Ann Toxicol Anal. 2009; 21(4): 197-202
© Société Française de Toxicologie Analytique 2009
DOI: 10.1051/ata/2009058

Available online at:
www.ata-journal.org

ANNALES DE TOXICOLOGIE ANALYTIQUE

Short paper / Article court

Driving under the effects of drugs of abuse: studies of oral fluid

Conduite sous l'influence de stupéfiants : détection des drogues dans la salive

Agustí Mora*, Amparo Arroyo, Maria Barbal, Mariona Palahí, Eneko Barbería, Marta Sánchez

Institut de Medicina Legal de Catalunya (Ciutat de la Justícia), Gran Via de les Corts Catalanes 111. Edifici G, 6 fl, 08014 Barcelona, Spain

Abstract – Introduction: This study includes the evaluation of the use of drugs of abuse ratio in leisure environments, in order to determine which drugs are the most widely used, which are normally used in poly-consumption. **Methods**: Total sample rise to 216 cases which were collected on the road by traffic police and analyzed by an on-the-road driving test of possible consumption. Positive samples were confirmed by GC/MS-MS. **Results**: It was detected positive samples for the studied drugs (cocaine, heroin metabolites (6-MAM), amphetamines/methamphetamines and cannabinoids (Δ^9-THC)). Sample shows a high degree of poly-consumption of different drugs differently distributed. The most widely detected drug of abuse as a single consumption has been cannabis and cocaine. The rest of the drugs were always mixed. The greater combination in poly-consumption is cannabis-cocaine. **Conclusions**: It is important to notice the high ratio of positive results in drivers. However, the tendency to poly-consumption of drugs of abuse mixed with alcohol is confirmed. Thus, it is necessary to consider the mixed/alternated use of ethylometers and on-the road detection kits of drugs of abuse in oral fluid to improve the traffic security.

Key words: Drugs of abuse, oral fluid, driving

Résumé – Introduction : Cet article étudie l'abus de stupéfiants drogues dans les zones de loisir, afin de déterminer les drogues les plus souvent utilisés. **Méthodes** : L'échantillon total comprend 216 cas rassemblés par la police de la route et analysés grâce à des tests immunochimiques effectués sur le bord de la route. Les échantillons positifs ont été ensuite confirmés par GC-MS/MS. **Résultats** : Nous avons détecté des échantillons positifs pour les composés étudiés (cocaïne, héroïne métabolites (6-MAM), amphétamine, methamphétamine et cannabis (Δ^9-THC)). Les résultats montrent un grand pourcentage de poly-consommation de drogues, différemment distribuées. La consommation de cannabis seul et la consommation de cocaïne seule sont les plus fréquentes. Les autres drogues sont toujours consommées en association. L'association la plus fréquente dans les cas de poly-consommation est l'association cannabis-cocaïne. **Conclusion** : Nous avons trouvé un grand nombre de résultats positifs chez les conducteurs et confirmé la poly-consommation de drogues, ainsi que l'association avec l'alcool. En conséquence, il faudrait, dans le cadre de la sécurité routière, utiliser à la fois les éthylomètres et les kits de détection de drogues dans la salive.

Mots clés : Abus de drogues, salive, circulation routière

Received 19 January 2009, accepted after revision 1st October 2009
Published online 17 December 2009

1 Introduction

Drug detection in legal medicine is mainly focused on driving under de influence of drugs (DUID) or alcohol where the importance related to legal repercussion shows a greater relevance [1–3]. During last years, concern about DUID as a risk factor and a direct cause of accidents has risen due to the fact that illegal drugs have a direct influence on driving performance. Opiates induce sedation, cocaine and other stimulants such as amphetamine and its derivatives (MDMA, MDEA) produces loss of concentration and cannabis has a direct influence in perception and psychomotor performance [4].

