RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2005; **19**: 1649–1656
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.1970

RCM

Quantification of the plant-derived hallucinogen Salvinorin A in conventional and non-conventional biological fluids by gas chromatography/mass spectrometry after *Salvia divinorum* smoking

Simona Pichini[1]*, Sergio Abanades[2,3], Magí Farré[2,3], Manuela Pellegrini[1], Emilia Marchei[1], Roberta Pacifici[1], Rafael de la Torre[2,4] and Piergiorgio Zuccaro[1]

[1]Drug Research and Evaluation Department, Istituto Superiore di Sanità. Rome, Italy
[2]Institut Municipal d'Investigació Mèdica (IMIM), Barcelona, Spain
[3]Universitat Autònoma, Barcelona, Spain
[4]Universitat Pompeu Fabra, Barcelona, Spain

Received 23 February 2005; Revised 12 April 2005; Accepted 12 April 2005

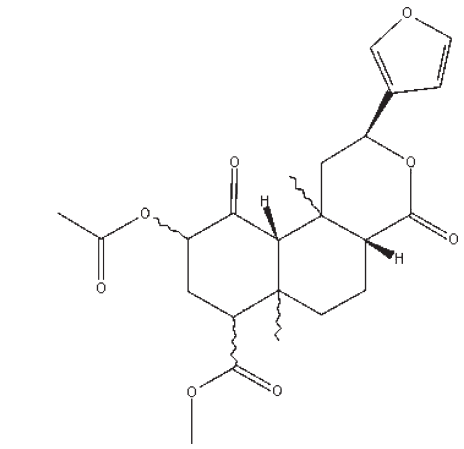
A gas chromatography method with mass spectrometric detection is described for the determination of Salvinorin A, the main active ingredient of the hallucinogenic mint *Salvia divinorum*. The method was validated in plasma, urine, saliva and sweat using 17-α-methyltestosterone as internal standard. The analytes were extracted from biological matrices with chloroform/isopropanol (9:1, v/v). Chromatography was performed on a 5% phenyl methyl silicone capillary column and analytes were determined in the selected ion monitoring mode. The method was validated over the concentration range 0.015–5 μg/mL plasma, urine and saliva and 0.01–5 μg/patch in the case of sweat. Mean recoveries ranged between 77.1 and 92.7% for Salvinorin A in different biological matrices, with precision and accuracy always better than 15%. The method was applied to the analysis of urine, saliva and sweat from two consumers after smoking 75 mg plant leaves to verify the presence of the active ingredient of *S. divinorum* in human biological fluids as a biomarker of plant consumption. Salvinorin A was detected in urine (2.4 and 10.9 ng/mL) and saliva (11.1 and 25.0 ng/mL), but not in sweat patches from consumers. Copyright © 2005 John Wiley & Sons, Ltd.

Salvia divinorum is a member of the mint family that has been used for centuries by the Mazatec people of Oaxaca, Mexico, in traditional medico-religious ceremonies. The plant is known to the Mazatec Indians as 'ska pastora' or 'ska Maria pastora', and more recently as 'magic mint'.[1,2] The abusers primarily consist of young adults and adolescents who frequent 'smart shops' (now spreading in Europe and selling hemp food, dietary supplements, plant extracts with supposed nutritional and health benefits) or internet websites promoting the drug.

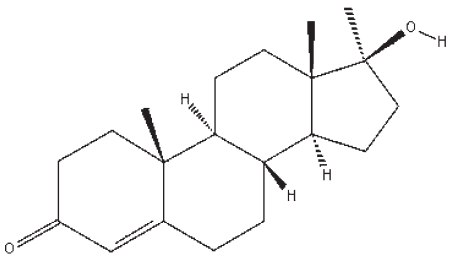
At present, in almost all countries, the use of *S. divinorum* is not banned because neither the plant nor or any of its constituents is listed in the controlled substances lists. In summer 2004, the plant and its main active principle, Salvinorin A, were added to Lists of Controlled Illicit Substances in Italy and sales are prohibited in Spain.[3,4] Abusers ingest the plant using various methods of administration. Fresh leaves can be chewed, or brewed and ingested after the preparation of a tea; dried leaves are chewed or smoked.[5] When converted into a liquid extract, *S. divinorum* can also be vaporized and inhaled.[6] Immediately after ingesting the drug, abusers typically experience vivid hallucinations and potent and intense hallucinatory effects, which, differently from other hallucinogens like LSD and PCP, last for up to an hour or less. High doses of the drug can cause unconsciousness and short-term memory loss.

