CUT-OFF CONCENTRATIONS FOR DRUGS OF ABUSE IN SALIVA FOR DUI, DWI OR OTHER DRIVING-RELATED CRIMES

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ABSTRACT: Investigation of the involvement of drugs in impaired driving would be facilitated by a roadside test for drugs in saliva such as currently exists for alcohol in breath. In the development of a hand-held on-site test for drugs in saliva, targets had to be set for appropriate cut-off values. This was done by consideration of the predominate drug appearing in saliva, the saliva/plasma ratio for that drug and the blood concentrations of the drug likely to cause impairment in driving. This paper describes the results for opiates, marijuana, amphetamines, benzodiazepines and cocaine. The theoretical target value is compared to the RapiScan Saliva Drug Test empirical cut-off values.

KEY WORDS: Saliva analysis; Drugs and driving; DUI; DWI.

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INTRODUCTION

The feasibility of detecting drugs in saliva samples obtained from impaired drivers was first investigated by Peel et al. in 1984 [6]. The foremost question in the application of saliva testing to forensic casework is "What is the relationship of saliva positive results to blood drug concentrations?" Drug concentrations in saliva reflect the free, unbound parent drug and lipophilic metabolites circulating in the blood. Since these are the forms of the drug which cross the blood-brain barrier and effect performance and behavior, saliva is a good specimen for detecting drug involvement in driving behavior or impairment of performance. An immunoassay for drugs in saliva must be able to detect the parent drug or lipophilic metabolites. It must also be able to detect the levels of drugs which appear in saliva.

Drug and lipophilic metabolite concentrations in saliva are a function of the drug's pK_a, plasma and saliva pH and the fraction of drug bound to saliva and plasma protein as shown by the following form of the Henderson-Hasselbach equation for saliva. S/P is the saliva to plasma ratio:

$$\frac{S}{P} = \frac{[1+10\cdot(pK_d - pH_s)]}{[1+10\cdot(pK_d - pH_p)]} + \frac{f_p}{f_s} \qquad \text{basic drugs}$$

$$\frac{S}{P} = \frac{[1+10\cdot(pH_s - pK_a)]}{[1+10\cdot(pH_p - pK_a)]} + \frac{f_p}{f_s} \qquad \text{acidic drugs}$$

Where S is the drug concentration in saliva and P is the drug concentration in plasma, pK_d is the log of the ionization constant for basic drugs, pK_a is the log ionization constant for acidic drugs and pH_s is the pH of saliva and pH_p is the pH of the plasma. f_p is the fraction of drug protein bound in plasma and f_s is the fraction protein bound in saliva. Dawes and Jenkins [2] demonstrated that saliva pH is inversely proportional to flow rate (Figure 1) and the reabsorption of sodium in the salivary tubules. At faster flow rates, less sodium is reabsorbed in the tubules on the way from the saliva glands to the saliva outlets

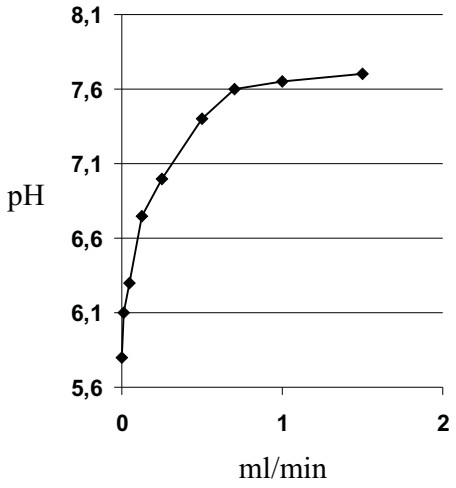

Fig. 1. The effect of flow rate on saliva pH [2].

in the mouth and the pH raises. For this reason unstimulated saliva has a low pH and stimulated saliva has a higher pH.

For drugs that have a pK_a of between 5.5 and 8.5, the saliva/plasma ratio can vary between stimulated and unstimulated saliva. This is true of many drugs of abuse. For this reason it is more conservative to use a cut-off value for drugs of abuse in saliva rather than to determine the absolute concentration.

