PO-CON1772E

SHIMADZU
Excellence in Science

Automatic, simultaneous and rapid analysis of 46 drugs of abuse in saliva by on line SPE and UHPLC-MS/MS

ASMS 2017 MP-714

Doriane TOINON[1], Aurore JAFFUEL[1], Alban HUTEAU[1]
1. SHIMADZU France, le Luzard II, bat A, Bd Allende, Noisiel, 77448 Marne La Vallée cedex 2, France

Automatic, simultaneous and rapid analysis of 46 drugs of abuse in saliva by on line SPE and UHPLC-MS/MS

Introduction

The drugs quantification of abuse in saliva offers information on the state of individual consumption by non-invasive and easy sample collection. Narcotic substances are detected in saliva from four different drugs family. Nevertheless, the concentrations of these drugs in saliva are lower than those found in blood or urine. Moreover, this analysis is based on the detection of parent compounds and not metabolites. Therefore, the analysis must be performed within 24 hours. So, it is necessary to use a very sensitive and rapid analysis method.

The objective is to have a robust and automatic analytical method allowing the analysis of all these substances for hundred samples per day.

Methods and Materials

The collection of saliva sample was made on special support the Floqswab®.
the workflow is :

- First step : extraction of this support with 2 mL of buffer pH6,4 during 5 minutes in an ultrasound bath (Fig 1). The standards drugs were doped in the extraction solution to make the different points of calibration.
- Second step :SPE Online for purification and automatically injection on SPE-LC-MS/MS (Fig 2).

The on-line SPE UHPLC-MS/MS system was coupled to triple quadrupole mass spectrometer (Nexera X2 with LCMS-8060, Shimadzu Corporation, Kyoto, Japan) offers the best solution for complete automatization between the SPE and UHPLC .

Separation was achieved using a ACE C18 AR column (100mmL., 2.1mmI.D., 2um particles) and the detection with electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode with ultra-fast polarity switching.

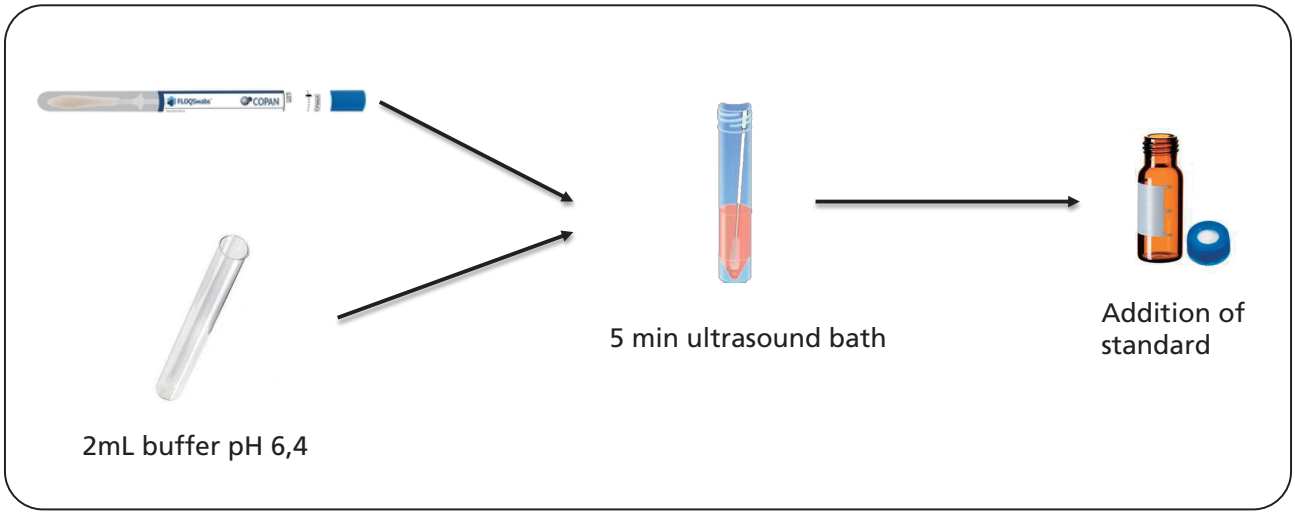

Figure 1 Extraction of saliva

Automatic, simultaneous and rapid analysis of 46 drugs of abuse in saliva by on line SPE and UHPLC-MS/MS

