TECHNICAL NOTE 73360

Quantification of drugs of abuse in human blood by TurboFlow chromatography coupled to tandem mass spectrometry for use in clinical research

Authors: Claudio De Nardi[1], Paolo Brambilla[2], Maura Brambilla[2], Chiara Fania[3]

[1]Thermo Fisher Scientific GmbH, Dreieich, Germany
[2]Ospedale di Desio, Desio, Italy
[3]Università degli Studi Milano-Bicocca, Milano, Italy

Keywords: Drugs of abuse, blood, TurboFlow, mass spectrometry, TSQ Quantis

Application benefits

- Simple pre-injection sample preparation
- Quantification of 14 different drugs of abuse in blood in less than five minutes
- Five-fold gain in sensitivity using Thermo Scientific™ TurboFlow™ online sample extraction

Goal

Implementation of an analytical method for the quantification of 14 drugs of abuse in human blood on a Thermo Scientific™ TSQ Quantis™ triple-stage quadrupole mass spectrometer using TurboFlow online sample extraction for clinical research.

Introduction

An analytical method for clinical research for the quantification of 14 drugs of abuse in human blood is reported. The list of analytes includes alkaloids [benzoylecgonine (BEG), cocaethylene, cocaine, and ecgonine methyl ester (EME)], amphetamines [amphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA), 3,4-methylenedioxymethamphetamine (MDMA), and methamphetamine], and opiates [6-monoacetylmorphine (6-MAM), codeine, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), methadone, and morphine].

Blood samples were first processed by offline protein precipitation with concomitant addition of the internal standards. Extracted samples were injected onto a Thermo Scientific™ Transcend™ TLX system using TurboFlow technology for online sample cleanup and analytical chromatography connected to a TSQ Quantis triple-stage quadrupole mass spectrometer with heated electrospray ionization operated in positive mode. Detection was performed by selected reaction monitoring (SRM) using seven deuterated internal standards for quantification. Method performance was evaluated using homemade calibrators and controls in terms of limits of quantification, linearity ranges, accuracy, and intra- and inter-assay precision.

Experimental

Target analytes

A list of analytes and corresponding internal standards are reported in Table 1.

Sample preparation

Homemade calibrators (seven levels including blank) and controls (two levels) were prepared by spiking blank whole blood with the proper amount of each analyte to cover a concentration range of 2.5 to 100 ng/mL. Samples were processed by adding 150 µL of a 0.1 M solution of zinc sulfate to 100 µL of blood sample followed by vortex-mixing and subsequent addition of 250 µL of methanol containing the internal standards. Precipitated samples were vortex-mixed again and centrifuged for 10 min at maximum speed; then the supernatant was transferred to a clean plate or vial.

Liquid chromatography

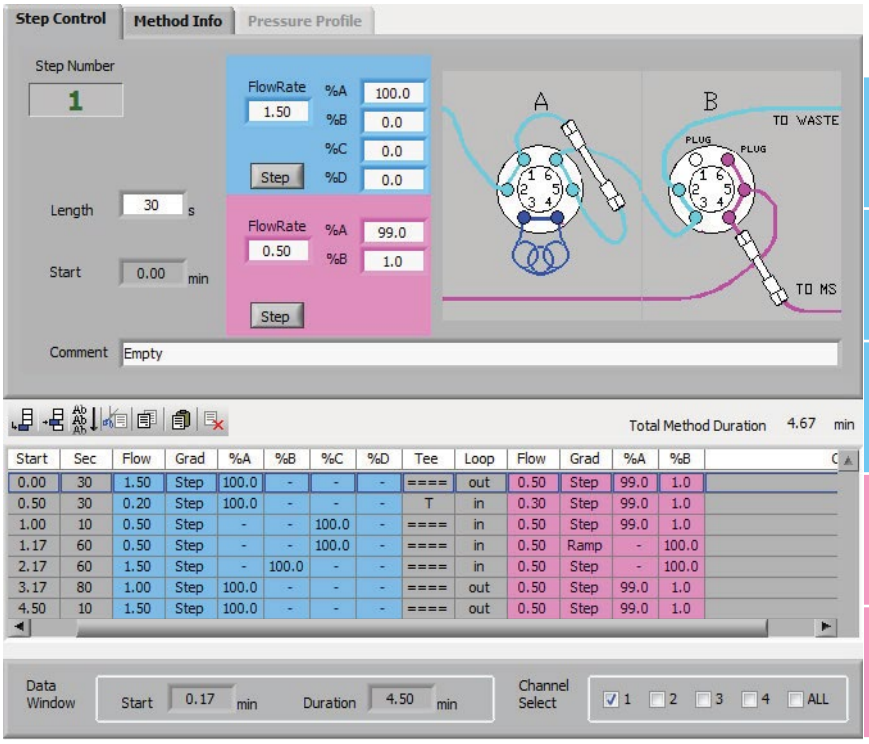
The supernatant was injected onto a Transcend TLX-2 system. Online sample cleanup was performed using a 0.5 × 50 mm Thermo Scientific™ TurboFlow™ Cyclone™ column. LC separation was achieved on a 50 × 2.1 mm (2.6 µm) Thermo Scientific™ Accucore™ biphenyl analytical column (P/N 17826-052130) kept at 30 ^{0}C. Details of the analytical method are reported in Figure 1. Total runtime was 4.7 minutes.

