Tox Explorer Collection for drugs of abuse screening and quantitation

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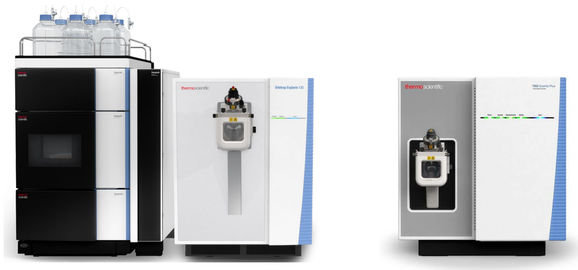
Introduction

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 reliable and trusted methods that can withstand the daily rigors of high-throughput drug screening and quantitation. Here we present a ready-to-use workstream for drugs of abuse testing implemented on both the Thermo Scientific™ TSQ Quantis™ Plus triple quadrupole mass spectrometer and Thermo Scientific™ Orbitrap Exploris™ 120 high-resolution accurate-mass (HRAM) mass spectrometer (Figure 1) that can be quickly and easily implemented for routine and reproducible drug testing. The Orbitrap Exploris 120 MS provides the added benefit of collecting high-resolution accurate-mass data which provides the ability to perform retrospective analysis.

The LC-MS/MS methods accommodate many drugs of different hydrophilicities and chemical structures and produce baseline separation of isomers. The expected retention times for each compound are provided along with the optimized SRM transitions for the triple quadrupole or exact mass and HRA M spectral library for the HRAM instrument. Data acquisition and processing methods are provided for Thermo Scientific™ TraceFinder™ software that allow for quick and easy use and adoption by the toxicology analyst.

Figure 1. Orbitrap Exploris 120 MS with Thermo Scientific™ Vanquish™ Flex UHPLC (A), TSQ Quantis Plus MS (B)



(A) (B)

Materials and methods

To verify performance, the 101 representative compounds of toxicological interest were analyzed in urine matrix using parameters taken from the Thermo Scientific™ Tox Explorer™ Collection methods. The compounds included opiates, benzodiazepines, anti-depressants, amphetamines, synthetic cannabinoids, cathinones, and other novel psychoactives. All compounds were analyzed at 14 concentration levels from 0.1 to 2,000 ng/mL.

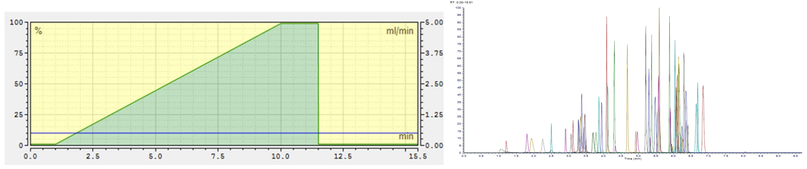
Sample preparation

Analytes were prepared in five mixes in Surine matrix. Subsequent dilutions were made to span a concentration range from 2,000 ng/mL down to 0.1 ng/mL, with the addition of eight internal standards. The samples were then diluted twenty-fold in water, and the calibration curves were analyzed in triplicate.

Liquid chromatography

Gradient elution was performed using a Vanquish Flex ultra-high performance liquid chromatography (UHPLC) system equipped with a Thermo Scientific™ Accucore™ phenyl hexyl 100 x 2.1 mm, 2.6 µm column. Mobile phases A and B were 2 mM ammonium formate with 0.1% formic acid in water and methanol:acetonitrile (1:1), respectively and run with the gradient shown in Figure 2.

Figure 2: HPLC gradient with combined chromatogram showing all compounds.



TSQ Quantis Plus MS method

The Thermo Scientific™ TSQ Quantis™ Plus mass spectrometer was used for the targeted screening and quantitation analysis (Figure 1B). Mass spectrometer source and experiment parameters are shown in Table 1.

Table 1. TSQ Quantis Plus Mass Spectrometer settings.

