/mg MDPA as I.S. SIM measurement using the masses given in Table 1.

Table 5
Precision and accuracy of the HS-SPME–GC–MS procedure

Compound	Expected conc. (ng/mg)	Response ratio[a] Mean ($n=15$)	Measured conc. (ng/mg) ($n=15$)	C.V. (%)		Relative recovery (%)
				Intra-day ($n=5$)	Inter-day ($n=15$)	
MDA	4	13.95	4.45	2.57	7.13	111.25
	8	19.61	7.40	2.77	6.53	92.51
	16	35.71	15.78	2.00	7.55	98.62
MDMA	4	27.47	4.16	2.83	6.82	104.00
	8	48.77	7.13	2.98	7.01	89.12
	16	112.65	16.02	1.54	8.39	100.12
MDE	4	36.81	3.79	1.62	4.31	94.75
	8	82.80	7.45	2.66	9.13	93.12
	16	191.37	16.07	1.54	8.39	100.43
MBDB	4	28.38	4.08	3.56	5.34	102.00
	8	62.81	7.27	2.98	5.44	90.87
	16	147.05	15.08	2.47	7.47	94.25

[a] The response ratio is the area derived from the total ionic current of a single compound in relation to the total ionic current of the internal standard.

relative recovery according to Kintz [16] and Jurado [24], and the intra- and inter-day precision evaluated for measurements on three consecutive days with five replicates for each concentration (at 4, 8 and 16 ng/mg MDA, MDMA, MDE and MBDB). As MDPA was used as the I.S. for all of the analytes, the relative recovery (the peak areas ratio versus the I.S.) was measured. This was reproducible and comprised between 89 and 112% when 15 replicates of each substance were analysed.

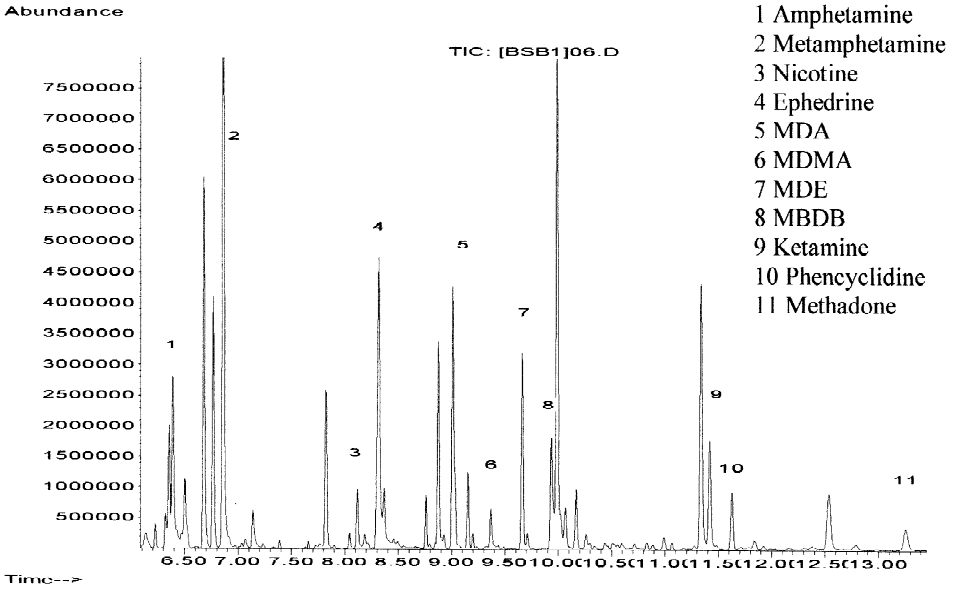

Fig. 2. HS-SPME–GC–MS-SCAN chromatogram of an aqueous solution spiked with 100 ng/ml amphetamine, metamphetamine, nicotine, ephedrine, MDA, MDMA, MDE, MBDB, ketamine, phencyclidine and methadone.

The parameters chosen for the extraction temperature (70 °C for 20 min), for the adsorption time in the headspace by the extraction fiber (5 min at 70 °C), and for thermal desorption inside the gas chromatograph (3 min) all provide an acceptable compromise between the sensitivity and efficiency of the method (data not shown). The analytical characteristics and reliability also make the procedure suitable for clinical and epidemiological purposes, mainly where regular drug abuse is suspected.