Salvinorin A (Fig. 1), the main active ingredient of *S. divinorum*, was first identified by Ortega and isolated by Valdes in 1982.[7,8] Salvinorin A is a neoclerodane diterpene and appears to be the only known psychoactive terpenoid of *S. divinorum*.[9] It has been reported as a potent, naturally occurring hallucinogen acting as a full agonist on the kappa opioid receptor.[10] To our knowledge, there are only few published descriptions of the effects of *S. divinorum*. Siebert[6] described the pharmacological effects that occurred after chewing herb leaves or by vaporizing and inhaling pure Salvinorin A. Profound hallucinations were experienced 5–10 min after chewing leaves with Salvinorin A concentrations ranging from 0.89–3.70 mg/g dry weight, and 30 s after inhalation of 200–500 μg pure substance. However, pharmacological

*Correspondence to: S. Pichini, Drug Research and Control Department, Istituto Superiore di Sanità', V.le Regina Elena 299, 00161 Rome, Italy.
E-mail: pichini@iss.it
Contract/grant sponsor: Presidenza del Consiglio dei Ministri, Dipartimento Nazionale per le politiche antidroga, and FIS (G03/005).



Salvinorin A



17-α-methyltestosterone (IS)

Figure 1. Chemical structures of Salvinorin A and 17-α-methyltestosterone.

effects were not associated with the presence of the psychoactive Salvinorin A in biological matrices.

This paper reports the development and validation of a sensitive and selective analytical method to determine Salvinorin A in conventional (plasma and urine) and non-conventional (saliva and sweat) biological matrices. The validated method was used to investigate the presence of the active substance in biological fluids of individuals who had consumed dry plant leaves.

EXPERIMENTAL

Materials

The pure standard of Salvinorin A (Fig. 1) was provided as a powder by Prof. Claudio Medana (Department of Analytical Chemistry, Torino University, Torino, Italy). 17-α-Methyltestosterone, which was used as internal standard (IS), was from Sigma (St. Louis, MO, USA). The PharmChek® sweat patches were provided by PharmChem Laboratories (Menlo Park, CA, USA). The patches consisted of a medical-grade cellulose blotter paper collection pad, with an area of 15.4 cm^2, covered by a thin layer of polyurethane and acrylate adhesives. Ultrapure water and all other reagents of analytical grade were obtained from Carlo Erba (Milan, Italy).

Biological samples

Drug-free biological samples (blood, urine, saliva and sweat) were obtained from healthy donors. Blood was immediately centrifuged and the obtained plasma was pooled, aliquoted into 1 mL plastic tubes, and stored at −20°C. Sweat was collected by sweat patches applied to the back of healthy donors, after the skin had been cleaned with a 70% isopropyl alcohol swab, and removed 2 h post-application. Saliva was obtained without any stimulation by spitting into polypropylene tubes; 5 mL of saliva were obtained from each volunteer. Saliva samples were pooled, aliquoted into 1 mL plastic tubes, and stored at −20°C. Different amounts of urine (100–400 mL) were obtained from healthy donors. These samples too were pooled, aliquoted into 1 mL plastic tubes, and stored at −20°C until analysis.

Dry leaves of *S. divinorum* were obtained in a 'Smart Shop' before legal prohibition.

Two subjects with previous experience in the use of hallucinogens (including *Salvia divinorum*) agreed to donate biological samples (urine, saliva and sweat) after smoking dry plant leaves. Saliva samples were collected before and 1 h after *ad lib* smoking 75 mg of dry leaves in a pipe for 3 min. Urine samples were collected at 0–1.5 h and 1.5–9.5 h after smoking. Two sweat patches were applied on the back of the two individuals and removed before (baseline) and 2 h after pipe smoking (when maximal drug recovery was expected taking into account the short duration of the effects). Samples were stored at −20°C until analysis.

Instrumentation

Analyte separation was achieved on a fused-silica capillary column (HP-5MS 30 m × 0.25 mm × 0.25 μm film thickness; Agilent Technologies, Palo Alto, CA, USA). The oven temperature was held at 70°C (for 3 min), raised at 30°C/min to 300°C, and held for 10 min. A splitless injection mode (3 μL) at an injector temperature of 260°C was used. Helium (purity 99%), at a flow rate of 1 mL/min, was the carrier gas.

Gas chromatography/mass spectrometry (GC/MS) analyses were carried out with a Hewlett-Packard (Agilent Technologies, Palo Alto, CA, USA) Series 6890 gas chromatograph equipped with a HP MSD Series mass spectrometer and HP 5973 N Series injector.

The mass spectrometer was operated in EI mode with selected ion monitoring (SIM) acquisition. The ionization was performed at 70 eV, and the ion source and interface temperature were set at 230 and 280°C, respectively. The ions monitored were *m/z* 432, 273 and 94 for Salvinorin A and *m/z* 302, 229 and 124 for the internal standard (IS), 17-α-methyltestosterone. The ions at *m/z* 94 for Salvinorin A and at *m/z* 302 for the IS were selected for the quantification measurement.

