Ann Toxicol Anal. 2008; 20(3): 131-136

DOI: 10.1051/ata/2009012

Available online at:
www.ata-journal.org

ANNALES DE TOXICOLOGIE ANALYTIQUE

Original article / Article original

Cozart® RapiScan Oral Fluid Drug Testing System validation by GC-MS/MS analysis

Validation du test salivaire Oral Fluid Drug Testing System (Cozart®) par analyse GC-MS/MS

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Abstract – Introduction : The increasing number of traffic deaths related to the use of drugs of abuse has made indispensable the use of roadside tests in an easy and fast way. The test most commonly used in Spain is the immunoassay-based Cozart® RapiScan Oral Fluid Drug Testing System. Due to the great number of analysis, it has become essential to validate this test by using more sensible methods. Accordingly, a test validation that combines analysis by gas chromatography and detection by mass spectrometry (GC-MS/MS) has been considered. **Methods**: The total number of samples examined in this study includes 216 cases collected on the road by traffic police and analyzed by a roadside test. Positive samples were confirmed by GC-MS/MS. **Results**: Among the 216 cases examined with the oral fluid test, 155 cases were positive and 61 were negative. In the analysis carried out by using GC-MS/MS, 158 were positive and 58 were negative. **Conclusion**: The results supports the reliability of the oral fluid test as a method for the detection of narcotic substances *in situ*, though later confirmation is recommended using a more sensitive method.

Key words: Oral fluid test, validation, legal medicine

Résumé – Introduction : L'accroissement du nombre de décès sur les routes liés à l'usage de stupéfiants a rendu indispensable l'usage de tests simples et rapides lors de contrôles routiers. En Espagne le test salivaire Oral Fluid Drug Testing System (Cozart®) est le plus couramment utilisé. Du fait de la grande quantité de tests pratiqués, une validation par des méthodes plus sensibles est devenue indispensable. Dans cette optique, une validation combinant analyse par chromatographie en phase gazeuse et spectrométrie de masse (GC-MS/MS) a été mise en œuvre. **Méthodes** : Les échantillons étudiés dans cette étude sont issus de 216 contrôles routiers effectués par les forces de l'ordre. **Résultats** : Sur les 216 cas considérés, 155 étaient positifs, et 61 négatifs. L'analyse par GC-MS/MS a révélé 158 tests positifs et 58 tests négatifs. **Conclusion** : Les résultats confirment la fiabilité du test salivaire pour la détection de narcotiques en situation réelle, moyennant confirmation par une méthode plus sensible.

Mots clés : Test salivaire, validation, médecine légale

Received 20 July 2008, accepted after revision 19 January 2009
Published online 5 March 2009

1 Introduction

In the last years there has been an increasing number of studies dealing with the detection of drugs in biological fluids and particularly with the detection of people driving under the effects of drugs (DUID) or alcohol [1, 2]. The importance of these studies stems from the necessity to minimize the deaths caused by the consumption of alcohol and drugs by the driver. In this context the availability of reliable and accurate roadside tests has become increasingly important. The urgent need for having reliable detection tests has been clearly recognized since the 1990s by both police and legislators [3–5], and accordingly different matrixes have been evaluated [6–8]. Among the earliest studies, oral fluid (OF) test has been considered as a valuable procedure to be used on-site by police officers.

OF is a complex biological matrix consisting not only of the products generated by the secretary glands (parotid, submandibular and sublingual) that form saliva, but also

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from secretions produced by minor glands (labial, buccal and palatal) as well as other substances such as bacteria, food debris, epithelial cells and gingival fluid [9–11]. The concentration of drugs in OF depends on the relative contribution of each gland since the transportation of drugs from plasma to saliva involves passive diffusion across lipid membranes, and different factors related to the chemical structure and nature of drugs play important roles in this mechanism [11–13].

