

# Times of Detection of Drugs of Abuse in Saliva: Study of Arrested Population

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## Abstract

**Aim:** Detection of drugs of abuse is interesting in legal procedures. The aims of the study were: 1: Evaluating the detection time of drugs of abuse in saliva in an arrested population, drugs consumers that are taken into judicial custody in a maximum time of 72 hours from their detention. 2- Verifying analytical results with self reported reference of the subjects.

**Participants:** 50 oral fluid samples coming from arrested people that are taken into judicial custody in police officer's courts of the city of Barcelona. The study was carried out in the Laboratory of the Institute of Legal Medicine of Catalonia. The design study was a cross sectional study. Drug tested were: cocaine, amphetamines and related compounds, cannabis and opiates. The time of previous consumption was of 1-3 days.

**Measurements:** The oral fluid samples were analysed by gas- chromatography-mass spectrometry.

**Findings:** Positive results were obtained in 40% of the samples. Cocaine was detected in the 73.9% of positive results to this drug. Cannabis was detected in the 28.5%, Opiates was detected in the 23, 1%. Amphetamines were not detected.

**Conclusions:** It is important to stand out the high rate of cocaine positive results in relation with the time passed and abstinence consumption of 1-3 days. Oral fluid may be a good sample for cocaine detection in drug consumers.

**Keywords:** Forensic sciences; Forensic toxicology; Drugs of abuse; Drug analysis; Oral fluid analysis; Legal medicine

## Introduction

At present detection of drugs of abuse is of interest in different fields of legal medicine. Among others, saliva is a fluid that has increased in the last years for this purpose and the publication of texts as the Proposed Revisions to Federal Mandatory Guidelines for Workplace Drug Testing Programs emphasizes its acceptance [1]. The advantage of this fluid is not being an invasive sample, easy to obtain and difficult to tamper with or to forge. In a progressive and routine form the saliva is analysed coming from samples of the workplace, criminal justice and rehabilitation centres. It is necessary to add the kits of detection that begun to be tested in 1983 on side of highways, to detect drivers who were driving under the influence of the drugs [2].

Schramm et al. [3] had provided an early review on detection times of drugs and early studies pre 1990s. Other reviews on the testing for drugs of abuse in saliva also exist [4].

Toxicologists are frequently asked about the duration of detection times of drugs, but is difficult to get approval for this kind of studies because illicit products have to be given to healthy volunteers and doses administered are low compared with street doses.

The aims of the study were: a) to evaluate detection times of drugs of abuse in saliva in a drug abuse population, arrested people who are taken into judicial custody. Drugs tested were; cannabis, cocaine, amphetamines, methamphetamines and related compounds (MDMA and MDEA) and opiates. Qualitative analysis was carried out. The time between the detention of arrested people and time of collecting saliva sample was 1-3 days. Oral fluid were collected from 50 individuals and analyzed by gas-chromatography-mass-spectrometry (GC-MS). b) To compare analytical results with the self-referred information by the arrested people related to the time on the last consumptions of toxics.

## Material and Methods

### Design of the study: cross-sectional study

**Size of the sample:** 50 oral fluid samples coming from arrested people that are taken into judicial custody in police officer's courts of the city of Barcelona. The study was carried out in the Laboratory of the Institute of Legal Medicine of Catalonia.

**Selection of the sample:** The participation was requested to the detainees that were addicted to drugs that used the right to be looked after by a forensic doctor according to the legal spanish normative [5]. All subjects had a history of chronic drugs abuse consumption. Inclusion criteria included self-reported use of smoked, inhaled or intravenous drugs at least for six months prior to the detention. The participants provided informed consent. Times of abstention consumption could not be accurately established, but ranged from 1-3 days during staying in the police setting. Additional information was gathered relative to the sex and age of the participants.

**Methods:** Obtaining of the sample: the sample of saliva was obtained directly by spitting in a polypropylene tube. Saliva flow was not stimulated. Following collection oral fluid was aliquoted into cryotubes and frozen at -20° until analysis.