Preparation of calibration standards and quality control samples

Stock standard solutions (1 mg/mL) of analytes were prepared in methanol. Working solutions at concentration of 100, 10, and 1 μg/mL were prepared by dilution of the stock standards with methanol and stored at −20°C until analysis. The IS working solution was used at a concentration of 10 μg/mL. Calibration standards containing 0.015 (0.010 for sweat patches), 0.05, 0.1, 0.5, 1.0 and 5.0 μg/mL (or μg/patch) were prepared daily for each analytical batch by adding suitable amounts of methanol solutions to pre-checked drug-free urine, plasma, saliva and sweat patches.

Quality control (QC) samples of 4.25 (high control), 2.0 (medium control) and 0.024 (low control) μg/mL and

μg/patch were prepared in drug-free biological matrices and stored at −20°C. These samples were included in each analytical batch to check calibration, precision, accuracy and stability of samples under storage conditions.

Protocol for preparation of plasma, urine, saliva and sweat patch samples

One mL of urine, serum, saliva and a sweat patch (cut into little pieces) with 50 μL of IS working solution were transferred into 15-mL screw-capped glass tubes and subjected to liquid-liquid extraction with 2 mL of chloroform/isopropanol (9:1). The mixture was homogenized by vortex for 2 min and centrifuged at 2500 rpm for 5 min. The organic layer was transferred to another tube and re-extracted with 2 mL organic mixture. The combined organic layers were evaporated under nitrogen at 40°C. The residue was dissolved in 50 μL ethyl acetate and 3 μL were injected into the GC/MS system.

Validation procedures

Prior to application to real biological samples, the method was tested in a 3-day validation protocol following the accepted criteria for validation of bioanalytical methods. Selectivity, recovery, matrix effect, linearity, precision, accuracy, and limits of detection (LOD) and quantification (LOQ) were evaluated.

Twenty different urine, plasma, saliva samples and ten sweat patches were extracted and analyzed for assessment of potential interferences from endogenous substances. The apparent responses at the retention times of the analyte under investigation and of the IS were compared with the responses of the analyte at the LOQ and of the IS at its lowest quantifiable concentration.

Potential interferences from principal drugs of abuse: opiates (6-monoacetylmorphine, morphine, morphine-3-glucuronide, morphine-6-glucuronide, codeine), cocaine and metabolites (benzoylecgonine and cocaethylene), cannabinoids (delta-9-tetrahydrocannabinol and 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid), benzodiazepines (clorazepate, diazepam, lorazepam, oxazepam, alprazolam), and antidepressants (imipramine, clomipramine, fluoxetine, norfluoxetine, paroxetine), also were evaluated by spiking 1 mL of pre-checked drug-free urine, plasma, saliva and a sweat patch with 1 μg of each of the aforementioned substances and carrying out the entire procedure. The potential for carryover was investigated by injecting extracted blank urine, plasma, saliva and sweat patch, with added IS, immediately after analysis of the highest concentration point of the calibration curve on each of the three days of the validation protocol and measuring the areas of the peaks present at the retention times of the analytes under investigation.

Analytical recoveries were calculated by comparing the peak areas obtained when calibration samples were analyzed by adding standard and IS in the extract of drug-free biological matrices tested prior to and after the extraction procedure. The recoveries were assessed at three QC concentration levels, using four replicates at each level.

For an evaluation of matrix effects, the peak areas of extracted blank samples spiked with standards at three QC concentration levels after the extraction procedure were compared with the peak areas of pure diluted substances.

Calibration curves were tested over the 0.015 (0.010 for sweat patches) to 5 μg/mL and μg/patch range for the analytes. Peak area ratios for analyte and IS were used for calculations. A weighted (1/concentration) least-squares regression analysis was used (SPSS, version 9.0.2 for Windows). Five replicates of blank samples were used for calculating the LODs and LOQs. The standard deviation (SD) of the analytical background response was used to determine the detection limit (LOD = 3 SD) and the quantification limit (LOQ = 10 SD). Once calculated, LOQ was tested for acceptance criteria (precision and accuracy coefficient of variation below 20%) and included in calibration curves.

Five replicates at each of three different concentrations of standards (4.25, 2.0, 0.024 μg/mL and μg/patch) added to drug-free samples, extracted as reported above, were analyzed for the determination of intra-assay precision and accuracy. The inter-assay precision and accuracy were determined for three independent experimental assays of the aforementioned replicates. Precision was expressed as the relative standard deviation (RSD) of concentrations calculated for QC samples. Accuracy was expressed as the relative error of the calculated concentrations.

The effect of three freeze/thaw cycles (storage at −20°C) on compound stability in tested biological matrices was evaluated by repeated analysis (n = 3) of QC samples (4.25, 2.0, and 0.024 μg/mL) after each of the three cycles. The stability was expressed as a percentage of the initial concentration of the analyte spiked in tested biological matrices and quantified just after preparation.