METHODS

Saliva was collected using the Cozart RapiScan collection pad and tube and tested using the RapiScan Saliva Drugs Test (Cozart Bioscience Ltd.). When placed in the mouth, the collection pad absorbs exactly 1 ml of saliva, which is indicated by development of a blue color in the indicator section of the handle. The pad was then placed in the tube, where it was diluted with 2 ml of run buffer fluid. A measured aliquot of the saliva/run fluid mixture was placed in the immunoassay cartridge by using a transfer pipette. The cartridge was inserted into the hand-held RapiScan instrument for incubation. The saliva/run fluid rehydrates gold-labeled anti-drug antibodies contained within the cartridge. This mixture travels by capillary action across an array of immobilized drug sites. Absence of color development at an immobilized drug position indicated drug presence. The quality control position contained anti-IgG to ascertain that complete lateral transfer of specimen had been achieved.

The test results are read electronically, processed by a computer chip and displayed as a written message (Figure 2). After ten minutes for the 5 panel test (2 minutes for the single tests), if the quality control was satisfied, the screen on the RapiScan reader, displayed the results as "positive" or "negative" for each of the five drug classes. The back-lit screen for reading results, timing, quality control and error messages is similar to those used in mobile phones, onsite glucose analyzers and hand-held computers. In addition to the message, if all results are negative, a green light appears above the power

Fig. 2. The RapiScan reader.

switch. If any of the results are positive a red light appears.

The collector pad has a dead volume of approximately 1 ml and the Cozart RapiScan cartridge requires between 0.22 and 0.26 ml for completion. The same volume is

required for single, dual or multiple drug panels. The excess volume of saliva/run fluid mixture was designed to allow confirmation to be performed on the collected sample.

Each cartridge can test for five drug classes: amphetamines, benzodiazepines, cannabis, cocaine, and opiates. The cut-off concentrations in the diluted saliva are: 10 ng/ml drug equivalents for morphine, amphetamine and benzoylecgonine, 20 ng/ml for THCA and 100 ng/ml for temazepam. This corresponds to 30 ng/ml in saliva for morphine, amphetamine and benzoylecgonine, 60 ng/ml for THCA and 300 ng/ml for temazepam.

The theoretical range of saliva/plasma ratios over a saliva pH range of 6.4–7.6 were calculated for cocaine, amphetamine, methamphetamine, 6-monoacetylmorphine, morphine, codeine, methadone and diazepam and compared to published saliva/plasma ratios. The lowest ratio values were multiplied by the lower limit plasma levels for therapeutic or recreational effects from Uges [7]. The resulting suggested cut-offs for saliva were: cocaine 60 ng/ml, amphetamine 56 ng/ml, methamphetamine 40 ng/ml, 6-MAM 30 ng/ml, morphine 40 ng/ml, codeine 66 ng/ml, methadone 50 ng/ml and diazepam 3.6 ng/ml. These were compared to the RapiScan specifications.

RESULTS

Amphetamines

Amphetamine, methamphetamine, MDMA, MDA and other amphetamine class drugs can be found in saliva. Parent drug rather than amphetamine metabolites are found in saliva. The saliva/plasma ratio for amphetamine is 2.76 and for methamphetamine is 3.98. After administration of 10 mg amphetamine, plasma levels ranged from 1 to 20 ng/ml and saliva concentrations ranged from 10 to 60 ng/ml [8]. Based on the therapeutic range of 20–150 ng/ml for amphetamine [7], positives at a cut-off value of 56 ng/ml amphetamine or greater would indicate pharmacologically significant levels of amphetamine drugs in blood. The RapiScan cut-off is 10 ng/ml amphetamine in diluted oral fluid or 30 ng/ml amphetamine or MDA in saliva. This would correspond to a plasma concentration of approximately 15 ng/ml amphetamine or MDA in plasma. The crossreactivity of the antibody is 1% for methamphetamine or MDMA so a concentration of 3000 ng/ml methamphetamine or MDMA in saliva would be required to read positive on the device. This would be equivalent to a plasma concentration of 755 ng/ml methamphetamine or MDMA.