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Descriptive analysis of categorical variable and media, range and standard deviation of quantitative variable were done. Identification of analytes were done by GC-MS. Quantification of analytes was not done.

**Sample preparation:** Sample preparation consisted in the addition of 1ml of oral fluid, without centrifugation, and the pH was readjusted with 1ml of phosphate buffer (pH=6). Once the pH is readjusted, 20µl of d3-cocaine, d3-6-MAM, d5-amphetamine, d9-methamphetamine and Δ<sup>9</sup>-THC are added for a final concentration of 10µg/ml.

Sample was homogenised for 10 min. and transferred in a Toxityube A<sup>®</sup> which was waved for 10 min and centrifuged (3500 rpm for 10 min). The organic phase was extracted, evaporated to dryness under nitrogen and derivatized with 40µl of BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide) -TMCS (trimethylchlorosilane) at 80°C for 20 min for Δ<sup>9</sup>-THC and 6-MAM or PFPA for amphetamines and methamphetamines at 50°C for 40 min.

### Gas chromatography-mass spectrometry confirmation

**Chemicals and materials:** Methanolic solutions with a concentration of 1 mg/mL of cocaine, Δ<sup>9</sup>-tetrahydrocannabinol (Δ<sup>9</sup>-THC), amphetamine, methamphetamine, dl-3, 4-methylendioxi-methamphetamine (dl-3, 4-MDMA), dl-3, 4-methylendioxiethamphetamine (dl-3, 4-MDEA) and 6-monoacetylmorphine (6-MAM) were purchased from Alltech-Applied Science (State College, PA, USA).

For derivatization of Δ<sup>9</sup>-THC and 6-MAM, BSTFA and TMCS used as BSTFA + 1%TMCS were provided by Supelco (Bellefonte, PA, USA) and 2,2,3,3,3-Pentafluoropropionic acid (PFPA) by Merck KGaA (Darmstadt Germany). Phosphate buffer (0.1 M) was prepared from NaH<sub>2</sub>PO<sub>4</sub> and adjusted to pH 6.0 with NaOH 0.1M.

**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 factor Four Capillary Column) and the injection temperature was 250°C. 2µl of the sample were injected in split-less. 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 done operating in MS/MS. Details of the procedure of detection applied are shown in Table 1.

The different substances and ions used in identification by MS-MS are shown in Table 2. The detection limits (LOD) and quantification limits (LOQ) values used in the GC-MS/MS confirmation and LOD and LOQ established for GC/MS-MS are shown in Table 3. No attempts were made to determine quantitative values in positive results samples.

Drug Target	Ionization	Waveform Type	Excitation Width (V)
Amphetamine-PFP	El <sup>a</sup>	Non resonant	64.0
Methamphetamine-PFP	El	Non resonant	60.0
MDMA-PFP	El	Non resonant	60.0
MDEA-PFP	El	Non resonant	57.0
Cocaine	El	Non resonant	45.0
Δ <sup>9</sup> -THC-TMS	El	Non resonant	61.0
6-MAM-TMS	El	Resonant	1.0

**Table 1: Drug targets and procedures used in GC-/MS/MS.**

Drug Target	tr (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 <sup>9</sup> -THC-TMS	17.43	386	371,315,330
6-MAM-TMS	20.11	399	356,340,287

**Table 2: Drug targets, retention times (tr) and ions selected for each studied drug.**

Drug Target	GC/MS-MS	
	LOD (ng/ml)	LOQ (ng/ml)
Amphetamine-PFP	5	20
Methamphetamine-PFP	5	20
MDMA-PFP	5	20
MDEA-PFP	5	20
Cocaine	2.5	10
Δ <sup>9</sup> -THC-TMS	2.5	10
6-MAM-TMS	5	20