Parameter	Value	Parameter	Value
Sheath Gas	50 Arb	Voltage (+/-)	3500/2500 V
Aux Gas	10 Arb	Source Temp	350 ºC
Sweep Gas	1 Arb	ITT Temp	325 ºC
Cycle Time	0.5 sec	Collision Gas	2 mTorr

A Compound Database (CDB) was developed using the above LC-MS methodology by infusing standard solutions into the mass spectrometer to obtain optimized precursor RF and selected-reaction monitoring (SRM) transitions with associated collision energies. Relevant parameters such as molecular formula, retention time, precursor RF, fragment *m/z* and collision energies were recorded in the CDB[1].

Orbitrap Exploris 120 MS method

Source parameters were kept consistent with those used for TSQ Quantis Plus MS listed in Table 1. Full scan and targeted data-dependent MS/MS scanning were used with an inclusion list for the targeted compounds. The inclusion list contains the exact mass of the compound, polarity, and retention time. Orbitrap Exploris 120 MS parameters are listed in Table 2.

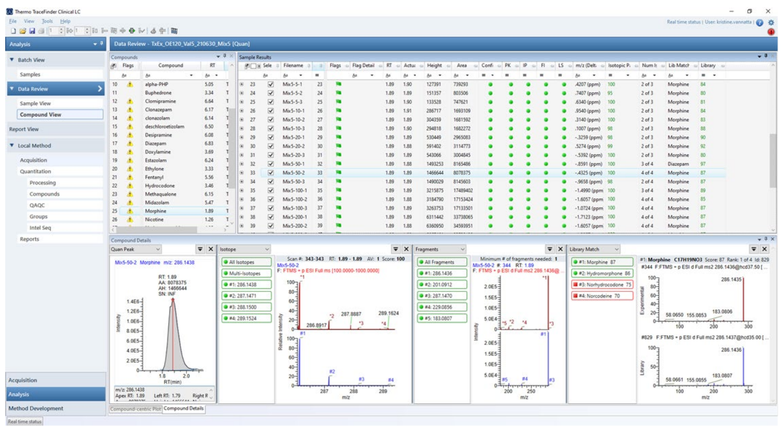
Data analysis

Data was acquired and processed with TraceFinder™ software, version 5.2 (Figure 4). For the TSQ Quantis Plus MS data, the TraceFinder CDB was used to populate the processing method. For the Oribtrap Exploris 120 MS data, the TraceFinder data analysis method used the HRAM CDB version which contained information for molecular formula, exact mass, retention time and fragments ions for all compounds of interest. Additionally, TraceFinder performed a library search of the MS2 fragmentation spectra against a HRAM spectral library.

Table 2. Orbitrap Exploris 120 Mass Spectrometer scan settings.

Scan Parameter	Value
GLOBAL SETTINGS	
Scan Type	Full MS / ddMSMS
RF %	80
Mass Calibration	Easy-IC™
Data Type	Profile
FULL SCAN	
Resolution	60,000
Max Injection Time	Auto
Scan Range	100 -1000
ddMSMS	
Intensity Threshold	1.0e5
Targeted Mass List Tolerance	≤ 5 ppm, +/- 0.5 min
Isolation Window	1.5 m/z
Stepped NCE	18.75, 37.5, 56.25
First Mass	40 m/z

Figure 4. Data review for screening of the analyte, morphine demonstrating retention time, isotope matching, fragment ions and library match for Orbitrap Exploris 120 MS.



Results

Verification for TSQ Quantis Plus MS and Orbitrap Exploris 120 MS

Limits of detection (LOD), limits of quantification (LOQ), and limits of confirmation (LOC) of identification (LOI) were determined for 101 compounds, covering a range of compound classes and polarities, in spiked urine (Table 4). Parameters for the limits are defined in Table 3.

Table 3. Definitions for Limits used in this experiment, based on triplicate injection of samples. LOI was used for Orbitrap Exploris 120 MS; LOC was used for TSQ Quantis Plus MS.

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 (TSQ Quantis Plus)	Ion ratio confirmation within 30% (relative) of target value
LOI = Limit of identification (Orbitrap Exploris 120)	IP = passing isotopic pattern score (70) FI = presence of diagnostic fragment ions LS = passing library score (70)

Table 4. Results for LOD, LOQ and LOI/LOC in ng/mL.