Encouraging results were obtained in preliminary tests when this procedure was applied to hair and saliva analysis for other drugs such as amphetamine, methamphetamine, nicotine, ephedrine, ketamine, phencyclidine and methadone (Figs. 2 and 3).

On the contrary, we were unable to detect Δ^9-tetrahydrocannabinol (THC), cannabinol and cannabidiol. According to other authors [10], these compounds require further pre-analytical steps, such as pH adjustment.

Table 6 shows the retention times and the ions monitored for the detection for each compound. As can be seen, the single step HS-SPME–GC–MS procedure, under the described conditions, offers a great advantage in the screening of many drugs in one run.

To check the procedure, it was applied to samples obtained from young people attending a disco in the Rome area. Twenty-seven subjects took part in this study. They were informed of the analytical methodology and were guaranteed anonymity for the study. They were asked to provide a sample of hair, collected by round point scissors, which was placed in a paper bag. When hair was not available, a sample of saliva was collected using a swab. We obtained 20 hair samples and seven saliva samples. For ethical reasons, each subject labeled his own sample with a code known only to himself. This approach, while respecting privacy, allowed each subject to single out his own data from the overall list of analytical results.

Table 6
List of other drugs detected by the HS-SPME–GC–MS procedure. Selected ions and retention times

Compound	R_t (min)	Ion m/z
Amphetamine	6.50	44, 91, 65
Metamphetamine	6.97	58, 91, 77
Nicotine	8.23	84, 133, 161
Ephedrine	8.53	58, 77, 105
Ketamine	11.47	180, 182, 209
Phencyclidine	11.66	200, 242, 243, 91
Methadone	13.20	72, 91, 223

Three hair samples (15%) were positive for ketamine (estimated concentrations 30–40 ng/mg) and one of these (5%) was also positive for MDMA at a concentration of 2.3 ng/mg. Fig. 4 refers to the hair sample positive both for ketamine and MDMA.

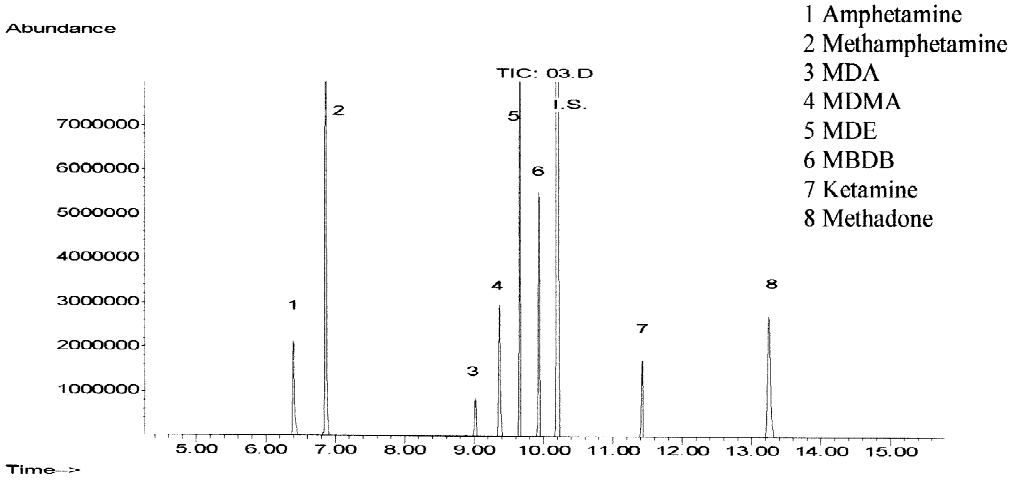

Fig. 3. HS-SPME–GC–MS-SIM chromatogram of a hair sample spiked with 10 ng/mg amphetamine, metamphetamine, MDA, MDMA, MDE, MBDB, ketamine and methadone.

