Quantification of drugs of abuse in oral fluid using online TurboFlow™ sample extraction

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ABSTRACT

Purpose

An analytical method for forensic toxicology for the quantification of drugs of abuse in oral fluid using online Thermo Scientific™ TurboFlow™ sample extraction is reported. Two approaches were developed, one for tetrahydrocannabinol and its metabolites and one for basic drugs. Both methods involve a protein precipitation step followed by online sample extraction using a Thermo Scientific™ Prelude™ SPLC system; a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer with heated electrospray ionization is used for detection by selected reaction monitoring (SRM). Method performance was evaluated using oral fluid sampled using Thermo Scientific™ OralEze™ Oral Fluid collection devices and spiked with the compounds of interest.

Methods

The reported analytical method for the quantification of 25 drugs of abuse in oral fluid includes two separate approaches. The first approach covers tetrahydrocannabinol and its metabolites; the second is used for basic drugs. Oral fluid from donors was sampled using OralEze oral fluid collection devices and spiked with the compounds of interest to generate the calibration curve. Sample clean-up is performed by a preliminary protein precipitation with internal standard addition followed by online TurboFlow sample extraction using a Thermo Scientific Prelude SPLC system. The Prelude SPLC system includes two extraction and separation channels working in parallel on the same mass spectrometer to optimize the throughput. Analytes and internal standards are detected by SRM on a TSQ Quantiva triple quadrupole mass spectrometer with heated electrospray ionization. Robustness was evaluated on the method for cannabinoids in terms of coefficient of variation for the peak area for tetrahydrocannabinol and its metabolites following 500 repeated injections of oral fluid extracts spiked with the cannabinoids at a concentration of 12 ng/mL each.

Results

Each method was run on a different channel, allowing to have the full panel of analytes run in less than 15 minutes per sample. Limits of quantification were obtained for each analyte as the lowest concentration with a bias between nominal and back-calculated concentration within ± 20% and a maximum standard deviation on three injections of 20%. Limits of quantification between 20 pg/mL and 5 ng/mL were obtained. A maximum coefficient of variation of 13% for the peak areas of the cannabinoids was obtained following 500 repeated injections of spiked oral fluid.

INTRODUCTION

An analytical method for forensic toxicology for the quantification of drugs of abuse in oral fluid using online Thermo Scientific TurboFlow sample extraction is reported. Two approaches were developed, one for tetrahydrocannabinol and its metabolites hydroxy- and carboxy-tetrahydrocannabinol, and one for basic drugs. Both methods involve a protein precipitation step followed by online TurboFlow sample extraction using a Thermo Scientific Prelude SPLC system; a Thermo Scientific TSQ Quantiva triple quadrupole mass spectrometer with heated electrospray ionization is used for detection by single reaction monitoring (SRM) using 15 isotopically labeled internal standards. Method performance was evaluated using oral fluid from donors sampled using Thermo Scientific OralEze oral fluid collection device and spiked with the compounds of interest. Limits of quantification and linearity ranges for each analyte as well as robustness of the method were evaluated.

MATERIALS AND METHODS

Sample Preparation

Saliva samples from donors were taken using OralEze oral fluid collection devices and spiked with the molecules of interest at different concentrations. A concentration range from the limit of quantification (LOQ - 20 pg/mL to 5 ng/mL depending on the analyte) up to 500 ng/mL was covered. Sample extraction was performed by adding 100 µL of acetonitrile containing a mix of 15 internal standards to 100 µL of saliva sample followed by vortex-mixing and centrifugation. The supernatant was transferred to a clean vial prior to injection onto the LC-MS system. A list of the analytes of interest, together with the corresponding internal standards and the covered concentration ranges, is reported in Table 1.

Table 1. Analytes of interest, internal standards and concentration ranges

Analyte	Calibration Type	Internal Standard	Calibration Range (ng/mL)
THC-COOH	Internal	THC-COOH-D3	0.2 – 500
THC	Internal	THC-D3	0.2 – 500
OH-THC	External	N/A	5 – 500
Methadone	External	N/A	0.05 – 200
Amphetamine	Internal	Amphetamine-D5	0.5 – 200
Metamphetamine	Internal	Metamphetamine-D5	0.1 – 200
MDA	Internal	MDA-D5	0.2 – 200
MDMA	Internal	MDMA-D5	0.2 – 200
MDEA	Internal	MDEA-D5	0.02 – 200
Mephedrone	External	N/A	0.1 – 200
Ephedrine	Internal	Ephedrine-D3	0.05 – 200
Pseudoephedrine	External	N/A	0.05 – 200
Ketamine	Internal	N/A	0.05 – 200
Heroin	External	N/A	0.2 – 200
Morphine	Internal	Morphine-D3	0.2 – 200
6-MAM	Internal	6-MAM-D3	0.05 – 200
Codeine	Internal	Codeine-D3	0.05 – 200
Pholcodine	External	N/A	0.02 – 200
Ethylmorphine	External	N/A	0.1 – 200
Dihydrocodeine	External	N/A	0.1 – 200
Buprenorphine	External	N/A	0.2 – 200
Cocaine	Internal	Cocaine-D3	0.1 – 200
Benzoylecgonine	Internal	Benzoylecgonine-D3	0.1 – 200
EME	Internal	EME-D3	0.2 – 200
Cocaethylene	Internal	Cocaethylene-D3	0.1 – 200

