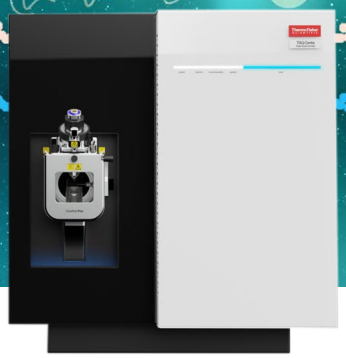
Technical note | 004362



Forensic toxicology

Beyond the box: A ready-to-run workflow for quantitating 80 drugs of abuse in whole blood with the TSQ Certis Triple Quadrupole MS

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Keywords

TSQ Certis MS, drugs of abuse, whole blood, toxicology, forensics

Application benefits

- A quantitative workflow for 80 drugs of abuse in whole blood featuring polarity switching and a 4.5-minute analysis equaling 320 samples per day on the TSQ Certis mass spectrometer
- Proven instrument robustness for high-throughput toxicology workflows in complicated matrices
- Streamlined sample preparation with efficient protein precipitation and filtration using INTip™ Filtration (DPX Technologies)

Goal

Develop a method for 80 drugs of abuse using a low porosity filtration tip for sample clean-up followed by a 4.5-minute method on the Thermo Scientific™ Vanquish™ Flex Ultra-High Performance Liquid Chromatography (UHPLC) System coupled with the Thermo Scientific™ TSQ Certis™ Triple Quadrupole Mass Spectrometer for toxicology in whole blood.

Introduction

The accurate and reliable quantitation of drugs of abuse in whole blood is critical for forensic toxicology and clinical research investigations. Analytical laboratories face ongoing challenges in meeting increasing demands for high-throughput, sensitive, and reproducible testing while maintaining compliance with regulatory standards. Advances in mass spectrometry technology have significantly enhanced analytical capabilities, enabling broader compound coverage and improved confidence in results.

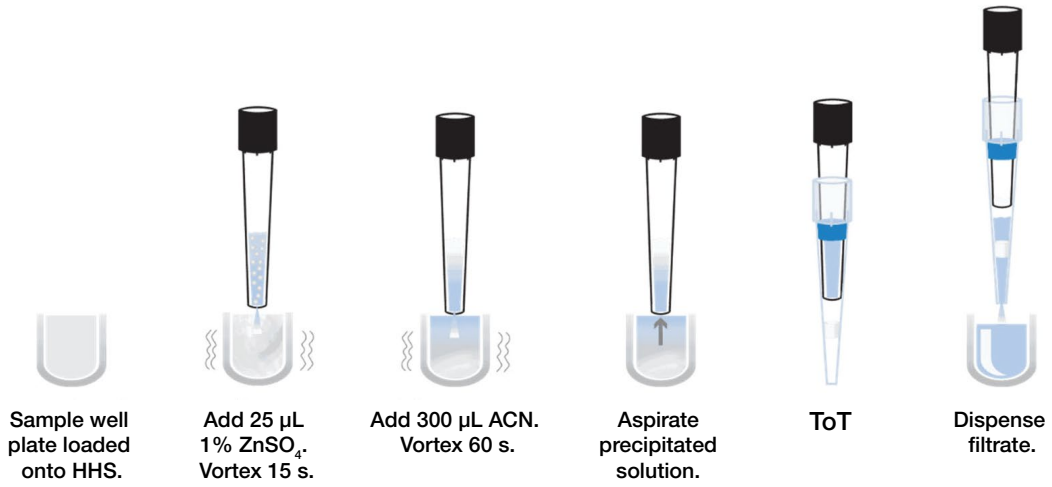

Figure 1. Schematic of the INTip™ Filtration extraction method. Artwork provided by DPX Technologies.

This technical note presents an end-to-end workflow for the quantitation of 80 commonly encountered drugs of abuse in whole blood using the TSQ Certis triple quadrupole mass spectrometer. The workflow integrates efficient sample preparation, robust chromatographic separation, and optimized mass spectrometric detection to deliver exceptional sensitivity, selectivity, and quantitative accuracy. Leveraging the latest innovations in instrument design of >900 SRM/second acquisition speed and less than 5 ms polarity switching, this method provides a powerful solution, enabling analysis of 320 samples/day for laboratories seeking to streamline operations and ensure consistent, high-quality data in complex biological and forensic matrices.

Experimental

Calibration standards and control samples

A total of 80 target analytes were prepared into a mix to be spiked into negative human whole blood. Calibration standards were then prepared at 13 levels ranging from 0.05 to 500 ng/mL in whole blood to a volume of 100 µL. Samples were spiked with 15 µL of internal standard (ISTD) stock comprising the labeled standards of each of the 80 drugs.

Dispersive solid phase extraction

A soft crash was performed by adding 25 µL of 1% zinc sulfate ($ZnSO_4$) and vortex mixing for 15 seconds. Then, 300 µL of acetonitrile was added for protein precipitation and the samples were vortexed for 60 seconds. Samples underwent INTip™ Filtration using Tip-on-Tip™ (ToT) technology from DPX Technologies (Figure 1). Using a regular 1,000 µL pipette tip, the crashed samples were aspirated. While aspirated, the pipette tip was positioned into a Low Porosity Filtration Tip. The samples were dispensed through the filtration tip into a clean test tube.

Samples were dried down at 50 °C for 14 minutes. Once dried, the samples were reconstituted with 20 µL of methanol (MeOH) + 0.1% formic acid + 2 mM ammonium formate and 80 µL of H_2O + 0.1% formic acid + 2 mM ammonium formate. Note that it is important to add the MeOH portion of the reconstitution solvent first and vortex before adding the remaining H_2O portion, as this will ensure THC remains in solution.

