

Clam- SPE on line- UHPLC-MS/MS : Fully automatic, simultaneous and quickly quantification of drugs of abuse in blood, plasma and urine.

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1. Introduction

Drugs of abuse are chemical, biochemical or natural compounds that can alter neuronal activities. Some drugs can be physically or psychologically addictive. The use of these may result in physical or mental disturbances.

A system for regulating the production, trade and consumption of drugs has been put in place. Controls and analysis have therefore multiplied. That's why it seemed necessary to develop a rapid and automatic analytical method for all drugs in various biological matrix.

2. Materials and Methods

Quantification of narcotics is performed on Shimadzu system with Clam-2000, SPE on-line UHPLC Nexera XR and on LCMS8060. Blood, plasma and urine are collected directly from patients and validated without the presence of CAOs and cannabinoids.

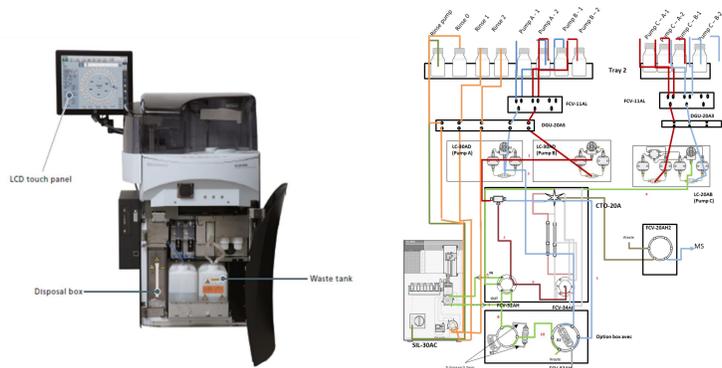


Figure 1. Schema of Clam-2000, SPE on-line UHPLC-MS/MS (Number of patent : WO 2016/098169 A1).

Clam precipitates the proteins, filters and dilutes the sample. Then, it's injected on the SPE on line LCMSMS.

The sample is cleaned on exchange SPE and target compounds are separated on C18 column and detected in 10min. This delay also includes system conditioning and internal/external rinsing of injector.

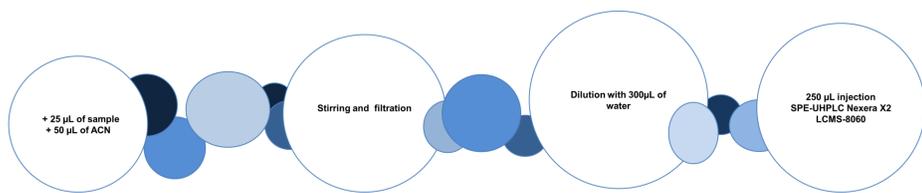


Figure 2. Sample workflow overview for the quantification of drugs of abuse.

3. Results

3-1. Typical chromatogram

The developed method is able to automatically quantify various drugs of abuse by an online sample preparation and cleaning with Clam-2000 and SPE. The impact of human handling is reduced to a minimum. Using this method, the different narcotics, 40 CAO et 3 cannabinoids are analyzed in a single run of 10 minutes. This allows worker and instrument to save time and money (less used solvent). Moreover, washes of the injector and SPE column are included in the same run ensuring a lack of carry-over and really good robustness of the method.

All targeted compounds are analyzed with 2 or 3 MRM in order to have enough quantification and qualification transitions. The calibration curve is determined between 5 ng/mL and 250 ng/mL for CAO and 0,5 to 25 ng/mL for cannabinoids.