**Table 3: Detection limits (LOD) and detection quantification limits (LOQ) values for GC-MS/MS analysis.**

Case	t	Analyte	Case	t	Analyte
Nº2	1	Cocaine + 6 MAM	Nº21	2	Codeine
Nº3	3	Cocaine	Nº23	1	Δ <sup>9</sup> tetrahydrocannabinol
Nº4	1	Cocaine	Nº27	3	Cocaine
Nº6	3	Cocaine	Nº28	3	Cocaine
Nº9	2	Cocaine +codeine	Nº29	2	Cocaine+ecgonidine-metilester
Nº10	2	Cocaine	Nº30	2	Cocaine+Δ <sup>9</sup> tetrahydrocannabinol
Nº11	2	Cocaine	Nº31	3	Cocaine+Δ <sup>9</sup> tetrahydrocannabinol
Nº15	2	Cocaine	Nº32	3	Cocaine
Nº18	2	Cocaine	Nº36	<1	Cocaine
Nº20	2	Δ <sup>9</sup> tetrahydrocannabinol	Nº48	2	Cocaine

t: time in days of previous consumption

**Table 4: Analytes confirmed by CG-MS in positive results (N=20).**

Total N=50	Self reported Drugs Consumption	Drugs Confirmed Analysis CG-MS
Cocaine	N= 23	N=17
Cannabis	N=14	N=4
Amphetamines	N=1	*ND
Opiates	N=13	N= 3
Methadone	N=14	N=13

\*ND: not detected

**Table 5: Analytical Results and self reported consumption of drugs.**

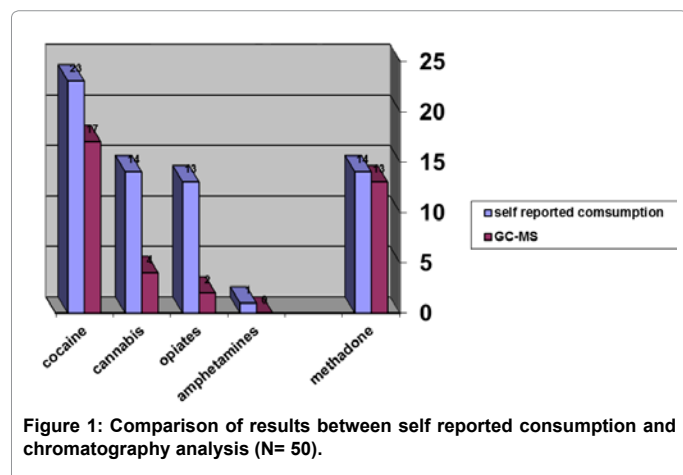
## Results

Middle age of subjects was 31, 6 years (range 38, max. 56, and min 18, SD 8.5).

Male gender was predominant. Female gender was 12% of the sample. Positive results to drugs were confirmed in 40% of the sample, 20 cases. In these cases drugs were confirmed for at least one analyte 80%, and two drugs were found in 20% of this group.

Cocaine was the principal analyte confirmed, 73, 9% although cocaine and ecgonidine- metilester was detected in two cases of this group. Δ<sup>9</sup> Tetrahydrocannabinol was confirmed in 28.5%. Opiates were confirmed in 23, 1% of the individuals who referred consumption (one case 6- MAM, 2 cases codeine) Amphetamines were not detected. Abstinence referred drugs consumption times in the 20 positive cases results were 20% one day, 50% 2 days, 30% three days. Cases and results positive to drugs are exposed in Table 4.

Results comparing self reported consumption cases and GC-MS confirmed cases are exposed in Table 5 and Figure 1. Methadone



was found in the 92.8% of the subjects who were in the maintenance program, although this drug was not specifically scheduled for detection.