Liquid chromatography

Drug analytes were separated with a Thermo Scientific™ Accucore™ Biphenyl Column (2.1 × 50 mm, 2.6 µm, Cat. No. 17826-052130) connected to a Vanquish Flex UHPLC system. Mobile phases consisted of 0.1% formic acid and 2 mM ammonium formate in water for mobile phase A and 0.1% formic acid and 2 mM ammonium formate in MeOH mobile phase B. One microliter of each standard was injected and chromatographic separation was accomplished using the gradient conditions in Table 1.

Table 1. LC gradient.

Time (min)	Flow rate (mL/min)	% A	% B	Curve
0.0	0.5	85	15	5
0.2	0.5	85	15	5
1.5	0.5	40	60	7
2.0	0.5	15	85	5
3.5	0.5	15	85	5
3.5	0.5	1	99	5
4.0	0.5	1	99	5
4.0	0.5	85	15	5
4.5	0.5	85	15	5

Mass spectrometry

Data was acquired on the TSQ Certis mass spectrometer using SRM mode for quantitative and confirming transitions for each target compound and a quantitative transition for the internal standards.

Table 2 highlights the source parameters and values. Q1 resolution was 0.7 FWHM, and Q3 resolution was 1.2 FWHM. The SRM list contained the retention times, precursor and product ions *m/z*, optimized RF lens voltages and collision energies, and retention times (Appendix 1).

Table 2. Source conditions.

Parameter	Value
Spray voltage positive ion (V)	3,500
Spray voltage negative ion (V)	2,500
Sheath gas (Arb)	50
Aux gas (Arb)	10
Sweep gas (Arb)	1
Ion transfer tube temp. (˚C)	325
Vaporizer temp. (˚C)	500

Figure 2 shows the instrument set-up with the Vanquish Flex UHPLC system and the TSQ Certis mass spectrometer. The dwell time per transition and the number of transitions per cycle are shown in Figure 3.

Figure 2. TSQ Certis mass spectrometer and Vanquish Flex UHPLC system.

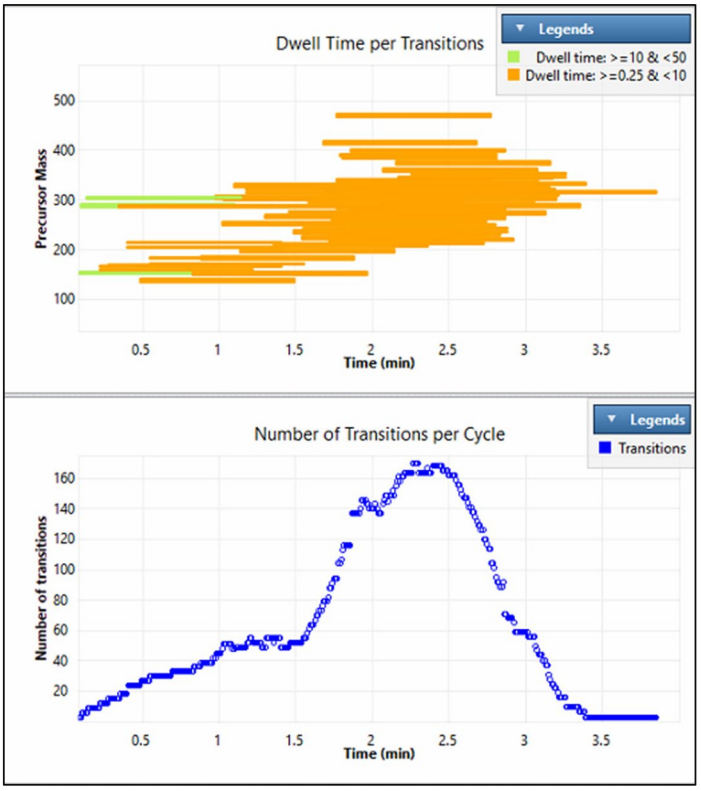

Figure 3. Dwell times of each transition and number of transitions per cycle. The minimum dwell time for any transition was 2.16 ms.

Data analysis

Data was acquired and processed with Thermo Scientific™ TraceFinder™ Software, version 5.2. In cases where no existing method parameter set is available, the Method Forge tool in TraceFinder software can be used to automatically build a complete target list directly from an instrument raw file. In this workflow, a raw file from one of the calibrators was loaded into the Method Forge tool, which extracted the precursor ions, product ions, retention times, and associated transition parameters defined in the instrument method (Figure 4).

The automated extraction tool created a fully populated master method without the need for manually entering compound information, and the resulting information was then saved into the TraceFinder software compound database, allowing the target list and associated transition parameters to be reused for future analyses. This newly created master method was subsequently applied to process all calibration and study samples.

