# Drugs of abuse: Reliable analysis through Orbitrap mass spec

A guide to rapid, easy and accurate screening and quantitation of targeted and untargeted compounds using high-resolution accurate mass LC-MS technology

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# Introduction

**Traditional testing methods to screen and quantify drugs using immunoassays endure numerous constraints, such as cross-reactivity, false positives and limited selectivity.**

With the growth of prescribed analgesics and misuse of opioids, clinical researchers and forensic toxicologists need simpler, more robust and reliable technologies that enable routine, reproducible and sensitive analysis of large toxicology panels within a single run to match their ever-increasing caseloads

High-resolution mass spectrometry using Thermo Scientific™ Orbitrap™ technology coupled with liquid chromatography (LC) enables forensic laboratories to produce sensitive and selective results for routine identification, screening, quantitation and confirmation of targeted and untargeted compounds. Unlike immunoassays, the Orbitrap technology allows laboratories to screen and detect multiple analytes in a compound class and offers the ability to perform retrospective analyses to enable data reassessment without having to re-analyze the sample.

Intuitive software further enables rapid LC-MS analyses in under 20 minutes, while allowing access to a database and spectral library of 1,800 compounds for targeted screening.

In this eBook, you will find a range of methods to quantify and screen drugs of abuse using the Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap™ series mass spectrometers.

## Contents



**• TARGETED QUANTITATION OF ABUSE DRUGS**

**Quantitation of barbiturates:** Learn how to quantitate barbiturates, namely, amobarbital, butalbital, pentobarbital, phenobarbital and secobarbital, in human urine using a simple dilute-and-shoot method. With limited matrix effects and high selectivity, the method performance meets toxicology lab requirements.

**Quantitation of opiates:** Discover a method for the quantitation of six opiates—morphine, codeine, hydromorphone, hydrocodone, oxymorphone, and oxycodone—in human urine down to low ng/mL levels. With quintuplicate replicates of quality control over three different days and assessment with 58 donor samples, this method demonstrates that toxicologists can perform quantitative confirmation of specific panels to industry-established limits using the Orbitrap mass spectrometer.

**• SCREENING OF ABUSE DRUGS**

**Fentanyl screening:** With a goal of developing a reliable and reproducible method for screening of fentanyl in urine, 14 different fentanyls were tested using a UHPLC system coupled to the Orbitrap high-resolution mass

spectrometer. All fentanyl compounds present in the spiked matrix were detected and confirmed at 0.5 – 5 ng/mL, validating this method for screening in clinical research or forensic toxicology.

**Forensic screening of drugs in urine:**
This method evaluates a urine screening method for ~300 compounds including drugs of abuse and environmental toxins. The combination of the Orbitrap mass analyzer and the identification workflow offered by Thermo Scientific™ ToxFinder™ software provides an economical solution to screen a virtually unlimited number of compounds in urine.

**Forensic screening of drugs in plasma:**
Generate a large targeted forensic screening panel in a short chromatographic run time of 15.5 minutes. The screening capabilities of the Orbitrap mass spectrometer coupled with the quantitation of 41 drugs in plasma provide a method to screen and semi-quantify compounds of interest in forensic toxicology including drugs of abuse, antidepressants, beta-blockers, antibiotics, pesticides and others.

**• CHOOSING A SCAN MODE**
The different scan modes available on the Q Exactive series of Orbitrap mass spectrometers offer researchers functionality comparable to triple quadrupole (QQQ) instruments. This white paper describes the various scan modes and applications for which they are most suitable. Get expert guidance for your method development based on three key considerations: sensitivity, selectivity and scan speed.

| | |
|---|---|
| **Full Scan MS** | A good starting point; often used for both applied and research and applications |
| **Full MS/AIF**<br>Full scan MS followed by an all ion fragmentation | Often used in small molecule applications |
| **t-SIM**<br>Targeted selected ion monitoring | Offers ultimate sensitivity; often used in small molecule applications |
| **PRM or targeted-MS2**<br>Parallel reaction monitoring or targeted MS/MS | Offers ultimate selectivity; often used for small molecule applications involving analyses of complex sample matrices |
| **Full MS/dd-MS2**<br>Full MS followed by data-dependent scans | Often used in proteomic applications for peptide and protein identification |
| **Targeted SIM/dd-MS2**<br>Targeted SIM followed by data-dependent scans | Most often used for small molecule applications |
| **DIA**<br>Data independent acquisition | Fragments “everything”; used in both applied and research applications |

# Analysis of Five Barbiturates in Urine Using an Affordable High-Resolution Mass Spectrometer

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## Key Words

Q Exactive Focus, barbiturates, forensic toxicology

## Goal

To develop a quantitative method for analysis of barbiturates in urine that meets forensic toxicology laboratory requirements.

