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Article · October 2014

DOI: 10.5455/ejfs.165152

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European Journal of Forensic Sciences
www.ejfs.co.uk
DOI: 10.5455/ejfs.165152

Original Research

Driving under the influence: Prevalence of drugs in oral fluid

Amparo Arroyo, Eneko Barberia, M. Teresa Marron, Jordi Medallo

Department of Laboratory, Institute of Legal Medicine of Catalonia, Gran Via de les Corts Catalanes, Barcelona, Spain

Address for correspondence:
Dr. Amparo Arroyo, Institute Legal Medicine of Catalonia, Gran Via de les Corts Catalanes, Barcelona, Spain. Tel.: +34 646846276, Fax: +34 936561154, E-mail: 8034aaf@comb.cat

Received: July 23, 2014
Accepted: September 18, 2014
Published: October 10, 2014

ABSTRACT

Nowadays, detection of drugs of abuse is a usual practice in the legal field due to its incidence in several proceedings. Saliva is a matrix of increasing utility as it is a non-invasive sample that has been tested in international projects such as ROSITA and drove under the influence of drugs (DUID). **Objectives:** The study focused on the study of prevalence of drugs of abuse in a sample population of drivers of motor vehicles 3468 oral fluid samples came from local police activities, during the years 2007 until June 2010 in Barcelona (Spain). **Materials and Methods:** Drivers suspected of DUIDs had to comply with an analytical road-side drug testing. A commercial kit immunoassay based was used (Cozart® DDS 801). Kits with positive results to any drug, 24.59%, were submitted to the Catalonia Institute of Legal Medicine, with an additional saliva sample (as indicated by Cozart® DDS 801 provider) for confirmation by gas-chromatography/mass-spectrometry (GC/MS). Drugs detected by the test Cozart® included: D9 tetrahydrocannabinol, cocaine (COC), opiates, methamphetamines (MAP) amphetamines (AMP). **Results:** After confirmation results showed a cannabis prevalence in 2064 samples (59.5%), COC in 1952 samples (56.2%) opiates in 258 (7.4%) AMP in 69 samples (1.9%) and MAP in 57 (1.6%). No quantitative analysis were achieved. **Conclusions:** Results show that cannabis is the most prevalent in the study, followed by COC. Data are valuable in order to initiate sanction proceedings in Spanish legislation and also as signs of recreational drugs consumption which provide information to both epidemiology and public health.

KEY WORDS: Forensic sciences, forensic toxicology, drug abuse, drugs analysis, saliva, traffic safety, toxicology

INTRODUCTION

In recent years, driving under the influence of the drugs (DUID) has been increasing, and many countries are dealing with this problem. Drugged driving puts at risk people who share the road DUID remains a persistent problem in traffic safety, despite the recent advances in traffic safety. The percentage of drug positive drivers is established in 1-15%, but the percentages ranged from 6 to 35% when accidents have a fatal outcome [1]. Different European countries such as Belgium [2], as well as Australia [3] and several US States [4] have modified the legislation and specific laws have been issued referring to traffic legislation. The prevalence of cannabis consume in the last year in Europe was 6.7% for adult Europeans, and 1.2% for cocaine (COC). Spanish legislation forbids driving under the influence of any toxic drug, narcotic substances or under the influence of prescription drugs that could affect the physical or mental capabilities thus making dangerous the fact of driving. Spain shows the highest prevalence in cannabis and COC [5]. In Spain, in 2010, 42.4% of drivers and 38.8% of pedestrians killed in road traffic crashes had a positive result to alcohol or drug presence; 12.5% of drivers killed had a positive result to drug of abuse, mainly COC and cannabis [6]. Statistical data related to drivers fatalities in traffic accidents in Catalonia (Spain) reports 6.9% of positive results to drugs presence [7]. Prevalence of recreational drugs in oral fluid (OF) of drivers in Catalonia has been reported previously in a sample of 632 drivers suspected of DUID. In this sample, 85% was positive for any drug [8].

During the year 2007, the Generalitat de Catalonia carried out drug detection tests in OF of drivers obtaining a positive result in two of every three tests. OF testing has revealed its usefulness in detecting recreational drugs in various studies [9,10] It is concluded that the detection of psychoactive substances in OF taken at the roadside is predictive for the detection of the corresponding drug or its metabolite in serums, since the pharmacokinetics of several drugs in blood and OF is quite similar [11,12]. The detection window of drugs in OF comprises several hours to 1-2 days depending on the drug and usually have similar time-courses to that in plasma; for that reason a recent consumption of drugs may be detected [13].