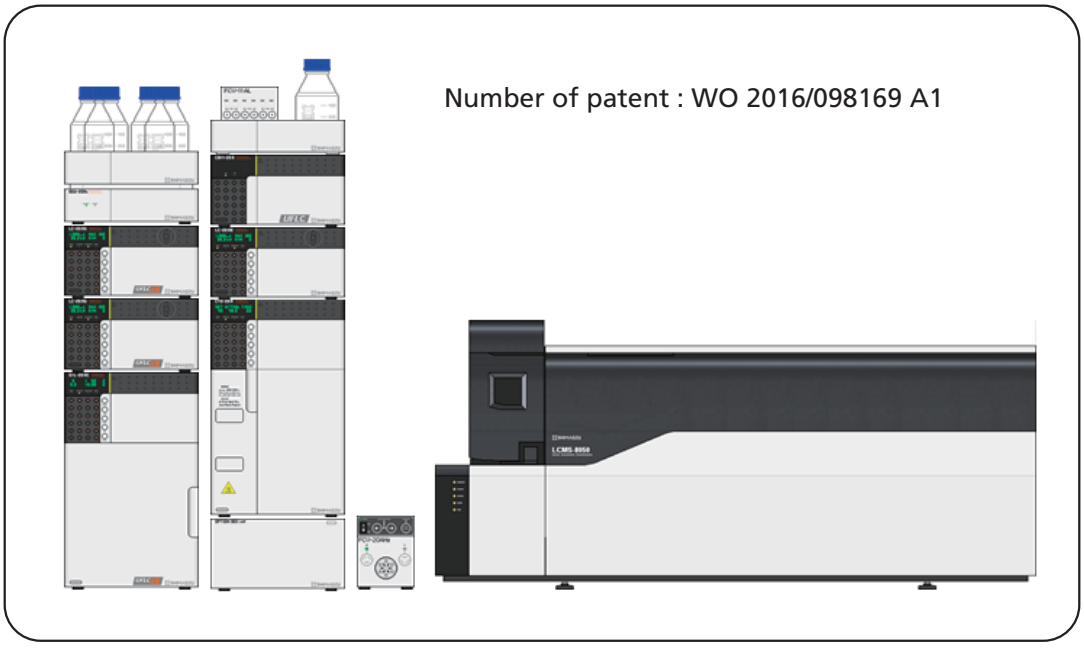

Figure 2 On-line SPE LCMS-8060

Results

Method development

UHPLC conditions (Nexera X2)	
Column	: ACE C18 AR 100mm×2.1mm, 2 um
Mobile phase A	: Water + 0.002% Formic Acid + 2mM Ammonium Formate
B	: 90/10 Methanol/ACN
Flow rate	: 0.6 mL/min
Injection vol.	: 80 uL
Column temperature	: 30 °C

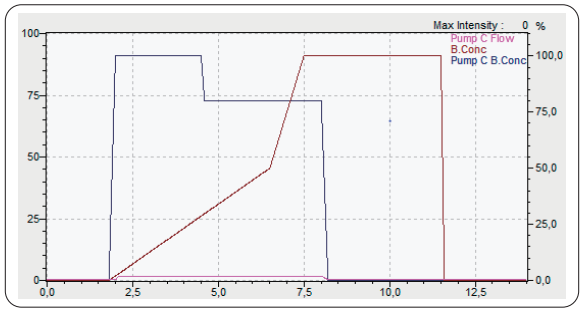

Figure 3 gradient

MS conditions (SHIMADZU LCMS-8060)
Ionization: ESI, Positive/Negative MRM mode

Automatic, simultaneous and rapid analysis of 46 drugs of abuse in saliva by on line SPE and UHPLC-MS/MS