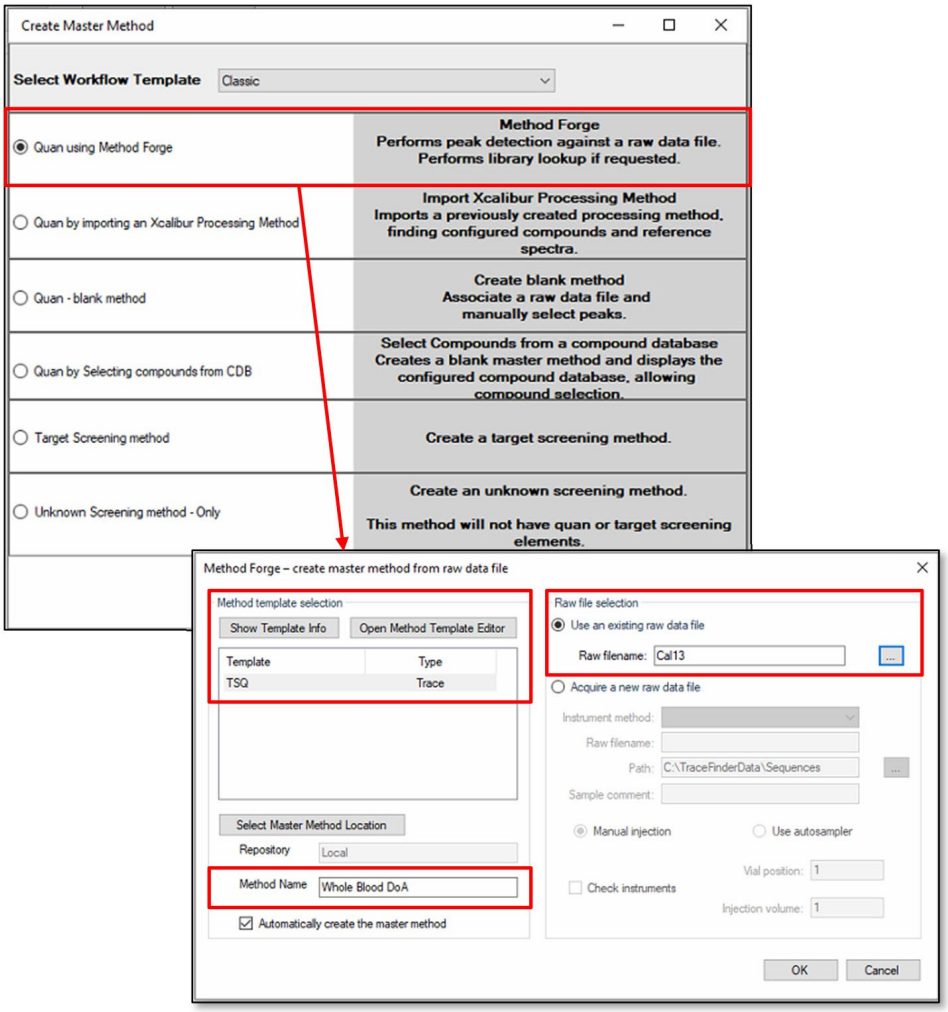

Figure 4. TraceFinder software master method creation using the Method Forge tool to seamlessly create a new quantitative master method from a single raw file.

Results and discussion

Chromatography efficiently separated all isomers including codeine/hydrocodone, morphine/hydromorphone, and methamphetamine/phentermine within a 4.5-minute run. Figure 5 shows the combined extracted ion chromatogram of the 80 drugs in this panel, which includes 75 positive and 5 negative polarity compounds.

For each of the 80 analytes, limit of quantitation (LOQ) and upper limit of linearity (ULOL) were evaluated. The criteria for these limits are shown in Table 3. Ion ratios were used to confirm each drug, and a tolerance allowance of ±20% was employed.

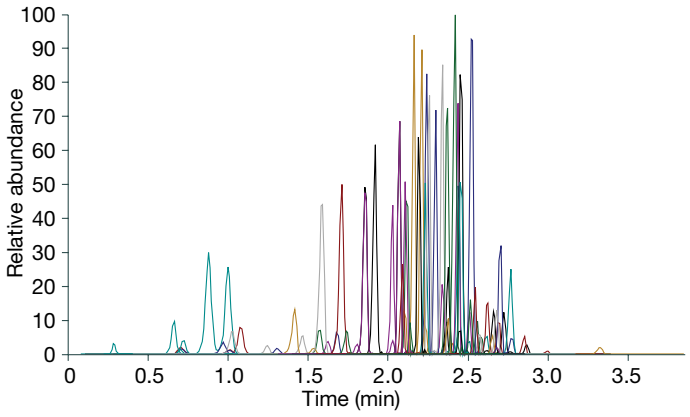

Figure 5. Combined extracted ion chromatogram of 80 drugs in whole blood.

Table 3. Criteria assigned in TraceFinder software for limits.

Parameter	Criteria
Limit of quantitation (LOQ)	Peak area CV of triplicate injections must be <20% Ion ratios tolerance must be <±20% of the average of the calibrators
Upper limit of linearity (ULOL)	Highest calibrator that achieves linearity

This workflow helped achieve LOQs as low as 0.05 ng/mL with ULOLs up to 500 ng/mL. The LOQ and ULOL of each analyte is shown in Table 4. The TSQ Certis mass spectrometer uses the Thermo Scientific™ OptaMax™ Plus HESI Source, which allows for increased vaporizer temperatures, improving the relative peak areas and overall sensitivity for drugs of abuse. Utilizing a vaporizer temperature of 500 °C helped achieve improved sensitivity. Figure 6 shows how increasing the vaporizer temperature of the source can increase peak areas up to 2-fold.

Figure 7 depicts the extracted ion chromatograms of three of the analytes—buprenorphine, THC, and secobarbital—at their respective LOQ concentrations with their corresponding calibration curves and internal standard %RSD.

Peak images and data come from TraceFinder software data analysis.

Table 4. Results table depicting LOQ and ULOL achieved for each drug in ng/mL.

