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Detection of Drugs in Saliva of Impaired Drivers

REFERENCE: Peel, H. W., Perrigo, B. J., and Mikhael, N. Z., **"Detection of Drugs in Saliva of Impaired Drivers,"** *Journal of Forensic Sciences*, JFSCA, Vol. 29, No. 1, Jan. 1984, pp. 185-189.

ABSTRACT: This study examined the feasibility of detecting drugs using saliva samples obtained from impaired drivers. Screening procedures on 1- to 2-mL samples were for cannabinoids, volatiles, benzodiazepines, and other acidic/neutral/basic drugs. Methodology consisted of enzyme multiple immunoassay technique (EMIT®) and temperature programmed gas chromatography with confirmation by gas chromatography/mass spectrometry (GC/MS). Fifty-six samples were obtained from drivers arrested for suspicion of impaired driving. Other than alcohol, the major drugs detected were cannabinoids and diazepam. Cocaine was found in one case.

KEYWORDS: toxicology, driving (motor vehicle operation), saliva, drug identification, cannabinoids, diazepam, cocaine

In recent years, there have been many reports concerning the use of saliva for drug monitoring, as applied to pharmacokinetic studies or to the management of patients in chronic drug therapy. The reports by Horning et al [*1*] and by Danhof and Breimer [*2*] showed the potential versatility of using saliva as a medium for monitoring various drugs. A more recent report by Idowu and Caddy [*3*] describes the use of saliva in the forensic detection of drugs. Although factors such as saliva pH, saliva flow, and individual drug characteristics can affect drug concentration in saliva, there is a relatively constant saliva/plasma ratio between individuals for many drugs [*1,2,4,5*].

The relatively high occurrence of drugs in impaired drivers [*6-8*] and in fatally injured drivers [*9*] has been reported previously. In all of these cases, blood or urine was used for analyses. However, in the study of impaired drivers, it is not easy to obtain a blood sample. A saliva sample is a relatively noninvasive technique which may be used to determine the occurrence of drugs. The purpose of this study was to determine if the analysis of saliva may be an effective means to detect drugs in the impaired driver.

Methods

A program was organized in cooperation with three local police forces in the Ottawa area to collect saliva samples from drivers who were suspected of driving while impaired. Once

Received for publication 18 April 1983; revised manuscript received 31 May 1983; accepted for publication 1 June 1983.

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the subject had completed the routine tests on the Breathalyzer® at the police station, he was asked to participate voluntarily in a research project related to driving safety. Containers for saliva collection consisted of a labelled glass vial (25 mL) with a Teflon®-lined screw cap. (Teflon liners replaced regular liners to reduce contamination.) A clear plastic vial with a snap cap was provided to hold the sample vial cleanly and safely. Chewing gum was provided as a chewing aid to stimulate saliva flow. The result of the Breathalyzer test and other information were provided with each sample.

The original analytical protocol was designed for a 3-mL sample of saliva, but had to be altered because of the smaller sample volumes received. All samples were centrifuged before analysis to remove any debris and mucus present in the saliva. The testing priority was as follows: enzyme multiple immunoassay technique (EMIT®) screen for cannabinoids (0.25 to 0.5 mL), base/neutral/acid compounds including benzodiazepines (1.0 mL), and volatile compounds (2 μL). Where possible confirmatory analysis using gas chromatography/mass spectrometry (GC/MS) was done.

Drug Analysis

Cannabinoid Screen—A previously described procedure for the determination of cannabinoids in blood using EMIT was followed [*10*] with the single modification of changing the relative volumes for initial extraction to 1 : 1 (saliva : methanol).

Basic/Neutral/Acid Drugs—One millilitre of saliva was mixed with 30 μL of aminochlorobenzophenone (100 mg/L, in methanol) and the solution made basic using ammonium hydroxide. The basic solution was extracted with 3 mL of *n*-butyl chloride and the aqueous portion retained. The organic extract was evaporated to dryness using nitrogen at room temperature, and the residue taken up in 50 μL of ethanol. Two microlitres of the extract containing basic and neutral drugs were injected into the gas chromatograph.