## Application Benefits

- Dilute-and-shoot method
- High selectivity
- Chromatographic separation of isobaric analyte
- Limited matrix effects
- Accurate chromatographic peak integration due to very low background
- Method performance meets toxicology lab requirements

## Introduction

High-resolution mass spectrometers are widely accepted in forensic toxicology laboratories for use in screening applications. Conventionally, triple quadrupole instruments have been used for quantitative methods. The high-resolution Thermo Scientific™ Orbitrap™ instruments can be utilized for both screening and quantitative confirmatory methods, as well as structural elucidation experiments. Their ability to perform over a wide range of applications makes them a highly versatile platform for use in toxicology labs. In this note, we show their ability to quantitate five barbiturates (amobarbital, butalbital, pentobarbital, phenobarbital and secobarbital) in human urine.

## Methods

### Sample Preparation

Sample preparation was a dilute-and-shoot technique. A 50 µL urine sample was diluted with 950 µL of water that contained internal standards at a concentration of 100 ng/mL.

### Calibrators and Quality Controls

Calibration standards in the range of 5 to 2000 ng/mL and quality control (QC) samples at concentrations of 25, 100, and 1000 ng/mL (LQC, MQC, HQC) were prepared in synthetic urine.

### Liquid Chromatography

A six-minute gradient elution was performed using a Thermo Scientific™ Dionex™ UltiMate™ 3000RS liquid chromatography pump with OAS autosampler. Mobile phases consisted of 5 mM ammonium acetate in water and acetonitrile (Fisher Chemical™ Optima™ grade) for mobile phases A and B, respectively. The column used was a Thermo Scientific™ Accucore™ C18, 2.6 µm, 50 x 2.1 mm column (P/N 17126-052130).

### Mass Spectrometry

Compounds were detected on a Thermo Scientific™ Q Exactive™ Focus quadrupole-Orbitrap mass spectrometer equipped with a Thermo Scientific™ Ion Max™ source and a heated electrospray (HESI-II) source. Data were acquired in parallel-reaction monitoring (PRM) mode. In this mode, a single precursor ion was selected in the quadrupole with an isolation width of 2.0 *m/z* and fragmented in the HCD cell using an optimized, compound-specific collision energy. The resulting MS/MS product ion spectrum was detected in the Orbitrap detector at a resolution of 35,000 (FWHM at *m/z* of 200).

### Method Performance Evaluation

The limits of quantitation (LOQ) and linearity ranges were evaluated by collecting calibration curve data in triplicate in three different batches. Method precision and accuracy were evaluated by running a triplicate calibration curve and quintuplicate replicates of QCs on three different days. Matrix effects were evaluated by spiking urine from seven different donors at concentrations of 10, 25, and 100 ng/mL and calculating recovery against the same concentrations prepared in water instead of urine. Matrix effects were also evaluated by analyzing 48 donor urine samples and calculating internal standards' recovery against a sample prepared in water instead of urine.

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### Data Analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software. For each analyte, the precursor exact ion mass in the MS/MS spectrum was used for quantification and the most abundant fragment was used for confirmation. The chromatograms were reconstructed with a mass accuracy of 5 ppm for quantification. Figure 1 shows representative MS/MS spectra for selected analytes with quantifying and confirming ions specified.