Compound	Detection		Quantitation		Confirmation and Identification	
	TSQ	OE	TSQ	OE	TSQ	OE
25B-NBOMe	0.5	0.1	5	0.5	5	1
25I-NBOMe	0.5	0.1	5	0.2	1	1
2C-B-FLY	2	0.2	5	1	2	5
4-ANPP	0.1	0.5	1	1	1	2
4-Cl-alpha-PVP	0.1	0.1	1	0.2	0.5	1
4-CMC	0.1	0.5	0.1	1	0.1	5
6-acetylmorphine	0.1	0.2	1	0.5	2	1
7-aminoflunitrazepam	0.1	0.1	0.1	0.2	0.1	0.2
AB-FUBINACA	0.2	0.1	0.2	1	5	5
a-hydroxyalprazolam	2	0.2	1	1	5	5
a-PHP	0.1	0.1	0.1	0.5	0.2	0.1
Alprazolam	0.5	0.1	0.5	0.2	0.5	2
Amiodarone	20	2	50	2	50	10
Amitriptyline	0.2	0.1	0.5	0.5	0.5	1
Amobarbital	20	20	20	100	20	500
Amoxapine	0.2	0.2	0.2	0.2	2	1
Amphetamine	0.5	5	1	5	2	10
Benzoylecgonine	0.1	0.1	0.2	0.5	1	0.1
Bromazepam	2	0.1	2	5	2	5
Buphedrone	0.2	5	0.2	5	0.5	5
Buspirone	0.1	0.1	1	0.2	1	0.5
Caffeine	1	0.1	2	0.5	10	0.1
Carisoprodol	1	2	1	2	1	10
Chlordiazepoxide	0.5	0.2	1	0.2	1	1
Chlorpheniramine	0.2	0.2	1	2	1	10
Clobazam	0.1	0.1	0.5	0.2	1	2
Clomipramine	0.1	0.2	0.5	0.5	0.5	2
Clonazepam	1	0.1	1	1	1	0.1
Clonazolam	0.5	0.1	1	0.1	2	0.5
Clozapine	0.1	0.1	0.5	0.2	0.2	0.5
Cocaethylene	0.1	0.1	0.2	0.2	0.1	0.2
Codeine	2	0.2	2	0.5	2	0.5
Cotinine	0.1	0.1	1	1	0.1	2
Cyamemazine	0.2	0.2	1	0.5	1	2
Delorazepam	5	0.5	5	1	5	10
Deschloroetizolam	0.5	0.2	0.5	0.2	0.5	0.5
Desipramine	0.2	0.2	0.2	0.2	2	0.5
Dextromethorphan	0.2	0.1	1	0.2	2	0.5
Dextrorphan	0.1	0.1	0.1	0.2	0.2	0.2
Diazepam	0.1	0.1	0.1	0.2	0.2	0.1
Dihydrocodeine	0.5	0.2	0.5	0.2	0.5	1
Diphenhydramine	0.1	0.5	1	0.5	0.5	2
Doxepin	0.2	0.1	0.2	0.2	0.2	1
EDDP	0.1	0.1	1	0.1	0.2	0.5
Estazolam	0.5	0.2	0.5	0.5	5	5
Ethylone	0.2	0.5	0.2	1	0.2	0.5
Fentanyl	0.2	0.1	5	0.2	0.5	0.1
Flunitrazepam	0.5	0.1	0.5	0.2	1	0.1
Fluoxetine	0.2	0.2	1	1	1	1
Fluvoxamine	0.5	0.2	1	0.2	2	1
Haloperidol	0.2	0.1	0.5	0.2	0.2	0.5
Hydrocodone	0.5	1	1	2	1	1
Hydromorphone	0.2	0.2	0.2	0.2	1	2
Hydroxytriazolam	5	0.5	10	1	20	10
Hydroxyzine	0.1	0.2	1	0.5	1	2
Imipramine	0.1	0.1	0.5	0.2	0.1	0.2
Lormetazepam	0.1	0.2	0.5	1	10	10
MDA	1	2	1	2	1	5
MDEA	0.1	0.2	0.1	0.5	0.1	0.5
MDMA	0.1	2	0.1	2	0.2	2
Mephedrone	0.1	1	0.5	2	0.2	1
Methadone	0.1	0.1	0.1	0.2	0.1	0.5
Methamphetamine	0.1	0.2	0.5	0.2	0.5	2
Methaqualone	0.5	0.1	0.5	0.1	0.2	0.1
Mianserin	0.2	0.1	0.2	0.2	0.2	0.2
Midazolam	0.2	0.1	0.2	0.2	0.5	1
Mitragynine	0.2	0.2	0.5	0.2	5	0.2
Morphine	1	0.2	1	0.5	1	1