Once OF was considered as a matrix in drug testing, its use has rapidly increased primarily due to the advantages that collection of samples from OF offers in comparison with other biological matrixes, such as blood and urine. The main advantages are the possibility of collection by non-medical personnel, the non-invasive character of this procedure and the difficulty to adulterate the sample [3]. OF is a complex biological matrix consisting not only of the products generated by the secretary glands (parotid, submandibular and sublingual) that form saliva, but also from secretions produced by minor glands (labial, buccal and palatal) as well as other substances such as bacteria, food debris, epithelial cells and gingival fluid [5–7]. The concentration of drugs in OF depends on the relative

* Correspondence: Agustí Mora, amora@gencat.cat

contribution of each gland since the transportation of drugs from plasma to saliva involves passive diffusion across lipid membranes, and different factors related to the chemical structure and nature of drugs play important roles in this mechanism [7–9].

Some years ago, one of the first publications described the immunoassay technique for drugs analysis in OF [10], although there were some difficulties in the analytical methods which sensibility were not improved yet. From the 1980s the number of publications related with the investigation of this subject has been risen and started the development of devices which objective was the screening of abuse drugs such as amphetamines, cocaine, opiates (morphine and codeine) and cannabinoids [11, 12].

Some Spanish investigation groups have taken part in international projects such as ROSITA (Roadside Testing Assessment) carried out in 16 European countries in 1999 and ROSITA 2 which was extended to 4 American countries [13], in which different kits of drugs detection in OF from drivers were evaluated.

The aims of this study are, on one side, to obtain data about abuse drugs consumption and, on the other side, observe both poly-consumption of abuse drugs and the most widely represented in this. Furthermore, from the obtained data, review the legal aspects related to DUID.

2 Observations

The study was carried out in Catalunya (NE Spain), in places near discotheques or raves by traffic police in four different areas (Barcelona, Tarragona, El Prat de Llobregat and Girona). The controls were executed at weekends (in general, Friday and Saturday nights). The selection of drivers was random and always carried out by the traffic police.

To 216 cases collected on the road by traffic police and analyzed by the roadside test Cozart® RapiScan Oral Fluid Drug Testing System. Positive samples were duplicated, for later confirmation. There was not a duplicate from all of the negative samples due to the legal impossibility to obtain a sample when the OF test was negative and the driver assent was not obtained.

3 Materials and methods

Chemicals and materials. Methanol solutions with a concentration of 1 mg/mL of cocaine, Δ^9-tetrahydrocannabinol (Δ^9-THC), amphetamine, methamphetamine, dl-3, 4-methylendioximethamphetamine (dl-3, 4-MDMA), dl-3, 4-methylendioxiethampheta-mine (dl-3, 4-MDEA) and 6-monoacetylmorphine (6-MAM) were purchased from Alltech-Applied Science (State College, PA, USA). For derivatization of Δ^9-THC and 6-MAM, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) used as BSTFA + 1%TMCS were provided from Supelco (Bellefonte, PA, USA) and 2,2,3,3,3-pentafluoropropionic acid (PFPA) from Merck KGaA (Darmstadt Germany). Phosphate buffer (0.1 M) was prepared from NaH_2PO_4 and adjusted to pH 6.0 with NaOH 0.1 M.

Table I. Drug targets and procedures used in GC/MS-MS.

Drug target	Ionization	Waveform type	Excitation width (V)
amphetamine-PFP	EI[a]	non resonant	64.0
methamphetamine-PFP	EI	non resonant	60.0
MDMA-PFP	EI	non resonant	60.0
MDEA-PFP	EI	non resonant	57.0
cocaine	EI	non resonant	45.0
Δ^9−THC-TMS	EI	non resonant	61.0
6-MAM-TMS	EI	resonant	1.0

[a] Electronic Impact.

Oral fluid samples. A total of 216 OF samples were obtained in roadside tests carried out by the police. Police officers were asked to collect sample in duplicate (one for roadside test and another for laboratory analysis) in those cases where the driver seemed to be under the effect of narcotic substances. One of the samples was analyzed *in situ* by using the Cozart® RapiScan Oral Fluid Drug Testing System in order to detect the possible consumption of drugs, and the duplicated sample was collected spitting in a plastic tube and stored at 4 °C to be analyzed in the laboratory with the GC-MS/MS technique.