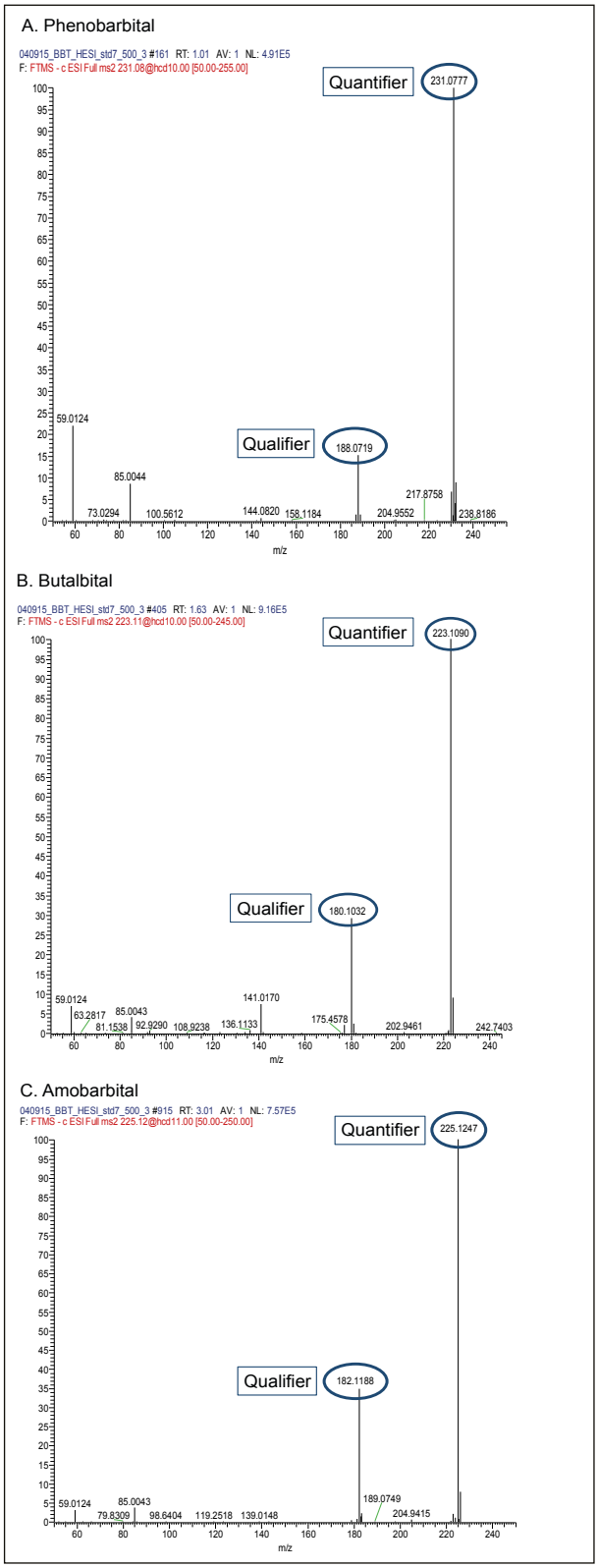


Figure 1. Representative MS/MS spectra for selected analytes with quantifying and qualifying ions specified.

## Results

LOQs were defined as the lowest concentrations that had back-calculated values within 20%, RSD for five QC replicates within 20%, and ion ratio within specified range. Using these criteria, the limit of quantitation for butalbital, pentobarbital, amobarbital, and secobarbital was 5 ng/mL, and for phenobarbital was 25 ng/mL.

The upper calibration range for all analytes was 2000 ng/mL. Figure 2 shows representative calibration curves for all five analytes, collected in triplicate, along with chromatograms for the lowest calibration standard. Calibration standards' precision and accuracy were better than 15%.

