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TECHNICAL NOTE 65138

Determination of cannabinoids in low-volume human whole blood samples by online extraction using turbulent flow chromatography and HRAM Orbitrap MS

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Keywords

Transcend II TLX-1, TurboFlow technology, Q Exactive Focus, HRAM, Orbitrap, whole blood, cannabinoids

Goal

Implementation of an analytical method for simple, fast, highly specific and sensitive, as well as robust determination of cannabinoids in low-volume human whole blood samples, using a Thermo Scientific™ Transcend™ II TLX-1 TurboFlow™ chromatography system coupled to a Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ mass spectrometer.

Introduction

Because of the common recreational use of cannabis in western countries, cannabinoids represent the most frequently detected drugs of abuse in samples of forensic toxicological interest. Their detection in blood demonstrates the recent intake of cannabis, as in the case of drivers suspected of driving under the influence of drugs (DUID), in drug-facilitated crimes (DFC), or in post-mortem (PM) toxicology cases.

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The analysis of cannabinoids in human whole blood is usually done by traditional MS techniques (GC-MS or LC-MS/MS), after sample cleanup via offline liquid-liquid extraction (LLE) or solid phase extraction (SPE), in addition to analyte derivatization in the case of GC-MS. These cleanup steps can be lengthy, laborious, and expensive. Therefore, a simple, fast, highly specific and sensitive, as well as robust analytical approach for cannabinoids determination in blood based on online sample cleanup and advanced MS techniques can be of utmost importance for toxicological laboratories.

Here we present a method for the determination of Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in low-volume human whole blood samples. It involves the addition of isotopically labeled internal standards (IS) followed by a simple offline protein precipitation step and online extraction using turbulent flow chromatography (Thermo Scientific™ TurboFlow™ technology). Analytes are detected by high-resolution, accurate-mass hybrid quadrupole Orbitrap™ mass spectrometry (HRAM Orbitrap MS), using positive/negative switching in Selected Ion Monitoring (SIM) acquisition mode.

Following the evaluation of performance, the method was applied to the analysis of hundreds of whole blood samples collected from drivers involved in road accidents, suspected of DUI of cannabis.

Experimental

Target analytes

- Δ^9-tetrahydrocannabinol (THC)
- Cannabidiol (CBD)
- Cannabinol (CBN)
- 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC)
- 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH)

Isotopically labeled internal standards

- d^9-Δ^9-tetrahydrocannabinol (d_9-THC)
- d_3-11-hydroxy-Δ^9-tetrahydrocannabinol (d_3-11-OH-THC)
- d_9-11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (d_9-THC-COOH)

Reagents

- Water, LC grade
- Acetonitrile, LC grade (ACN)
- Methanol, LC grade (MeOH)
- Formic acid, LC grade (HCOOH)
- Acetone
- 2-propanol

Sample preparation

After the addition of 25 µL of the IS solution mix (d_9-THC, d_3-11-OH-THC, d_9-THC-COOH, each 10 ng/mL) 50 µL of whole blood samples (samples of drivers suspected of DUID, calibrators, controls) were subjected to protein precipitation by adding, while vortexing, 150 µL of ACN/MeOH 2:1 (v/v) at 4 °C. Precipitated samples were centrifuged at 2800 rpm, and 120 µL of the supernatants were transferred to glass inserts contained in 2 mL autosampler vials and injected onto the LC-MS system.

Calibrators at 1, 2, 4, 10, 20, 50, and 100 ng/mL for each analyte, as well as “low” controls at 3 ng/mL (THC, 11-OH-THC, THC-COOH) or 4 ng/mL (CBD, CBN) and “high” controls at 10 ng/mL (THC, CBD, CBN) or 15 ng/mL (11-OH-THC, THC-COOH) were used for performance evaluation. These were generated using blank whole blood fortified with analytical standards sourced from LGC Standards (Lancashire, UK).

THC, 11-OH-THC, and THC-COOH were quantified using their corresponding isotopically labeled internal standards, whereas CBD and CBN were quantified using d_9-THC.

