



Drugs in oral fluid Part II. Investigation of drugs in drivers

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Abstract

As part of the European project, Impaired Motorists, Methods of Roadside Testing and Assessment for Licensing, otherwise known as IMMORTAL (Deliverable R4.2), the University of Glasgow was required to analyse 1396 oral fluid samples, collected from drivers, for a wide range of drugs. A previously described method to include 49 drugs and metabolites was used. To include cannabis in the study a separate extraction method was required because of interferences caused by the collection device. The study group included drivers who were stopped at random and participation was entirely voluntary. The results showed that out of the 1396 samples tested, 16.8% were positive for at least one drug. In the majority of positive cases (85%), monodrug use was found and the most commonly detected drug was 3,4-methylenedioxymethamphetamine. This study showed that a significant number of the driving population are positive for at least one drug.

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1. Introduction

Over the past decade a marked increase in the number of individuals misusing drugs in the West of Scotland has been recorded. As a result of this, the number of drug related fatalities and road traffic cases involving drugs has increased over this period [1–3]. However, this increase in the number of people driving under the influence of drugs is not unique to this country as demonstrated by the number of international publications [4–7].

Oral fluid analysis for drugs of abuse has become of great interest to police forces for roadside drug testing. The main benefit of such a sample is its non-invasive collection procedure compared with blood, or the inconvenience/embarrassment of urine sampling which requires the observation of a police officer. This has prompted many com-

mercial companies to manufacture devices which can be used to screen drugs in oral fluid at the roadside; a drug equivalent to the alcohol breath test device [8]. This means that samples would have to be transported to the laboratory for confirmation. At present, none of these devices have the sensitivity or specificity to successfully detect an extensive range of drugs.

There are several devices currently available for the collection of oral fluid [9]. Only one, however, collects a known volume of oral fluid. The Omni-Sal[®] device uses a cotton pad at the end of a stick to collect 1 mL of oral fluid which is then diluted in 2 mL of buffer. A modification of this device was used in this study to ensure collection of a known volume. As part of the European project, Impaired Motorists, Methods of Roadside Testing and Assessment for Licensing, otherwise known as IMMORTAL (Deliverable R4.2), the University of Glasgow was required to analyse oral fluid samples, collected from drivers, for a wide range of drugs. Drivers were stopped at random and asked to parti-

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cipate in this study. A total of 1396 oral fluid samples were collected and analyzed by a previously described method [10]. Interferents from the buffer in the collection device caused problems with the cannabis analysis in the extracts. For this study, therefore, enzyme immunoassay followed by an alternate extraction method using gas chromatography-mass spectrometry (GC–MS) analysis was required for cannabis confirmation.

2. Materials and methods

2.1. Specimens

Random check points were set-up in Glasgow by Strathclyde Police. The areas of sample collection were selected by the high number of accidents associated with a particular area. Traffic was stopped randomly by a police officer and the driver was requested to participate in the study. Consent was obtained from the driver prior to their participation in the study. An independent researcher was involved in the process and it was their duty to request an oral fluid sample from the driver. Participation was on a voluntary basis and if an individual declined the police would request a reason and the driver then permitted to leave. Sample collection was carried out over a 24 h period [11]. Oral fluid samples were collected using Omni-Sal[®] devices with proprietary modifications as supplied by Cozart Biosciences Ltd., Abingdon, UK. The oral fluid samples were sent to Forensic Medicine and Science, University of Glasgow for analysis.

2.2. Chemicals and reagents

HPLC grade methanol, ethyl acetate and hexane were purchased from BDH (Poole, England). Glacial acetic acid was also purchased from BDH. Sodium acetate trihydrate and bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA) was obtained from Sigma–Aldrich (Dorset, UK).