Once OF was considered as a matrix in drug testing, its use has rapidly increased primarily due to the advantages that collection of samples from OF offers in comparison with other biological matrixes, such as blood and urine. The main advantages are the possibility of collection by non-medical personnel, the non-invasive character of this procedure and the difficulty to adulterate the sample [14]. These advantages have made OF to be a particularly promising matrix among the possible kinds of matrixes used in toxicological laboratories. Therefore, it is not surprising that the number of publications related to detection of drugs using OF samples has increased since 1980, leading to the development of devices aimed at the screening of abuse drugs such as amphetamines, cocaine, opiates (morphine and codeine) and cannabinoids [15, 16].

The aim of this study is to validate the roadside test most commonly used in Spain, which relies on an immunoassay test performed on OF samples. Due to the large number of analyses carried out in the last years, there is an urgent need to validate the reliability of this test by resorting to the comparison with more sensible, accurate methods. In fact, this is motivated by the fact that the analytes identified by the immunoassay-based OF test are Δ^9-THC, 6-monoacetylmorphine, cocaine, amphetamine and methamphetamine with cut-off values of 31 ng/mL for Δ^9-THC, 30 ng/mL for cocaine and 50 ng/mL for 6-acetylmorphine, methamphetamine and amphetamine. The technique used to validate the immunoassay-based OF test was gas chromatography and detection by mass spectrometry (GC-MS/MS), which has been extensively used for the analysis of a wide range of drugs after suitable derivatization [17].

2 Observations

To 216 cases collected on the road by traffic police and analyzed by the roadside test Cozart® RapiScan Oral Fluid Drug Testing System. Positive samples were duplicated, for later confirmation. There was not a duplicate from all of the negative samples due to the legal impossibility to obtain a sample when the OF test was negative and the driver assent was not obtained.

3 Materials and methods

Chemicals and materials. Methanol solutions with a concentration of 1 mg/mL of cocaine, Δ^9-tetrahydrocannabinol (Δ^9-THC), amphetamine, methamphetamine, dl-3, 4-methylendioximethamphetamine (dl-3, 4-MDMA), dl-3, 4-methylendioxiethampheta-mine (dl-3, 4-MDEA) and 6-monoacetylmorphine (6-MAM) were purchased from Alltech-Applied Science (State College, PA, USA). For derivatization of Δ^9-THC and 6-MAM, N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) used as BSTFA + 1%TMCS were provided from Supelco (Bellefonte, PA, USA) and 2,2,3,3,3-Pentafluoropropionic acid (PFPA) from Merck KGaA (Darmstadt Germany). Phosphate buffer (0.1 M) was prepared from NaH_2PO_4 and adjusted to pH 6.0 with NaOH 0.1 M.

Oral fluid samples. A total of 216 OF samples were obtained in roadside tests carried out by the police. Police officers were asked to collect sample in duplicate (one for roadside test and another for laboratory analysis) in those cases where the driver seemed to be under the effect of narcotic substances. One of the samples was analyzed *in situ* by using the Cozart® RapiScan Oral Fluid Drug Testing System in order to detect the possible consumption of drugs, and the duplicated sample was collected spitting in a plastic tube and stored at 4 °C to be analyzed in the laboratory with the GC-MS/MS technique.

Sample preparation. Sample preparation consisted of the addition of 1 mL oral fluid to 1 mL phosphate buffer (pH = 6). Once the pH was readjusted, 20 µL of d3-cocaine, d3-6-MAM, d5-amphetamine, d9-methamphetamine and d9-Δ^9-THC were added for a final concentration of 10 µg/mL [18]. The sample was transferred in a Toxitube A®, stirred by using an orbital stirrer (in order to mix the immiscible phases) for 10 min and centrifuged (3500 rpm for 10 min). The organic phase was extracted, evaporated to dryness under nitrogen and derivatized with 40 µL of BSFTA-TMCS at 80 °C for 20 min for Δ^9-THC and 6-MAM or PFPA for amphetamines and methamphetamines at 50 °C for 40 min.

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 factorFour Capillary Column) and the injection temperature was 250 °C. 2 µL of the sample were injected in splitless mode. The oven was programmed from 90 °C for 1 min; ramped at 20 °C/min up 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 performed operating in MS/MS. Details of the detection procedure are shown in Table I, and the different substances and ions identified by MS/MS are shown in Table II. The MS/MS spectrums from the standards of the studied drugs are shown in Figure 1.