The development of on-site testing devices for recreational drugs started in the 1990s [14] and has been developed for detecting drugs in OF, urine and sweat. The utility of OF as a sample matrix for the detection of cannabis abuse is largely due to collection advantages, since it can be used in various settings and situations, such as roadside drug testing. It is widely recognised as a non-invasive and tamper-resistant screening method, which can identify drug use as accurately

as blood testing, given the relative good correlation between the two fluids, specially regarding impairment when comparing to urine.

The aims of this study were to examine the trends and prevalence of recreational drugs in OF in a sample population of drivers of motor vehicles. The subjects were screened by Catalonian Police [15] for drugs use during 2007 until June 2010.

MATERIALS AND METHODS

Sample Selection

OF samples were collected from drivers suspected of being DUIDs but not involved in any infraction or accident. The selection of drivers was random and always carried out by the traffic police in Catalonia (Spain). Samples were collected in leisure environment places, near discotheques or raves, at night during weekends (in general, Friday and Saturday nights). In order to collect the OF samples, traffic police were trained by the Cozart provider team but no data about inter or intra observatory error was supplied. Screening tests carried out in the period 2007-until June 2010 were 14410. From the total, 3468 OF presumptive positive samples for any drug were sent to the laboratory for confirmatory analysis. Samples were taken in the geographic area of Catalonia, mainly Barcelona and near surroundings.

Immunoassay Test

Screening test of OF samples was performed by applying Cozart® DDS 801 test (Bioscience Laboratories Ltd., Abingdon, Oxfordshire, United Kingdom 2006) [16] The test detects the following families of drugs: Amphetamines (AMP), methamphetamines (MAP), cannabinoids, opiates and COC. No other drugs are investigated. The Catalonia Project was initiated with Cozart® DDS 801 device and has not been changed as the mentioned drugs are the most representative ones in Spain. A swab sample collector was introduced into the oral cavity until the indicator turned completely blue. The swab was then placed into the dropper bottle that contained the extraction buffer. The contents of the bottle were mixed completely. Once the sample and the buffer were mixed, the dilution factor obtained was 1:3. Four drops of the mixed contents were applied across the sample well of the test cartridge into the reacting well. The volume of the sample to developing the reaction is 220 μL according to specifications provided by the manufacturer. According to these instructions the time required to complete the test was 5 min, and the control line has been checked in order to consider it as correct. The tests were interpreted in situ. Visual inspection has considered positive results and when they were positive, an OF additional sample was immediately collected in order to confirm results. This second additional sample was a neat OF sample, obtained directly by spitting of the driver in a new tube supplied by the manufacturer. No buffer is provided by the manufacturer for this second sample. Buffer solution is added to the second sample to diminish the viscosity of the sample when confirmatory analysis is achieved in the laboratory. Nevertheless no considerations of dilution factors or weighing tubes corrections have to be taken in account as no quantification of qualitative results was done. The cut-off values of Cozart® DDS 801 test were: COC family 30 ng/ml, Δ9 tetrahydrocannabinol (THC) family 31 ng/ml, opiates and AMP family 50 ng/ml and MAP family 300 ng/ml.

Chemicals and Reagents

Methanol solutions with a concentration of 1 mg/ml of COC, benzoylecgonine, ecgonine methyl ester (EME), THC, 11-nor-9-carboxy-Δ9 -tetrahydrocannabinol (THC-COOH), AMP, MAP, dl-3,4-metylendioxymehylamphetamine (MDMA), dl-3,4-metylendioxiethylamphetamine (MDEA), codeine (COD), morphine (MOR) and 6-monoacetylmorphine (6-MAM) were purchased from Alltech-Applied Science® (State College, PA, USA). Methanol solutions with a concentration of 1 mg/ml of deuterated analogues of the drugs (d3-COC, d3-THC, d3-THC-COOH, d6-AMP, d9-MAP, d5-MDMA, d5-MDEA, d3-MOR and d3-6-MAM) were purchased from Alltech-Applied Science (State College, PA, USA).

Due to the fact that in most of the cases the derivatization of the drug in the sample is necessary, N,O-bis-(trimethylsilyl) trifluoroacetate (BSTFA) and trimethylchlorosilane (TMCS) used as a dissolution of BSTFA + 1% TMCS were used for derivatization of THC, THC-COOH, 6-MAM, MOR, COD, BEG and EME both in the normal and in the deuterated form. BSFTA and TMCS were provided by Supelco (Bellefonte, PA, USA). 2, 2, 3, 3, 3-Pentafluoropropionic acid (PFPA) was used for derivatization of AMP, MAP, MDMA, MDEA both in the normal and the deuterated form. PFPA were purchased from Merck KGaA (Darmstadt Germany). In the analysis of COC, the use of derivatization agents is not necessary.