Mass spectrometry

Analytes and internal standards were detected by SRM on a TSQ Quantis triple-stage quadrupole mass spectrometer with heated electrospray ionization operated in positive mode. Two SRM transitions for each analyte were included in the acquisition method for quantification and confirmation, respectively. Mass spectrometric conditions are reported in Table 2.

Table 1. Analytes and corresponding internal standards

Class	Analyte	Internal standard	Concentration range (ng/mL)
Alkaloids	BEG	d_3-BEG	2.5–100
	Cocaethylene	d_3-morphine	
	Cocaine	d_3-morphine	
	EME	d_3-BEG	
Amphetamines	Amphetamine	d_3-amphetamine	
	MDA	d_3-MDMA	
	MDEA	d_3-MDMA	
	MDMA	d_3-MDMA	
	Methamphetamine	d_9-methamphetamine	
Opiates	6-MAM	d_3-morphine	
	Codeine	d_3-codeine	
	EDDP	d_3-methadone	
	Methadone	d_3-methadone	
	Morphine	d_3-morphine	



Mobile Phases	
A	Water + 0.1% NH_4OH
B	Water / Methanol 40 / 60 (v/v) + 0.2% formic acid
C	Acetonitrile Methanol 2-propanol 40 / 40 / 20 (v/v/v)
A	Water + 10 mM ammonium formate + 0.1 % formic acid
B	Methanol + 10 mM ammonium formate + 0.1 % formic acid

Figure 1. LC method description

Table 2. MS settings

Parameter	Value
Source type	Heated electrospray ionization (H-ESI)
Vaporizer temperature	350 °C
Capillary temperature	350 °C
Spray voltage (positive mode)	3500 V
Sheath gas	45 AU
Sweep gas	0 AU
Auxiliary gas	10 AU
Data acquisition mode	Selected-reaction monitoring (SRM)
Collision gas pressure	1.5 mTorr
Cycle time	0.250 s
Q1 mass resolution (FWMH)	0.7
Q3 mass resolution (FWMH)	0.7

Method evaluation

The method performance was evaluated in terms of linearity of response within the calibration range, carryover, accuracy, and intra- and inter-assay precision. Carryover was calculated in terms of percentage ratio between the peak area of the highest calibrator and a blank sample injected just after it. Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations on the homemade quality control samples at two levels prepared and analyzed in replicates of five on three different days. Intra-assay precision for each day was evaluated in terms of percentage coefficient of variation (%CV) using the controls at two different levels in replicates of five (n=5). Inter-assay precision was evaluated as the %CV on the full set of samples (control samples at two levels in replicates of five prepared and analyzed on three different days).

An evaluation of the best achievable limit of quantification (LOQ) was made by diluting the lowest calibrator down to 100-fold and injecting the maximum injectable volume for each diluted calibrator with and without online sample extraction using TurboFlow technology, keeping the same LC method. The LOQ in both cases was evaluated as the lowest diluted calibrator having a percentage bias between nominal and back-calculated concentration within ±20%.