TurboFlow and liquid chromatography

Fifty microliters of sample supernatant following deproteinization were injected onto a Thermo Scientific™ Transcend™ II TLX-1 system, equipped with a 50 × 0.5 mm Thermo Scientific™ TurboFlow™ Cyclone-P™ column. Mobile phase A was 0.1% (v/v) aqueous HCOOH; mobile phase B was 75:25:0.1 (v/v/v) ACN/MeOH/HCOOH; and mobile phase C was 45:45:5:5:0.1 (v/v/v/v) ACN/MeOH/acetone/2-propanol/HCOOH. Analytes were

separated on a Thermo Scientific™ Hypersil GOLD™ (50 × 2.1 mm, 5 µm) C18 analytical column, thermostatted at 30 °C. A gradient method was employed with a flow rate of 400 µL/min. Mobile phase A was 0.1% (v/v) aqueous HCOOH and mobile phase B was 0.1% (v/v) HCOOH in ACN. Mobile phase gradients are reported in Figure 1. Total run time was 10 minutes.

Mass spectrometry

Analytes and internal standards (IS) were detected using a Thermo Scientific™ Q Exactive™ Focus MS system equipped with a heated electrospray ionization (HESI-II) source operated in polarity switching mode. MS detection was performed in SIM mode using a resolution of 70,000 (FWHM) at *m/z* 200. Five specific acquisition windows, according to the retention times of analytes, were utilized (Table 1).

Table 1. Acquisition windows.

Positive Mode Acquisition Window	Analyte
4.25–4.85 min	11-OH-THC, *m/z* 331.22677 d3-11-OH-THC, *m/z* 334.24560
5.50–6.50 min	CBD, *m/z* 315.23186
6.50–7.35 min	CBN, *m/z* 311.20056
7.00–8.20 min	THC, *m/z* 315.23186 d9-THC, *m/z* 324.28834
Negative Mode Acquisition Window	**Analyte**
4.50–5.20 min	THCCOOH, *m/z* 343.19148 d9-THC-COOH, *m/z* 352.24797