Compound	RT (min)	LOQ	ULOL
11-hydroxy THC	2.89	2.5	500
2-hydroxyethylflurazepam	2.58	5	500
6-MAM	1.60	0.05	500
7-aminoclonazepam	2.13	0.25	500
7-aminoflunitrazepam	2.27	0.25	500
a-hydroxyalprazolam	2.61	2.5	500
a-hydroxytriazolam	2.57	0.5	500
Alprazolam	2.71	0.25	500
Amphetamine	0.99	0.5	500
Benzoylecgonine	2.08	0.1	500
Bromazolam	2.76	0.25	500
Buprenorphine	2.27	0.25	50
Bupropion	2.09	0.5	100
Butabarbital	2.22	25	500
Butalbital	2.25	25	500
Carisprodol	2.36	0.25	500
Chlordiazepoxide	2.36	0.25	500
Clonazepam	2.56	0.5	500
Cocaethylene	2.19	0.25	500
Cocaine	2.12	0.1	500
Codeine	1.53	0.05	500
Cyclobenzaprine	2.36	0.5	500
Desalkylflurazepam	2.56	0.25	500
Dextromethorphan	2.30	0.05	100
Diazepam	2.85	0.25	500
Dihydrocodeine	1.48	1	500
Diphenhydramine	2.24	2.5	500
Doxylamine	1.96	0.1	100
EDDP	2.36	0.1	50
Ephedrine	0.78	0.1	500
Fentanyl	2.27	0.05	500
Flualprazolam	2.65	0.1	500
Flunitrazepam	2.69	1	500
Flurazepam	2.29	0.1	500
Gabapentin	1.05	0.5	500
Hydrocodone	1.70	0.1	500
Hydromorphone	0.85	0.1	500
Ketamine	2.05	0.25	500
Lorazepam	2.51	0.5	500
LSD	2.17	0.1	500

Compound	RT (min)	LOQ	ULOL
MDA	1.38	0.25	500
MDEA	1.86	0.1	500
MDMA	1.64	0.25	500
Meprobamate	2.42	0.5	500
Methadone	2.43	0.1	500
Methamphetamine	1.33	0.25	500
Methylphenidate	2.07	0.25	500
Midazolam	2.36	0.25	500
Mirtrazepine	2.15	0.1	50
Mitragynine	2.36	0.25	50
Morphine	0.60	0.25	500
Norbuprenorphine	2.18	0.25	500
Norcodeine	1.19	0.5	500
Nordiazepam	2.63	0.5	500
Norfentanyl	1.99	0.1	500
Norpseudoephedrine	0.58	0.5	500
O-desmethyltramadol	1.52	0.1	500
ortho-desmethylvenlafaxine	1.80	0.1	500
Oxazepam	2.65	0.25	250
Oxycodone	1.68	0.1	500
Oxymorphone	0.64	0.1	500
PCP	2.30	1	500
Pentobarbital	2.33	10	500
Phenobarbital	2.21	10	500
Phentermine	1.46	0.5	100
Pregabalin	0.72	1	500
Pseudoephedrine	0.90	0.25	500
Psilocin	0.90	0.5	50
Quetiapine	2.31	0.05	500
Secobarbital	2.38	25	500
Sertraline	2.43	0.5	500
Temazepam	2.70	0.25	500
THC	3.35	0.25	500
THC-COOH	2.76	2.5	500
Tramadol	1.80	0.5	500
Trazodone	2.66	0.25	500
Triazolam	2.67	0.25	500
Venlafaxine	2.36	0.25	500
Xylazine	2.04	0.1	500
Zolpidem	2.22	0.05	500

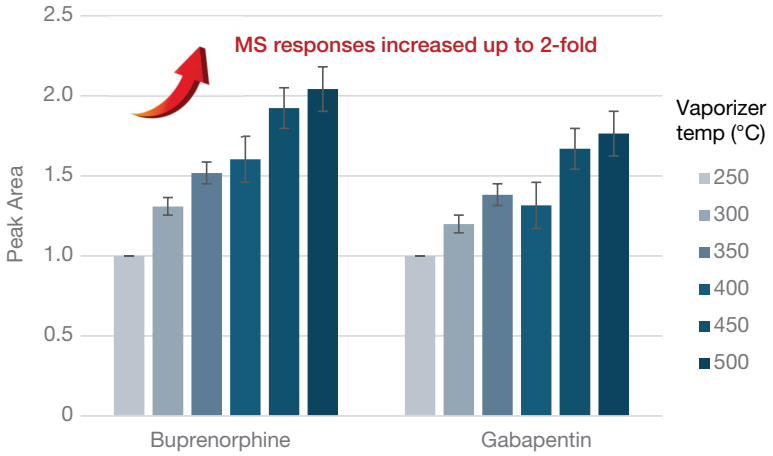

Figure 6. Up to a 2-fold increase in peak area for drugs of abuse was achieved utilizing the higher vaporizer temperatures of the OptaMax Plus HESI source.