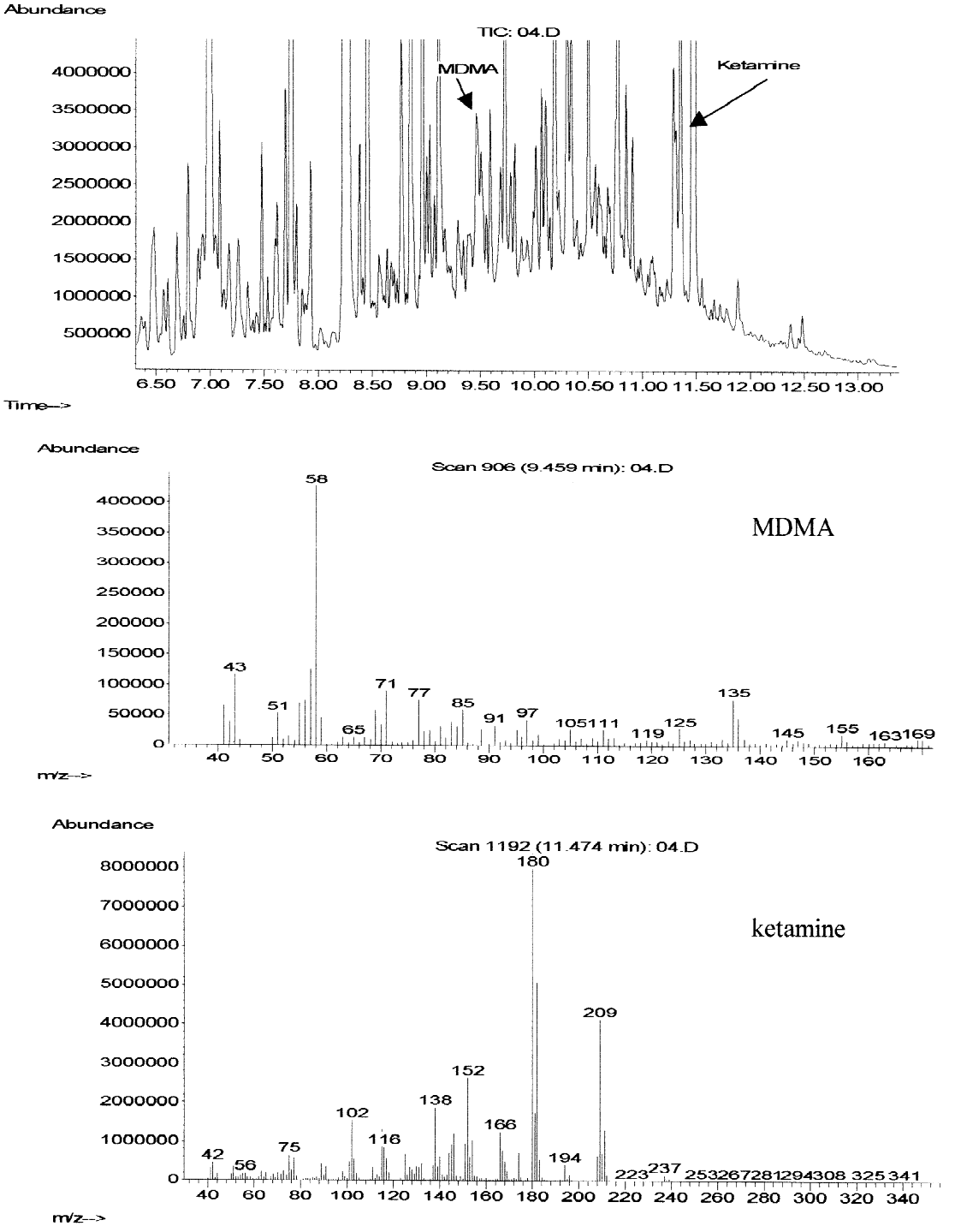

Fig. 4. HS-SPME–GC–MS-SCAN chromatogram (top) and relative mass spectrum (bottom) of a real hair sample positive for MDMA (2.3 ng/mg) and ketamine.