Delta-9-tetrahydrocannabinol (Δ^9 -THC) and trideuterated delta-9-tetrahydrocannabinol (Δ^9 -THC- d_3) were produced by Radian International and purchased from Promochem Limited (Herts, UK) as 100 $\mu\text{g}/\text{mL}$ solutions in methanol. A 1 $\mu\text{g}/\text{mL}$ working standard was prepared by measuring 100 μL of stock standard Δ^9 -THC into a 10 mL volumetric flask and making up to the volume with methanol. This solution was used to spike blank oral fluid collected using the modified Omni-Sal[®] device.

The deuterated standard was used as an internal standard and a working standard was prepared in the same way.

Immunoassay kits for cannabinoids in oral fluid were supplied by Cozart Bioscience Ltd. (Oxfordshire, UK). Bond Elut Certify[®] II LRC solid phase extraction cartridges (200 mg, 10 mL) were produced by Varian and purchased from Promochem Limited (Herts, UK).

2.3. Solutions

2.3.1. 0.1 M sodium acetate buffer with 5% methanol

An amount of 6.8 g of sodium acetate was dissolved in 400 mL deionised water, the pH was adjusted to 7.0 with 1 M hydrochloric acid and the total volume was made up to 500 mL with deionised water. 25 mL of the solution was removed and replaced with 25 mL of methanol.

2.4. Instrumentation

A European Diagnostic Products Mark 5 robotic pipette with Dynatech Laboratories AM60 multi-reagent washer and MRX Microplate reader was used to screen for cannabinoids.

All software and equipment was supplied by Thermo-Finnigan, San Jose, CA. The GC–MS instrument consisted of a Trace 2000 Series Gas Chromatograph and Mass Spectrometer. Data was acquired and analysed using Xcalibur Software, version 1.2.

2.5. Overall method

Two extractions were used to cover a wide range of drugs. The first extraction method has been published prior to this article and permits the analysis of 49 drugs and metabolites of interest with the exception of Δ^9 -THC [10]. All of the samples were screened for Δ^9 -THC using enzyme immunoassay and confirmed by GC–MS. An in-house extraction method currently used for analysis of Δ^9 -THC in whole blood was validated for oral fluid samples.

2.6. Cannabis method

2.6.1. Microplate enzyme immunoassay

The assay procedure provided by the manufacturers was followed using the calibrators provided 0, 5, 10, 50 ng/mL. The oral fluid sample, controls and calibrators were diluted 1 to 2 in the buffer provided with the kit and 50 μL of each was added to a well, followed by 100 μL of enzyme conjugate. This was incubated for 30 min and the plate then washed four times with 350 μL of wash buffer. Substrate solution (100 μL) was added to each well, incubated for 30 min and finally 100 μL of stop solution was added. The absorbance was measured at 450 nm within 15 min. The cut-off for the assay was 5 ng/mL.

2.6.2. Sample preparation for GC–MS

Drug free oral fluid was collected using the modified Omni-Sal[®] device and measured out into 0.5 mL aliquots.

Δ^9 -THC was added to the diluted oral fluid at concentrations 2, 5, 10, 25, 50, 100, and 200 ng/0.5 mL oral fluid/buffer mix, followed by 200 ng/0.5 mL of Δ^9 -THC- d_3 and 2 mL of 0.1 M sodium acetate buffer, pH 7 with 5% methanol. This solution was vortex mixed and centrifuged at 2500 rpm for 5 min.

2.6.3. Extraction for GC–MS

Bond Elut Certify[®] II columns were conditioned with 2 mL methanol and 2 mL of 0.1 M sodium acetate buffer, pH 7 with 5% methanol. The sample was then added to the column and drawn through at 1 mL/min. The column was washed with 1 mL of 0.1 M sodium acetate buffer with 5% methanol and dried under full vacuum for 5 min. Δ^9 -THC was eluted using 2 mL of hexane:ethyl acetate (95:5) solution. The eluate was evaporated to dryness and derivatized with 30 μ L of BSTFA at 70 °C for 15 min.

