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Toxicology

All-in-one LC-MS/MS toxicology solution for drugs of abuse quantitation using the TSQ Quantis Plus mass spectrometer

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Keywords

Tox Explorer Collection, TSQ Quantis Plus mass spectrometer, Vanquish Flex UHPLC, Accucore Phenyl-Hexyl column, TraceFinder software, drugs of abuse, quantitation, toxicology, sports anti-doping, anti-doping, forensics, pain management, drug monitoring, LC-MS, drug screening, illicit drugs

Application benefits

- Complete LC-MS/MS workflow from data acquisition to reporting for quick and easy adoption
- Over 1,200 optimized, ready-to-use SRM transitions on a Thermo Scientific™ TSQ Quantis Plus™ triple quadrupole mass spectrometer
- Robust, reliable quantitation of drugs of abuse with the accuracy and precision of triple quadrupole technology
- Analysis of compounds of different drug classes with a wide range of hydrophobicities and polarities in one comprehensive method

Goal

Demonstrate the utility of the Thermo Scientific™ Tox Explorer™ Collection workflow-based method for fast, reliable quantitation on the TSQ Quantis Plus triple quadrupole mass spectrometer for quick and easy adoption by the toxicology laboratory.

Introduction

Toxicology laboratories are overwhelmed by the ever-increasing number of drugs that need to be rapidly screened, confirmed, and quantified. There is a significant demand to have a reliable and trusted method that can withstand the daily rigors of high-throughput drug screening and quantitation. Here we present a ready-to-use LC-MS/MS solution for drugs of abuse testing on the TSQ Quantis Plus triple quadrupole mass spectrometer.

The Tox Explorer Collection for the TSQ Quantis Plus triple quadrupole mass spectrometer provides the liquid chromatography method for separation of more than 1,200 drugs of abuse with wide ranging chemical properties, the expected retention times for each compound, the optimized SRM transitions for MS/MS analysis, and

Figure 1. Chromatographic gradient

the Thermo Scientific™ TraceFinder™ software data processing method for quick and easy use and adoption by the toxicology analyst. The Tox Explorer Collection on the TSQ Quantis Plus mass spectrometer benefits from a scan speed of 600 SRM/s and can achieve sensitive levels of detection. The compound database provided enables the user to easily select and build their own custom-made methods based on their needs.

Experimental

Sample preparation

Over a hundred compounds covering a wide range of drug classes, hydrophobicities, and polarities were prepared in five mixes in urine matrix with eight internal standards. Calibration standards were prepared to span a concentration range of 2,000 ng/mL down to 0.1 ng/mL and were diluted twenty-fold in water and analyzed in triplicate.

Liquid chromatography

Gradient elution was performed using a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system equipped with a Thermo Scientific™ Accucore™ Phenyl-Hexyl column (2.6 µm, 100 x 2.1 mm, P/N 17926-102130). Mobile phases A and B were 2 mM ammonium formate and 0.1% formic acid in water and acetonitrile:methanol 50:50 (v/v), respectively. The method duration for liquid chromatography separation was 15.5 minutes, with the LC gradient shown in Figure 1 at a flow rate of 0.5 mL/minute. The Tox Explorer Collection provides specified Thermo Scientific™ Viper™ tubing and fittings for zero dead volume connections within the LC and for specified retention times for all compounds included in the method. The provided retention times allow for high-throughput, high-confidence screening and quantitation.

Mass spectrometry

The TSQ Quantis Plus triple quadrupole mass spectrometer was used for quantitation of over 100 compounds contained in the Tox Explorer Collection. The drugs of abuse that were selected from the Tox Explorer Collection menu spanned a wide range of drug classes with diverse chemical properties known to elute at different retention times across the 15.5 min runtime. Mass spectrometer source settings are listed in Table 1.