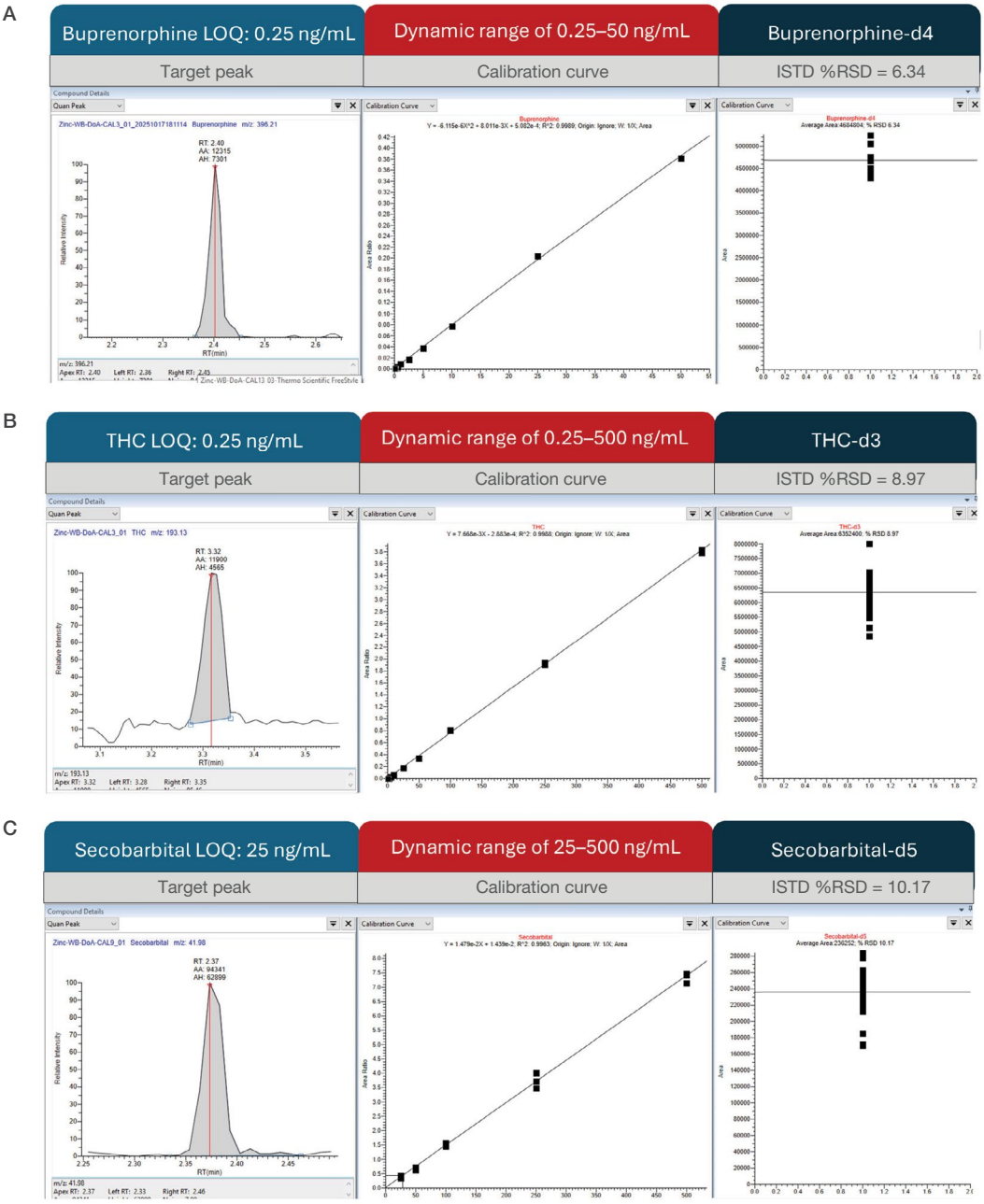

Figure 7. Extracted ion chromatograms of (A) buprenorphine, (B) THC, and (C) secobarbital at their LOQ along with their respective calibration curves and internal standard %RSD of peak area.

A robustness study was performed by injecting extracted whole blood spiked with drugs of abuse for over 450 injections. Peak areas were monitored from injection to injection. Figure 8 depicts lorazepam peak areas over time well within a ±20% RSD for peak area.

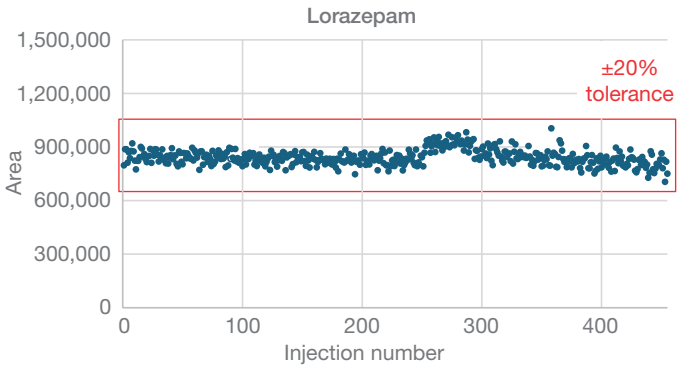

Figure 8. Peak areas of lorazepam over 450 injections.

Conclusion

The developed workflow enables rapid, accurate, and reproducible quantitation of 80 drugs of abuse in whole blood using the TSQ Certis triple quadrupole mass spectrometer. With low limits of quantitation down to 0.05 ng/mL, excellent linearity, and stable performance across more than 450 injections, the method demonstrates strong sensitivity and robustness. Additionally, TraceFinder software further enhances this workflow by streamlining and automating data processing method creation and data analysis with a comprehensive set of tools that are applicable to a broad range of toxicology workflows. By combining efficient sample preparation, fast chromatographic separation, and reliable SRM detection, this workflow offers a practical and high-performance solution for forensic and clinical toxicology laboratories seeking confident, high-throughput results.

Appendix

Appendix 1 (part 1). MS parameters for all target analytes and internal standards.