RESULTS AND DISCUSSION

Validation results

Representative chromatograms obtained following the extraction of 0.5 μg of Salvinorin A and 17-α-methyltestosterone spiked in 1 mL of drug-free urine, plasma, saliva and in a drug-free sweat patch are shown in Figs. 2 and 3. Since neither deuterated Salvinorin A, nor other terpenoids structurally similar to the tested analyte but not contained in the plant leaves, was available at the time of the study, 17-α-methyltestosterone was chosen as the internal standard (IS). Its physicochemical characteristics and molecular structure are similar to those of Salvinorin A and it is readily available.

Each chromatographic run was completed in 25 min. No additional peaks were observed from any endogenous substances that could have interfered with the detection of the compound of interest. None of the drugs of abuse or aforementioned medications, carried through the entire procedure, interfered with the assay. Blank samples injected after the highest point of the calibration curve did not present any traces of carryover. The recoveries (mean ± SD) for Salvinorin A, obtained after liquid extraction of urine, plasma, saliva and sweat, are shown in Table 1. These results suggested that recoveries from urine samples were the highest, with no relevant differences in extraction recovery for plasma, saliva and sweat patches.

With respect to the matrix effect, the comparison between peak areas of analytes spiked in extracted blank samples and

RCM

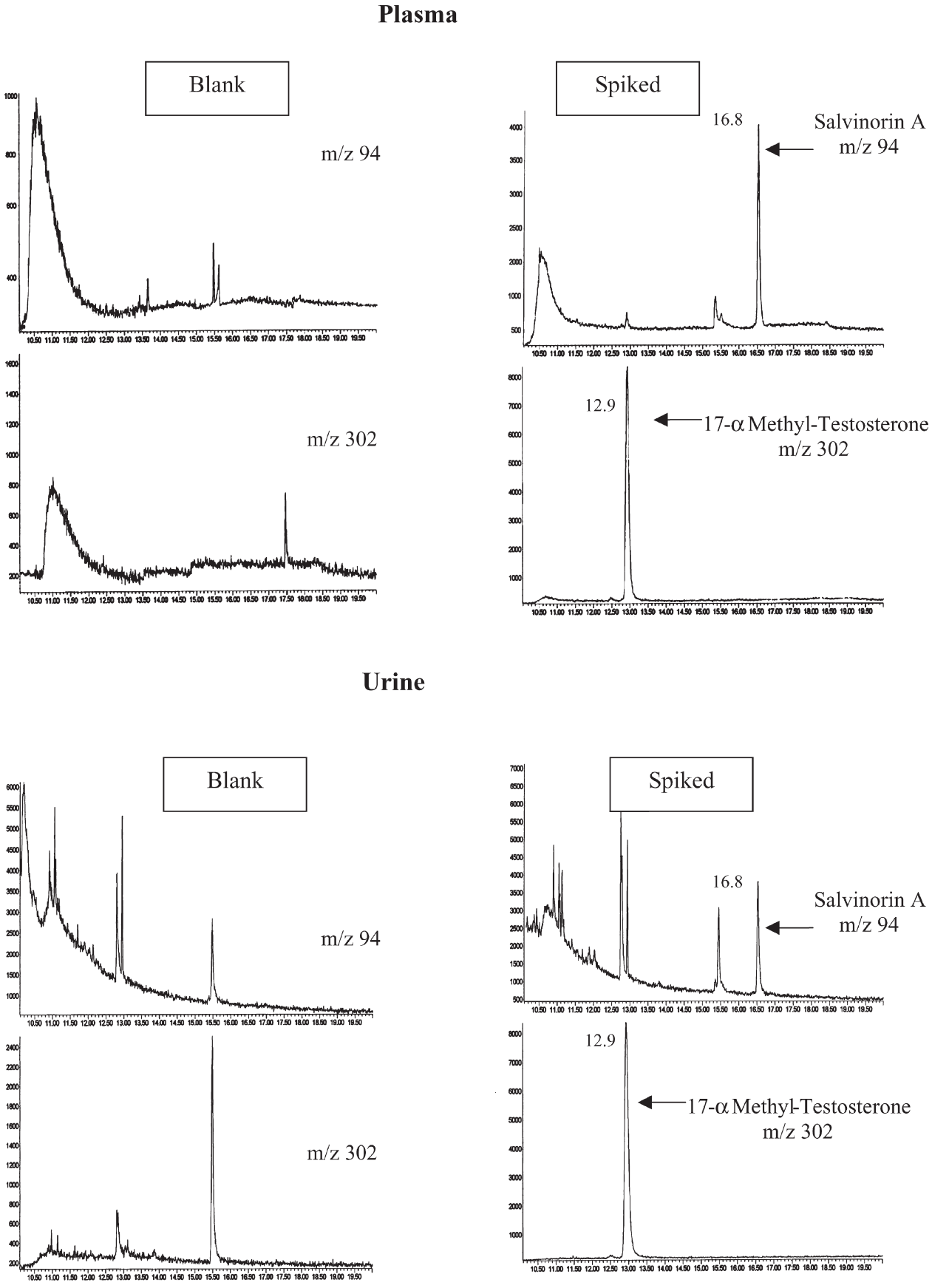


Figure 2. SIM chromatogram of extracts of: 1 mL drug-free plasma sample (left); 1 mL drug-free plasma sample spiked with 0.05 μg Salvinorin A (right), and 1 mL drug-free urine sample (left) with 1 mL drug-free urine sample spiked with 0.05 μg Salvinorin A (right).