Data analysis

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

Results and discussion

The method proved to be linear in the calibration range covered by the calibrators with a correlation factor (R^2) always above 0.99. Representative chromatograms of BEG and metamphetamine at the lowest calibration level together with their internal standards are reported in Figure 2. Representative calibration curves for the same analytes are reported in Figure 3.

No significant carryover was observed, with no signal detected in the blank injected just after the highest calibrator.

The data presented in this report demonstrate excellent accuracy of the method with a percentage bias between nominal and average back-calculated concentration for the used control samples ranging between -1.0% and 6.9% (Table 3). The %CV for intra-assay precision was always below 7.3%. The maximum %CV for inter-assay precision was 7.8%. Results for intra- and inter-assay precision are reported in Table 4 and Table 5, respectively.

A gain in sensitivity up to 5-fold was obtained due to the use of online sample cleanup using TurboFlow technology when compared to an LC-only approach. Results of the comparison between the two approaches are reported in Figure 4 and Table 6.

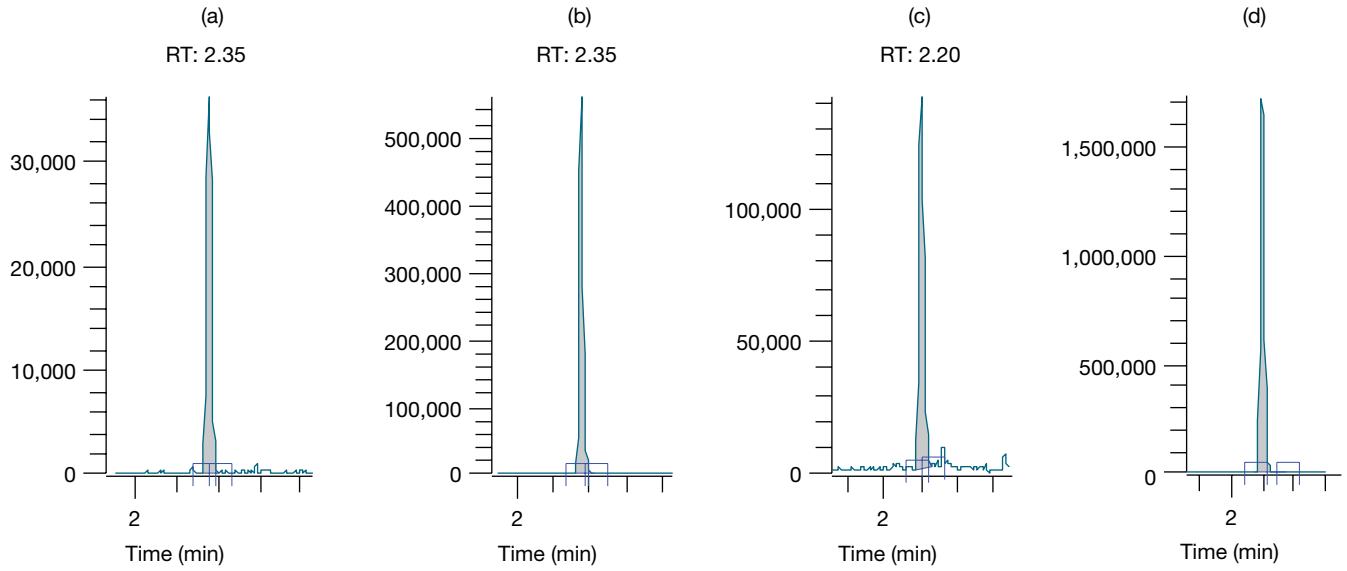


Figure 2. Representative chromatograms of the LLOQ for (a) BEG, (b) d_3-BEG, (c) methamphetamine, and (d) d_9-methamphetamine