Compound	Retention time (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
11-hydroxy THC	2.87	Positive	331.229	193.122	24	40
11-hydroxy THC	2.87	Positive	331.229	201.091	24	40
11-hydroxy THC-d3	2.87	Positive	334.246	316.220	15	40
2-Hydroxyethylflurazepam	2.58	Positive	333.080	109.040	30	80
2-Hydroxyethylflurazepam	2.58	Positive	333.080	211.200	37	80
2-Hydroxyethylflurazepam-d4	2.58	Positive	337.105	113.070	30	80
6-MAM	1.68	Positive	328.160	165.130	38	60
6-MAM	1.68	Positive	328.160	211.130	25	60
6-MAM-d3	1.68	Positive	331.173	165.125	38	60
7-Aminoclonazepam	2.23	Positive	286.112	121.113	32	80
7-Aminoclonazepam	2.23	Positive	286.112	222.125	25	80
7-Aminoclonazepam-d4	2.23	Positive	290.099	226.130	25	80
7-Aminoflunitrazepam	2.34	Positive	284.160	135.050	28	60
7-Aminoflunitrazepam	2.34	Positive	284.160	227.130	25	60
7-Aminoflunitrazepam-d7	2.34	Positive	291.163	138.050	28	60
a-Hydroxyalprazolam	2.61	Positive	325.090	279.068	24	80
a-Hydroxyalprazolam	2.61	Positive	325.090	297.130	26	80
a-Hydroxyalprazolam-d5	2.61	Positive	330.116	302.100	26	80
a-Hydroxytriazolam	2.57	Positive	359.010	175.920	27	80
a-Hydroxytriazolam	2.57	Positive	359.010	331.000	28	80
a-Hydroxytriazolam-d4	2.57	Positive	363.071	335.060	28	80
Alprazolam	2.71	Positive	309.090	205.080	41	80
Alprazolam	2.71	Positive	309.090	281.070	27	80
Alprazolam-d5	2.71	Positive	314.122	286.100	27	80
Amphetamine	1.06	Positive	136.110	91.070	18	30
Amphetamine	1.06	Positive	136.110	119.130	9	30

Appendix 1 (part 2). MS parameters for all target analytes and internal standards.

Compound	Retention time (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
Amphetamine-d5	1.06	Positive	141.143	96.070	18	30
Benzoylecgonine	2.08	Positive	290.139	105.071	31	30
Benzoylecgonine	2.08	Positive	290.139	167.768	20	30
Benzoylecgonine-d8	2.08	Positive	298.190	109.970	31	30
Bromazolam	2.76	Positive	353.040	274.100	32	60
Bromazolam	2.76	Positive	353.040	325.000	32	60
Buprenorphine	2.39	Positive	468.310	396.210	39	100
Buprenorphine	2.39	Positive	468.310	414.200	39	100
Buprenorphine-d4	2.39	Positive	472.338	400.220	39	100
Bupropion	2.09	Positive	240.152	131.113	27	30
Bupropion	2.09	Positive	240.152	184.042	13	30
Bupropion-d9	2.09	Positive	249.171	184.967	13	30
Butabarbital	2.22	Negative	211.108	42.000	17	60
Butabarbital	2.22	Negative	211.108	168.103	11	60
Butabarbital-d5	2.22	Negative	216.140	173.134	11	60
Butalbital	2.25	Negative	223.109	42.000	17	60
Butalbital	2.25	Negative	223.109	180.100	11	60
Butalbital-d5	2.25	Negative	228.140	185.130	11	60
Carisoprodol	2.36	Positive	261.180	97.070	16	30
Carisoprodol	2.36	Positive	261.180	176.130	9	30
Carisoprodol-d7	2.36	Positive	268.225	183.170	9	30
Chlordiazepoxide	2.61	Positive	300.090	227.050	25	40
Chlordiazepoxide	2.61	Positive	300.090	282.100	25	40
Chlordiazepoxide-d5	2.61	Positive	305.121	232.000	25	40
Clonazepam	2.56	Positive	316.050	213.970	38	60
Clonazepam	2.56	Positive	316.050	269.970	26	60
Clonazepam-d4	2.56	Positive	320.073	274.000	25	60
Cocaethylene	2.19	Positive	318.170	82.050	30	60
Cocaethylene	2.19	Positive	318.170	196.130	20	60
Cocaethylene-d3	2.19	Positive	321.189	199.300	20	60
Cocaine	2.12	Positive	304.156	82.054	29	40
Cocaine	2.12	Positive	304.156	182.125	20	40
Cocaine-d3	2.12	Positive	307.173	85.125	29	40
Codeine	1.62	Positive	300.160	152.130	55	60
Codeine	1.62	Positive	300.160	165.050	42	60
Codeine-d3	1.62	Positive	303.178	165.054	42	60
Cyclobenzaprine	2.44	Positive	276.262	215.090	42	60
Cyclobenzaprine	2.44	Positive	276.262	231.117	25	60
Cyclobenzaprine-d3	2.44	Positive	279.193	215.090	42	60
Desalkylflurazepam	2.56	Positive	289.054	140.042	28	80
Desalkylflurazepam	2.56	Positive	289.054	226.250	29	80
Desalkylflurazepam-d4	2.56	Positive	293.079	140.042	28	80
Dextromethorphan	2.38	Positive	272.201	171.080	38	60
Dextromethorphan	2.38	Positive	272.201	215.140	24	60
Dextromethorphan-d3	2.38	Positive	275.220	171.080	38	60
Diazepam	2.85	Positive	285.080	154.040	27	80
Diazepam	2.85	Positive	285.080	193.030	32	80
Diazepam-d5	2.85	Positive	290.110	198.120	32	80
Dihydrocodeine	1.56	Positive	302.175	128.060	61	60
Dihydrocodeine	1.56	Positive	302.175	199.071	33	60

Appendix 1 (part 3). MS parameters for all target analytes and internal standards.