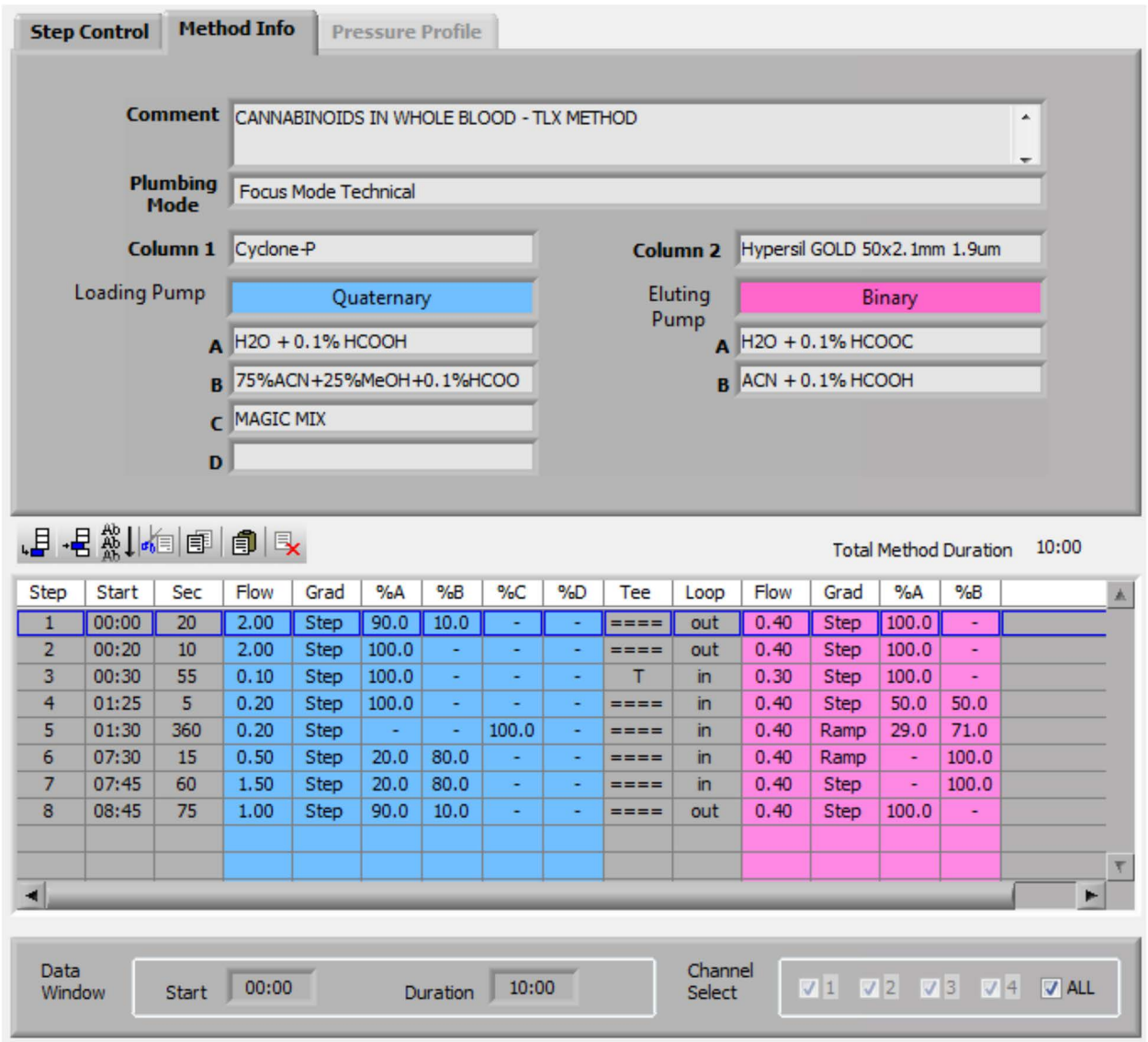


Figure 1. TurboFlow and LC method description.

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software version 3.3.

Method evaluation

Limits of quantitation, linearity ranges, accuracy, and intra- and inter-assay precision were calculated for each analyte. Analytical accuracy was evaluated in terms of trueness of measurement using the “low” and “high” controls prepared and analyzed on five different days in single runs each day. Intra-assay precision was evaluated in terms of percentage coefficient of variation (%CV) using the “low” and “high” controls in replicates of eight (n=8), prepared and analyzed in one batch. Inter-assay precision was evaluated on the same controls in replicates of two (n=2), prepared and analyzed on five different days. Method performance was evaluated also using the Medidrug® DOA-I VB Kit from Medichem (containing THC at 15.1 ± 0.6 ng/mL, 11-OH-THC at 15.6 ± 1.1 ng/mL, and THC-COOH at 102 ± 3 ng/mL levels).

Results and discussion

The developed method proved to be fast, highly specific and sensitive, and reliable.

Total run time, including TurboFlow online sample extraction and HRAM Orbitrap MS analysis, was 10 minutes.

The online extraction using TurboFlow technology allows injection of relatively high amounts, in this case 50 µL, of protein precipitated samples. This feature, together with the very high analytical specificity and sensitivity attainable with HRAM Orbitrap MS in SIM acquisition mode, enabled analysis of whole blood samples starting from very low volumes (50 µL).

Lower limits of quantitation (LLOQ) were 0.5 ng/mL for THC, CBN, and THC-COOH, and 1.0 ng/mL for CBD and 11-OH-THC. Representative ion chromatograms for the lowest calibrator level are reported in Figure 2. The method proved to be linear in the covered calibration ranges with correlation factors (R^2) above 0.99 for all analytes. Representative calibration curves for all analytes are shown in Figure 3.

