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Cozart Rapiscan System: our experience with saliva tests

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Abstract

International literature has devoted many contributions to the evaluation of alternative biological matrices (such as saliva) as diagnostic tools in drug testing. The immunoassay Cozart Rapiscan saliva drug system, has been studied in recent years. In the present paper we report our experience with saliva collection and the quali–quantitative determination of drugs of abuse. Fifty-nine saliva samples were collected by the Cozart Rapiscan pad. Qualitative analyses were carried out by Cozart Rapiscan System and the results were confirmed by a solid-phase microextraction–GC–MS technique. Quantitative determinations were performed for methadone and its metabolite by GC–MS technique. The Cozart System provides collection and transfer procedures more easily than other systems, requiring minimal operator intervention.

Keywords: Cozart Rapiscan system; Saliva

1. Introduction

The use of saliva as a diagnostic tool in drug testing [1–3] has been increasing in recent years. There is much interest in standardizing both saliva collection and analytical methods for the detection of drugs of abuse.

Compared with other biological fluids saliva offers specific advantages, when the information desired relates to the pharmacological state of the individual at the time of testing. Hence the use of saliva is justified when simplicity of collection is required, for example in road testing [4,5].

Many systems for saliva collection have been developed [6–8] to obtain easy and reproducible sampling. In recent years the Cozart Rapiscan system (Cozart Bioscience, Ltd., Abingdon, Oxford, UK) was suggested as a suitable tool for saliva collection [9,10]. Moreover, the system offers the opportunity to screen drugs of abuse with a multiple on-site immunoassay. High sensitivities for many compounds are reported by Cozart Bioscience.

The present paper deals with our experience with the Cozart Rapiscan System, applied to saliva samples obtained both from heroin addicts therapeutically treated with methadone, and from discotheque-goers.

Data obtained by the screening were then con-

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firmed by GC–MS technique. Because of the small amount of sample available, a solid-phase microextraction (SPME) system was chosen [11–13].

2. Experimental

2.1. Samples

Twenty drug-free saliva samples were collected from volunteers.

They were first tested with the immunoassay to evaluate hypothetical false positive results. To check false negatives, the same samples were spiked with some drugs at two different concentrations (50 and 500 ng/ml for amphetamine, 3,4-methylenedioxyamphetamine (MDA), 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium perchlorate (EDDP), methadone, cocaine and cocaethylene; 1000 and 2000 ng/ml for methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA).

Saliva samples were obtained from 25 heroin addicts therapeutically treated with methadone, coming from a hospital centre. The methadone dosage is known. They are often polydrug abusers, hence other substances could be found in the samples.

Fourteen saliva specimens were also obtained from young people at the exit of a discotheque. These volunteers only gave information about their usual kind of drug intake.

2.2. Saliva collection

Saliva samples were collected with the Cozart Rapiscan collection pad and tube, according to Cozart Bioscience instructions.

When placed in the mouth, the collection pad absorbs 1 ml of saliva as indicated by a blue indicator in the handle. The saliva-soaked pad is then placed in the tube containing the elution fluid and separated from the plastic handle. The saliva on the cellulose pad undergoes a dilution with 2 ml of run buffer fluid to a final volume of 3 ml.

2.3. Immunochemical screening

The immunochemical screening was performed using Cozart Rapiscan five-drug panel cartridges designed to detect five main groups of abused drugs (opiates, benzodiazepines, methadone, cocaine and amphetamines).

The Cozart Rapiscan is a qualitative detection system for the on-site analysis of drugs in saliva. The system simultaneously analyses the sample for multiple drugs.

Saliva specimens, collected by the swab, are placed into a test cartridge together with the run fluid. After a few minutes, red lines appear on the cartridge: the presence of a line means the result is negative, no line means a positive result. The test is valid if a coloured line appears on the control zone. The cartridge is then placed into the instrument for the electronic reading. The Cozart Rapiscan interprets the results from multiple immunoassays within the cartridge and gives a digital readout for each drug tested.

In our experience, the visual reading gives lower sensitivity compared to the electronic reader, because of some problems related to colour perception. In Table 1 sensitivities related to reading without the instrument are reported and compared with Cozart.

2.4. SPME extraction

All the samples were tested by GC–MS to confirm the results obtained by screening.

Because of the small volume of saliva available, the SPME technique was chosen to simultaneously detect methadone, EDDP, amphetamine, methamphetamine, MDA, MDMA, cocaine, cocaethylene, cannabidiol, tetrahydrocannabinol and cannabinol.