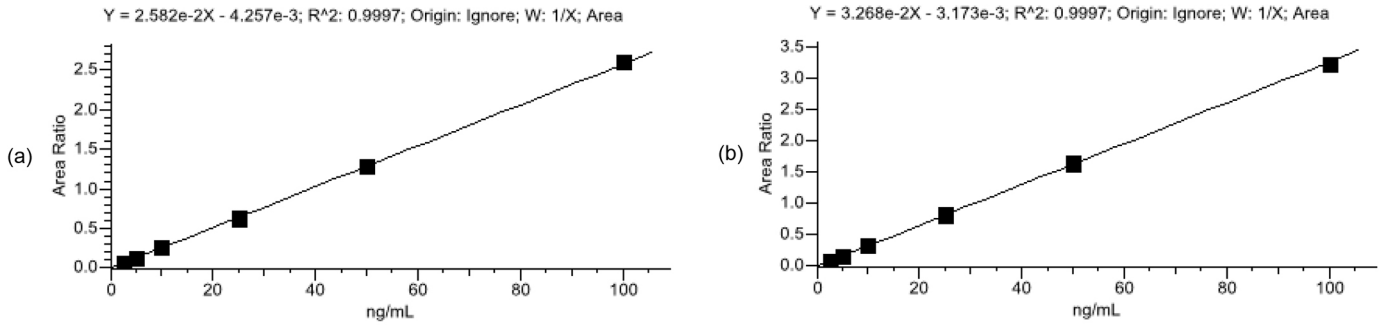


Figure 3. Representative calibration curves for (a) BEG and (b) methamphetamine – day 3

Table 3. Analytical accuracy results

Analyte	Control 1			Control 2		
	Nominal concentration (ng/mL)	Average calculated concentration (ng/mL)	Bias (%)	Nominal concentration (ng/mL)	Average calculated concentration (ng/mL)	Bias (%)
BEG	5.00	5.28	5.5	50.0	51.9	3.9
Cocaethylene	5.00	5.35	6.9	50.0	52.6	5.2
Cocaine	5.00	5.29	5.9	50.0	52.0	3.9
EME	5.00	4.95	-1.0	50.0	50.9	1.9
Amphetamine	5.00	5.10	2.0	50.0	52.4	4.8
MDA	5.00	5.17	3.5	50.0	51.2	2.5
MDEA	5.00	5.31	6.1	50.0	51.6	3.2
MDMA	5.00	5.22	4.3	50.0	51.4	2.9
Methamphetamine	5.00	5.27	5.4	50.0	52.1	4.2
6-MAM	5.00	5.26	5.2	50.0	50.8	1.6
Codeine	5.00	5.12	2.4	50.0	53.0	6.0
EDDP	5.00	5.31	6.2	50.0	51.6	3.3
Methadone	5.00	5.29	5.9	50.0	51.2	2.4
Morphine	5.00	5.14	2.7	50.0	50.6	1.1

Table 4. Intra-assay precision results

Analyte	Control 1						Control 2					
	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3	
	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)
BEG	5.21	4.4	5.28	0.8	5.35	0.7	50.7	2.2	53.0	0.8	52.1	1.9
Cocaethylene	5.30	1.6	5.36	1.6	5.39	0.4	53.6	2.7	51.6	2.2	52.6	1.8
Cocaine	5.37	1.9	5.23	1.2	5.28	1.0	52.6	2.3	51.7	1.4	51.5	2.6
EME	4.47	3.6	5.04	1.6	5.34	1.1	51.7	3.0	49.7	2.0	51.4	2.6
Amphetamine	4.86	2.7	5.26	2.3	5.17	3.1	50.9	1.0	52.5	1.7	53.8	1.4
MDA	4.89	4.8	5.29	2.5	5.34	1.1	50.3	3.6	51.2	0.9	52.2	2.4
MDEA	5.18	2.9	5.40	0.9	5.34	1.1	50.2	4.7	53.0	1.7	51.6	2.5
MDMA	5.15	1.7	5.32	1.1	5.18	0.6	50.3	2.0	51.9	1.8	52.1	1.5
Methamphetamine	5.13	3.4	5.33	1.1	5.35	0.5	50.6	3.4	52.4	1.1	53.3	1.2
6-MAM	5.14	6.1	5.33	1.4	5.32	1.6	50.6	3.7	48.9	3.8	53.0	1.4
Codeine	5.12	4.8	5.10	3.0	5.15	4.6	52.9	2.1	53.3	1.7	52.9	0.7
EDDP	5.28	2.6	5.34	1.7	5.31	1.6	50.1	3.6	52.6	3.0	52.3	2.6
Methadone	5.24	1.4	5.32	2.0	5.31	1.1	49.9	2.2	51.6	1.7	52.0	3.5
Morphine	5.11	6.6	5.09	4.3	5.22	3.6	49.0	7.3	51.5	3.4	51.2	4.3