Table 1. Mass spectrometer settings

Parameter	Value	Parameter	Value
Sheath gas	50 Arb	Voltage (+/-)	3,500/2,500 V
Aux gas	10 Arb	Source temp.	350 °C
Sweep gas	1 Arb	ITT temp.	325 °C
Cycle time	0.5 s	Collision gas	2 mTorr

Compound Database generation

A Compound Database (CDB) was developed by infusing standard solutions into the mass spectrometer to obtain optimized precursor RF, single-reaction monitoring (SRM) fragments, and their associated collision energies. The compounds were then analyzed using the LC-MS/MS method described here to obtain retention times for each compound. The resulting information was used to make a CDB in Thermo Scientific™ TraceFinder™ software containing relevant parameters such as molecular formula, retention time, precursor RF, fragment *m/z* and collision energies. This database was used to quickly and easily generate the SRM table for the final MS method.

Two SRM transitions were used for each of the 101 analytes analyzed, except tramadol, which has only one relevant transition. The SRM settings were taken directly from the TraceFinder software CDB. The SRM transitions for a given compound were monitored in a one-minute window around the nominal retention time.

Software

Data acquisition, processing, and reporting were all completed using a single integrated software platform, TraceFinder software, version 5.1. Additionally, the TraceFinder CDB was used to quickly create the SRM table for MS acquisition. It is recommended that the CDB of more than 1,200 compounds be used as a menu for the analyst to quickly select their compounds of interest and implement into their own personalized methods.

Results and discussion

Targeted quantitation and screening limits

Limits of detection (LOD), limits of quantitation (LOQ), and limits of confirmation (LOC) were determined for all 101 compounds, covering a range of compound classes and polarities, in spiked urine. Parameters for setting limits of detection, quantitation, and confirmation are defined in Table 2.

Table 2. Definitions for limits used in the experiments, based on triplicate injection of the standards

Parameter	Passing criteria
LOD = limit of detection	Presence of peak at correct retention time with %RSD < 30% for triplicate injections
LOQ = limit of quantitation	Back-calculated concentration within < 30% of mean for triplicate injections
LOC = limit of confirmation	Ion ratio confirmation within 30% (relative) of target value

Results for 101 compounds

All 101 compounds were successfully detected in a single LC-MS/MS method using the Tox Explorer Collection. A representative composite chromatogram showing all 101 compounds analyzed by this method is shown in Figure 2. Results obtained on the TSQ Quantis Plus mass spectrometer were shown to be fully quantitative with linear calibration curves obtained for all 101 compounds tested and limits of detection, quantitation, and confirmation established. Concentrations for these limits ranged from 0.1 to 200 ng/mL and are listed in Table 3 for each compound. Results were based on investigating each of the chromatographic peaks and replicate data for each compound as seen in Figures 3 through 5 for MDMA.