Sample preparation. Sample preparation consisted of the addition of 1 mL pure oral fluid to 1 mL phosphate buffer (pH = 6). Once the pH was readjusted, 20 µL of d3-cocaine, d3-6-MAM, d5-amphetamine, d9-methamphetamine and d9-Δ^9-THC were added for a final concentration of 10 µg/mL [18]. The sample was transferred in a Toxitube A®, stirred by using an orbital stirrer (in order to mix the immiscible phases) for 10 min and centrifuged (3500 rpm for 10 min). The organic phase was extracted, evaporated to dryness under nitrogen and derivatized with 40 µL of BSFTA-TMCS at 80 °C for 20 min for Δ^9-THC and 6-MAM or PFPA for amphetamines and methamphetamines at 50 °C for 40 min.

GC-MS/MS conditions. A Varian Inc. (Palo Alto, USA) 3800 gas chromatograph coupled to a 4000 mass selective ion trap detector (MSD) operating in electron impact mode was used for analysis (GC-MS/MS). The gas chromatographic column was 5% phenyl-95% methyl silicone DB-5, 0.25 mm ID, 0.25 µm thickness, 30 m length (Varian factorFour Capillary Column) and the injection temperature was 250 °C. 2 µL of the sample were injected in splitless mode. The oven was programmed from 90 °C for 1 minute; ramped at 20 °C/min to 240 °C; then ramped at 5 °C/min to 300 °C where it remained for two minutes. The transfer line was held at 280 °C. The total run time was 23.5 min. Detection was performed operating in MS/MS. Details of the detection procedure are shown in Table I, and the different substances and ions identified by MS/MS are shown in Table II. The MS/MS spectrums from the standards of the studied drugs are shown in Figure 1.

Limit of detection-Limit of quantification (LOD-LOQ). Since cut-offs for the roadside OF test were different from the LOD and LOQ values used in the GC-MS/MS confirmation, OF test cut-offs and LOD and LOQ are shown for GC-MS/MS are shown in Table III.