A 200-μl amount of samples was spiked with deuterated internal standard analogous of all the

Table 1

	Cozart sensitivity (ng/ml)	Laboratory sensitivity (ng/ml)
Amphetamine	10	50
Methamphetamine	1000	1000
MDA	10	50
MDMA	1000	1000
EDDP	Not referred	50
Methadone	10	50
Cocaine	25	50
Cocaethylene	Not referred	50

substances examined (obtained from Radian LLC, Austin, TX, USA).

SPME technique was carried out using SPME fibre 30-μm polydimethylsiloxane coating (Supelco, Bellefonte, PA, USA). The sampling was performed by direct immersion modality, placing the sample in a 300-μl insert combo microtube; after addition of sodium chloride and 0.1 *M* NaOH to reach pH 8.0, the fibre was directly submersed in the microvial at room temperature for 30 min.

2.5. GC–MS conditions

After the sampling, thermal desorption of analytes from the fibre was performed directly in the GC injection port at 250°C for 1 min. A Hewlett-Packard model 5890 gas-chromatograph fitted with split–splitless injector was equipped with a 12 m×0.2 mm I.D. capillary column; 0.33 μm film thickness methylsilicone. The following temperature program was used: initial temperature set at 40°C, held for 1 min, then increased in 10°C/min increments to 260°C, held for 1 min, then increased at 20°C/min increments to 280°C and held for 5 min.

The injector temperature was set at 250°C; helium was used as carrier gas at a flow-rate of 3 ml/min.

The capillary column was connected to a mass analyzer (HP5971A) operating by electronic impact (70 eV) in the selected ion monitoring (SIM) mode.

Calibration curves for methadone and EDDP were prepared between 10 and 500 ng/ml, adding pure standards and deuterated analogous to saliva free samples.

Straight lines with good correlation coefficients were obtained during the calibration.

3. Results and discussion

The sampling is a critical step in saliva analysis for drug detection. The Cozart Rapiscan pad allows a reproducible collection, and is easy and quick; moreover, drug abusers carry out their own sampling and the transfer of the pad requires minor handling by the operator compared with other systems. In our experience, all volunteers (drug abusers included) accepted the sampling without any problems, while maintaining their privacy.

The availability of an immunoassay for the screening of drugs in saliva, is very important to obtain preliminary information on the patient. The screening should be sensitive, specific and reproducible. Cozart Rapiscan on-site immunoassay has these characteristics; in fact this drug test is sensitive enough to detect the low amounts often encountered in saliva samples [14]. Moreover it allows the simultaneous detection of some drugs with acceptable specificity, employing a very small amount of sample. However, the dilution factor of saliva with the run fluid decreases the sensitivity three times.

For the evaluation of false positive and/or false negative, 20 drug-free saliva samples were collected. We never obtained false positive results. To evaluate false negative, amphetamine, methamphetamine, MDA, MDMA, EDDP, methadone, cocaine, cocaethylene, were added to the drug-free samples. Only with the low concentrations were some uncertain results observed, probably due to the low colour intensity variation, linked to the eye appreciation. These uncertain results disappear with the use of the instrument. However, the sensitivity obtained by the visual reading of Rapiscan cartridge, appears to be lower than that with the electronic reader (Table 1).

To confirm the results, SPME extraction followed by GC–MS analysis was employed. High sensitivities were obtained for all the substances examined (about 10–20 ng/ml). In Fig. 1 the chromatographic pattern of a drug-free saliva sample added with all the compounds examined, and submitted to the SPME–GC–MS analysis is reported. A complete separation was obtained. In Table 2, the chromatographic characteristics of the substances are listed.

The results obtained for the other 39 saliva samples, are shown in Tables 3 and 4.

In the drug-abuser's group we never detected opiates or benzodiazepines; only one sample showed the presence of cocaine with the screening technique, confirmed by GC–MS; in two cases the cocaine detected by GC–MS was not picked up by the immunoassay, probably because of the low salivary concentration.