Morphine-3ß-gluc	1	2	2	2	20	20
Morphine-6ß-gluc	1	5	1	5	50	100
Naloxone	0.5	0.5	0.5	2	2	2
Nicotine	1	5	10	10	2	10
Norclobazam	2	1	2	2	5	5
Norcodeine	5	0.5	5	1	5	2
Nordiazepam	0.2	0.1	0.5	0.1	1	0.5
Norfentanyl	0.1	0.1	0.5	0.5	0.5	0.5
Norhydrocodone	0.5	0.5	1	0.5	2	2
Normorphine	5	1	5	2	5	5
Noroxycodone	1	0.2	1	0.5	1	1
Noroxymorphone	1	0.5	1	0.5	2	2
Nortriptylline	0.2	0.1	0.2	0.2	0.5	1
Olanzepine	20	2	50	5	20	10
Oxazepam	0.5	0.2	0.5	0.5	0.5	5
Oxycodone	0.1	0.5	0.5	1	0.5	5
Oxymorphone	0.2	0.5	1	0.5	0.5	5
Phenazepam	2	0.1	2	0.5	2	0.5
Phenobarbital	200	50	200	50	200	500
Phentermine	0.5	1	0.5	1	2	1
Prazepam	0.1	0.1	0.2	0.1	0.1	0.2
Pregabalin	1	2	2	10	2	5
Protriptyline	0.2	0.1	0.2	0.2	0.5	1
Pyrazolam	0.5	0.5	0.5	1	1	2
Ritalinic Acid	0.1	0.2	0.5	0.2	1	1
Secobarbital	200	50	200	100	200	1000
Sufentanil	0.2	0.2	1	0.5	1	5
Temazepam	0.2	0.1	0.2	0.1	2	2
Tramadol	0.1	0.1	0.1	0.1	NA	0.5
Triazolam	0.2	0.1	0.5	0.1	2	1
Trimipramine	0.2	0.1	0.5	0.2	0.5	0.5
Zolpidem	0.1	0.1	0.1	0.1	0.1	0.5
Zopiclone	0.5	1	1	1	0.5	10

Conclusions

- Tox Explorer workflow was used to detect, identify, and quantitate 101 drugs of abuse by TSQ Quantis Plus MS and Orbitrap Exploris 120 MS .
- Implementing this high-capacity screening method on a more economical HRAM MS platform makes this powerful capability accessible to more forensic toxicological laboratories.
- The TSQ Quantis Plus MS and Orbitrap Exploris 120 MS can both screen and quantitate compounds in one platform and injection.
- A method capable of screening very large toxicology panels from a supplied database of 1100 compounds on a triple quadrupole LC-MS/MS system and over 1800 compounds on a HRAM orbitrap mass spectrometer.
- TraceFinder software with its integrated compound database enables data acquisition, processing for both screening and quantitation and reporting in one platform.

References

1. Technical Note 001256: All-In-One LC-MS/MS Toxicology Solution for Drugs of Abuse Quantitation Using the TSQ Quantis Plus Mass Spectrometer.
2. Technical Note 73771: LC-MS/MS Toxicology Platform and Method for High-resolution, Accurate Mass (HRAM) Detection, Screening, and Quantitation of Drugs.

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