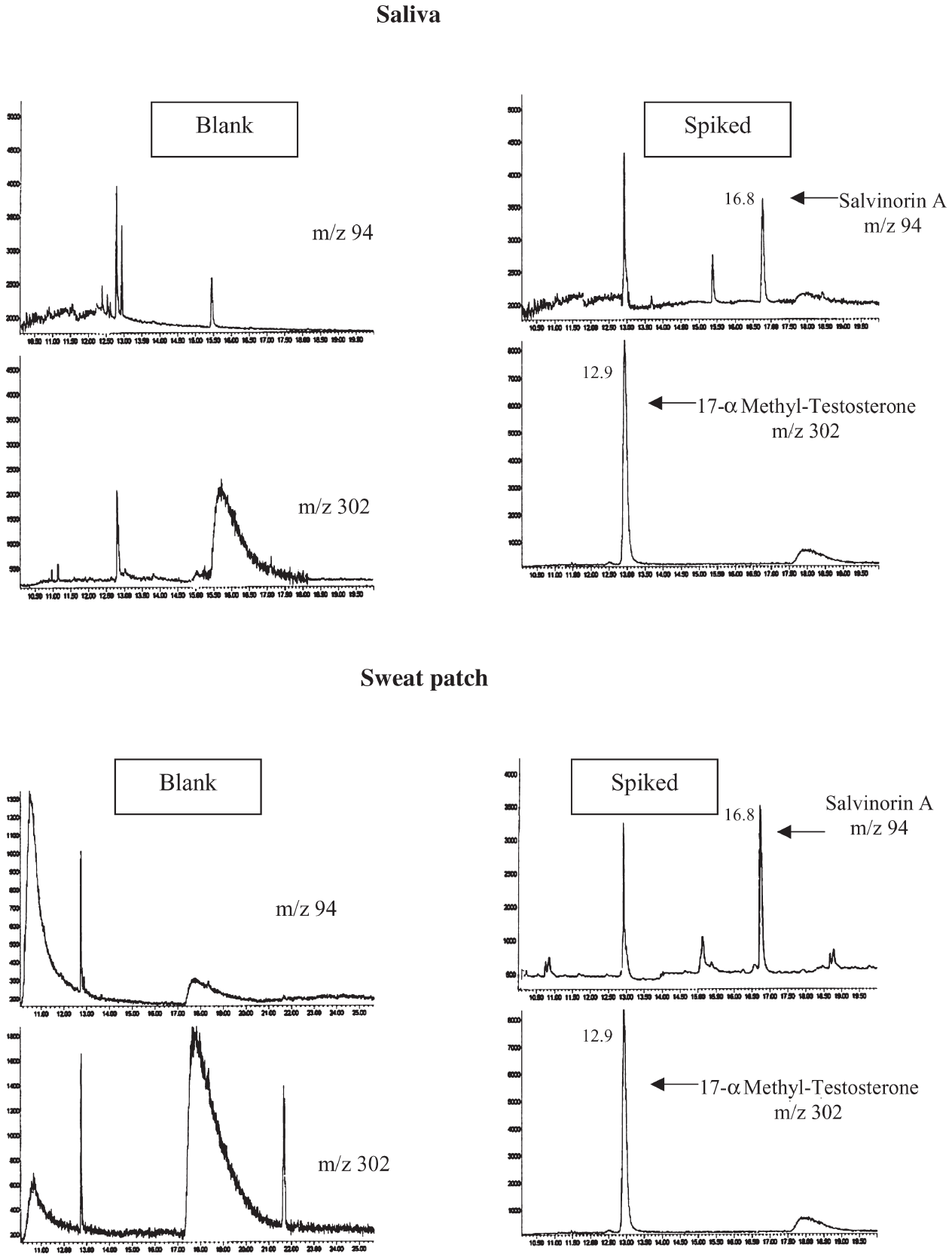


Figure 3. SIM chromatogram of extracts of: 1 mL drug-free saliva sample (left); 1 mL drug-free saliva sample spiked with 0.05 μg Salvinorin A (right), and a drug-free sweat patch sample (left) with sweat patch sample spiked with 0.05 μg Salvinorin A (right).