Phosphate buffer (0.1 M) was prepared from NaHPO4 (VWR Prolab) and adjusted to pH 6.0 with NaOH 0.1M (Panreac Química SA).

Sample Preparation

The OF samples were stored at +4°C. The delay between the OF sampling and the analysis varied from 24 to 48 h of the reception of the samples. The samples were kept refrigerated at 4°C in police departments until its delivery to the laboratory and custody chain was guaranteed. Sample preparation consisted of the addition of 1 μl of OF to 4 ml of phosphate buffer (pH 6). Once the pH was readjusted samples were homogenized for 15 s, centrifuged for 10 min at 10000 rpm and transferred into a liquid phase extraction toxitube A cartridge (Varian). The toxitube contains both, an aqueous and an organic phase. Agitation is performed during 10 min and once again centrifuged for 10 min at 10000 rpm to separate both phases. The organic phase is extracted, evaporated to dryness under nitrogen and reconstituted with the derivatization agents BSTFA-TMCS for 25 min to 70° and amphetamines analysis with PFPA for 40 min to 40°C.

Gas-chromatography/Mass-spectrometry (GC/MS) Analysis

The detection limits for confirmatory analysis were: Amphetamine, MAP and opiates family 5 ng/ml, COC and THC family 2.5 ng/ml.

A Varian Inc. (Palo Alto, USA) 3800 gas chromatographs coupled to a 4000 ion trap mass spectrometer operating in electron impact ionisation mode was used for analysis. The gas chromatographic column was 5% phenyl-95% methyl silicone VF-5ms, (Varian Factor Four Capillary Column) and the injection temperature was 250°C. 2 μl of the sample were injected in split-less mode. The oven was programmed from 90ºC for 1 min; ramped at 20°C/min up to 240°C; then ramped at 5°C/min to 300°C where it remained for 2 min. The transfer line was held at 280°C. The total run time was 23.5 min. Details of the detection procedure are shown as well as the different substances and qualifier ions identified by GC/MS [Table 1]. Confirmation of the results obtained by the immunoassay test has been performed by using GC/MS in full scan mode. Validation of the GC/MS method has not been published but more than 11000 proofs have been processed at the laboratory.

RESULTS

Drivers positive for recreational drugs consisted mainly of young men (mean age 29.31 year, standard deviation: 8.382, range 15-68 years); participants sex was 93.1% men, 4.2 women. No data available 2.7%. In Catalonian Project, the distribution and the total number of screening tests carried out is shown in Figure 1. Comparison of results Cozart DDS versus GC/MS by year are shown in Graphic 1. Regarding drugs prevalence, distribution and percentage of recreational drugs by year in the confirmatory analysis, are shown in Table 2. Cannabis was positive in 2064 (59.5%) COC in 1952 (56.2%), opiates 258 (7.4%) amphetamines 69 (1.9%) and MAP 57 (1.6%). Polyuse of drugs and multipositive samples by year is shown in Graphic 2.

Table 1: Spectrometric GC/MS conditions for target drugs

Drug target	Drug family	Qualifier ions	RT min
COC	COC-F	182, 82, 303	17.40
BEG-TMS	COC-F	240, 82, 362	17.78
EME-TMS	COC-F	272, 82, 182	11.68
Δ9THC-TMS	THC-F	386, 371, 315	18.47
11-nor-9-carboxy-THC-TMS	THC-F	473, 371, 73	20.75
MDMA-PFP	MA-F	204, 162, 135	11.84
MDEA-PFP	MA-F	218, 190, 135	12.00
AMP-PFP	AMP-F	190, 91, 118	8.86
MAP-PFP	AMP-F	204, 91,160	9.86
6-acethylmorphine-TMS	OPI-F	399, 340, 224	19.88
COD-TMS	OPI-F	371, 73, 178	19.08
MOR-TMS	OPI-F	429, 73, 146	19.33