2.6.4. GC–MS conditions

The gas chromatograph was fitted with an HP-1 column, 30 m \times 0.25 mm \times 0.25 μ m film thickness. The oven temperature was increased from 100 to 280 °C at 10 °C/min and held at 280 °C for 2 min. The injector temperature was 225 °C and the carrier gas was helium. The injection volume was 1 μ L. Selected ion monitoring was used and ions monitored were m/z 371 and 386 for Δ^9 -THC and m/z 374 for Δ^9 -THC- d_3 .

3. Results

3.1. Validation of cannabis method

The linearity of the method was evaluated for Δ^9 -THC using six concentrations 5, 10, 25, 50, 100 and 200 ng/

0.5 mL oral fluid/buffer mixture. The regression line was calculated by the method of least squares and expressed by the correlation coefficient (R^2). This value was 0.999.

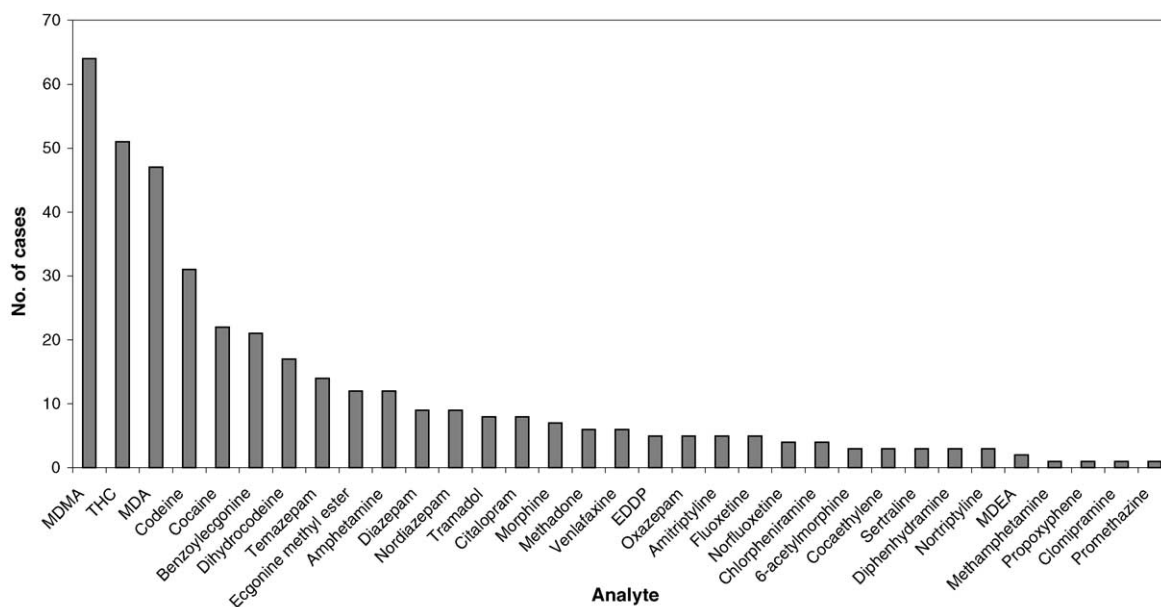
The limit of detection (LOD) was evaluated as the concentration with a signal-to-noise ratio of 3, while the limit of quantitation (LOQ) was defined as the concentration with a signal-to-noise ratio of 10. The LOD and LOQ were 0.3 and 0.5 ng/mL, respectively.

The relative recovery of Δ^9 -THC was obtained by extracting a 200 ng/mL standard, five times. The mean recovery was calculated by comparing the ratio of peak areas obtained from spiked oral fluid extracts with the ratio of peak areas obtained from unextracted standards at an equivalent concentration. The recovery of Δ^9 -THC was 85%.