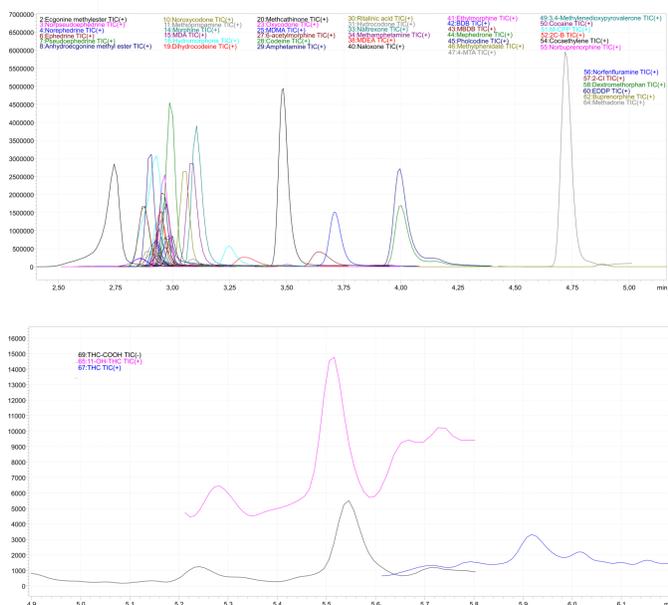


Figure 3. Typical chromatogram and a zoom on cannabinoids .

3-2. Calibration

The calibration standards were performed by spiking the blood matrix. The regression coefficients were calculated and are greater than 0,99. The achieved limits of quantification are at least 10 ng/mL for all compounds, 1 ng/mL for 11-OH-THC, THC-COOH and THC in blood.

The achieved results on the calibration curve in blood have a good accuracy between 85 and 115%. The ion ratio stays stable with relative response which doesn't exceed 30%.

Compound	Accuracy average (%)	Set ion ratio (%)
11-OH-THC	100	7
2C-B	98	46
2-CI	98	35
3,4-Methylenedioxypropylverone	97	86
4-MTA	106	85
6-acetylmorphine	97	142
Amphetamine	98	73
Anhydroecgonine methyl ester	93	103
BDB	106	27
Benzoyllecgonine	99	28
Buprenorphine	97	242
Cocaine	104	378
Cocaine	100	19
Cocaine	99	72
Dextromethorphan	97	99
Dihydrocodeine	94	82
Ecgonine methylester	98	52
EDDP	99	58
Ephedrine	95	31
Ethylmorphine	102	84
Hydrocodone	98	49
Hydromorphone	103	79
MBDB	100	40
M-CPP (meta-Chlorophenylpiperazine)	99	129

Compound	Accuracy average (%)	Set ion ratio (%)
MDA	99	73
MDEA	102	70
MDMA	106	89
Mephedrone	100	105
Methadone	101	58
Methamphetamine	95	28
Methcathinone	100	87
Methiopropamine	98	48
Methylphenidate	96	24
Morphine	99	43
Naloxone	99	23
Naltrexone	98	18
Norbuprenorphine	103	92
Norephedrine	99	157
Norfenfluramine	97	37
Noroxycodone	103	37
Norpseudoephedrine	101	180
Oxycodone	101	26
Pholcodine	102	26
Pseudoephedrine	96	28
Ritalinic acid	99	17
THC	106	58
THC-COOH	111	26

Table 1. Accuracies and ion ratios average for the different levels of calibration .

3-3. Method Performances

Analytical performances of the method were monitored using 3 blood, 3 plasma and 3 urine samples spiked with 10 ng/ml of CAO and 1 ng/mL of cannabinoids. These samples spiked, directly in primary tube, are arranged in the Clam-2000 to be analysed.

The repeatability and accuracy are determined by the quantification of these samples with the calibration curve in blood.

Compound	Repeatability urine (n=3) (%)	Repeatability plasma (n=3) (%)	Repeatability blood (n=3) (%)	Repeatability total (n=9) (%)
11-OH-THC	3	8	8	7
2C-B	7	8	3	9
2-CI	1	7	3	5
3,4-Methylenedioxypropylverone	13	14	3	10
4-MTA	10	2	6	8
6-acetylmorphine	8	4	6	9
Amphetamine	10	6	4	10
Anhydroecgonine methyl ester	12	12	10	10
BDB	3	2	12	9
Benzoyllecgonine	1	1	1	4
Buprenorphine	3	6	6	14
Cocaine	1	1	2	11
Cocaine	1	2	0	8
Cocaine	12	1	11	9
Dextromethorphan	3	3	9	15
Dihydrocodeine	15	17	10	13
Ecgonine methylester	2	4	8	8
EDDP	1	8	0	7
Ephedrine	4	20	4	11
Ethylmorphine	18	16	16	13
Hydrocodone	13	10	14	11
Hydromorphone	3	11	12	13
MBDB	1	15	12	14
M-CPP	11	8	2	10