Liquid Chromatography

A Prelude SPLC system was used for online TurboFlow sample extraction and chromatographic separation. The Prelude SPLC system is an HPLC front-end made of two independent extraction and separation channels working in parallel on the same mass spectrometer using either identical of different methods. In this case, channel #1 was used for the cannabinoids, channel #2 for the basic drugs. A detailed description of the analytical conditions for both online sample extraction and chromatographic separation are reported in Figure 1 and 2.

Table 2. MS conditions for (a) the cannabinoids and (b) the basic drugs

	(a)	(b)
Source type	HESI with polarity switching	HESI in positive mode
Spray voltage	3500 V positive mode - 2900 V negative mode	
Vaporizer temp	350ºC	
Ion transfer tube temp	350ºC	
Sheath gas	35 AU	60 AU
Sweep gas	1 AU	2 AU
Auxiliary gas	17 AU	25 AU
Data acquisition mode	SRM	
Chrom filter peak width	3.0 s	
Collision gas pressure	1.5 mTorr	
Cycle time	0.400 s	
Q1 (FWMH)	0.7	
Q3 (FWMH)	0.7	

Figure 1. LC method details for cannabinoids

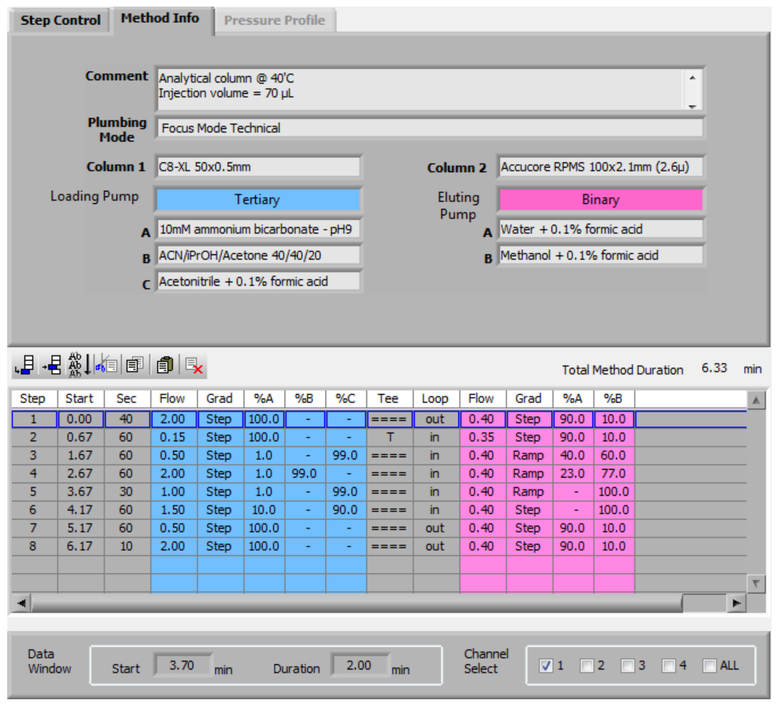
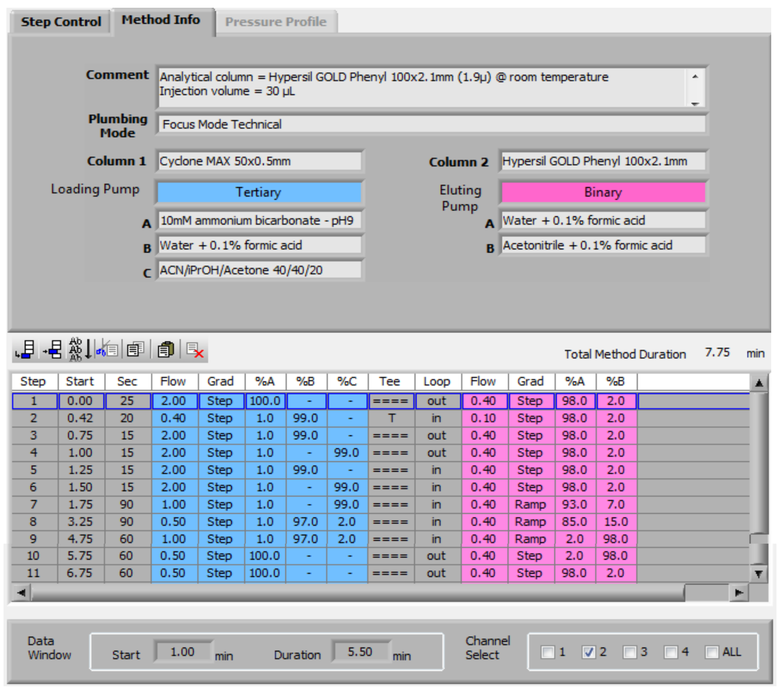