Table 1. Method calibration in different biological matrices

Biological matrix	Calibration curve slope (n = 3)	Calibration curve intercept (n = 3)	Correlation coefficient (r^2)	LOD (μg/mL)	LOQ (μg/mL)	Recovery (%mean ± SD; n = 4)
Plasma	0.785 ± 0.249	0.042 ± 0.021	0.997 ± 0.003	0.005	0.015	84.6 ± 4.1
Urine	1.192 ± 0.202	0.067 ± 0.012	0.999 ± 0.001	0.005	0.015	93.7 ± 6.7
Saliva	0.721 ± 0.256	0.002 ± 0.015	0.996 ± 0.002	0.005	0.015	84.2 ± 2.4
Sweat*	0.719 ± 0.290	0.016 ± 0.025	0.999 ± 0.002	0.003	0.010	77.1 ± 4.4

* Collected by sweat patches.

Table 2. Intra-day precision and accuracy obtained for Salvinorin A in different biological matrices

Biological matrix	n	Concentration	Estimated mean ± SD	Precision (RSD)	Accuracy (Error %)
		(μg/mL)	(μg/mL)		
Plasma	5	0.024	0.022 ± 0.002	8.6	8.3
	5	0.10	0.091 ± 0.012	13.1	9.0
	5	4.25	3.915 ± 0.198	5.0	7.8
		(μg/mL)	(μg/mL)		
Urine	5	0.024	0.021 ± 0.002	9.5	12.5
	5	0.10	0.097 ± 0.010	10.3	3.0
	5	4.25	3.991 ± 0.210	5.2	8.4
		(μg/mL)	(μg/mL)		
Saliva	5	0.024	0.021 ± 0.002	10.0	12.5
	5	0.10	0.095 ± 0.011	11.5	5.0
	5	4.25	3.895 ± 0.189	4.8	8.3
		(μg/patch)	(μg/patch)		
Sweat	5	0.024	0.022 ± 0.002	10.4	8.3
	5	0.10	0.098 ± 0.010	10.2	2.0
	5	4.25	3.935 ± 0.185	4.7	7.4

those for pure diluted standards showed less than 10% analytical signal suppression due to co-eluting endogenous substances.

The method was linear in the concentration range assayed for all tested biological matrices, with the correlation coefficients (r^2) being higher than 0.99 in all cases. Calibration curve slope values were homogeneous for all the tested biological matrices (t-test with p always >0.05). LODs and LOQs were considered adequate for the purposes of the present study (Table 1).

Tables 2 and 3 show the results obtained for intra-assay and inter-assay precision and accuracy calculations. These results satisfactorily meet internationally established acceptance criteria.[11,12]

With reference to the freeze/thaw stability assays for QC samples, no relevant degradation was observed after three freeze/thaw cycles, with differences from initial concentrations lower than 10%.

Application to analyses of biological samples from plant leaves consumers

This method was used to investigate the presence of Salvinorin A in biological matrices from two volunteers who had smoked dry leaves of *S. divinorum* (Table 4, Fig. 4).

Table 3. Inter-day precision and accuracy for Salvinorin A in different biological matrices obtained for five replicates assayed in three different batches

Biological matrix and compound	n	Concentration	Estimated mean ± SD	Precision (RSD)	Accuracy (Error %)
Plasma		(μg/mL)	(μg/mL)		
Salvinorin A	15	0.024	0.021 ± 0.002	9.5	12.5
	15	0.10	0.095 ± 0.013	13.6	5.0
	15	4.25	3.835 ± 0.199	5.1	9.7
Urine		(μg/mL)	(μg/mL)		
Salvinorin A	15	0.024	0.022 ± 0.002	10.4	8.3
	15	0.10	0.098 ± 0.010	10.2	2.0
	15	4.25	3.935 ± 0.185	4.7	7.4
Saliva		(μg/mL)	(μg/mL)		
Salvinorin A	15	0.024	0.023 ± 0.002	9.1	4.1
	15	0.10	0.099 ± 0.013	13.1	1.0
	15	4.25	3.895 ± 0.189	4.8	8.3
Sweat		(μg/patch)	(μg/patch)		
Salvinorin A	15	0.024	0.021 ± 0.002	10.4	12.5
	15	0.10	0.097 ± 0.014	14.4	3.0
	15	4.25	3.995 ± 0.185	4.6	6.0

Table 4. Salvinorin A concentration in biological fluids from the two subjects after smoking *S. divinorum*

Subject	Dose of Salvinorin A smoked (mg)	Saliva* (ng/mL)	Urine** (ng/mL)		Total amount excreted in urine (ng)		
		1 h post-administration	0–1.5 h	1.5–9.5 h	0–1.5 h	1.5–9.5 h	Sweat patch
A	0.58	25.0	10.9	N.D	7085 (1.2% initial dose)	N.D	N.D
B	0.58	11.1	2.4	N.D	2400 (0.4% initial dose)	N.D	N.D

* Values obtained with 2 mL saliva.
** Values obtained with 10 mL urine.
N.D: not determined.