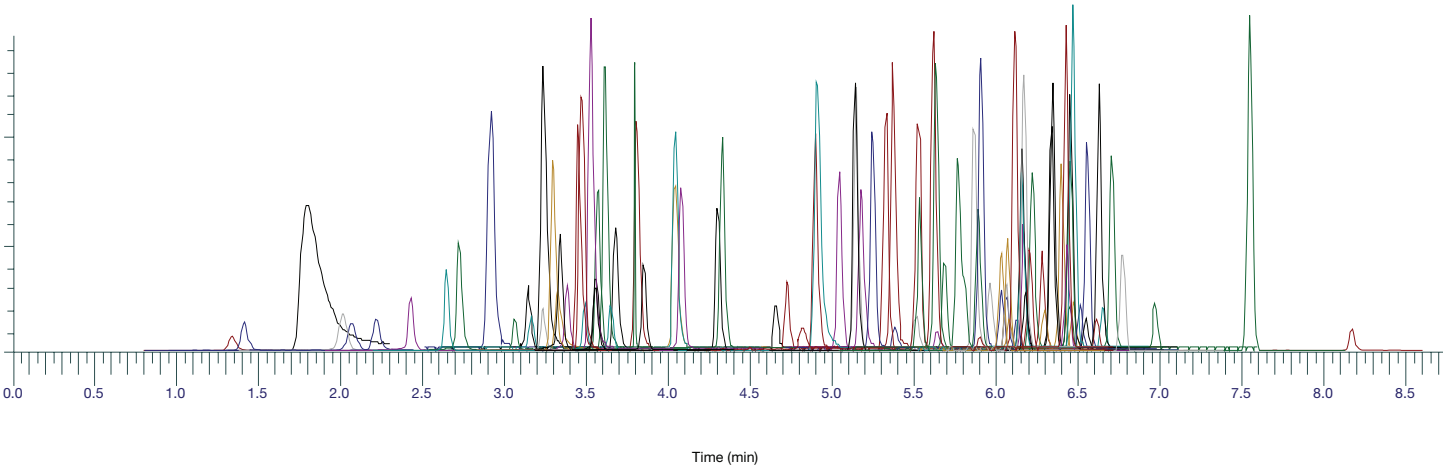

Figure 2. Representative combined chromatogram of 101 analytes

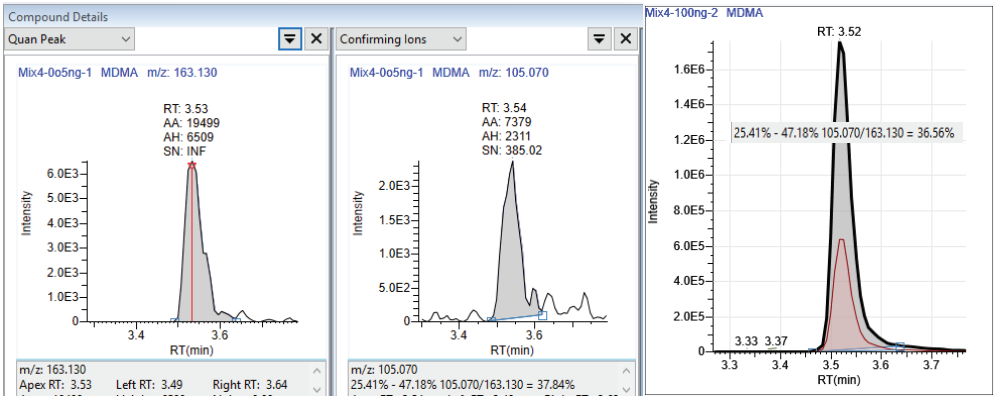

Figure 3. Chromatogram results for MDMA at its LOC showing quantifying and confirming peaks and ion overlay