Figure 2. LC method details for basic drugs



Mass Spectrometry

A TSQ Quantiva triple quadrupole mass spectrometer with a heated electrospray source was used as a detector. Data were acquired in selected reaction monitoring (SRM) mode. An internal calibration approach thanks to the corresponding isotopically labeled internal standards was used for 15 analytes; external calibration was used for the remaining compounds. A detailed description of MS conditions and SRM transitions are reported in Table 2 and Table 3, respectively.

Table 3. SRM transitions with RF lens and collision energy values

Analyte	Polarity	Precursor (m/z)	RF Lens (V)	Product (m/z)	Collision Energy (V)
THC-COOH	Negative	343.2	75	245.2	28
THC-COOH-D3	Negative	346.3	88	302.3	22
THC	Positive	315.2	62	193.0	21
THC-D3	Positive	318.0	62	196.0	21
OH-THC	Positive	331.3	58	201.1	22
Methadone	Positive	310.2	52	265.1	14
Amphetamine	Positive	136.2	30	91.0	17
Amphetamine-D5	Positive	141.2	30	93.1	17
Metamphetamine	Positive	150.2	33	91.0	18
Metamphetamine-D5	Positive	155.2	31	92.0	18
MDA	Positive	180.0	47	163.1	10
MDA-D5	Positive	185.2	30	168.0	10
MDMA	Positive	194.3	35	163.1	10
MDMA-D5	Positive	199.2	38	165.0	10
MDEA	Positive	208.2	42	163.0	12
MDEA-D5	Positive	213.2	40	163.1	13
Mephedrone	Positive	178.2	36	160.0	10
Ephedrine	Positive	166.2	30	115.1	25
Ephedrine-D3	Positive	166.2	30	148.0	10
Pseudoephedrine	Positive	166.2	30	115.1	25
Ketamine	Positive	238.2	43	124.9	27
Heroin	Positive	370.2	82	268.1	27
Morphine	Positive	286.2	72	201.1	24
Morphine-D3	Positive	289.0	71	165.0	39
6-MAM	Positive	328.2	78	165.0	38
6-MAM-D3	Positive	331.2	77	211.1	25
Codeine	Positive	300.2	73	165.1	41
Codeine-D3	Positive	303.2	73	165.1	42
Pholcodine	Positive	399.3	82	114.1	32
Ethylmorphine	Positive	314.2	74	165.0	42
Dihydrocodeine	Positive	302.2	72	199.0	32
Buprenorphine	Positive	468.4	98	396.2	38
Cocaine	Positive	304.2	60	182.1	18
Cocaine-D3	Positive	307.2	62	185.0	18
Benzoylecgonine	Positive	290.2	58	168.1	18
Benzoylecgonine-D3	Positive	293.2	60	171.1	18
EME	Positive	200.2	50	182.1	16
EME-D3	Positive	203.2	51	185.1	16
Cocaethylene	Positive	318.2	62	196.1	18
Cocaethylene-D3	Positive	321.3	62	199.1	18

Data Analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software.