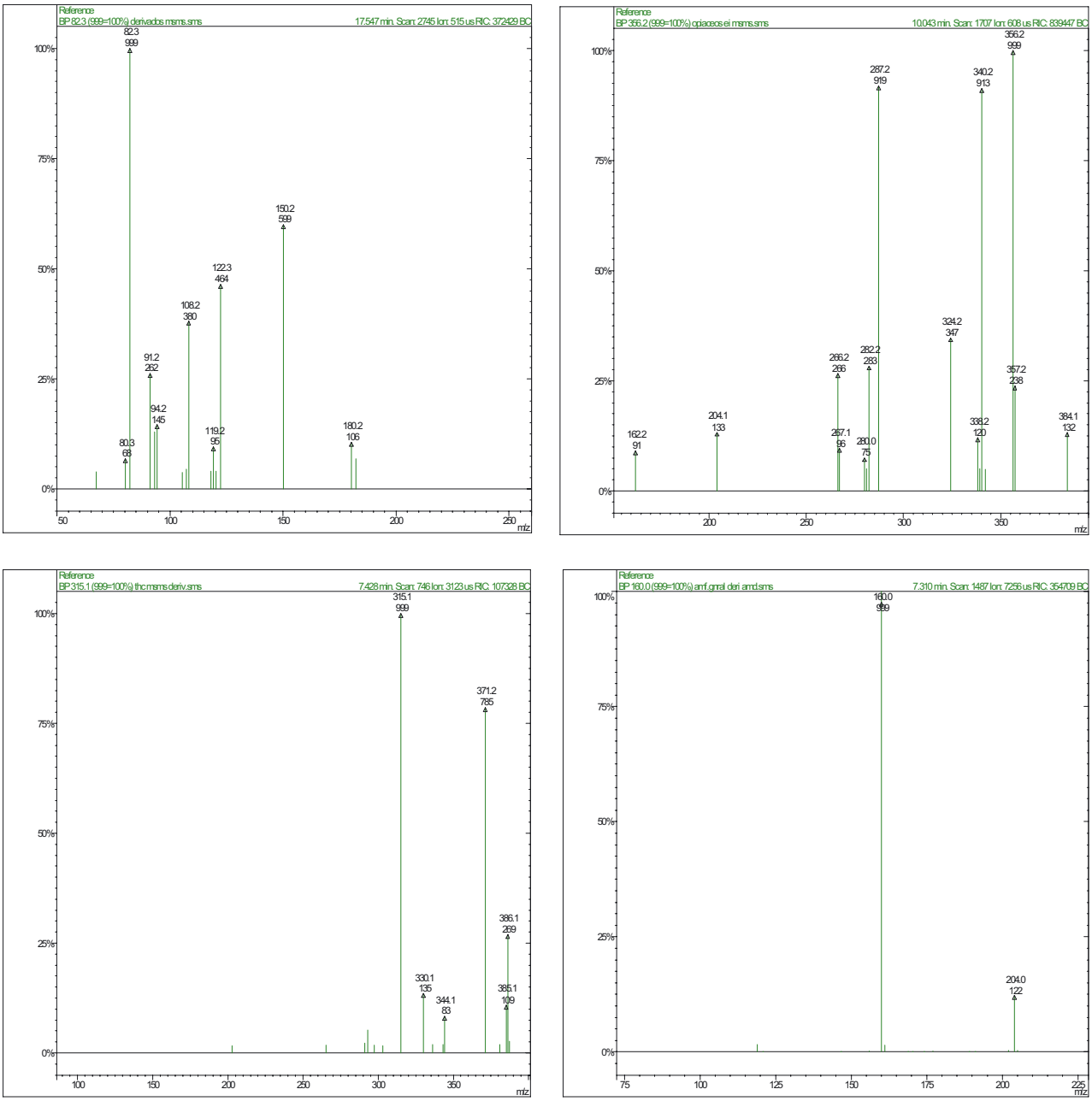

Fig. 1. MS-MS Spectrum from cocaine, 6-MAM-TMS, Δ^9−THC-TMS, methamphetamine-PFP, MDMA-PFP, MDEA-PFP, amphetamine-PFP, respectively showed from up to down and from left to right. X-axis shows the ion *m/z* value and Y-axis and numbers under the ions show the ion abundances.

Table II. Drug targets, retention times (Rt) and ions selected for each studied drug.

Drug target	Rt (min)	Precursor ion (*m/z*)	Qualifier ions (*m/z*)
amphetamine-PFP	5.92	190	118,91,119
methamphetamine-PFP	6.81	204	160,119
MDMA-PFP	10.53	204	160,161
MDEA-PFP	11.07	218	190,146,163
cocaine	16.85	182	150,82,122
Δ^9−THC-TMS	17.43	386	371,315,330
6-MAM-TMS	20.11	399	356,340,287

4 Results

Samples were considered as positive when confirmed by using the described GC/MS-MS procedure. Among the 216 cases analyzed, the 73.1% were detected as positive samples for the studied drugs (cocaine, opioids derived from heroin (6-MAM), amphetamines/methamphetamines and cannabinoids (Δ^9-THC)).

Results show degree of poly-consumption (Fig. 2A) higher than 30% which can be considered as high. The distribution of the total amount of positive samples is different depending on