Compound	Repeatability urine (n=3) (%)	Repeatability plasma (n=3) (%)	Repeatability blood (n=3) (%)	Repeatability total (n=9) (%)
MDA	11	9	6	8
MDEA	6	8	7	8
MDMA	6	6	9	5
Mephedrone	6	21	14	14
Methadone	2	9	2	7
Methamphetamine	4	10	8	11
Methcathinone	6	18	11	13
Methiopropamine	12	9	5	14
Methylphenidate	7	11	3	11
Morphine	6	9	8	11
Naloxone	11	15	5	12
Naltrexone	6	2	9	10
Norbuprenorphine	8	16	10	10
Norephedrine	4	12	2	12
Norfenfluramine	9	8	2	13
Noroxycodone	5	5	10	11
Norpseudoephedrine	1	10	6	10
Oxycodone	4	15	3	9
Pholcodine	8	8	4	11
Pseudoephedrine	2	19	3	11
Ritalinic acid	1	14	4	13
THC	ND	4	5	4
THC-COOH	4	6	9	4

Table 2. Repeatabilities on QC in blood, plasma and urine.

Compound	Accuracy urine (n=3) (%)	Accuracy plasma (n=3) (%)	Accuracy blood (n=3) (%)	Accuracy total (n=9) (%)
11-OH-THC	105	95	97	99
2C-B	105	94	90	97
2-CI	99	95	92	96
3,4-Methylenedioxypropylverone	97	95	97	96
4-MTA	105	117	107	109
6-acetylmorphine	104	93	88	95
Amphetamine	102	99	84	95
Anhydroecgonine methyl ester	88	90	95	91
BDB	108	116	98	107
Benzoyllecgonine	102	99	92	97
Buprenorphine	84	104	83	90
Cocaine	99	118	93	103
Cocaine	95	110	94	100
Cocaine	104	95	92	97
Dextromethorphan	110	82	96	96
Dihydrocodeine	97	92	84	91
Ecgonine methylester	97	104	87	96
EDDP	97	104	91	97
Ephedrine	91	92	94	92
Ethylmorphine	100	102	110	104
Hydrocodone	95	97	95	96
Hydromorphone	117	101	89	103
MBDB	117	98	99	105
M-CPP	107	95	92	98

Compound	Accuracy urine (n=3) (%)	Accuracy plasma (n=3) (%)	Accuracy blood (n=3) (%)	Accuracy total (n=9) (%)
MDA	100	103	99	101
MDEA	96	109	100	102
MDMA	107	113	109	110
Mephedrone	103	96	105	101
Methadone	98	107	94	100
Methamphetamine	102	87	84	91
Methcathinone	110	103	88	100
Methiopropamine	108	90	86	95
Methylphenidate	103	87	101	97
Morphine	109	95	89	97
Naloxone	106	92	96	98
Naltrexone	112	95	89	99
Norbuprenorphine	109	100	106	105
Norephedrine	106	105	83	98
Norfenfluramine	110	89	88	96
Noroxycodone	97	113	91	100
Norpseudoephedrine	114	103	94	103
Oxycodone	99	102	93	98
Pholcodine	105	108	86	100
Pseudoephedrine	95	95	94	95
Ritalinic acid	109	89	105	101
THC	ND	110	114	112
THC-COOH	116	115	115	115

Table 3. Accuracies average on QC in blood, plasma and urine.

The accuracy average is contained between 80-120% and the repeatability is less than 15%.

Conclusions & Discussion

The presented method allows quick, sensitive and simultaneous analysis of different families of drugs (cannabinoid, amphetamine, opiate and cocaine compounds). It is fully automated with efficient sample preparation and processing without human intervention. This method allows the quantification of 3 biological samples blood, plasma and urine, from a single calibration curve made in blood.

In a second way, this sample preparation with Clam-2000 and SPE one-line will be adapted to also allow the analysis of saliva extracted from Floqswab® and Quantisal® collectors.