As expected, most samples showed the presence of methadone, always confirmed by GC–MS; quantitative determinations of methadone and its metabolite EDDP were also carried out, using the cali-

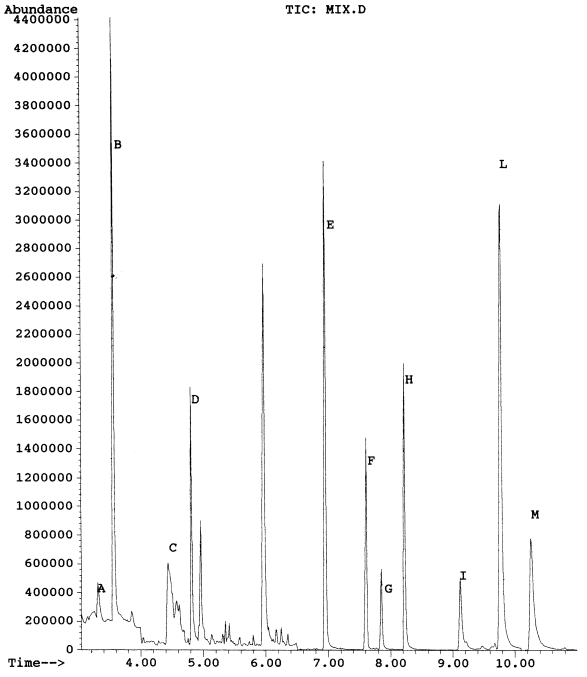

Fig. 1. Chromatographic pattern of a saliva drug-free sample spiked with amphetamine (A), methamphetamine (B), MDA (C), MDMA (D), EDDP (E), methadone (F), cocaine (G), cocaethylene (H), CBD (I), THC (L), CBN (M), analysed by DI-SPME followed by GC–MS.

bration curves. The results are summarized in Table 5.

Some negative samples analysed with the on-site test, gave methadone concentrations lower than 50 ng/ml with the chromatographic technique.

Amphetamine immunochemical screening showed 12% of positive results always confirmed; MDA in one case and MDMA in the other two samples, were identified.

In 20% of samples, cannabinoids were found, indicating the polydrug abusers statement.

The volunteers asked for a saliva samples at the exit of a discotheque, gave some information about their usual drug intake. Neither opiates, nor methadone, nor benzodiazepines were found in the samples with the on-site test. Two positive immunochemical results for cocaine, were both confirmed by GC–MS. In one sample, however, low amounts of cocaine noted by GC, were not detected by the Cozart cartridge. Five samples showed positive results for amphetamine class, not always declared by the volunteers; in four cases these results were confirmed, and MDMA was identified. Hence in one case we did not confirm the positive immunochemical result, probably due to a false positive.

Table 2
Chromatographic characteristics

Substances	Retention time	Target ion	Qualifiers
Amphetamine	3.30	44	91–135
Amphetamine-D5	3.30	60	92–134
Methamphetamine	3.55	58	91–134
Methamphetamine-D5	3.55	62	92–139
MDA	4.60	56	135–191
MDA-D5	4.60	60	136–196
MDMA	4.80	58	135–193
MDMA-D5	4.80	62	136–198
EDDP	7.05	277	276–262
EDDP-D3	7.05	280	279–265
Methadone	7.70	294	223–309
Methadone-D3	7.70	297	226–312
Cocaine	7.94	182	303–198
Cocaine-D3	7.94	185	306–201
Cocaethylene	8.31	196	317–272
Cocaethylene-D3	8.31	199	320–275
Cannbidiol	9.20	231	246–314
Tetrahydrocannabinol	9.80	299	231–314
Tetrahydrocannabinol-D3	9.80	302	234–317
Cannbinol	10.33	295	296–310

A high percentage of cases (~43%) showed the presence of cannabinoids in this group too.

The declared ketamine and LSD were not investigated, because they are not included in the Cozart kit.

In conclusion, saliva displays a number of advantages compared to the body fluids, such as blood, traditionally used as materials for therapeutic drug monitoring. It is obtained by a painless and non-invasive sampling method, it does not require specially trained personnel, it is readily available, it contains the free fraction of drugs and, therefore it is a better indicator of intoxication states [2].

The use of saliva for the analysis of drugs of abuse is useful in forensic field, when blood sampling is forbidden, for example in heroin abusers, or in impaired drivers [4,5].