Table 1. MRM transition of drugs

Compound name	MRM	Compound name	MRM
11-OH-THC (+)	331,1>313,3	Hydromorphone (+)	286,2>185,1
2-CB (+)	260,1>243,1	M-CPP (+)	197,2>154,1
2C-I (+)	308,1>276,1	MBDB (+)	208,2>177,1
4-MTA (+)	182,2>117,2	MDA (+)	180,3>105,2
Acide ritalinique (+)	220,2>84,2	MDEA (+)	208,2>163,2
Acétyl-6-morphine (+)	328,2>165,2	MDMA (+)	194,2>163,1
Amphétamine (+)	136,2>91,0	MDPV (+)	276,2>126,2
Anhydroecgonine méthylester (+)	182,2>118,1	Methadone (+)	310,2>265,1
BDB (+)	195,2>136,2	Methiopropramine (+)	155,9>97,1
Benzoylecgonine (+)	290,2>168,3	Morfenfluramine (+)	204,2>159,2
Buprenorphine (+)	468,0>55,0	Morphine (+)	286,1>152,2
Cocaéthylène (+)	318,2>82,2	Méphédrone (+)	178,0>160,2
Cocaine (+)	304,0>82,1	Méthadone (+)	310,1>265,3
Dextromethorphan (+)	272,3>215,2	Méthamphétamine (+)	150,2>91,1
Dihydrocodéine (+)	302,0>199,1	Méthcathinone (+)	164,2>131,3
EDDP (+)	278,1>234,3	Méthylmorphine (+)	300,2>165,1
Ecgonine methylester (+)	200,2>182,2	Méthylphénidate (+)	234,2>84,1
Ephédrine (+)	166,2>91,1	Naloxone (+)	328,2>310,1
Ethyl amphétamine (+)	164,3>131,2	Naltrexone (+)	342,2>324,1
Ethylmorphine (+)	314,3>153,2	Noroxycodone (+)	302,2>284,1
Hydrocodone (+)	300,2>199,1	Noréphédrine (+)	152,2>134,2
Oxycodone (+)	316,2>298,1	THC (+)	315,1>123,0
Pholcodine (+)	399,1>114,1	THC-COOH (-)	343,2>299,2

Automatic, simultaneous and rapid analysis of 46 drugs of abuse in saliva by on line SPE and UHPLC-MS/MS

Quantitative Results

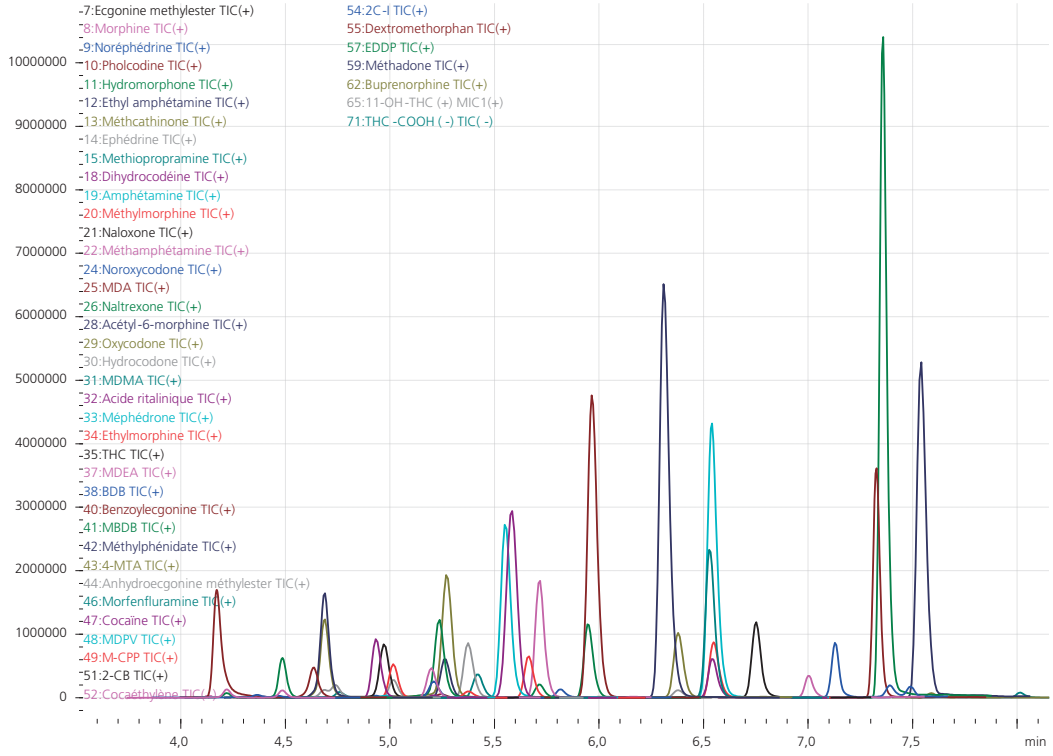

Figure 3 Mass Chromatgrams of 46 drugs of abuse

All saliva samples was pretreated with buffer solution and injected by SPE on-line coupled to UHPLC –MS/MS