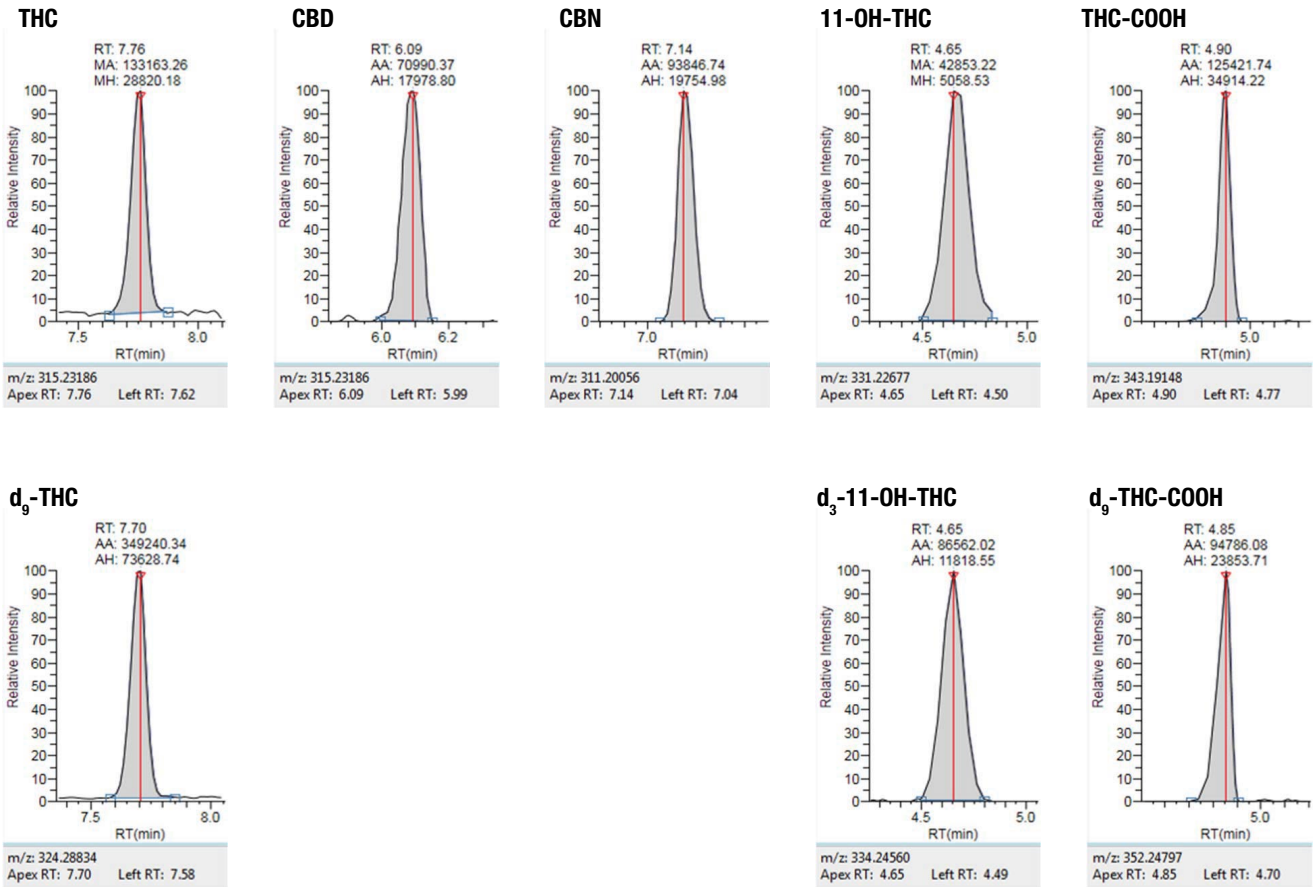
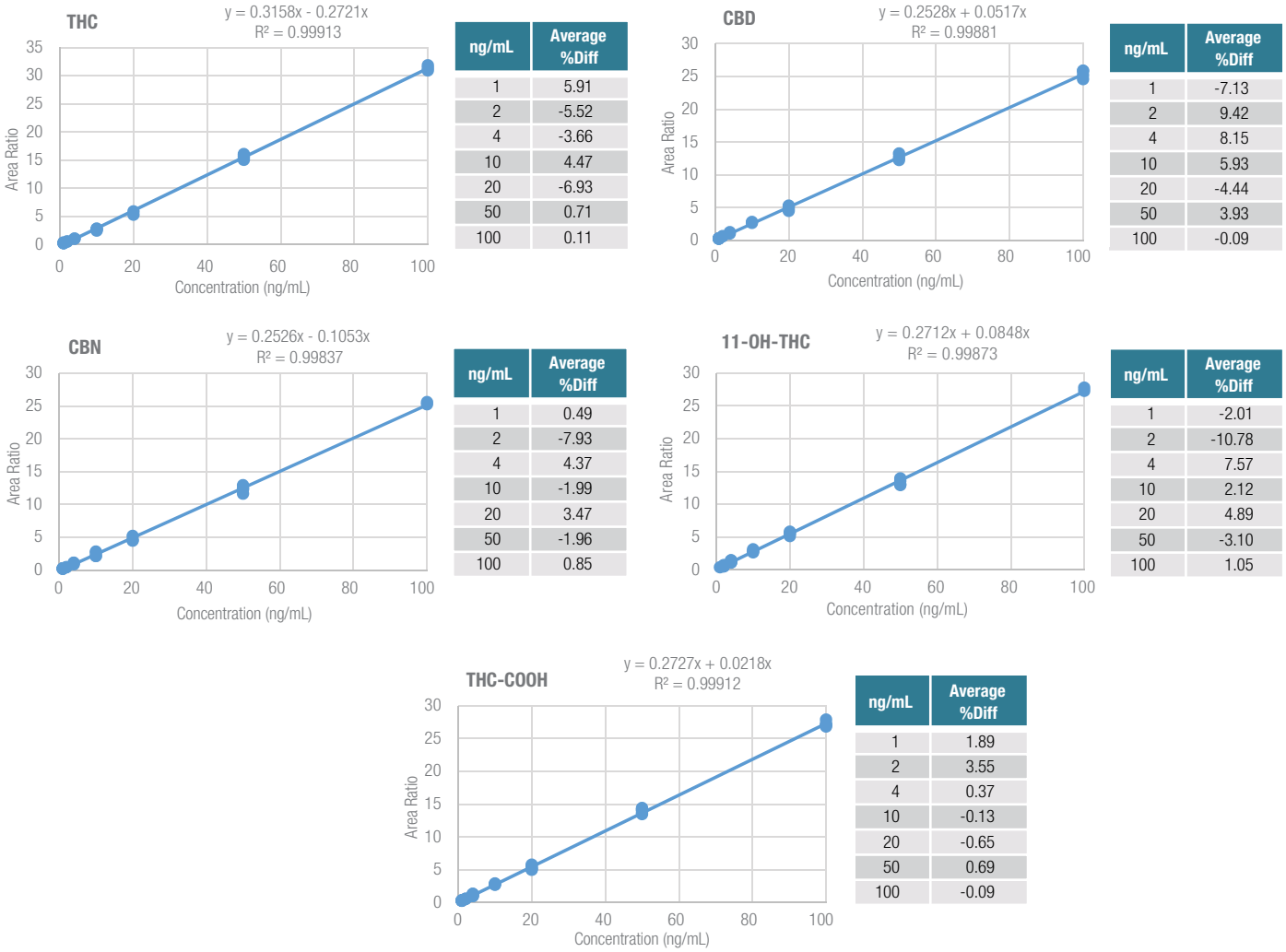