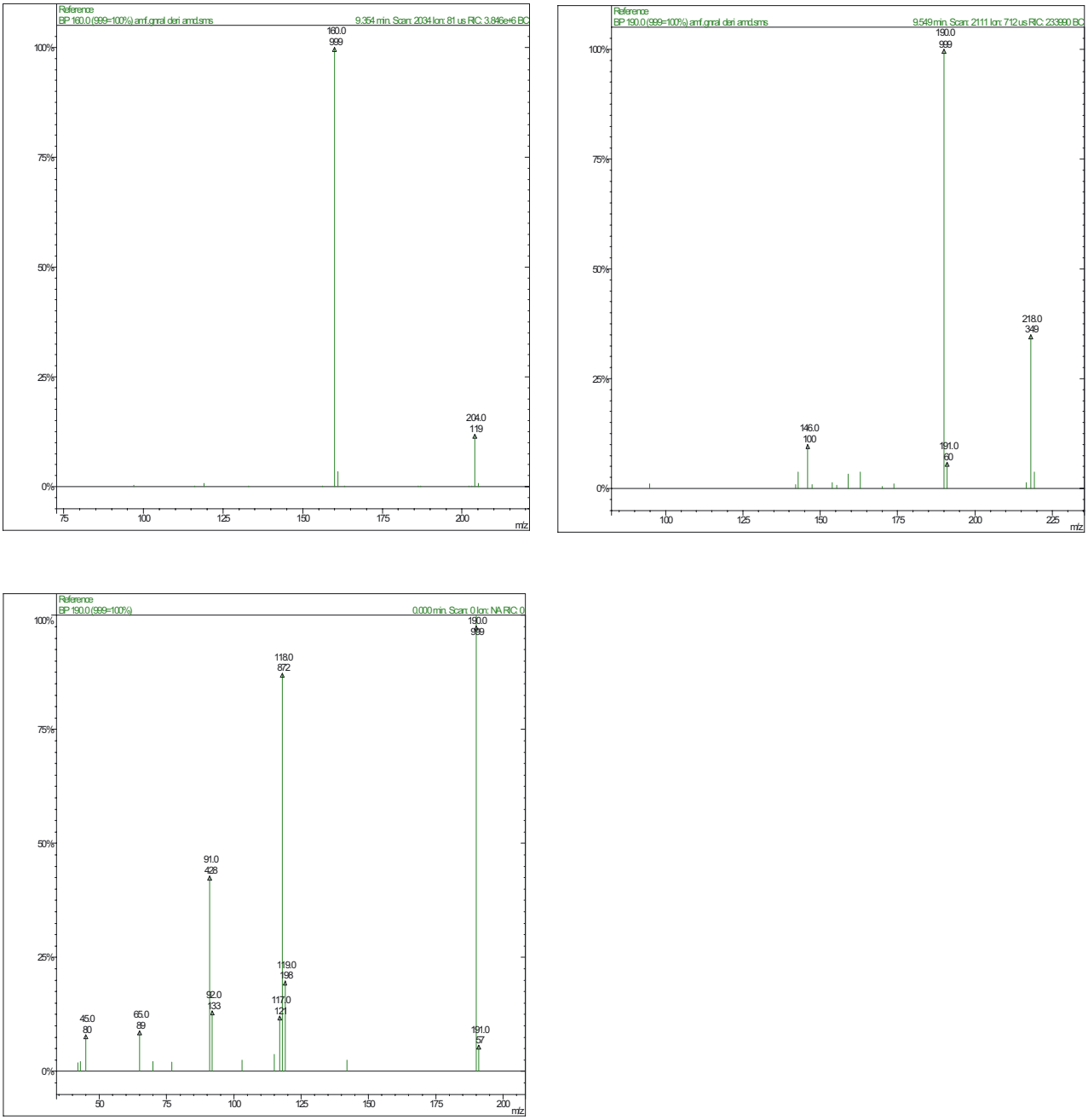

Fig. 1. Continued.

the number of drugs detected: one drug (64.5%), two drugs (30.4%), or three or more drugs (5.1%). The most widely detected drugs when a single drug is consumed are equally distributed between cannabinoids in a 51% and cocaine in a 48% (Fig. 2B) while the other drugs were always mixed. The greater combination in poly-consumption was cannabinoids-cocaine reaching an 89.5% of the cases (Fig. 2C).

It is important to notice the high ratio of positive samples to opiates related to the kind of population from where samples were obtained, what is said, there are some areas reported as opiates consumption and distribution. Thus, when the traffic control is carried out in one of these areas, the number of positive samples to opiates increases (Tab. IV). It should be taken into account that in most cases traffic controls were carried out in conflictive points and, according to that, in some cases results could be higher than expected. Furthermore, opiate consumption seems to be related up to a point with the density of population.