Intra-day variability was ascertained by analysing five replicate 200 ng/mL standards and inter-day variability was evaluated over five days within a 2-month period. Intra-day precision was 201 ng/mL (%R.S.D. = 1.3) and inter-day precision was 202 ng/mL (%R.S.D. = 0.8). All validation data for other analytes involved in this study has been presented in a previous article [10].

3.2. Application

Of 1436 drivers who were asked to participate in this study, there was an acceptance rate of 97.2%. Of the 1396 oral fluid samples received into the laboratory, 16.8%



^aMDMA = 3,4-methylenedioxyamphetamine, THC = delta-9-tetrahydrocannabinol, MDA = 3,4-methylenedioxyamphetamine, EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, MDEA = 3,4-methylenedioxyethylamphetamine

Fig. 1. Frequency of drugs detected in oral fluid samples collected at the roadside ($n = 235$).

($n = 235$) were found positive for at least one drug. 84.7% ($n = 199$) of these cases showed a single drug group and in 15.3% ($n = 36$) of cases polydrug use was found. Of this group, the presence of 2, 3, 4 or 6 drugs was shown in 29, 4, 2 and 1 cases respectively. Fig. 1 shows the frequency of drugs detected in oral fluid positive cases. Out of the total number of positive cases, stimulants were the most frequently detected drug group. 3,4-Methylenedioxyamphetamine was detected in 4.6% ($n = 64$) of all cases and cocaine in 1.6% ($n = 22$). Δ^9 -THC was the second most frequently detected drug in 3.7% ($n = 51$) of cases.

Table 1 shows the range of drug concentrations detected in oral fluid samples. These concentrations were found to range from the limits of quantitation to concentrations associated with oral contamination. When a drug is taken orally, a residue of the drug has the potential to remain in the mouth for a period of time. If an oral fluid sample is collected immediately after oral administration

of a drug then the detected concentration will be a combination of that in the oral fluid and drug residue in the mouth. However, the median concentration of most drugs was within therapeutic ranges. Fig. 2 shows that monodrug use was prevalent in the positive oral fluid cases.

4. Discussion

In a German roadside survey, from 1992 to 1994, oral fluid was collected from an unselected driver population. In this study, a comparable acceptance rate to the present study (93.1%) was found. Benzodiazepines were the most commonly detected drug group with 2.7% of cases found to be positive, followed by opiates with 0.7%. Cannabis was the most frequently used illicit drug, 0.6%, and stimulant drugs had the least number of positive cases, 0.09%. Similar to this

Table 1
Drug findings in oral fluid samples

Drug	<i>n</i>	Mean (ng/mL)	Median (ng/mL)	Range (ng/mL)
MDMA ^a	64	244	107	9–3144
Δ^9 -THC ^a	51	506	82	7–4538
MDA ^a	47	48	24	4–275
Codeine	31	139	50	4–1504
Cocaine	22	1001	80	4–11110
Benzoylcegonine	21	1188	94	5–11471
Dihydrocodeine	17	371	190	8–1315
Temazepam	14	37	14	4–189
Ecgonine methylester	12	337	76	4–1520
Amphetamine	12	2231	433	12–16414
Diazepam	9	15	15	5–28
Nordiazepam	9	46	16	4–221
Tramadol	8	1571	1056	28–5289
Citalopram	8	200	84	15–775
Morphine	7	1119	61	9–7442
Methadone	6	1578	667	8–6949
Venlafaxine	6	400	280	45–845
EDDP ^a	5	18	14	9–38
Oxazepam	5	14	10	4–33
Amitriptyline	5	34	22	12–86
Fluoxetine	5	123	77	8–260
Norfluoxetine	4	74	87	24–98
Chlorpheniramine	4	73	59	7–168
6-Acetylmorphine	3	2541	12	10–7600
Cocaethylene	3	61	39	25–119
Sertraline	3	22	22	20–24
Diphenhydramine	3	331	162	27–805
Nortriptyline	3	59	52	21–104
MDEA ^a	2	14	14	13–14
Methamphetamine	1	98	98	n/a ^b
Propoxyphene	1	179	179	n/a ^b
Clomipramine	1	36	36	n/a ^b
Promethazine	1	383	383	n/a ^b

^a MDMA: 3,4-methylenedioxyamphetamine; Δ^9 -THC: delta-9-tetrahydrocannabinol; MDA: 3,4-methylenedioxyamphetamine; EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; MDEA: 3,4-methylenedioxyethylamphetamine.