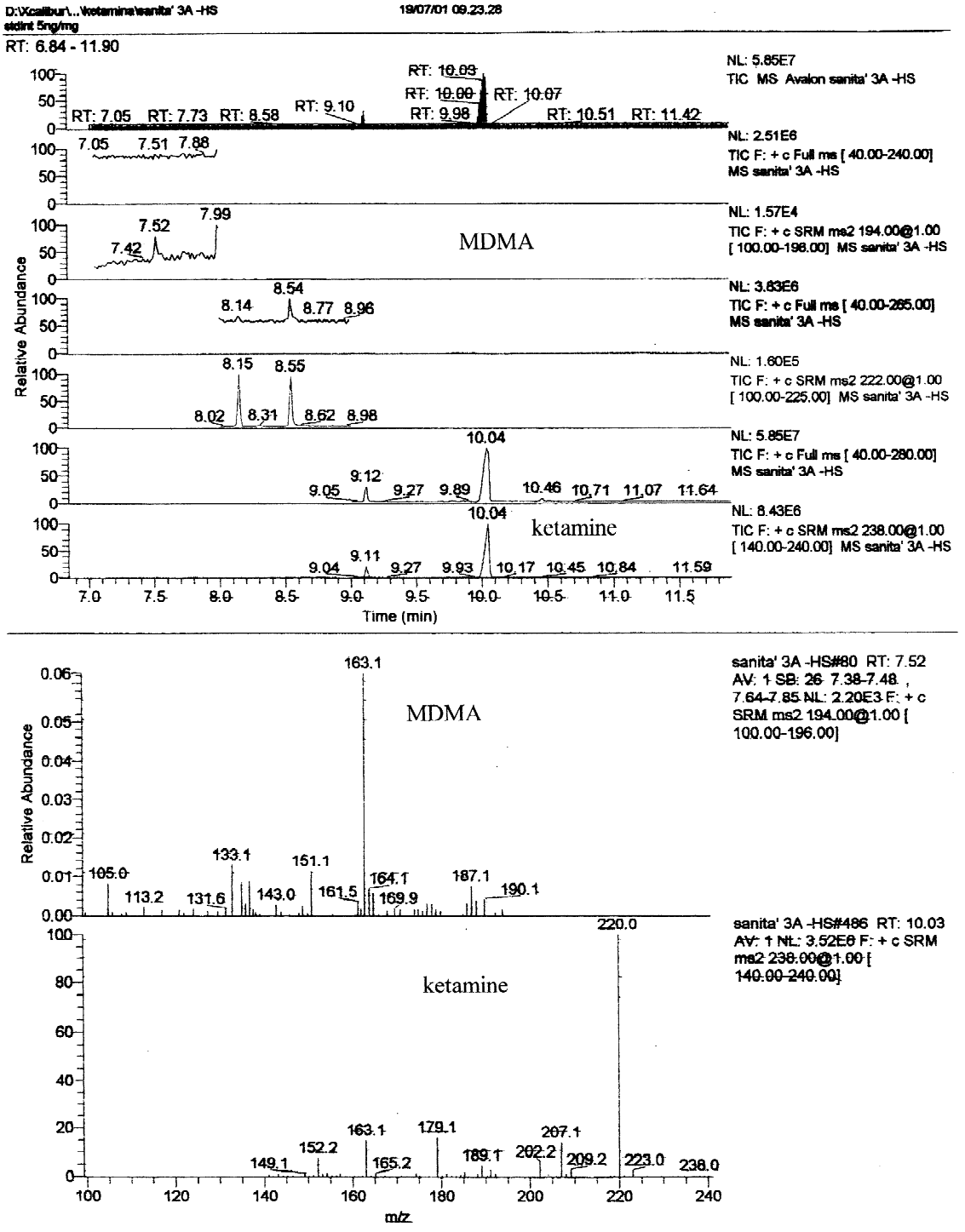

Fig. 5. Confirmatory PCI GC–MS^2 analysis carried out on one of the real hair samples positive for the presence of MDMA and ketamine. Upper panel: MDMA, with a retention time of 7.52 (monitoring the product ion which results from the precursor ion m/z 194). Ketamine, with a retention time of 10.04 (monitoring the product ion which results from the precursor ion m/z 238). Lower panel: the SRM-MS^2 mass spectra for MDMA and ketamine, respectively, obtained using our experimental conditions.

When we tested the seven saliva samples, six of them (86%) were positive for ketamine, with only one also positive for MA, MDMA and MDA. The decreasing use of MDMA among young people in the Rome area partly accounts for the few samples positive for this drug.

Our procedure was established for hair analysis, therefore only the positive hair samples were confirmed using the PCI GC–MS^2 technique with a LOQ of 1 ng/mg. Fig. 5 shows chromatograms and MS fragmentation spectra obtained during confirmatory analysis of a positive hair sample.