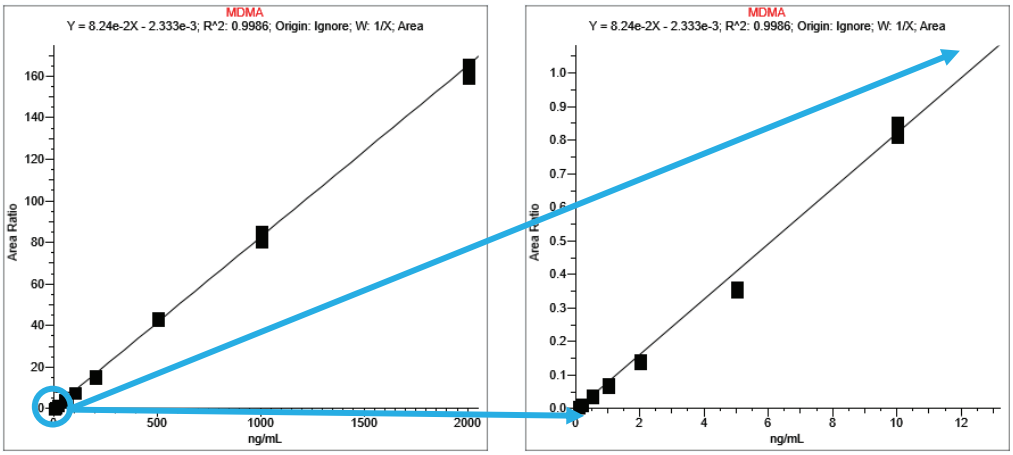

Figure 4. MDMA calibration curve with zoomed view of lower concentrations

Sample Results

	Sele	Filename		Flags	Flag Detail	IR	Actual	Area	Calc	%Diff	%RSD	% CV	Sampl	Status
2	☑	Mix4-0o1ng-2	2		I,CPF		3.53	3575	0.100	0.42	11.41	15.14	Calibrator	
3	☑	Mix4-0o1ng-3	3		I,CPF		3.53	4312	0.118	18.41	11.41	15.14	Calibrator	
4	☑	Mix4-0o2ng-1	4		I,CPF		3.52	6997	0.165	-17.62	14.94	17.82	Calibrator	
5	☑	Mix4-0o2ng-2	5				3.52	8030	0.205	2.41	14.94	17.82	Calibrator	
6	☑	Mix4-0o2ng-3	6				3.53	6194	0.156	-22.17	14.94	17.82	Calibrator	
7	☑	Mix4-0o5ng-1	7				3.53	19499	0.475	-4.94	9.10	9.65	Calibrator	
8	☑	Mix4-0o5ng-2	8				3.52	22406	0.548	9.56	9.10	9.65	Calibrator	
9	☑	Mix4-0o5ng-3	9				3.53	18716	0.465	-7.01	9.10	9.65	Calibrator	
10	☑	Mix4-1ng-1	10				3.52	35289	0.842	-15.83	6.16	6.36	Calibrator	
11	☑	Mix4-1ng-2	11				3.52	38114	0.950	-5.04	6.16	6.36	Calibrator	
12	☑	Mix4-1ng-3	12				3.53	37479	0.920	-8.05	6.16	6.36	Calibrator	
13	☑	Mix4-2ng-1	13				3.52	76765	1.682	-15.88	3.63	3.69	Calibrator	
14	☑	Mix4-2ng-2	14				3.52	80677	1.809	-9.55	3.63	3.69	Calibrator	
15	☑	Mix4-2ng-3	15				3.52	75038	1.742	-12.88	3.63	3.69	Calibrator	
16	☑	Mix4-5ng-1	16				3.52	206619	4.447	-11.05	2.55	2.57	Calibrator	
17	☑	Mix4-5ng-2	17				3.52	198006	4.290	-14.21	2.55	2.57	Calibrator	

Figure 5. Quantitation and confirmation results in TraceFinder software. The result grid shows easy-to-review pass/fail flags along with details on each result.

Table 3. Result summary detailing LOD, LOQ and LOC for compounds analyzed in this technical note. All concentrations are in ng/mL.