5 Discussion

The high prevalence of cannabis and cocaine matches with the current tendency in drug consumption, while the opiates

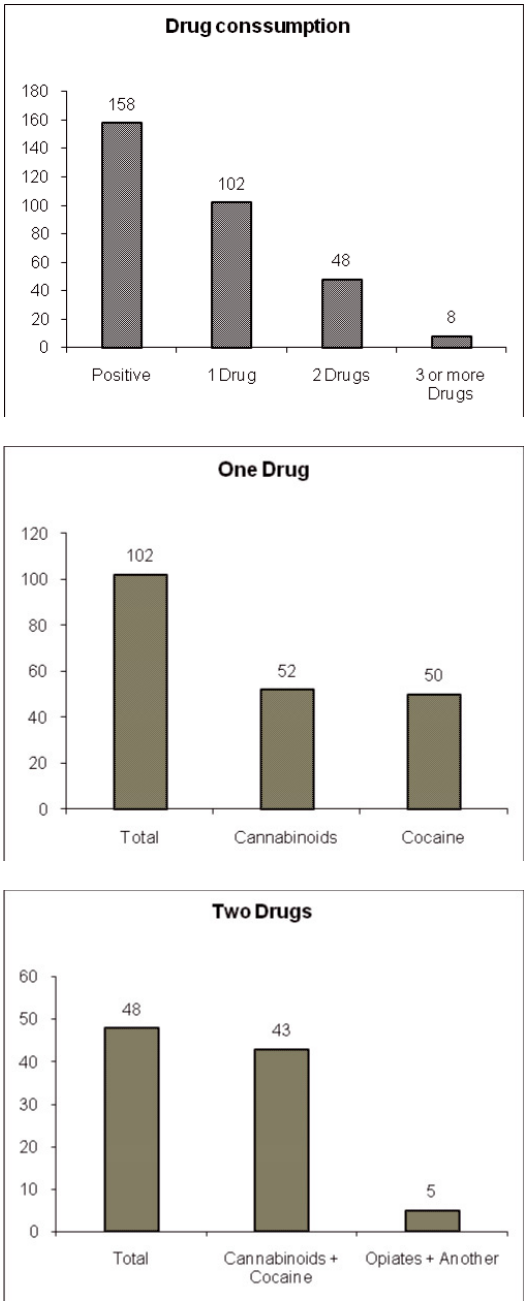

Fig. 2. Results of drug consumption from total positive samples (A) depending on number of drugs consumed. Distribution when one single drug has been consumed (B) and when two drugs have been mixed (C).

consumption has decreased [7, 14], as confirmed in our results where the presence of amphetamines, methamphetamines and opiates were low. These results also confirm that both cannabis and cocaine are strongly related with leisure activities and environments.

Although it should be taken into account that the sample was not a random sample since roadside controls were done at concrete days and hours, the study provides valuable statistical data related to consumption prevalence of an interesting social group considering this people as a significant part of drivers.

Table III. Cut-off values of the immunoassay OF test used in roadside controls and LOD and LOQ values for GC-MS/MS analysis.

Drug target	OF test	GC/MS-MS	
	cut-off (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
amphetamine-PFP	50	5	20
methamphetamine-PFP	50	5	20
MDMA-PFP	50	5	20
MDEA-PFP	50	5	20
cocaine	30	2.5	10
Δ^{9-}THC-TMS	31	2.5	10
6-MAM-TMS	50	5	20

Table IV. Opiate ratios depending on the collection area of the sample. Area 1 corresponds to Barcelona; area 2, Tarragona; area 3, El Prat de Llobregat and area 4, Girona.

Total opiates samples	Area 1	Area 2	Area 3	Area 4
100%	28,6%	0%	57,1%	14,3%

These results could be compared to other results obtained from population studies in order to decide how our biased population could affect the results of the study [10, 15, 16]. These studies describe that when sample is higher and there is no bias in the sampling procedure the ratios of positive samples decreases.

It is our aim to follow this project in order to achieve more representative results with a greater amount of samples and to evaluate the application of roadside drug tests together with alcohol detection, and the quantification of drugs both in saliva and in blood. These results could open new possibilities of promoting legal changes related to DUID. The most important conclusion could be the high prevalence of drugs in drivers, which makes strongly recommendable the use of both drug tests and ethylometers in road preventive controls.