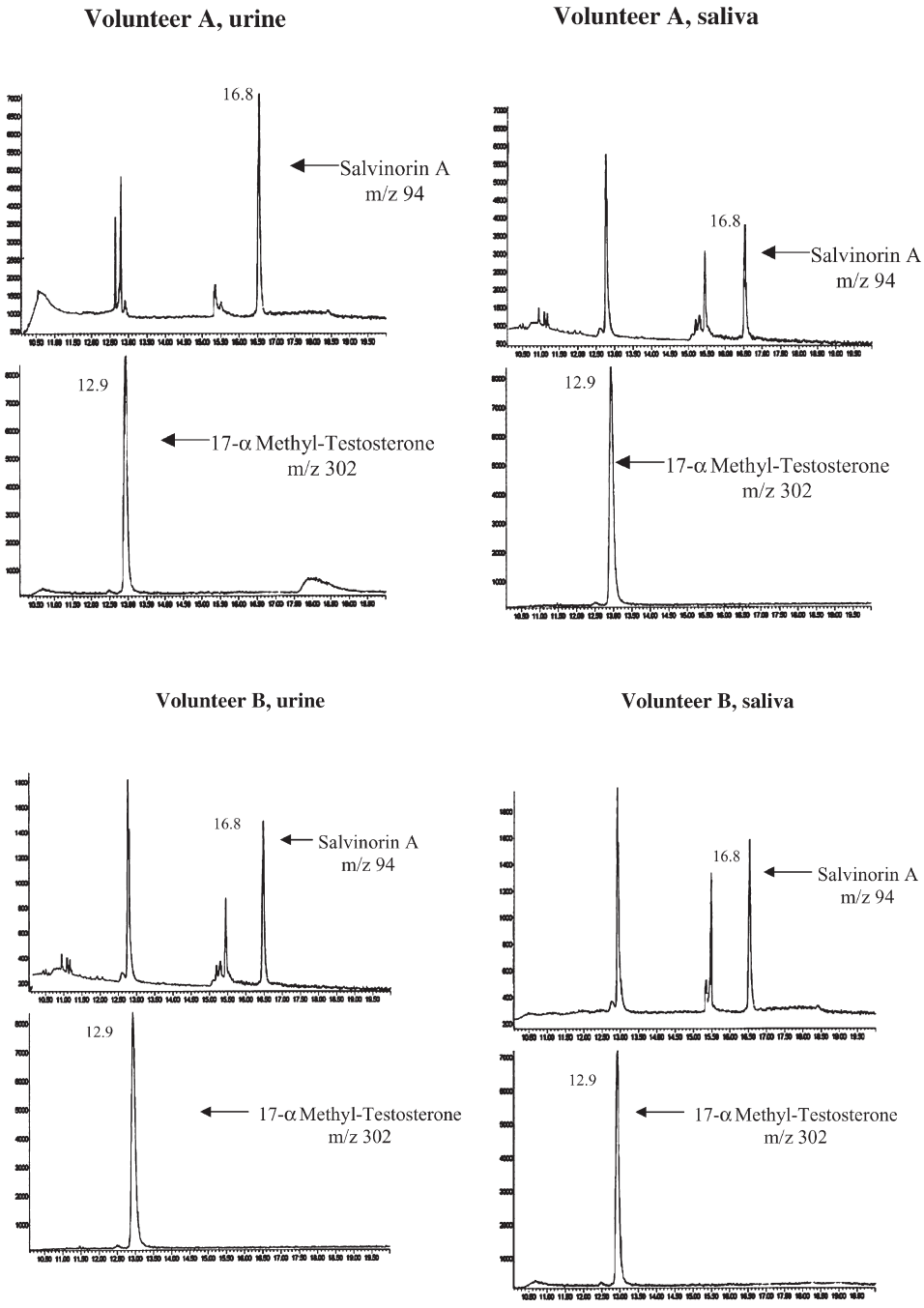


Figure 4. SIM chromatograms of extracts of urine and saliva samples containing 10.9 and 25 ng/mL Salvinorin A, respectively, obtained from two volunteers.

Unfortunately, although the methodology was validated for the different biological matrices that could have been obtained by the two individuals, blood collection was not possible within this experiment. Both volunteers experienced intense hallucinations that started 30 s after inhaling, peaking after 3–5 min, and lasting for about 15–20 min. During that period, blood drawing was considered unsafe and, at the end of the experience, the subjects refused blood collection. Urine, saliva and sweat patch specimens were obtained and Salvinorin A was detected in saliva and urine but not in sweat patches.