Benzodiazepines

Benzodiazepines have an unfavorable saliva to plasma ratio (S/P = 0.01–0.08). However, cases of driving under the influence of benzodiazepines often occur with persons who, with long term over use of benzodiazepines, have developed tolerance and present with blood levels greater therapeutic levels. In several cases the authors have

seen diazepam levels greater than 1 μg/ml. This would correspond to a saliva concentration of 30 ng/ml which would not be detected by immunoassay. The RapiScan cutoff is 100 ng/ml temazepam in diluted oral fluid or 300 ng/ml in saliva. Benzodiazepine positives in saliva are probably due to detection of residual pill fragments in the mouth indicating very recent oral administration.

Cannabinoids

Tetrahydrocannabinol (THC) and tetrahydrocannabinoic acid (THCA) are excreted in only trace amounts in saliva. Idowu and Caddy [3] calculated a theoretical saliva/plasma ratio of 0.099–0.129 for Δ-9- tetrahydrocannabiniol and of 0.060–0.099 for 11-OH-Δ-9 -tetrahydrocannabiniol. The measured saliva/plasma ratio after intravenous injection of labeled cannabidiol is 0.0012 [5]. However the measured saliva/plasma ratio for THC after smoking marijunana is 10 and is a function of the time since smoking [1]. Cannabinoids in saliva are due to residuals left in the mouth during ingestion or smoking of marijuana or marijuana products.

A rather high cut-off concentration of 100 ng/ml THC is recommended based on literature reports. Cone et al. found saliva levels of greater than 100 ng/ml for an hour after smoking a 3% THC marijuana cigarrette. Menkes et al. [4] found that saliva levels of correlated with rapid heart rate and psychological feelings of "high" (Figure 3). At a cut-off concentration of greater than 100 ng/ml, a positive saliva result for THC will correspond to blood levels which produce these common physiological symptoms which indicate recent smoking of marijuana. The RapiScan cut-off is 20 ng/ml THCA in diluted oral fluid or 60 ng/ml THCA in saliva. Since the crossreactivity of the RapiScan cartridge antibody to THC is 10% relative to THCA, this is equivalent to a cut-off of 600 ng/ml for THC in saliva. From the relationship published by Menkes, this would indicate recent use (1–2 hours) or ingestion of very potent marijuana (Figures 4, 5).

Cocaine

In unstimulated saliva cocaine is trapped in saliva and the saliva/plasma ratio is greater than 5. In stimulated saliva the saliva/plasma ratio ranges from 0.5 to 3.0. Benzoylecgonine is also found in saliva at concentrations approximately equal to those in blood (Figure 6). The RapiScan uses a cut-off of 30 ng/ml benzoylecgonine or 150 ng/ml cocaine in saliva. If this is caused by the inactive metabolite benzolylecgonine, it is unlikely that the person was driving under the influence of cocaine. The RapiScan antibody has a 20% crossreactivity with cocaine. A concentration of greater than 150 ng/ml cocaine in saliva would most likely correspond to pharmacologicaly significant concentrations of cocaine in blood, or in excess of approximately 50 to 300 ng/ml cocaine in plasma.

Fig. 3. Correlation of saliva THC concentrations and marijuana effects [4].
HR = heart rate, bpm = beats per minute.

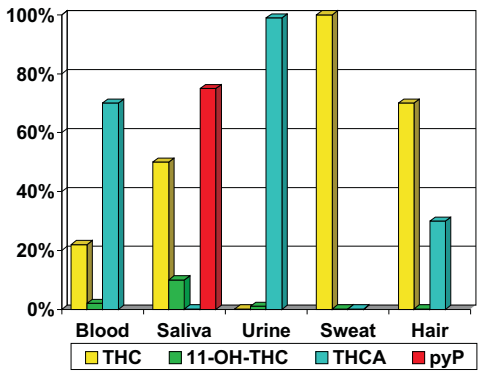

Fig. 4. Distribution of THC or THCA in body fluids and tissues.