Compound	Retention time (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
Dihydrocodeine-d6	1.56	Positive	308.213	202.100	33	60
Diphenhydramine	2.30	Positive	256.170	165.050	40	30
Diphenhydramine	2.30	Positive	256.170	167.140	13	30
Diphenhydramine-d3	2.30	Positive	259.188	167.140	13	30
Doxylamine	2.20	Positive	271.180	167.000	34	40
Doxylamine	2.20	Positive	271.180	182.000	16	40
Doxylamine-d5	2.20	Positive	276.212	187.100	16	40
EDDP	2.44	Positive	278.190	234.160	31	80
EDDP	2.44	Positive	278.190	249.140	24	80
EDDP-d3	2.44	Positive	281.209	234.160	31	80
Ephedrine	0.88	Positive	166.123	117.069	17	30
Ephedrine	0.88	Positive	166.123	148.112	10	30
Ephedrine-d3	0.88	Positive	169.142	151.113	10	30
Fentanyl	2.33	Positive	337.227	105.070	37	60
Fentanyl	2.33	Positive	337.227	188.140	24	60
Fentanyl-d5	2.33	Positive	342.259	188.140	25	60
Flualprazolam	2.65	Positive	327.081	223.070	38	80
Flualprazolam	2.65	Positive	327.081	299.100	32	80
Flualprazolam-d4	2.65	Positive	331.106	303.125	32	80
Flunitrazepam	2.69	Positive	314.094	239.160	35	80
Flunitrazepam	2.69	Positive	314.094	268.130	27	80
Flunitrazepam-d7	2.69	Positive	321.138	246.198	35	80
Flurazepam	2.36	Positive	388.159	315.070	23	60
Flurazepam	2.36	Positive	388.159	317.090	19	60
Flurazepam-d4	2.36	Positive	392.184	319.100	23	60
Gabapentin	0.94	Positive	172.133	95.090	23	30
Gabapentin	0.94	Positive	172.133	137.100	16.5	30
Gabapentin-d10	0.94	Positive	182.196	147.160	16.5	30
Hydrocodone	1.79	Positive	300.160	128.060	55	80
Hydrocodone	1.79	Positive	300.160	199.050	29	80
Hydrocodone-d6	1.79	Positive	306.197	202.100	29	80
Hydromorphone	1.00	Positive	286.144	157.070	42	60
Hydromorphone	1.00	Positive	286.144	185.050	31	60
Hydromorphone-d3	1.00	Positive	289.163	185.050	31	60
Ketamine	2.10	Positive	238.099	125.020	29	30
Ketamine	2.10	Positive	238.099	179.060	16	30
Ketamine-d4	2.10	Positive	242.124	129.040	29	30
Lorazepam	2.51	Positive	321.019	163.006	30	60
Lorazepam	2.51	Positive	321.019	229.050	31	60
Lorazepam-d4	2.51	Positive	325.044	279.044	22	60
LSD	2.22	Positive	324.207	207.226	44	60
LSD	2.22	Positive	324.207	223.208	25	60
LSD-d3	2.22	Positive	327.226	226.100	24	60
MDA	1.44	Positive	180.102	105.050	23	30
MDA	1.44	Positive	180.102	135.040	11	30
MDA-d5	1.44	Positive	185.133	110.125	23	30
MDEA	1.90	Positive	208.130	105.070	26	30
MDEA	1.90	Positive	208.130	163.100	13	30
MDEA-d5	1.90	Positive	213.165	163.080	13	30

Appendix 1 (part 4). MS parameters for all target analytes and internal standards.

Compound	Retention time (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
Meprobamate	2.14	Positive	219.134	97.100	14	20
Meprobamate	2.14	Positive	219.134	158.155	9	20
Meprobamate-d3	2.14	Positive	222.153	161.140	9	20
Methadone	2.52	Positive	310.220	105.030	28	30
Methadone	2.52	Positive	310.220	265.210	15	30
Methadone-d3	2.52	Positive	313.235	268.180	15	30
Methamphetamine	1.39	Positive	150.130	91.070	19	30
Methamphetamine	1.39	Positive	150.130	119.090	11	30
Methamphetamine-d5	1.39	Positive	155.159	92.071	19	30
Methylphenidate	2.07	Positive	234.186	56.050	43	40
Methylphenidate	2.07	Positive	234.186	84.080	20	40
Methylphenidate-d4	2.07	Positive	238.174	88.000	20	40
Midazolam	2.75	Positive	326.090	223.070	38	80
Midazolam	2.75	Positive	326.090	291.000	27	80
Midazolam-d4	2.75	Positive	330.111	295.142	27	80
Mirtazapine	2.25	Positive	266.165	71.982	20	60
Mirtazapine	2.25	Positive	266.165	195.054	26	60
Mirtazapine-d3	2.25	Positive	269.185	75.099	20	60
Mitragynine	2.44	Positive	399.230	174.090	29	60
Mitragynine	2.44	Positive	399.230	226.140	22	60
Mitragynine-d3	2.44	Positive	402.247	177.100	31	60
Morphine	0.73	Positive	286.144	152.060	58	80
Morphine	0.73	Positive	286.144	165.070	38	80
Morphine-d6	0.73	Positive	292.181	165.070	38	80
Norbuprenorphine	2.18	Positive	414.260	83.040	46	80
Norbuprenorphine	2.18	Positive	414.260	101.096	46	80
Norbuprenorphine-d3	2.18	Positive	417.283	83.040	46	80
Norcodeine	1.29	Positive	286.144	152.050	55	60
Norcodeine	1.29	Positive	286.144	268.140	20	60
Norcodeine-d3	1.29	Positive	289.163	271.153	20	60
Nordiazepam	2.63	Positive	271.060	140.070	28	80
Nordiazepam	2.63	Positive	271.060	208.130	28	80
Nordiazepam-d5	2.63	Positive	276.095	140.070	28	80
Norfentanyl	1.99	Positive	233.165	56.050	27	30
Norfentanyl	1.99	Positive	233.165	84.080	18	30
Norfentanyl-d5	1.99	Positive	238.200	84.080	18	30
Norpseudoephedrine	0.66	Positive	152.107	117.070	17	20
Norpseudoephedrine	0.66	Positive	152.107	134.096	10	20
Norpseudoephedrine-d3	0.66	Positive	155.126	137.116	10	20
O-Desmethyltramadol	1.52	Positive	250.180	42.050	70	30
O-Desmethyltramadol	1.52	Positive	250.180	58.050	17	30
O-Desmethyltramadol-d6	1.52	Positive	256.218	64.090	17	30
O-Desmethylvenlafaxine	1.80	Positive	264.196	58.054	19	30
O-Desmethylvenlafaxine	1.80	Positive	264.196	246.208	12	30
O-Desmethylvenlafaxine-d6	1.80	Positive	270.234	64.092	19	30
Oxazepam	2.53	Positive	287.060	104.050	35	60
Oxazepam	2.53	Positive	287.060	241.070	23	60
Oxazepam-d5	2.53	Positive	292.090	246.080	23	60
Oxycodone	1.68	Positive	316.150	241.130	29	60