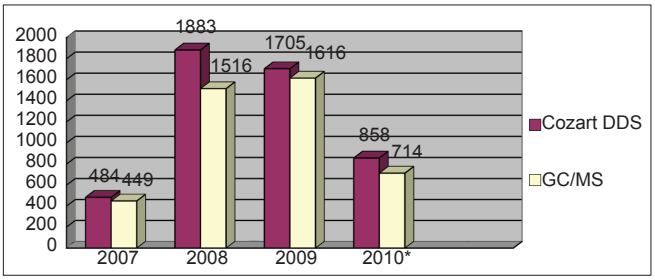
THC: Tetrahydrocannabinol, TMS: Trimethylsilane, PFP: Pentafluor opropionic, MDMA: dl-3,4-Metylendioxymehylamphetamine, MDEA: dl-3,4-metylendioxiethylamphetamine, GC/MS: Gas-chromatography/mass-spectrometry, RT: Retention time, AMP: Amphetamine, BEG: Benzoylecgonine, EME: Ecgonine methyl ester, COD: Codeine, MOR: Morphine, MAP: Methamphetamines, COC: Cocaine

This data indicates that consumption of opiates and amphetamine is inferior compared to cannabis and COC.

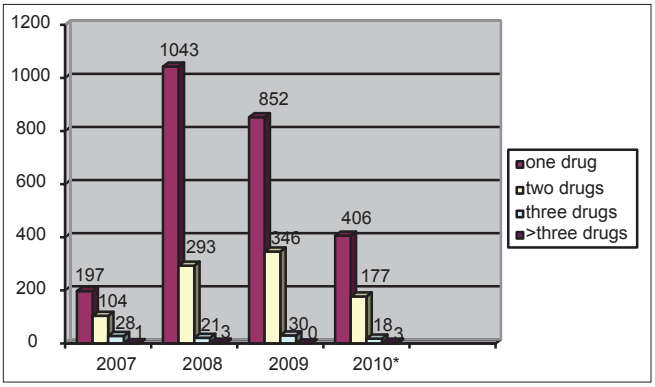
DISCUSSION

A high predominance of cannabis and COC was represented. Among the drivers, the association of recreational drugs cannabis and COC were the most frequently found.

We have not found reports with the same characteristics of our population, drivers suspicious of being under the influence, samples collected at night time, on weekends and near leisure places. Some of the publications deal with samples selected from the population driving at night time [17]. In this population, 14% of the drivers were positive for drugs where half of them positive for cannabis (7.6%). Beirness and Beasley [18], in a random sample of night drivers in British Columbia, detected 10.4% of subjects tested positive for drugs, where cannabis and COC were also the drugs most frequent The study was



Graphic 1: Comparison of results Cozart DDS versus gas-chromatography/mass-spectrometry by year.*Until June 2010



Graphic 2: Samples multipositive by year: *N* = 3468. *Until June 2010

Table 2: Prevalence results of confirmatory analysis

	2007	2008	2009	2010*	Total
	N=330 *n* (%)	*N*=1306 *n* (%)	*N*=1228 *n* (%)	*N*=604 *n* (%)	*N*=3468 *n* (%)
Cannabis	211 (63.9)	722 (55.2)	775 (63.1)	356 (58.9)	2064 (59.5)
COC	213 (64.5)	653 (50.0)	700 (57.0)	386 (63.9)	1952 (56.2)
Opiates	9 (5.7)	103 (7.8)	106 (8.6)	30 (4.9)	258 (7.4)
AMP	15 (4.5)	9 (0.6)	23 (1.8)	22 (3.6)	69 (1.9)
Methamphet	5 (1.5)	30 (2.2)	12 (0.9)	10 (1.6)	57 (1.6)

*Data until June 2010, *N*=Total positive samples by year, *n*=Positive samples by drugs and year. AMP: Amphetamines, COC: Cocaine