Compound	LOD	LOQ	LOC	Compound	LOD	LOQ	LOI
25B-NBOMe	0.5	5	5	Haloperidol	0.2	0.5	0.2
25I-NBOMe	0.5	5	1	Hydrocodone	0.5	1	1
2C-B-FLY	2	5	2	Hydromorphone	0.2	0.2	1
4-ANPP	0.1	1	1	Hydroxytriazolam	5	10	20
4-Cl-alpha-PVP	0.1	1	0.5	Hydroxyzine	0.1	1	1
4-CMC	0.1	0.1	0.1	Imipramine	0.1	0.5	0.1
6-acetylmorphine	0.1	1	2	Ketamine	0.1	0.2	1
7-aminoflunitrazepam	0.1	0.1	0.1	Lormetazepam	0.1	0.5	10
AB-FUBINACA	0.2	0.2	5	MDA	1	1	1
α-hydroxyalprazolam	2	1	5	MDEA	0.1	0.1	0.1
α-PHP	0.1	0.1	0.2	MDMA	0.1	0.1	0.2
Alprazolam	0.5	0.5	0.5	Mephedrone	0.1	0.5	0.2
Amiodarone	20	50	50	Methadone	0.1	0.1	0.1
Amitriptyline	0.2	0.5	0.5	Methamphetamine	0.1	0.5	0.5
Amobarbital	20	20	20	Methaqualone	0.5	0.5	0.2
Amoxapine	0.2	0.2	2	Mianserin	0.2	0.2	0.2
Amphetamine	0.5	1	2	Midazolam	0.2	0.2	0.5
Benzoylecgonine	0.1	0.2	1	Mitragynine	0.2	0.5	5
Bromazepam	2	2	2	Morphine	1	1	1
Buphedrone	0.2	0.2	0.5	Morphine-3ß-gluc	1	2	20
Buspirone	0.1	1	1	Morphine-6ß-gluc	1	1	50
Caffeine	1	2	10	Naloxone	0.5	0.5	2
Carisoprodol	1	1	1	Norclobazam	2	2	5
Chlordiazepoxide	0.5	1	1	Norcodeine	5	5	5
Chlorpheniramine	0.2	1	1	Nordiazepam	0.2	0.5	1
Clobazam	0.1	0.5	1	Norfentanyl	0.1	0.5	0.5
Clomipramine	0.1	0.5	0.5	Norhydrocodone	0.5	1	2
Clonazepam	1	1	1	Normorphine	5	5	5
Clonazolam	0.5	1	2	Noroxycodone	1	1	1
Clozapine	0.1	0.5	0.2	Noroxymorphone	1	1	2
Cocaethylene	0.1	0.2	0.1	Nortriptylline	0.2	0.2	0.5
Codeine	2	2	2	Olanzepine	20	50	20
Cotinine	0.1	1	0.1	Oxazepam	0.5	0.5	0.5
Cyamemazine	0.2	1	1	Oxycodone	0.1	0.5	0.5
Delorazepam	5	5	5	Oxymorphone	0.2	1	0.5
Deschloroetizolam	0.5	0.5	0.5	Phenazepam	2	2	2
Desipramine	0.2	0.2	2	Phenobarbital	200	200	200
Dextromethorphan	0.2	1	2	Phentermine	0.5	0.5	2
Dextrorphan	0.1	0.1	0.2	Prazepam	0.1	0.2	0.1
Diazepam	0.1	0.1	0.2	Pregabalin	1	2	2
Dihydrocodeine	0.5	0.5	0.5	Protriptyline	0.2	0.2	0.5
Diphenhydramine	0.1	1	0.5	Pyrazolam	0.5	0.5	1
Doxepin	0.2	0.2	0.2	Ritalinic Acid	0.1	0.5	1
EDDP	0.1	1	0.2	Secobarbital	200	200	200
Estazolam	0.5	0.5	5	Sufentanil	0.2	1	1
Ethylone	0.2	0.2	0.2	Temazepam	0.2	0.2	2
Fentanyl	0.2	5	0.5	Tramadol	0.1	0.1	NA
Flunitrazepam	0.5	0.5	1	Triazolam	0.2	0.5	2
Fluoxetine	0.2	1	1	Trimipramine	0.2	0.5	0.5
Fluvoxamine	0.5	1	2	Zolpidem	0.1	0.1	0.1
				Zopiclone	0.5	1	0.5

Conclusion

The Tox Explorer Collection is an all-in-one, standardized LC-MS/MS approach that provides a complete solution from data acquisition to data reporting for drugs of abuse testing. It was developed first as a screening method for high-resolution accurate mass (HRAM) Orbitrap™ detection by the Thermo Scientific™ Q Exactive™ Plus mass spectrometer and then the Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer. Tox Explorer Collection has now been expanded to include the TSQ Quantis Plus mass spectrometer to give the analyst a quantitative solution with optimized SRM transitions and retention times provided for over 1,200 compounds and is one of the largest experimentally tested toxicology compound databases for triple quadrupole mass spectrometers to date. Incorporating the TSQ Quantis Plus mass spectrometer for drugs of abuse testing by the Tox Explorer Collection workflow enables sensitive and reproducible analytical measurement needed for confirmation. It has been tested for 101 drugs of abuse to determine the limits of detection, quantitation, and confirmation on 20-fold diluted urine, and the results presented here demonstrate a 15.5-minute method that allows the toxicology analyst to navigate with ease and solve complex analytical challenges while increasing the laboratory's productivity.

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