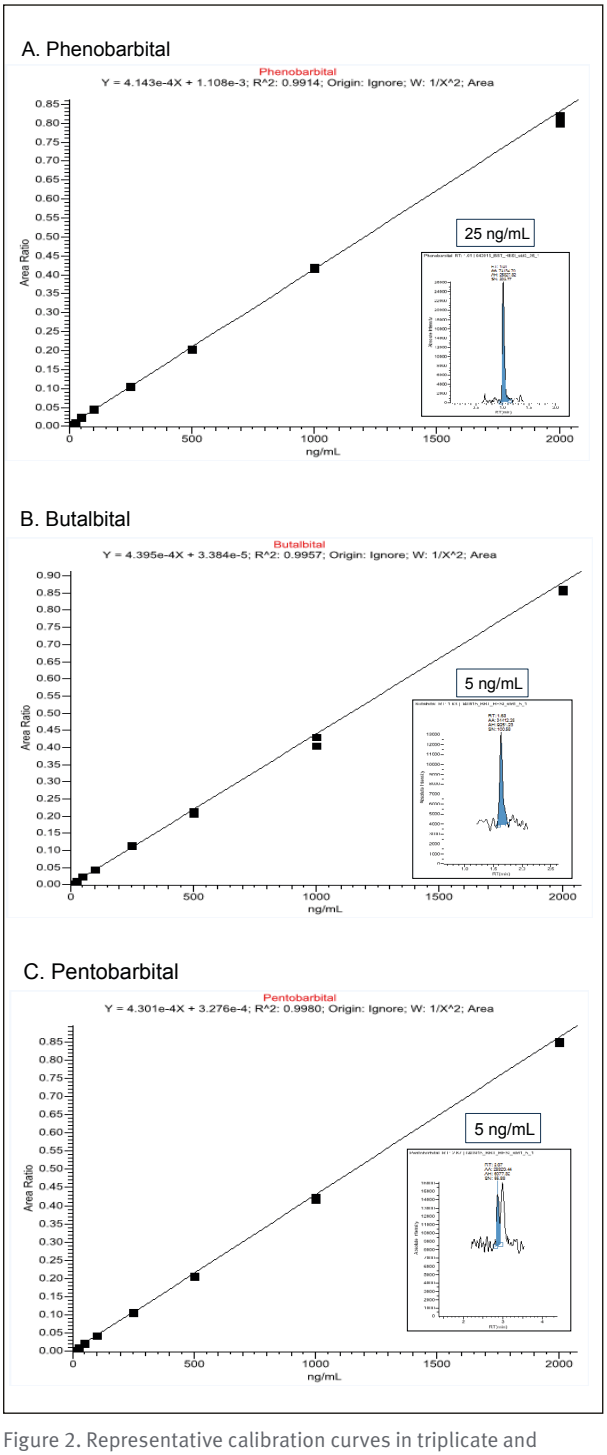


Figure 2. Representative calibration curves in triplicate and chromatograms for the lowest calibration standard. Accuracy of all calibration standards is within 15%.

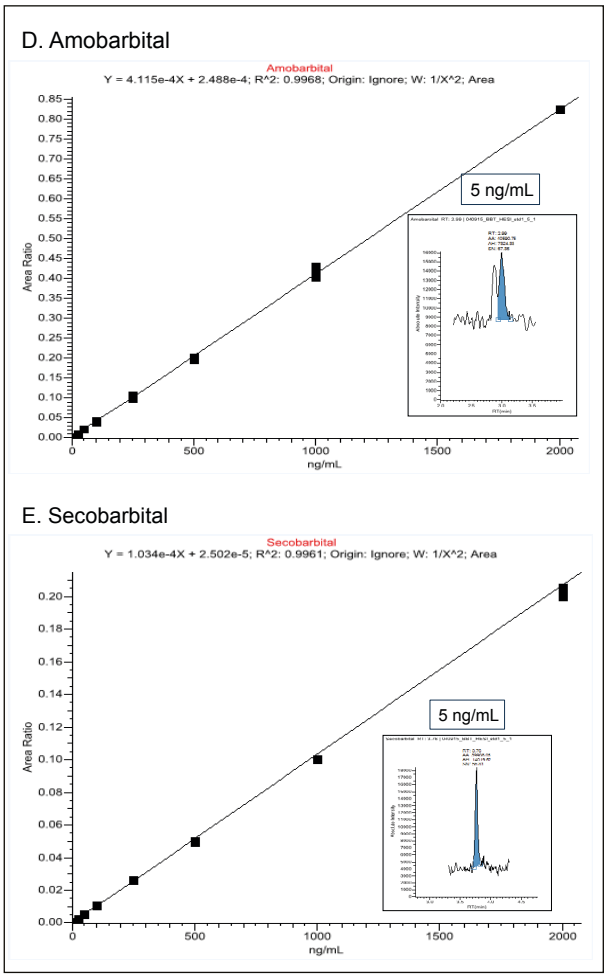


Figure 2 (continued). Representative calibration curves in triplicate and chromatograms for the lowest calibration standard. Accuracy of all calibration standards is within 15%.

Intra-assay precision was better than 8% (Table 1), and inter-assay precision was better than 10% (Table 2) for all analytes.

Table 1. Intra-assay precision.

| Analyte | LQC | MQC | HQC |
|---|---|---|---|
| | %RSD | | |
| **Phenobarbital** | 3.5–7.2 | 2.6–4.6 | 3.0–3.5 |
| **Butalbital** | 3.0–5.2 | 2.0–2.8 | 1.9–3.8 |
| **Pentobarbital** | 2.5–8.0 | 0.74–2.6 | 2.0–4.0 |
| **Amobarbital** | 3.6–6.8 | 2.6–4.3 | 1.6–2.8 |
| **Secobarbital** | 2.9–4.8 | 2.2–2.8 | 1.7–3.3 |

Table 2. Inter-assay precision.