RESULTS

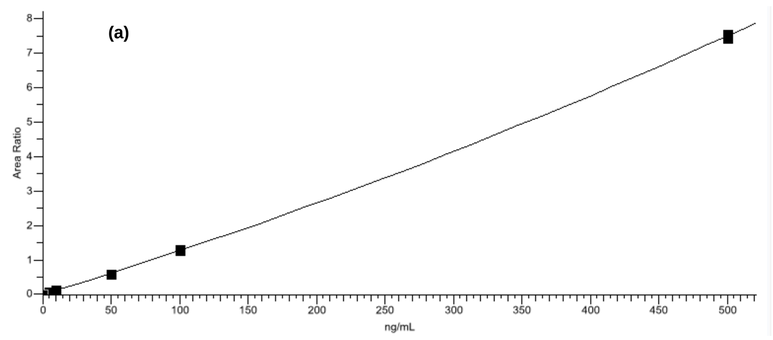
The limit of quantification for each analyte was established as the lowest calibrator with an average percentage bias between nominal and back-calculated concentration of less than 20% and a maximum standard deviation of 20% following triplicate injections. LOQ values between 20 pg/mL and 5 ng/mL were obtained using linear or quadratic fitting. 1/x weighing was used for the cannabinoids, $1/x^2$ for the basic drugs. The correlation factor for the calibration curves (R^2) was always above 0.99. Details are reported in Table 3.

Table 3. Calibration fitting, LOQ, calibration range and correlation factor

Analyte	Calibration Fitting	R^2	LOQ (pg/mL)	Calibration Range (ng/mL)
THC-COOH	Quadratic	0.999	200	0.2 – 500
THC	Linear	0.998	200	0.2 – 500
OH-THC	Linear	0.998	5000	5 – 500
Methadone	Linear	0.994	50	0.05 – 200
Amphetamine	Linear	0.997	500	0.5 – 200
Metamphetamine	Linear	0.995	100	0.1 – 200
MDA	Linear	0.996	200	0.2 – 200
MDMA	Linear	0.997	200	0.2 – 200
MDEA	Linear	0.994	20	0.02 – 200
Mephedrone	Linear	0.994	100	0.1 – 200
Ephedrine	Quadratic	0.993	50	0.05 – 200
Pseudoephedrine	Linear	0.993	50	0.05 – 200
Ketamine	Linear	0.992	50	0.05 – 200
Heroin	Linear	0.996	200	0.2 – 200
Morphine	Linear	0.997	200	0.2 – 200
6-MAM	Linear	0.990	50	0.05 – 200
Codeine	Linear	0.993	50	0.05 – 200
Pholcodine	Quadratic	0.995	20	0.02 – 200
Ethylmorphine	Linear	0.991	100	0.1 – 200
Dihydrocodeine	Quadratic	0.994	100	0.1 – 200
Buprenorphine	Linear	0.995	200	0.2 – 200
Cocaine	Linear	0.994	100	0.1 – 200
Benzoylecgonine	Linear	0.996	100	0.1 – 200
EME	Linear	0.996	200	0.2 – 200
Cocaethylene	Linear	0.996	100	0.1 – 200

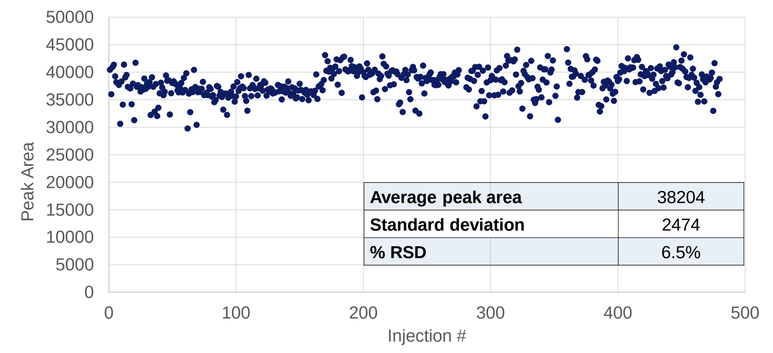
Representative calibration curves for (a) THC-COOH and (b) morphine are reported in Figure 3.

Figure 3. Representative calibration curves for (a) THC-COOH and (b) morphine



The robustness of the method was evaluated for the cannabinoids at a concentration of 12 ng/mL each in terms of % RSD on the peak area following 500 injections of a spiked saliva extract. A maximum % RSD value of 13% was obtained. A representative plot of the peak area for THC through 500 injections is reported in Figure 4.

Figure 4. Peak area for THC at 12 ng/mL in extracted saliva - 500 injections



CONCLUSIONS

Two analytical methods for the quantification of 25 drugs of abuse in oral fluid have been implemented on a Prelude SPLC system coupled to a TSQ Quantiva. Both methods showed excellent sensitivity in line with requirements from forensic toxicology laboratories for all of the compounds taken into consideration. The use of a dual channel LC system running the two methods in parallel on the same mass spectrometer made it possible to reduce the analysis time to less than 10 minutes to quantify the whole panel of analytes of interest in one sample. Moreover, the use of online sample extraction by TurboFlow technology allowed to obtain an increased robustness for the method.

TRADEMARKS/LICENSING

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