## Discussion

The most important information of our results is the detection of cocaine in a high percentage of the sample, after an interval of abstention from consumption among 1-3 days, 17 cases. Only a few investigators have evaluated the pharmacokinetics of orally administered cocaine and even less has been published about the pharmacokinetics of chronic cocaine administration [6,7,8]. These results do not match with previous data known about the rapid decrease of cocaine and acceptance that drugs in saliva follows the same metabolic course that in blood. Some studies indicate that the cocaine in saliva can be detected up to 17 hours with a correlation of 3 with the plasma [9, 10]. Nevertheless, it is important to emphasize some works where longest times of detection in chronic consumers are described. Cone and Weddington [11] provided evidence of prolonged excretion of cocaine in oral fluid specimens collected from heavy cocaine users during abstinence and postulated that cocaine can be excreted in low concentration over long period of time. Cocaine is a lipophilic compound that can be stored in bodily tissues to a greater extent than the more water soluble metabolite benzoilecgonine, following repeated dosing and result in accumulation in bodily tissues. It is likely slower elimination phase for the drug and extended elimination times upon cessation of use. Although cocaine has an extremely short half-life of approximately 1 hour, accumulation in tissues could conceivably result in prolongation of effects, amelioration of withdrawal, and alteration of detection times. These authors indicate an average life of elimination of cocaine in saliva between 21.6-110.4 h. In chronic users even it can reach 10 days with a LOD 0, 5 ng/ml. Other authors agree with this statement [7]. It remains unclear how repeated dosing alters or prolongs detection times.

Our population belongs to a group of drug dependent chronic consumers and it would justify the prolongation of time of detection of cocaine what agrees with data published by the mentioned authors.

Only confirmation of the cannabis was obtained in 4 of 14 cases where a previous consumption was reported. According to Niedbala the cannabis can be detected in saliva up to 30 hours after the consumption to certain doses [12]. According to other authors is detected between 2-10 hours [13,14]. Laloup et al. [15] found good correlation between the THC in saliva and plasma in 139 subjects what agrees with other publications though protocols of analysis and values of cut-off

established in every study can be different and may affect the results [16].

Only one case of amphetamines consumption was revealed but not confirmed in the analytical chromatographic study. Amphetamines in saliva, since they are basic drugs, can present higher concentrations than in plasma [17]. Actually very sensitive methods have been published for its detection and quantification [18]. Having had only one sample we cannot get conclusions. Opiates were only confirmed in 3 of 13 cases although codeine was the analyte found in two cases. The detection of opiates can arrive up 24 h, according to administered dose [19] though Speckl et al. point out that time of detection can be situated between 1-4 days [20].

In our sample we have detected methadone in 13 of 14 self reported consumption cases. It was not one of our objectives because of methadone analysis is not so routinely investigated since its administration belongs to sanitary established programs.

Detection times are generally determined in drug administration studies performed with a small number of individuals who are housed in a close setting. Most frequently detection times are determined in studies in which a single dose is administered. Some caution is needed because of inherent limitations on detection times based in these studies that may not be truly representative of frequent drugs users. The detection time is influenced by many factors: the dose that was taken, the preparation and route of administration, acute versus chronic use, the choice of the matrix, the detection time or cut-off of the analytical technique, the nature of the molecule or the metabolite sought, the pH and concentration of oral fluid and the interindividual variation in metabolism [21]. Unfortunately there is a paucity of data on drug detection times following repeated use. These studies, under controlled conditions are exceedingly difficult and expensive to perform and few studies have focused on the detection time. Volunteers' studies are based in controlled administration in subjects imprisoned or admitted for detoxification. The work that gathers a good number of experimental studies in volunteers' saliva is Drummer's review that describes 23 studies [22]. The analysis were carried out after short intervals of time from the administration of the compound, up to the capture of the sample of saliva; the detection of the drugs was not evaluated in long times.

The limitations of our study and of others published on the matter are the difficulties of carrying out controlled studies in a big sample and the scanty quantity of sample that is possible to obtain, since many drugs originate dryness of mouth.

Secondly confusion can exist in the information reported by the subjects about drugs abuse consumption. Concordance was not full between the self reported data and the analytical results. In relation with cocaine, in 3 detected cases the consumption was not referred, and in 8 cases where the consumption was referred the results were not confirmed.

In our study it was not possible to specify the quantities of administered substances which is controlled in the experimental studies. For that reason quantitative analysis of these substances in oral fluid was not done and our objective was only to confirm a qualitative result.

In conclusion detection times related to opiates and cannabis are not reliable in a period of 1-3 days but cocaine time detection may be longer and detectable in chronic drugs users, which agrees with other published data but a larger sample must be investigated in order to corroborate our results.

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