Concentrations were in the range of a few ng per mL of biological fluid and saliva (Table 4). Nonetheless, it has to be considered that the amount of consumed preparation (and consequently the amount of the active compound) was extremely low. Indeed, when examining the Salvinorin A content in the dry leaves smoked by the volunteers by a validated methodology,[2] a total amount of 0.58 mg of the active compound (7.7 mg/g dry leaves) was found. Hence, if one accepts the hypothesis that the entire calculated amount was smoked by the two individuals, the concentrations found in saliva and urine samples in the first hour (or hour and half) post-consumption would be in a range compatible with the consumed dose of the drug. The total amount of Salvinorin A excreted in urine represents from 0.4–1.2% of the theoretically administered dose although the true inhaled dose is unknown because one part may have been lost in the combustion and some to sidestream. Salvinorin A was not detected in urine samples collected from 1.5–9.5 h after smoking, probably because of a dilution effect, which yielded concentrations below the LOD obtainable with this methodology. Another possible explanation could be related to fast elimination of the compound, but the pharmacokinetics parameters of Salvinorin A have not been reported. Conversely, regarding the absence of Salvinorin A in sweat patches, previous experience with other drugs of abuse administered as hundred mg doses under controlled conditions and quantified in the range of ng/patch[13–15] led us to conclude that the low amount of administered substance coupled with the small volume of sweat collected in the patch area over such a short wear period resulted in minimal excretion of Salvinorin A.

Nonetheless, sweat was only collected for 2 h after drug administration, while normal sweat patch application involves wearing the patch for 1 week. However, this is normally done to check exposure to illicit drugs in subjects who are not under medical supervision. Thus, the moment of exposure to drugs is not known. This is not the case with controlled drug administration. It seems that Salvinorin A is rapidly absorbed after smoking of dry leaves (maximum effects occur in 3–5 min after consumption), and the fact that it appears only in urine samples collected up to 1.5 h also suggests a rapid elimination (or else an extensive metabolism to unknown substances) and thus a theoretical passage to sweat in the first hours after administration. On the other hand, the non-polar nature of the compound, which does not favor excretion through sweat, and the low concentration of the active principle in the consumed plant leaves are consistent with the lack of detection of this substance in patches worn for 2 h after drug consumption.

CONCLUSIONS

A simple and reliable GC/MS method is reported for the analysis of Salvinorin A in conventional and non-conventional biological matrices. The method was validated according to internationally accepted criteria; it consists of an easy sample preparation by liquid extraction, followed by chromatographic separation on a capillary column and detection in SIM mode. For the first time, the presence of the psychoactive ingredient of *Salvia divinorum*, Salvinorin A, was detected in the urine and saliva of two subjects in the first 1.5 h after its consumption. Pharmacological effects experienced by subjects were intense and short-lived, in agreement with a previous report.[6]

Acknowledgements

This study was supported by Presidenza del Consiglio dei Ministri, Dipartimento Nazionale per le politiche antidroga, and FIS (G03/005).

REFERENCES

1. Valdes LJ. *J. Psychoactive Drugs* 1994; **26**: 277.
2. Giroud C, Felber F, Augsburger M, Horisberger B, Rivier L, Mangin P. *Forensic Sci. Int*. 2000; **112**: 143.
3. Available: www.ministerosalute.it/imgs/C_17_normativa_484_allegato.pdf.
4. Available: www.boe.es (number 32, February 6, 2004).
5. Available: www.sagewisdom.org.
6. Siebert DJ. *J. Ethnopharmacol*. 1994; **43**: 53.
7. Ortega A, Blount JF, Manchand PS. *J. Chem. Soc. Perkin Trans*. 1982; **1**: 2505.
8. Valdes LJ, Butler WM, Hatfield GM, Paul AG, Koreeda M. *J. Org. Chem*. 1984; **49**: 4716.
9. Gruber JW, Siebert DJ, Der Marderosian AH, Hock RS. *Phytochem. Anal*. 1999; **10**: 22.
10. Roth BL, Baner K, Westkaemper R, Siebert D, Rice KC, Steinberg S, Ernsberger P, Rothman RB. *Proc. Natl. Acad. Sci. USA* 2002; **99**: 11934.
11. Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, May 2001. Available: http://www.fda.gov/cder/guidance/4252fnl.htm.
12. ICH Topic Q 2 B Validation of Analytical Procedures: Methodology, The European Agency for the Evaluation of Medicinal Products, ICH Technical Coordination: London. Available: http://www.emea.eu.int/htms/human/ich/quality/ichfin.htm.
13. Pichini S, Navarro M, Pacifici R, Zuccaro P, Ortuno J, Farre M, Roset PN, Segura J, de la Torre R. *J. Anal. Toxicol*. 2003; **27**: 294.
14. Saito T, Wtsadik A, Scheidweiler KB, Fortner N, Takeichi S, Huestis MA. *Clin. Chem*. 2004; **50**: 2083.
15. Uemura N, Nath RP, Harkey MR, Henderson GL, Mendelson J, Jones RT. *J. Anal. Toxicol*. 2004; **28**: 253.