Figure 2. Representative chromatograms for the lowest calibrator for target analytes and isotopically labeled internal standards.



ng/mL	Average %Diff
1	5.91
2	-5.52
4	-3.66
10	4.47
20	-6.93
50	0.71
100	0.11

ng/mL	Average %Diff
1	-7.13
2	9.42
4	8.15
10	5.93
20	-4.44
50	3.93
100	-0.09

ng/mL	Average %Diff
1	0.49
2	-7.93
4	4.37
10	-1.99
20	3.47
50	-1.96
100	0.85

ng/mL	Average %Diff
1	-2.01
2	-10.78
4	7.57
10	2.12
20	4.89
50	-3.10
100	1.05

ng/mL	Average %Diff
1	1.89
2	3.55
4	0.37
10	-0.13
20	-0.65
50	0.69
100	-0.09

Figure 3. Representative calibration curves for each compound in this method.

Results reported in Table 2 show remarkable accuracy of the method with the percentage bias between nominal and average concentration for two levels control samples ranging between -6.7% and 5.0%. The %CV for intra-assay precision was always below 10.0% for all analytes at “low” and “high” levels (Table 3). The maximum %CV for inter-assay precision ranged from 3.6% to 14.8% (Table 4).

The described method was applied to the analysis of hundreds of whole blood samples collected from drivers involved in road accidents, suspected of DUI of cannabis. The same samples were analyzed by a laboratory-validated GC-MS method for THC and THC-COOH detection. Highly correlated results were obtained with the two methods.

Table 2. Analytical accuracy results (n=5).

Analyte	Control	Nominal Concentration (ng/mL)	Measured Concentration (ng/mL)	Bias (%)
THC	Low	3.0	2.9	-3.3
	High	10.0	9.6	-4.0
CBD	Low	4.0	4.1	2.5
	High	10.0	10.1	1.0
CBN	Low	4.0	4.2	5.0
	High	10.0	9.9	-1.0
THC-COOH	Low	3.0	2.8	-6.7
	High	15.0	14.8	-1.3
11-OH-THC	Low	3.0	2.8	-6.7
	High	15.0	15.3	2.0

Table 3. Intra-assay precision results (n=8).