Limit of detection-Limit of quantification (LOD-LOQ). Since cut-offs for the roadside OF test were different from the LOD and LOQ values used in the GC-MS/MS confirmation, OF test cut-offs and LOD and LOQ for GC-MS/MS are shown in Table III.

4 Results

Among the 216 cases analyzed by using the oral fluid test, a total of 155 cases were positive and 61 were negative (Table IV). From the 155 positive samples, 152 were confirmed by

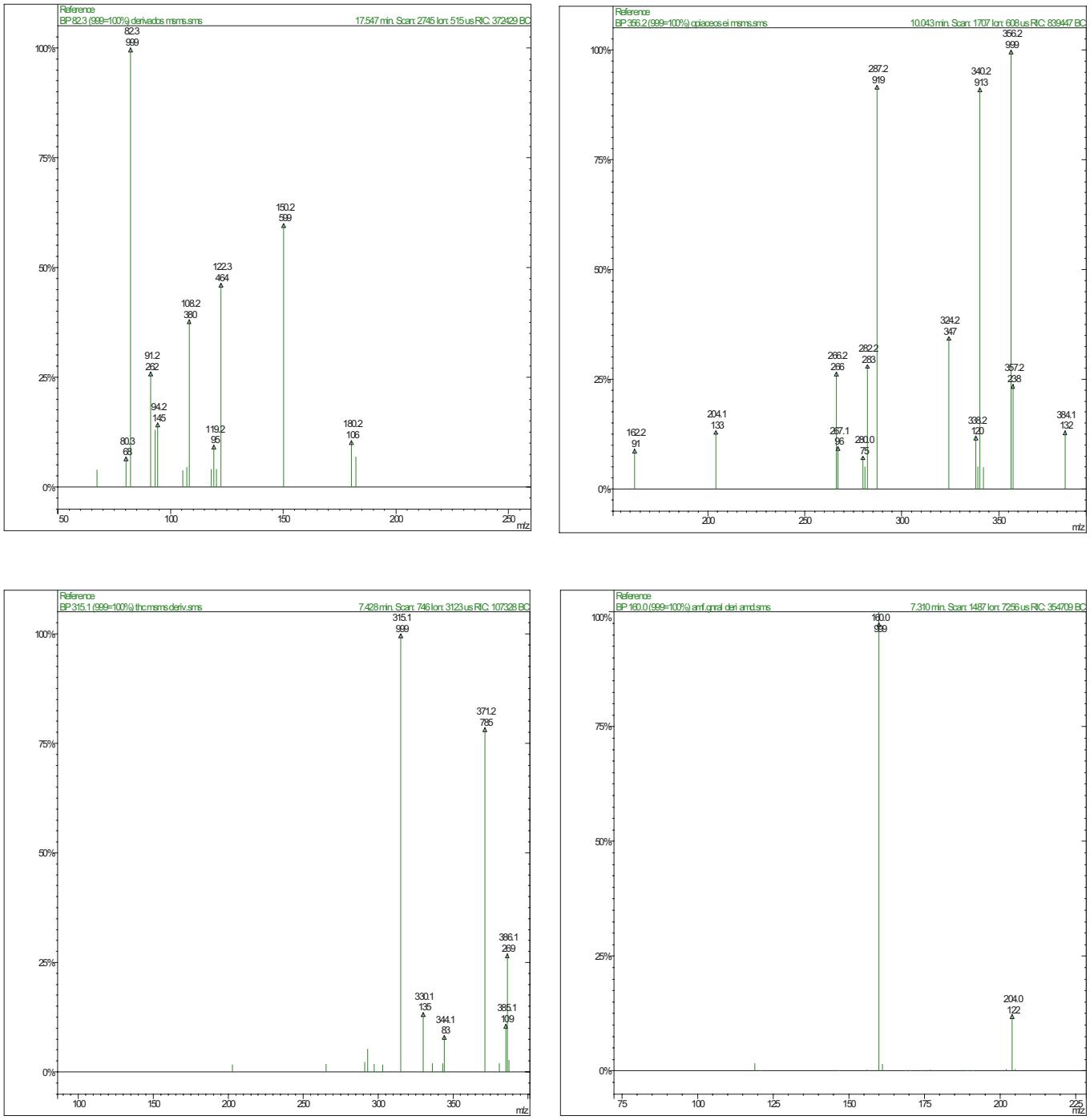

Fig. 1. MS/MS Spectrum from cocaine, 6-MAM-TMS, Δ^9-THC-TMS, methamphetamine-PFP, MDMA-PFP, MDEA-PFP, amphetamine-PFP, respectively showed from up to down and from left to right. *X*-axis shows the ion m/z value and *Y*-axis and numbers under the ions show the ion abundances.

GC-MS/MS as positive and 3 were false positive, and from the 61 negative samples only 19 were confirmed by GC-MS/MS, from which 6 were positive, all of them to cannabis. Thus, in the analysis by GC-MS/MS 158 cases were positive and 58 were negative (Table V).

Based on these results, it can be stated that both methods are in agreement for 98.1% of the positive cases, but only 68.4% of the negatives ones.

5 Discussion

This work examines the reliability of commercial and available devices for the detection of drugs in OF samples. The importance of this subject is supported by the large number of studies referred to commercial tests and the correlation with analytical methods in order to achieve higher detection levels of drugs in OF samples.