Conflict of interest. The authors declare they have no conflict of interest.

Acknowledgements. We are grateful to O. Huertas for making this job easier and for his technical assistance.

References

1. Tunbridge R. Conclusions and recommendations of the seminar Pompidou Group. In: Road Traffic and Psychoactive Substances. Strasbourg: Council of Europe Publishing 2004: 377–378.
2. Krüger HP, Bud Perrine MW, Mettke M, Huessy FB. Illicit drugs in road traffic. Overview of the legal provisions, difficulties faced by police, and analysis of prevention attempts in selected European countries, P-PG/Circrout (98) 4 rev. Council of Europe, Pompidou Group. Strasbourg: 1999: 1–29.
3. Verstraete A. Oral fluid testing for driving under the influence of drugs. History, recent progress and remaining challenges. Forensic Sci Int. 2005; 150(2-3): 143–150.

4. Maes V, Charlier C, Grenez O, Verstaete A. Drugs and medicines that are suspected to have a detrimental impact on road user performance, in: Verstraete A (Project coordinator), ROSITA: Roadside Testing Assessment, Ghent University, Ghent, Belgium 2000: 5–44 (ROSITA Contract No. DGVII PL 98-3032).

5. Höld M, de Boer D, Zuidema J, Maes R. Saliva as an analytical tool in toxicology. Int J Drug Testing. 1996; 1: 1–36.

6. Mandel ID. The diagnosis use of saliva. J Oral Pathol Med. 1990; 19: 119–125.

7. Gorodischer R, Koren G. Salivary excretion of drugs in children: theoretical and practical issues in therapeutic drug monitoring. Dev Pharmacol Ther. 1992; 19: 161–168.

8. Kaufman E, Lamster I. The diagnostic applications of saliva – a review. Crit Rev Oral Biol Med. 2002; 13: 197–202.

9. Haeckel R. Factors influencing the saliva/plasma ratio of drugs. Ann N Y Acad Sci. 1993; 694: 128–142.

10. Leute R, Ullman E, Golstein A. Inmunoassay of opiate narcotics in urine and saliva. J Am Med Assoc. 1972; 221: 1231–1234.

11. Peel H, Perrigo B, Mikhael N. Detection of drugs in saliva of impaired drivers. J Forensic Sci. 1984; 29: 185–189.

12. Wals J, Flegel R, Crouch D, Cangianelli L, Baudys J. An evaluation of rapid point of collection oral fluid drugs-testing devices. J Anal Toxicol. 2003; 27(7): 429–439.

13. (a) Möller M, Steinmeyer S, Aberl F. Deliverable D3: Operational, user and legal requirements across EU members states for roadside drug testing equipment. In: ROSITA deliverables. Gent. ROSITA Consortium.1999; D3. (b) VerstraeteA. 'Results of the Certified and Rosita projects'. In: Road traffic and psychoactive substances: basic documents, conclusions and recommendations – Report on the seminar in September 2003, p. 323, Pompidou Group, Strasbourg. (c) Verstraete A and Raes E (2006), 'Rosita-2 project: final report' (available from www.rosita.org).

14. Campora P. La espectrometría de masas por impacto electrónico e ionización química en el análisis de drogas de abuso en saliva. Tesis Doctoral 2001. Universidad de Santiago de Compostela. Facultad de Medicina. Instituto de Medicina Legal.

15. Observatorio Español de Drogas. Informe n° 6. Plan nacional de Drogas. Ministerio de Sanidad 2003.

16. Starner G, Mascord D, Tattam B, Zeleny R. Analysis for drugs in saliva. CR 141 Australian government publishing service. Canberra 1994: 1-76.

17. Krugger HP, Shcultz E, Magerl H. The German roadside survey 1992-1994. Saliva analyses from an unselected driver population : licit and illicit drugs. In: Kloeden CN, Wada AJ, McLean A (Eds). Alcohol, Drugs and safety- T 95NHMRC Road accident research unit. Adelaide: University of Adelaide 1995: 55–62.