| Analyte | LQC | MQC | HQC |
|---|---|---|---|
| | %RSD | | |
| **Phenobarbital** | 5.5 | 5.6 | 4.1 |
| **Butalbital** | 5.7 | 6.1 | 5.4 |
| **Pentobarbital** | 6.5 | 6.2 | 6.1 |
| **Amobarbital** | 9.0 | 9.7 | 7.0 |
| **Secobarbital** | 5.1 | 5.7 | 5.1 |

Limited matrix effects were observed. Recovery in seven donor samples, calculated as ratio between analyte peak area in urine matrix and analyte peak area in solvent matrix, ranged from 85.8% to 115% (Table 3). Figure 3 presents a quantifying ion chromatogram of donor urine spiked with all analytes at a concentration of 25 ng/mL.

Internal standards recovery in 48 donor urine samples ranged from 76% to 108% (Figure 4).

Table 3. Matrix effects in spiked donor urine samples.

| Analyte | 10 ng/mL | 25 ng/mL | 100 ng/mL |
|---|---|---|---|
| | %recovery | | |
| **Phenobarbital** | 92.3–104 | 85.8–105 | 99.3–108 |
| **Butalbital** | 99.7–110 | 93.0–105 | 99.1–109 |
| **Pentobarbital** | 99.1–112 | 89.2–102 | 96.3–108 |
| **Amobarbital** | 93.8–113 | 97.8–112 | 102–111 |
| **Secobarbital** | 92.3–102 | 85.3–104 | 99.7–110 |

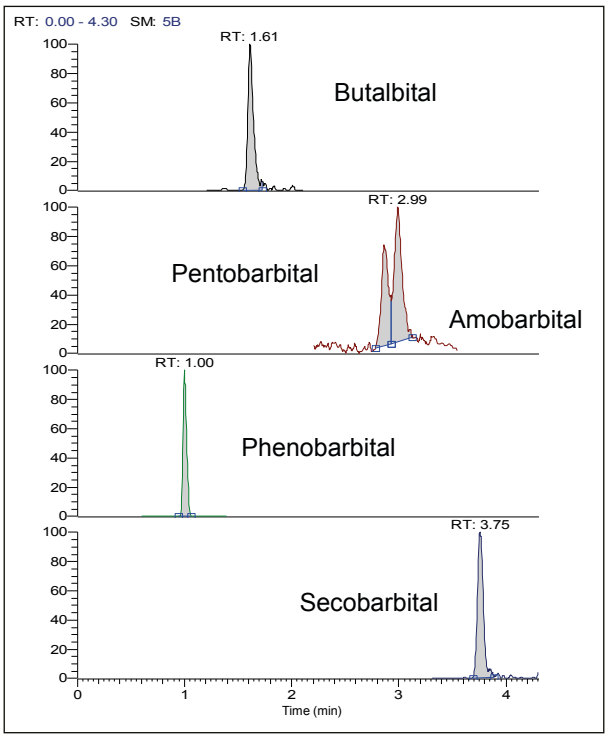


Figure 3. Chromatogram of donor urine spiked with all analytes to a concentration of 25 ng/mL. Chromatogram is reconstructed with a mass accuracy of 5 ppm.