The previous aqueous solution was made acidic with a few drops of 50% hydrochloric acid and was extracted with 4 mL of diethylether. The ether extract was evaporated to dryness at room temperature under nitrogen, and the residue taken up in 50 μL of ethanol. Two microlitres of the extract (containing acidic drugs) were injected into the gas chromatograph.

A gas chromatograph equipped with a nitrogen phosphorous (NP) detector and a capillary column—DB-1[3] (0.25 μm), fused silica 15 m by 0.315 mm was used. The injection port and detector temperatures were 250 and 300°C, respectively. The oven temperature was programmed from 120 to 280°C at 8°C/min with a final hold at 280°C. Helium was used as the carrier gas at a flow rate of 1.5 mL/min and was split 20 : 1. Helium was also used as a detector makeup gas (20 mL/min). Hydrogen and air flow rates were 5 and 100 mL/min, respectively. Attenuation was set so the internal standard gave at least 50% full scale deflection.

Benzodiazepines—The previous extract containing basic and neutral compounds was used for the benzodiazepine screen. A 2-μL injection was made into a gas chromatograph equipped with an electron capture (EC) detector and using a 10% OV-1 column [*11*].

Volatiles—Two microlitres of saliva and 1 μL of *n*-propanol solution (1 mg/mL) were injected into a gas chromatograph equipped with a flame ionization detector and using a column (180 cm by 2 mm) packed with 2% Carbowax 1500 on Poropak Q (80-100). The injection port, detector, and oven temperatures were 200, 250, and 150°C, respectively. The nitrogen flow rate was adjusted so the internal standard (*n*-propanol) eluted at about 4.2 min. Solutions of methanol, ethanol, acetone, isopropanol, and ethyl acetate (1 mg/mL) were used as controls.

[3]DB-1 Durabond is a chemically bonded liquid phase available from J & W Scientific Inc., Rancho Cordova, CA.

Confirmation Procedures

The positive findings from the basic, neutral, and acidic drug screen and the benzodiazepine screen were confirmed using a Finnegan model 4510 capillary gas chromatograph-quadrupole mass spectrometer in the electron impact mode (36 eV). The instrument used a Grob injector and a 15-m DB-5 wide bore column. The following temperature settings were used: injection port; column, 100°C for 1 min, programmed to 290°C at 12°C/min; GC/MS transfer line, 250°C; and ion source, 150°C.

The cannabinoid results were confirmed by GC/MS analysis for only tetrahydrocannabinol (THC) following a modification of the method of Foltz et al [*12*]. The modifications consisted of extracting the saliva solutions only with hexane/ethyl acetate (9:1), using hexahydrocannabinol as an internal standard and using the mass spectrometer in the electron impact mode.[4] The fluoroacetate derivatives of THC and hexahydrocannabinol were monitored at the ion peaks 367, 395, 410 and 369, 412, respectively. The detection limit for this modified procedure is about 2 ng/mL of Δ^9-tetrahydrocannabinol.

Results and Discussion

The analytical methods were selected to detect common drugs that might be anticipated to cause an effect upon, or be encountered in drivers. The lower limits of detection for compounds in saliva were approximately as follows: cannabinoids via the EMIT procedure, 10 ng/mL as 11-nor-Δ^9-THC-9 carboxylic acid; basic and neutral compounds via GC/NP, 0.1 μg/mL; acid compounds, 1 μg/mL; benzodiazepines GC/EC, 10 ng/mL; and volatiles, 50 μg/mL. As the EMIT assay is cross-reactive to other cannabinoids, it is not considered to be indicative of only the acid metabolite. It is less sensitive to Δ^9-tetrahydrocannabinol than to 11-nor-Δ^9-THC carboxylic acid. The temperature programmed procedure using the capillary column included compounds ranging from amphetamine (retention index 1120) to thioridazine (retention index 3180) and encompassed common psychoactive drugs and narcotics.

A total of 56 saliva samples were obtained from 445 drivers suspected of impaired driving during the sample period. A majority of the samples were obtained by a relatively few breath test operators. The general quality of the samples was good. However the individual volumes were somewhat limited and ranged from 1 to 1.5 mL. Shortly after the program began, it was realized that a chewing aid was necessary to stimulate saliva flow in the alcohol impaired subject. A recent report confirms that saliva flow is decreased by alcohol consumption [*13*].