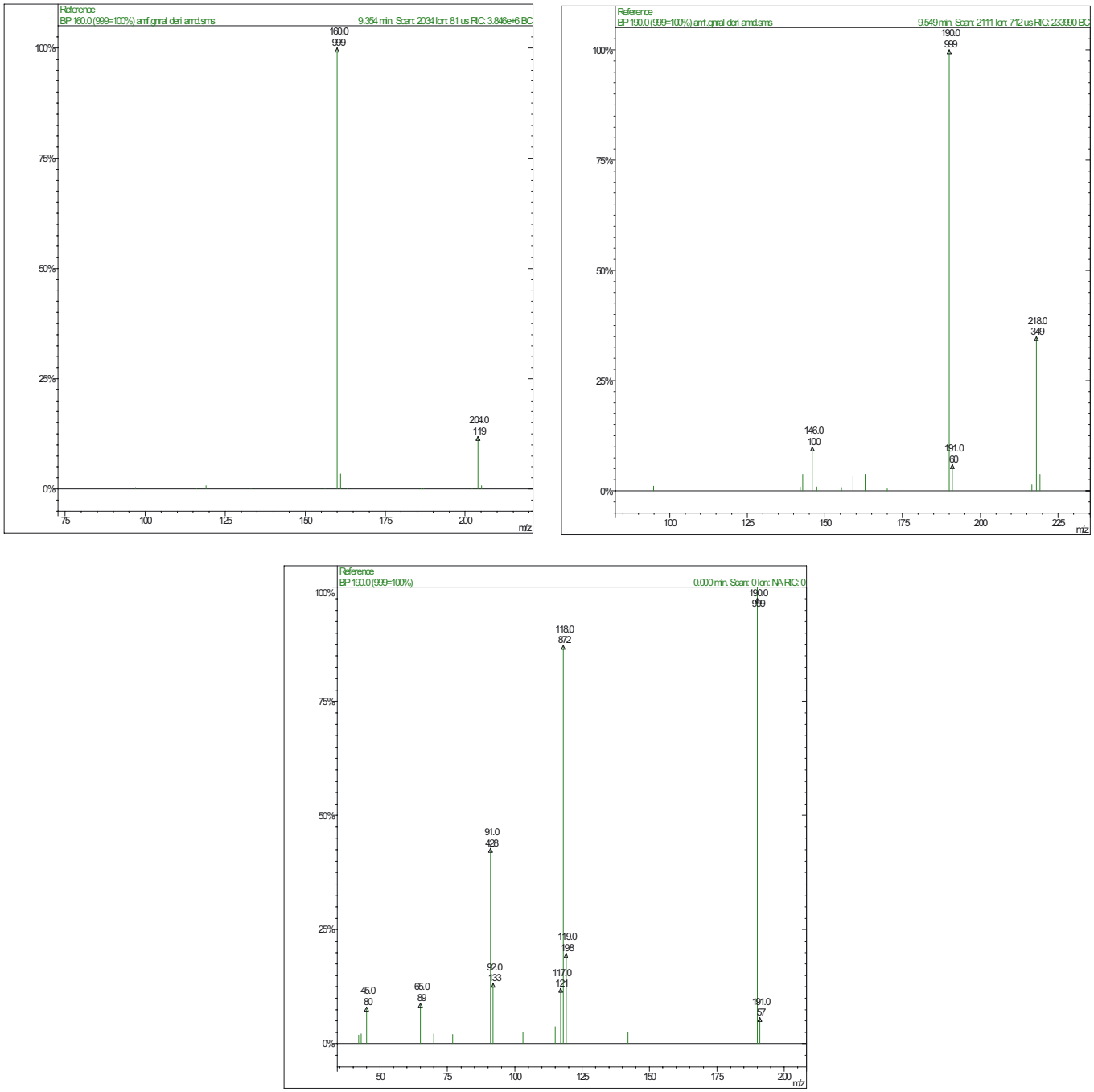

Fig. 1. Continued.

The results obtained here indicate that positive cases can be detected confidently by the OF test. However, the random sampling of 19 negatives cases of the immunoassay-based test showed a 31.6% of false negatives, which would raise the total number of positive cases to cannabinoids. The difficulty associated with the detection of the different metabolites of cannabis is well-known [19, 20], and this fact should be taken into account in order to approve the immunoassay-based OF test for routine roadside tests.

The reliability of the OF test is supported by the large agreement (>95%) found with the results obtained from a highly sensitive method such as GC/MS-MS. This test, nevertheless, should be rejected for the analysis of Δ^9-THC, since it gives rise to an error of *ca*. 30% in negative samples for cannabis, although all the available kits show similar results for Δ^9-THC and the error is also minimized by the fact that all of them are false negatives instead of false positives.

Overall, the OF test can be considered to be a valid method for the detection of narcotic substances *in situ*, which also combines a short analysis time and a practical, easy procedure to collect samples. However, confirmation by more sensible methods is recommended to detect drugs at lower

Table I. Drug targets and procedures used in GC-MS/MS.

Drug target	Ionization	Waveform type	Excitation width (V)