^b n/a = not applicable.

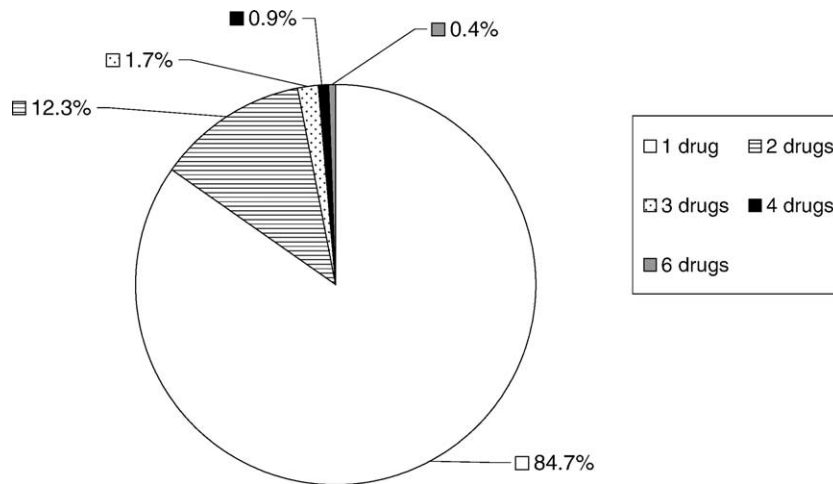


Fig. 2. Number of drugs per sample.

present study, the majority of opiate positive cases were suspected to have been through codeine use [12].

In another study, oral fluid samples from 896 Danish drivers were collected at random and analyzed for benzodiazepines and illegal drugs. The findings showed that cannabis was the most frequently confirmed drug in 0.8% of cases, followed by the benzodiazepines in 0.7% of cases and opiates in 0.3% of cases. Stimulants were present in only 0.2% of cases. The number of positive benzodiazepine cases was assumed to be low because the screening technique did not include some of the frequently used benzodiazepines [13]. Similar populations were used in each of these studies. However, the prominent drug group differed in these and in the current study.

The results of this present study also contrast with another in the West of Scotland, where blood samples were received from drivers suspected to be impaired through the use of drugs. In this case benzodiazepines were the most commonly encountered drug group, followed by morphine and cannabis. Unlike the oral fluid study, polydrug use was prevalent [3].

Further studies have investigated drugs in non-random categories of drivers. In Finland, benzodiazepines were the most frequently found drug group in drivers suspected of driving under the influence of alcohol and/or drugs [4]. Also, in the Netherlands, benzodiazepines and cocaine were the two most commonly used drugs in impaired drivers. 42% of those tested were polydrug users with cocaine present in the most frequent combinations [7]. In Switzerland, cannabinoids followed by opiates were the most commonly found drug groups among suspected impaired drivers. Stimulants were much less frequent [5].

Previous reports have shown that drug group frequency in non-random driving populations differs between countries. The drug group frequency in random driving populations has also been shown to vary between countries as supported by this study.

5. Conclusion

A method to determine cannabis in oral fluid was successfully validated. Using this method and a previously reported method [10], 1396 oral fluid samples were analysed for 50 drugs and metabolites. In this study, drivers were stopped randomly and requested to give an oral fluid sample for drugs analysis. The outcome showed that 16.8% of these were positive for at least one drug and the most commonly detected drugs were stimulants. These results differ from other studies involving random and non-random driver populations.

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