The high reproducibility required by saliva sampling can be reached by the Cozart Rapiscan System for the presence of the blue indicator of end-sampling and for rapidity and ease of collection. More-

Table 3

HEROIN ADDICTS			COZART RAPISCAN SYSTEM					GC/MS RESULTS
SAMPLE	SEX	METHADONE MG/DAY	OPI	COC	METHAD	AMPH	BDZ	
1	M	160	N	N	*POS*	N	N	METHADONE, EDDP
2	M	40	N	N	*POS*	N	N	METHADONE, EDDP
3	F	10	N	N	N	N	N	
4	M	25	N	N	N	N	N	
5	M	45	N	N	N	*POS*	N	METHADONE, EDDP, MDA
6	M	40	N	N	N	N	N	METHADONE, EDDP
7	M	60	N	N	*POS*	N	N	METHADONE, EDDP
8	M	60	N	N	*POS*	N	N	METHADONE, EDDP, CBN
9	F	180	N	N	*POS*	N	N	METHADONE, EDDP
10	M	196	N	N	*POS*	*POS*	N	METHADONE, EDDP, COCAINE, MDMA
11	F	90	N	N	*POS*	N	N	METHADONE, EDDP
12	M	40	N	N	N	N	N	
13	M	130	N	N	*POS*	N	N	METHADONE, EDDP
14	M	100	N	N	*POS*	N	N	METHADONE, EDDP, CBD, CBN, THC
15	M	250	N	N	*POS*	N	N	METHADONE, EDDP
16	M	45	N	N	N	N	N	
17	M	20	N	N	N	N	N	METHADONE, CBD, THC, CBN
18	M	130	N	N	*POS*	N	N	METHADONE, CBD, THC, CBN
19	M	90	N	*POS*	*POS*	*POS*	N	METHADONE,EDDP, COCAINE, MDMA,
20	F	25	N	N	N	N	N	METHADONE
21	M	196	N	N	*POS*	N	N	METHADONE, EDDP, COCAINE
22	M	90	N	N	*POS*	N	N	METHADONE, EDDP
23	M	46	N	N	*POS*	N	N	METHADONE, CBN
24	M	40	N	N	N	N	N	METHADONE, EDDP
25	M	40	N	N	N	N	N	METHADONE

OPI, opiaces; COC, cocaine; METHAD, methadone; AMPH, amphetamines; BDZ, benzodiazepines; N, negative; POS, positive.

Table 4

DISCOTHEQUE-GOERS			COZART RAPISCAN SYSTEM					GC/MS RESULTS
SAMPLE	SEX	DECLARED INTAKES	OPI	COC	METHAD	AMPH	BDZ	
1	M	COCAINE	N	*POS*	N	N	N	COCAINE
2	F	MDMA - KETAMINE	N	N	N	*POS*	N	MDMA
3	M	MDMA	N	N	N	*POS*	N	MDMA, CBD, THC, CBN
4	F	KETAMINE	N	N	N	N	N	CBD, THC, CBN
5	M	KETAMINE	N	N	N	*POS*	N	MDMA, CBD, THC
6	F	KETAMINE	N	N	N	N	N	CBD, COCAINE
7	M	KETAMINE - LSD	N	N	N	N	N	CBD, THC
8	M		N	N	N	N	N	
9	M		N	N	N	N	N	
10	F	COCAINE	N	*POS*	N	N	N	COCAINE
11	F	KETAMINE	N	N	N	*POS*	N	
12	M	CANNABIS	N	N	N	N	N	
13	M	CANNABIS	N	N	N	*POS*	N	MDMA, CBD, THC, CBN
14	M	CANNABIS	N	N	N	N	N	

OPI, opiaces; COC, cocaine; METHAD, methadone; AMPH, amphetamines; BDZ, benzodiazepines; N, negative; POS, positive.

Table 5

Sample	Methadone dosage (mg/day)	Methadone (ng/day)	EDDP (ng/day)
1	160	74	16
2	40	76	16
3	10	Neg	Neg
4	25	Neg	Neg
5	45	30	10
6	40	45	14
7	60	134	18
8	60	164	22
9	180	644	60
10	196	343	38
11	90	280	25
12	40	Neg	Neg
13	130	400	35
14	100	112	20
15	250	1592	183
16	45	Neg	Neg
17	20	30	Neg
18	130	250	Neg
19	90	431	50
20	25	40	Neg
21	196	527	50
22	90	206	20
23	46	121	Neg
24	40	30	10
25	40	30	Neg

over, this method offers some advantages for the possibility to screen samples using a small amount of saliva and giving simultaneous results with high sensitivity for different drugs.

Obviously the results must be confirmed by a GC technique. The use of SPME [12] extraction enables the simultaneous analysis of many substances using just a small amount of sample, while preserving the specimen for other analyses.

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