Amphetamine-PFP	EI[a]	Non resonant	64.0
Methamphetamine-PFP	EI	Non resonant	60.0
MDMA-PFP	EI	Non resonant	60.0
MDEA-PFP	EI	Non resonant	57.0
Cocaine	EI	Non resonant	45.0
Δ^{9-}THC-TMS	EI	Non resonant	61.0
6-MAM-TMS	EI	Resonant	1.0

[a] Electronic Impact.

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

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^{9-}THC-TMS	17.43	386	371, 315, 330
6-MAM-TMS	20.11	399	356, 340, 287

Table III. Cut-off values of the immunoassay OF test used in roadside controls and LOD and LOQ values for GC-MS/MS analysis.

Drug target	OF test	GC-MS/MS	
	Cut-off (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
Amphetamine-PFP	50	5	20
Methamphetamine-PFP	50	5	20
MDMA-PFP	50	5	20
MDEA-PFP	50	5	20
Cocaine	30	2.5	10
Δ^{9-}THC-TMS	31	2.5	10
6-MAM-TMS	50	5	20

Table IV. Comparative results between roadside test and GC-MS/MS.

	Roadside test	GC-MS/MS
Positive	155	158
Negative	61	58

concentrations [21–23]. Furthermore, it would be strongly recommended the use of both drug tests and ethilometers in road preventive controls.

Acknowledgements. We are grateful to O. Huertas for making this job easier and for his technical assistance.

Table V. Results of GC-MS/MS.

	GC-MS/MS results	
	Confirmed positive	False positive
	152	3
	False negative	Confirmed negative
	6	19 (55)[a]
Total	158	58

[a] 19 of the total negative samples were confirmed.

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