Drugs, other than alcohol, were detected in 10 of the 56 cases received. The results shown in Table 1, describe the subjects (all male), breath alcohol concentration (from the police report), and the drug found in the saliva. Aside from alcohol, the most common drugs found were cannabinoids and diazepam. The screen for volatiles showed alcohol was present in all the 56 samples. Six saliva samples were found to have cannabinoids present in combination with alcohol levels ranging from 140 to 240 mg/100 mL. Three of the six cannabinoid users admitted using the drug sometime before arrest for impaired driving, whereas the other three drivers did not admit to taking the drug. Cannabinoids in saliva may not be indicative of a blood concentration of the drug [*14*]; however, their presence in saliva is indicative of recent consumption of the drug, perhaps sequestered there during smoking. The saliva/plasma ratio of Δ^9-tetrahydrocannabinol and metabolite 11-hydroxy-Δ^9-tetrahydrocannabinol is calculated to be 0.099 to 0.129 and 0.06 to 0.099, respectively [*3*]. The age of the drivers who had consumed the cannabinoid with alcohol ranged from 21 to 26 years.

Diazepam was found in four cases, three of which included a trace amount of the metabolite nordiazepam. Case 13 (Table 1) contained cannabinoids, prazepam, diazepam, and nor-

[4] B. J. Perrigo, D. J. McClure, and D. J. Ballantyne, personal communication.

TABLE 1—*Drugs in saliva of impaired drivers.*

Case	Drug	Alcohol, mg/dL	Age, years
4	diazepam (nordiazepam)	140	47
6	diazepam (nordiazepam)	170	43
9	cannabinoids	150	26
12	cannabinoids	140	21
13	diazepam (nordiazepam) cannabinoids prazepam	180	25
19	cocaine	150	23
20	cannabinoids	240	23
25	diazepam	160	39
27	cannabinoids	150	22
29	cannabinoids	240	25

diazepam, as well as alcohol. (Although this driver said he was taking an indomethacin product, none was detected.) The ages of drivers found taking diazepam were 25, 39, 43, and 47 years. Cocaine was easily detected using the screening procedure for base/neutral drugs. There was no benzoylecgonine detected in this case. The calculated saliva/plasma ratio for cocaine is one of the few to exceed unity [*15*]. The alcohol influence report for this 23-year-old driver did not include any unusual comments related to impairment.

The analyses for volatile substances did not reveal any compounds, aside from ethyl alcohol. Caffeine and nicotine were also detected in the base/neutral extraction procedure of the saliva samples. Although not listed in Table 1, nicotine was detected in 29 samples and caffeine in 14 samples.

Information included in the alcohol influence reports showed 12 of the 56 subjects admitted to taking some kind of drug including ethical medicaments and illicit substances (Table 2). Cannabinoid positive tests were obtained in those samples where the subject admitted consumption, although specific samples were not identified during the batch taking. Although it was anticipated that the analytical procedures would have detected low concentration of indomethacin, cimetidine, and lorazepam, none was found. It is not established if those are significantly excreted into saliva.

TABLE 2—*Incidence of alleged drug consumption.*

Case	Alleged Drug	Drug Found
4	"antibiotics"	diazepam
13	indomethacin	diazepam, nordiazepam, prazepam, cannabinoids
17	"arthritic drug"	...
20	hashish, 2 to 3 joints	cannabinoids
21	cimetidine	...
25	meclizine	...
21	marihuana	...
29	hashish, 1 joint	cannabinoids
35	"skin creme"	...
41	"penicillin"	...
52	lorazepam	...
54	"decongestant"	...

Conclusion

The results of this investigation have shown that it is possible to obtain and analyze samples of saliva from drivers who have been suspected of impaired driving. This investigation indicates the potential versatility of using saliva as a noninvasive technique of determining the occurrence and frequency of drug use in impaired drivers.

Acknowledgments

The authors thank Officers of the Nepean City Police, Ottawa City Police, and the Ontario Provincial Police (Kanata Detachment) for their interest and cooperation in this project. Mr. D. McClure of Royal Canadian Mounted Police provided the mass spectral analyses.

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