thermo scientific

Table 5. Inter-assay precision results

Analyte	Control 1		Control 2	
	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)
BEG	5.28	2.6	51.9	2.5
Cocaethylene	5.35	1.4	52.6	2.6
Cocaine	5.29	1.8	52.0	2.2
EME	4.95	7.8	50.9	3.0
Amphetamine	5.10	4.2	52.4	2.6
MDA	5.17	4.9	51.2	2.8
MDEA	5.31	2.5	51.6	3.7
MDMA	5.22	1.8	51.4	2.4
Methamphetamine	5.27	2.7	52.1	3.0
6-MAM	5.26	3.8	50.8	4.5
Codeine	5.12	3.9	53.0	1.5
EDDP	5.31	1.9	51.6	3.7
Methadone	5.29	1.6	51.2	3.0
Morphine	5.14	4.7	50.6	5.3

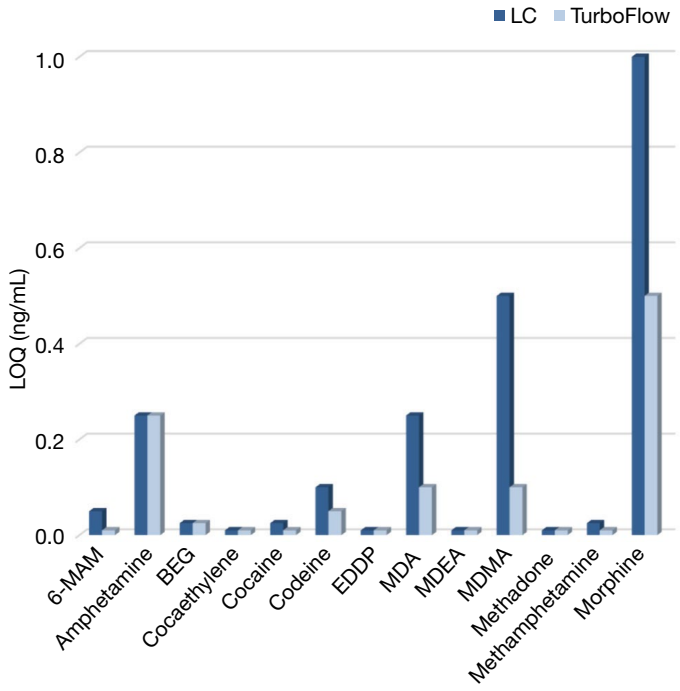


Figure 4. Comparison between the obtainable LOQs using an LC-only or a TurboFlow approach

Table 6. Comparison between the obtainable LOQs using an LC-only or a TurboFlow approach

Analyte	LC (ng/mL)	TurboFlow (ng/mL)
6-MAM	0.05	0.01
Amphetamine	0.25	0.25
BEG	0.025	0.025
Cocaethylene	0.01	0.01
Cocaine	0.025	0.01
Codeine	0.1	0.05
EDDP	0.01	0.01
MDA	0.25	0.1
MDEA	0.01	0.01
MDMA	0.5	0.1
Methadone	0.01	0.01
Methamphetamine	0.025	0.01
Morphine	1.0	0.5

Conclusions

A robust, reproducible, and sensitive liquid chromatography-tandem mass spectrometry method for clinical research for the quantification of 14 drugs of abuse in human blood was implemented on a Transcend TLX system coupled to a TSQ Quantis triple-stage mass spectrometer. The use of TurboFlow online sample extraction reduces offline sample preparation to a minimum and provides better sensitivity when compared to an LC-only approach. The data obtained with the described method, which highlights the performance of the Accucore biphenyl analytical column, successfully met sensitivity, reliability, accuracy, and precision expectations typically demanded by clinical research laboratories.

Find out more at **thermofisher.com/clinicalresearch**

ThermoFisher SCIENTIFIC