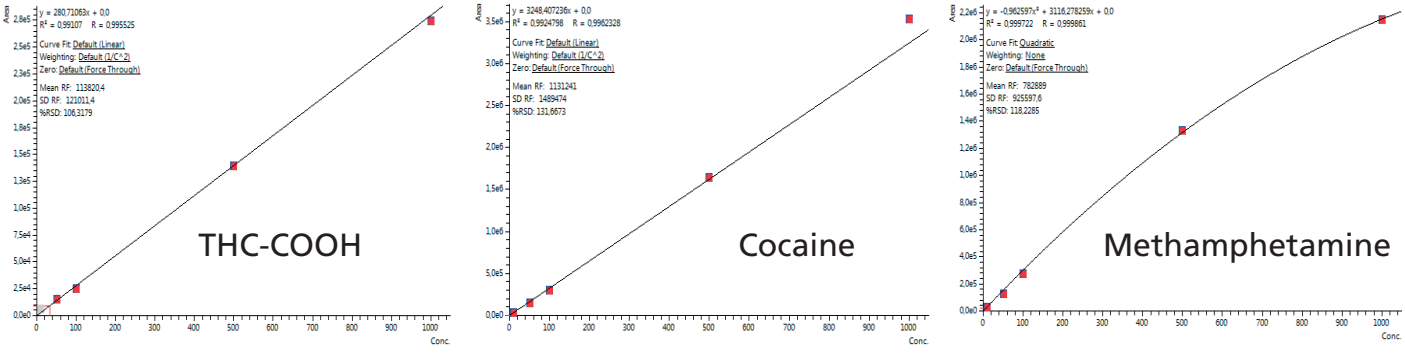

Figure 4 Representative calibration curve (THC-COOH, Cocaine, Methamphetamine)

Automatic, simultaneous and rapid analysis of 46 drugs of abuse in saliva by on line SPE and UHPLC-MS/MS

Table2 Quantification range (without inclusion of Floqswab® efficiency extraction) and accuracies on this range

Compound name	Range (pg/mL)	Range accuracy (%)	Compound name	Range (pg/mL)	Range accuracy (%)
11-OH-THC (+)	500-1000	98-102	Hydromorphone (+)	10-1000	87-115
2-CB (+)	10-1000	90-114	M-CPP (+)	10-1000	91-112
2C-I (+)	10-1000	88-115	MBDB (+)	10-1000	80-115
4-MTA (+)	10-1000	80-109	MDA (+)	50-1000	92-111
Acide ritalinique (+)	10-1000	96-105	MDEA (+)	10-1000	86-112
Acétyl-6-morphine (+)	10-1000	85-113	MDMA (+)	10-1000	86-115
Amphétamine (+)	500-1000	94-107	MDPV (+)	10-1000	85-113
Anhydroecgonine méthylester (+)	10-1000	87-107	Methadone (+)	10-1000	92-107
BDB (+)	50-1000	93-104	Methiopropramine (+)	50-1000	86-110
Benzoylecgonine (+)	10-1000	85-111	Morfenfluramine (+)	10-1000	89-106
Buprenorphine (+)	50-1000	92-107	Morphine (+)	50-1000	87-115
Cocaéthylène (+)	10-1000	93-110	Méphédrone (+)	10-1000	80-114
Cocaine (+)	10-1000	90-109	Méthadone (+)	10-1000	94-105
Dextromethorphan (+)	10-1000	86-115	Méthamphétamine (+)	10-1000	85-101
Dihydrocodéine (+)	10-1000	89-112	Méthcathinone (+)	10-1000	98-105
EDDP (+)	10-1000	91-106	Méthylmorphine (+)	10-1000	94-105
Ecgonine methylester (+)	500-1000	89-111	Méthylphénidate (+)	10-1000	94-118
Ephédrine (+)	50-1000	88-112	Naloxone (+)	10-1000	91-108
Ethyl amphétamine (+)	10-1000	91-105	Naltrexone (+)	10-1000	99-101
Ethylmorphine (+)	10-1000	86-110	Noroxycodone (+)	50-1000	92-104
Hydrocodone (+)	10-1000	92-110	Noréphédrine (+)	100-1000	97-103
Oxycodone (+)	10-1000	93-115	THC (+)	500-1000	95-109
Pholcodine (+)	10-1000	94-110	THC-COOH (-)	50-1000	91-112

Automatic, simultaneous and rapid analysis of 46 drugs of abuse in saliva by on line SPE and UHPLC-MS/MS

Conclusions

The method implementation allows to quantify the 46 targeted compounds in 15 minutes, including injector cleaning, column rinsing, samples treatment and separation. This method can be used on saliva and blood, For blood the Floqswab® extraction is replaced by a protein precipitation.

For Research Use Only. Not for use in diagnostic procedures. Not available in the USA, Canada and China.

First Edition: June, 2017

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/