Appendix 1 (part 5). MS parameters for all target analytes and internal standards.

Compound	Retention time (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
Oxycodone-d6	1.68	Positive	322.192	262.170	26	60
Oxymorphone	0.80	Positive	302.130	198.090	45	60
Oxymorphone	0.80	Positive	302.130	227.090	28	60
Oxymorphone-d3	0.80	Positive	305.158	230.110	28	60
Pentobarbital	2.33	Negative	224.986	42.000	18	60
Pentobarbital	2.33	Negative	224.986	182.120	13	60
Pentobarbital-d5	2.33	Negative	230.156	187.100	13	60
Phencyclidine	2.36	Positive	244.206	86.125	12	30
Phencyclidine	2.36	Positive	244.206	159.116	12	30
Phencyclidine-d5	2.36	Positive	249.237	86.100	12	30
Phenobarbital	2.21	Negative	231.080	42.000	16	60
Phenobarbital	2.21	Negative	231.080	188.070	10	60
Phenobarbital-d5	2.21	Negative	236.109	193.100	10	60
Phentermine	1.50	Positive	150.130	65.040	40	60
Phentermine	1.50	Positive	150.130	91.050	20	60
Phentermine-d5	1.50	Positive	155.159	96.080	20	60
Pregabalin	0.72	Positive	160.130	55.050	22	40
Pregabalin	0.72	Positive	160.130	83.090	17	40
Pregabalin-d6	0.72	Positive	166.171	103.140	16	40
Pseudoephedrine	1.00	Positive	166.123	115.050	27	30
Pseudoephedrine	1.00	Positive	166.123	117.070	20	30
Pseudoephedrine-d3	1.00	Positive	169.142	115.050	28	30
Psilocin	0.99	Positive	205.134	58.065	13	30
Psilocin	0.99	Positive	205.134	160.100	13	30
Psilocin-d10	0.99	Positive	215.197	66.100	13	30
Quetiapine	2.43	Positive	384.174	221.110	38	60
Quetiapine	2.43	Positive	384.174	253.083	23	60
Quetiapine-d8	2.43	Positive	392.224	258.100	23	60
Secobarbital	2.38	Negative	237.120	41.976	21	80
Secobarbital	2.38	Negative	237.120	194.050	12	80
Secobarbital-d5	2.38	Negative	242.156	199.150	12	80
Sertraline	2.52	Positive	306.081	158.900	28	30
Sertraline	2.52	Positive	306.081	275.040	13	30
Sertraline-d3	2.52	Positive	309.100	158.900	28	30
Temazepam	2.70	Positive	301.070	177.020	39	60
Temazepam	2.70	Positive	301.070	255.050	23	60
Temazepam-d5	2.70	Positive	306.105	260.100	23	60
THC	3.35	Positive	315.233	123.054	33	60
THC	3.35	Positive	315.233	193.125	23	60
THC-d3	3.35	Positive	318.251	196.125	23	60
THC-COOH	2.76	Positive	345.206	299.205	21	40
THC-COOH	2.76	Positive	345.206	327.163	17	40
THC-COOH-d9	2.76	Positive	354.263	336.133	17	40
Tramadol	2.06	Positive	264.196	58.000	17	30
Tramadol	2.06	Positive	264.196	264.200	0	30
Tramadol-13C,d3	2.06	Positive	268.219	58.000	17	30
Trazodone	2.45	Positive	372.159	148.050	33	80
Trazodone	2.45	Positive	372.159	176.080	24	80
Trazodone-d6	2.45	Positive	378.196	182.100	24	80

Appendix 1 (part 6). MS parameters for all target analytes and internal standards.

Compound	Retention time (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)
Triazolam	2.67	Positive	343.050	239.040	43	80
Triazolam	2.67	Positive	343.050	308.080	26	80
Triazolam-d4	2.67	Positive	347.076	312.106	26	80
Venlafaxine	2.19	Positive	278.211	58.107	20	30
Venlafaxine	2.19	Positive	278.211	121.060	28	30
Venlafaxine-d6	2.19	Positive	284.249	64.100	21	30
Xylazine	2.04	Positive	221.111	90.042	22	60
Xylazine	2.04	Positive	221.111	164.054	26	60
Xylazine-d6	2.04	Positive	227.148	90.042	22	60
Zolpidem	2.40	Positive	308.126	235.100	35	80
Zolpidem	2.40	Positive	308.126	263.120	26	80
Zolpidem-d6	2.40	Positive	314.213	235.100	36	80

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