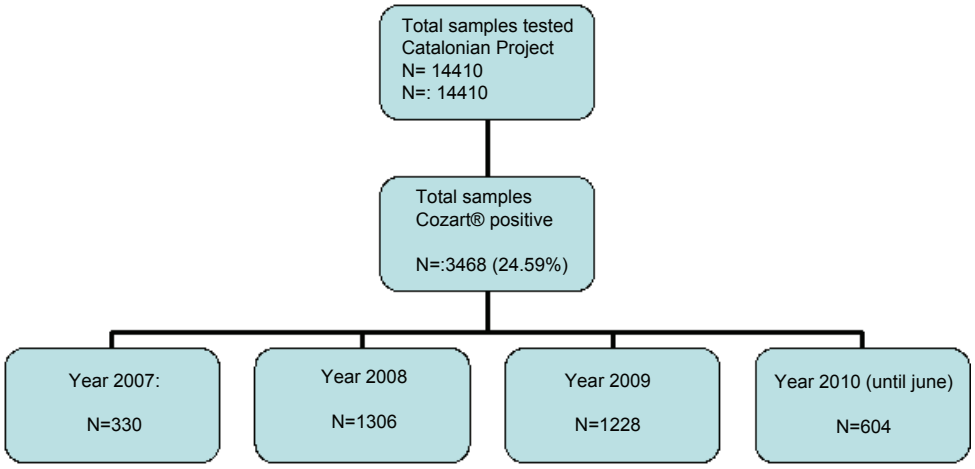


Figure 1: Total sampled tested: Total positive screening samples distribution screening positive samples by year

conducted between the hours 9.00 p.m. and 03.00 a.m., on Wednesday through Saturday nights, although drivers were selected from the traffic stream, not from leisure places. On weekend nights, another study have shown 10.5% of drivers using illegal drugs [19] and, in California 14.4% of weekend night-time drivers tested positive for illegal drugs, 8.5% for cannabis [20].

In our country, Gómez-Talegón *et al*. [21] have also reported data of illegal drugs alcohol and psychoactive substances in drivers in a sample of 3407 cases were cannabis and COC prevalence was 7.7% and 3.5% respectively. The study shows differences in the prevalence of positive cases of alcohol, cannabis and COC, in relation to the period of the week; in three cases the highest prevalence seen in night time. In a similar context, nightlife, studies provide data more related to traffic risk, traffic accidents and risk behaviours [22,23].

All these studies share some variables matching with the design of our study: Sample collection on weekends, at night time and nightlife environments. Overall, we want to stand out the specific characteristics of our sample, the dependent and independent variables, the sampling technique and the analytical methodology and cut-off established. For that reason comparison with other samples may bias the interpretation of results of our sample and the prevalence of drug use among the general Spanish drivers population.

Limitations of the study may be considered. The presence of other substances, alcohol or benzodiazepines, have not been previously evaluated, and no information was collected relating to medication taken that may account for the opiate positive results. The reason is that we have not designed the initial methodology of the study; the study was scheduled and initiated by Police Department in Catalonia referring to recreational drugs of abuse in OF of drivers and not alcoholic proofs were done at the same time in this population. So we can not provide any data. This study was carried out in Spain with an important number of subjects and at present it is going on. Partial report of results has been published by us in a small sample where clinical effects of drugs and correlation with impairment were also considered [8].

Screening devices for detecting drugs in OF provide an indirect aid to the traffic control. It can be assumed that the ability of the police to screen for drug users in police checkpoints might determine drugged persons from driving and hence improve road safety. In Catalonia, more than 25000 tests have been applied, using the Cozart® DDS 801 test, from 2006 until now. Statistical parameters of efficiency of the test have not been attended in this study, but at present a paper dealing with performance of the Cozart DDS 801 diagnostic test and parameters of sensibility, specificity, positive and negative predictive values for COC and cannabis has been published by our group [24].

If OF screening is positive, administrative measures are taken, and sanctionary proceedings are initiated after confirmatory analysis of the suspect. An initial suspicion of impairment is established using a drug recognition test, based on external signs of recreational substance use, although symptoms of drug use are not always clearly recognizable. At present, in Spain more Police Departments are spreading the screening tests to other parts of the country. Nevertheless referring to recreational and illegal drugs, Spanish legislation is not as well defined as the alcohol one and not concentration values are yet scheduled as legal limits for safety driving

In Spain, drivers who give a positive test with any toxic substance are administratively punished, according to A°27 and A°28 of the Spanish Traffic Regulation [25]. With a 500 € fine and incur demerit points against their driver' licence. If they get 12 demerit points, they lose the driving licence. Besides, if they get involved in a car collision, they are penally punished by losing their driving licence, receiving an x sentence that can be substituted by benefits of community service and the corresponding fine. Recently a new Spanish legislation has been published related to traffic regulation including driving under the influence of alcohol and drugs [26]. The fine has been elevated to 1000 € and six demerit points if driving when the presence of illegal drugs is detected in the subject.

CONCLUSION

In conclusion, data provided by this study and Catalonia Project are valuable as signs of drugs consumption that provide information to both epidemiology and public health.

ACKNOWLEDGMENTS

To Mr. Aizpun for writing assistance and to the Forensic Laboratory team for their collaboration.

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Source of Support: Nil, Conflict of Interest: None declared.