Analyte	Control	Average Concentration (ng/mL)	CV (%)
THC	Low	2.9	2.9
	High	9.7	5.3
CBD	Low	4.2	9.9
	High	9.9	5.5
CBN	Low	4.1	7.8
	High	9.8	5.1
THC-COOH	Low	2.9	2.6
	High	4.8	2.4
11-OH-THC	Low	2.9	6.0
	High	15.4	3.4

Table 4. Inter-assay precision results (n=10).

Analyte	Control	Average Concentration (ng/mL)	CV (%)
THC	Low	2.8	4.4
	High	9.6	7.9
CBD	Low	4.3	14.8
	High	9.7	8.2
CBN	Low	3.8	11.7
	High	9.8	7.7
THC-COOH	Low	3.2	3.9
	High	4.9	3.6
11-OH-THC	Low	2.7	9.0
	High	15.2	5.1

Conclusions

Turbulent flow chromatography coupled to HRAM Orbitrap MS allowed highly specific and sensitive quantitation of three cannabinoids and two metabolites (THC, CBD, CBN, 11-OH-THC, and THC-COOH) from a small volume of whole blood. The use of a simple protein precipitation step followed by online extraction using turbulent flow chromatography provided a simpler, faster, and more cost-effective sample pre-treatment compared to traditional LLE or SPE workup procedures. As a matter of fact, online extraction has the benefit of freeing up technical staff from mundane repetitive tasks, and total run time per sample is about 10 min, compared to 40–60 min using offline LLE or SPE. The described analytical approach may be useful for various forensic toxicology applications (DUID, DFC, PM), as well as for clinical toxicology due to the small matrix volume required, the simple procedure, and the fast analytical run time. Lastly, the opportunity for an on-site upgrade to a Transcend II multi-channel (TLX-2 or TLX-4) system can further improve the overall throughput of this analytical procedure.

References

- United Nations Office on Drugs and Crime, World Drug Report 2017 (ISBN: 978-92-1-148291-1, eISBN: 978-92-1-060623-3, United Nations publication, Sales No. E.17.XI.6). Vienna, Austria, May 2017.
- European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). European Drug Report 2017, EMCDDA, Lisbon, Portugal, 2017.
- Zamengo, L.; Frison, G.; Bettin, C.; Sciarrone, R. Cannabis potency in the Venice area (Italy): update 2013. *Drug Test Anal.* **2015** Mar, *7*(3), 255–8.
- Zamengo, L.; Frison, G.; Bettin, C.; Sciarrone, R. Variability of cannabis potency in the Venice area (Italy): a survey over the period 2010–2012. *Drug Test Anal.* **2014** Jan-Feb, *6*(1–2), 46–51.
- Strand, M.C.; Gjerde, H.; Mørland, J. Driving under the influence of non-alcohol drugs--An update. Part II: Experimental studies. *Forensic Sci Rev.* **2016** Jul, *28*(2), 79–101.
- Tiscione, N.B.; Miller, R.; Shan, X.; Sprague, J.; Yeatman, D.T. An Efficient, Robust Method for the Determination of Cannabinoids in Whole Blood by LC-MS-MS. *J Anal Toxicol.* **2016** Oct, *40*(8), 639–648.
- Schwope, D.M.; Scheidweiler, K.B.; Huestis, M.A. Direct quantification of cannabinoids and cannabinoid glucuronides in whole blood by liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem.* **2011** Sep, *401*(4), 1273–83.
- Helfer, A.G.; Michely, J.A.; Weber, A.A.; Meyer, M.R.; Maurer, H.H. Liquid chromatography-high resolution-tandem mass spectrometry using Orbitrap technology for comprehensive screening to detect drugs and their metabolites in blood plasma. *Anal Chim Acta.* **2017** May 1, *965*, 83–95.
- Roche, L.; Pinguet, J.; Herviou, P.; Libert, F.; Chenaf, C.; Eschalier, A.; Authier, N.; Richard, D. Fully automated semi-quantitative toxicological screening in three biological matrices using turbulent flow chromatography/high resolution mass spectrometry. *Clin Chim Acta.* **2016** Apr 1, *455,* 46–54.

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TN65138-EN 0118S