It should be noted that the proposed HS-SPME–GC–MS procedure also allows easy qualitative detection of ketamine. This feature is very useful, as the increasing abuse of ketamine has long been suspected, though never proven, because appropriate analytical procedures for routine and epidemiological purposes were lacking.

The proposed HS-SPME–GC–MS procedure also allows detection of a considerable number of amphetamine-like drugs which most of the available screening tests cannot detect. Diagnostics and research can gain advantage from this.

The small number of samples tested in this first methodological application does not allow an epidemiological analysis. Nevertheless, this procedure represents a suitable tool for the simple, rapid, reliable and reproducible detection of many abused drugs in only one test run. This procedure could easily be applied to both biological and mineral matrices to gain information on what is currently available on the illicit market, independent of what the users believe [29].

Our preliminary data on the application of the proposed procedure to real samples suggest that ketamine could represent an emerging problem to be dealt with in the health and analytical fields. The ever-increasing habit among opiate addicts of using ketamine as an occasional substitute for heroin, and the widespread use of this substance for recreational purposes, support this hypothesis.

It was not within the scope of this study to address all current, unresolved analytical problems. The main purpose was to achieve a cost–benefit equilibrium between reliability and practicability while minimizing sample manipulation and the number of analytical steps. All these represent practical advantages for new drug abuse studies.

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References

[1] P. Griffiths, L. Vingoe, K. Jensen, J. Lewis, R. Harnoll, M. Nelson, Insights, EMCDDA Series, No. 1, November 1997.
[2] Ministry of Interior, Italian Focal Point for the ''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 l'Alcolismo XVII (1995) 16.
[4] EMCDDA (European Monitoring Centre for Drug Addiction), Risk assessment of ketamine in the framework of the joint action on synthetic drugs (manuscript in preparation).
[5] D.M. 8 febbraio 2001, Gazzetta Ufficiale 22 febbraio 2001, No. 44.
[6] M. Yashyki et al., Forensic Sci. Int. 76 (1995) 169.
[7] I. Koide et al., J. Chromatogr. B 707 (1998) 99.
[8] F. Sporkert, F. Pragst, Forensic Sci. Int. 107 (2000) 129.
[9] J. Liu et al., J. Chromatogr. B 758 (2001) 95.
[10] F. Sporkert, F. Pragst, J. Anal. Toxicol 24 (2000) 316.
[11] Y. Nakahara, Forensic Sci. Int. 70 (1995) 135.
[12] S. Strano Rossi, M. Chiarotti, J. Anal. Toxicol. 23 (1999) 7.
[13] A.M. Bermejo et al., J. Anal. Toxicol. 24 (2000) 66.
[14] N.H. Snow, J. Chromatogr. A 885 (2000) 445.
[15] G. Theodoridis et al., J. Chromatogr. B 745 (2000) 49.
[16] P. Kintz et al., J. Chromatogr. B 670 (1995) 162.
[17] M.R. Moeller, Ther. Drug Monit. 18 (1996) 444.
[18] R. Kikura et al., Forensic Sci. Int. 84 (1997) 165.
[19] M. Rothe et al., Forensic Sci. Int. 89 (1997) 11.
[20] J. Röhrich, G. Kourt, Forensic Sci. Int. 84 (1997) 179.
[21] P. Kintz et al., Forensic Sci. Int. 84 (1997) 151.
[22] H. Sachs, P. Kintz, J. Chromatogr. B 713 (1998) 147.
[23] P. Kintz, N. Samyn, J. Chromatogr. B 733 (1999) 137.
[24] C. Jurado et al., J. Anal. Toxicol. 24 (2000) 11.
[25] O. Quintela et al., Forensic Sci. Int. 107 (2000) 273.
[26] R. Wennig, Forensic Sci. Int. 107 (2000) 5.
[27] J.C. Miller, J.N. Miller, in: E. Horwood (Ed.), Statistics for Analytical Chemistry, 1988, p. 115, Chapter 5.
[28] US Pharmacopoeial Convention (1995), United States Pharmacopoeia 23/National Formulary 18, Rockville, MD (1225), 1982.
[29] J.D. Ramsey, M.A. Butcher, M.F. Murphy, T. Lee, A. Johnston, D.W. Holt, Br. Med. J. (2001) 323.