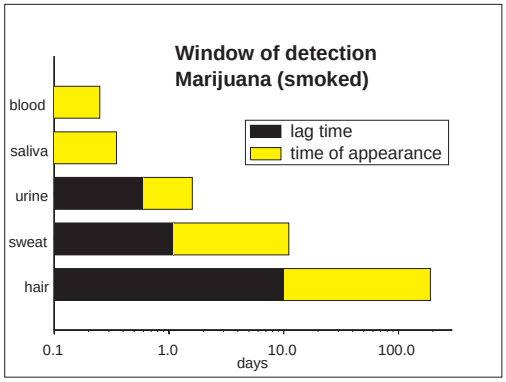

Fig. 5. Window of detection of THC or THCA after smoking marijuana for various specimens.

Opiates

The major metabolite found in saliva after heroin use is 6-monoacetyl morphine (6-MAM, S/P ratio of 6) (Figure 7). After codeine administration, codeine is found in saliva with a saliva to plasma ratio of 3.3 and after morphine administration, morphine may be found in a saliva to plasma ratio of 0.2. The RapiScan cut-off is 10 ng/ml morphine in diluted oral fluid or 30 ng/ml morphine equivalents in saliva and the RapiScan antibody is equally crossreactive with morphine, 6-MAM, heroin, dihydrocodeine and codeine (Figure 8). An opiate positive in saliva with the RapiScan would indicate the recent use of heroin or codeine or the use of a large amount of dihydrocodeine, pholcodeine or morphine. For example, a saliva opiate positive due to 6-MAM at a cut-off of 10 ng/ml in dilution or 30 ng/ml in saliva corresponds to a greater than 5 ng/ml concentration of 6-MAM in plasma. A saliva morphine concentration in

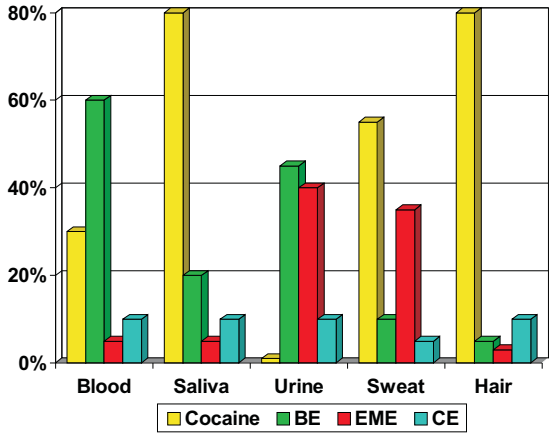

Fig. 6. Distribution of cocaine and cocaine metabolites in body fluids and tissues.

excess of 30 ng/ml corresponds to a plasma concentration of greater than 150 ng/ml free morphine.

CONCLUSIONS

The RapiScan provides positive saliva test results for opiates, amphetamine, marijuana and cocaine which correspond to blood levels which can be predicted to cause driving impairment. The test does not correspond to blood levels of marijuana or benzodiazepines but can readily react with residual drug deposited from benzodiazepine pills or smoking of marijuana or hashish. RapiScan positives with these high cut-offs for marijuana or for benzodiazepines indicate very recent drug use and a high probability of driving impairment by these substances.

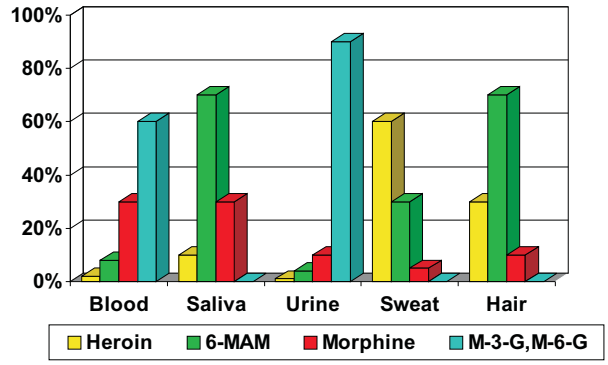

Fig. 7. Distribution of heroin and heroin metabolites in body fluids and tissues.

Fig. 8. The crossreactivity of the RapiScan saliva opiates test.

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