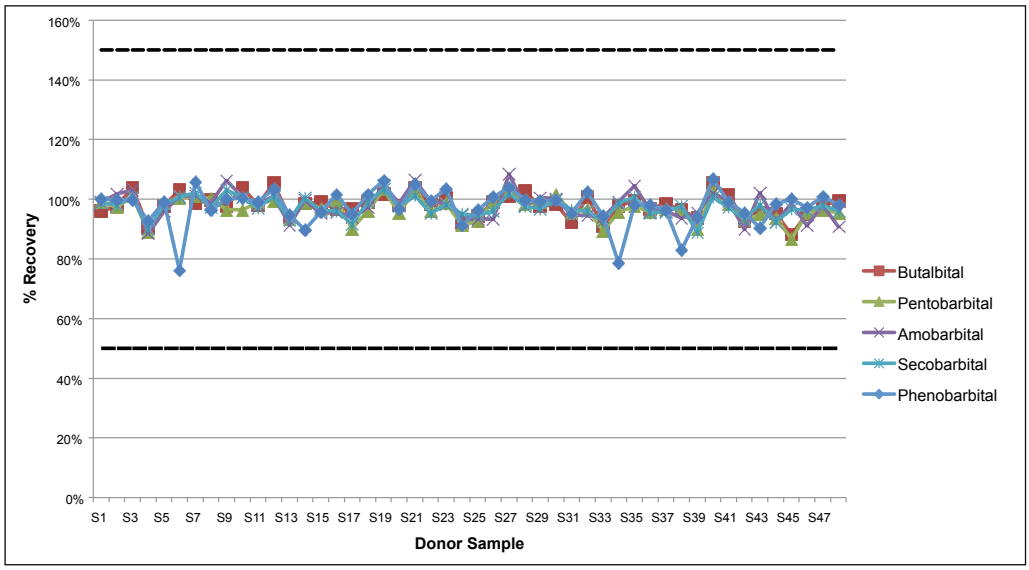


Figure 4. Internal standards % Recovery in donor urine samples.

## Conclusion

We demonstrated a simple dilute-and-shoot method for the analysis of five barbiturates in urine implemented on a Q Exactive Focus high-resolution mass spectrometer. The method performance evaluation data indicate that the method can be implemented in forensic toxicology laboratories and show the versatility of Orbitrap technology for these laboratories.

For forensic toxicology use only.





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# Quantitation of Opiates to Low ng/mL Levels in Urine for Forensic Use Using an Affordable, High-Resolution, Accurate-Mass Mass Spectrometer

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## Key Words

Q Exactive Focus, opiates, morphine, codeine, hydromorphone, hydrocodone, oxymorphone, oxycodone, TraceFinder, forensic toxicology, drugs of abuse, PRM, parallel reaction monitoring

## Goal

To evaluate the performance of the Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ mass spectrometer as a quantitative platform for HPLC-MS analysis of opiates in human urine to low ng/mL levels for forensic toxicology.

## Introduction

Forensic toxicologists need an economical instrument capable of both screening a large number of compounds and quantifying smaller panels to industry-established limits. Here we present a method for quantitation of six opiates—morphine, codeine, hydromorphone, hydrocodone, oxymorphone, and oxycodone—in human urine down to low ng/mL levels. This work was performed on a Q Exactive Focus hybrid quadrupole-Orbitrap mass spectrometer.

## Methods

### Sample Preparation

Samples were processed by enzymatic hydrolysis followed by urine dilution. Briefly, an aliquot of urine was spiked with stable-isotope-labeled internal standards and incubated with ß-glucuronidase enzyme. The resulting mixture was centrifuged and further diluted before an aliquot was analyzed by gradient HPLC and a Q Exactive Focus MS. Calibrators and controls were prepared by spiking compounds into blank synthetic urine in the range of 1 to 5000 ng/mL.

### Liquid Chromatography

Gradient elution was performed using a Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system with OAS autosampler (Figure 1). Mobile phases consisted of 10 mM ammonium acetate with 0.1% formic acid in water and methanol (Fisher Chemical brand) for solvents A and B, respectively. The column used was a Thermo Scientific™ Accucore™ PFP, 2.6 µm particle size, 50 x 2.1 mm fused core (p/n 17426-052130). The

Figure 1. Q Exactive Focus MS with UltiMate 3000 RSLC HPLC pump and UltiMate 3000 OAS autosampler.

gradient was run from 0 to 70% mobile phase B over 3.3 minutes followed by a column wash at 100% B and re-equilibration to starting conditions. The total run time was 5.3 minutes.

### Mass Spectrometry

Compounds were detected on a Q Exactive Focus MS equipped with a Thermo Scientific™ Ion Max™ source and a heated electrospray ionization (HESI-II) sprayer. Data was acquired in parallel reaction monitoring (PRM) mode. In this mode, a single precursor ion is selected in the quadrupole with an isolation width of 3.0 *m/z* and fragmented in the HCD cell. The resulting MS/MS product ions are detected in the Orbitrap detector at a resolution of 35,000.

### Method Evaluation

The method precision and accuracy were evaluated by running a calibration curve and quintuplicate replicates of quality controls on three different days. Additionally, internal-standard response was assessed in 58 donor samples obtained from a collaborator laboratory and compared to a sample prepared in water to determine matrix effects.

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### Data Analysis

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software. Two product ions were selected as the quantifying and confirming ions for each compound. The resulting chromatograms were extracted and reconstructed with a mass accuracy of 5 ppm for quantification and ion ratio confirmation. Because the entire MS/MS spectrum was collected, multiple confirming ions could be chosen. Figure 2 shows a representative MS/MS spectrum for oxymorphone, highlighting the quantifying and confirming ions with corresponding reconstructed chromatograms.

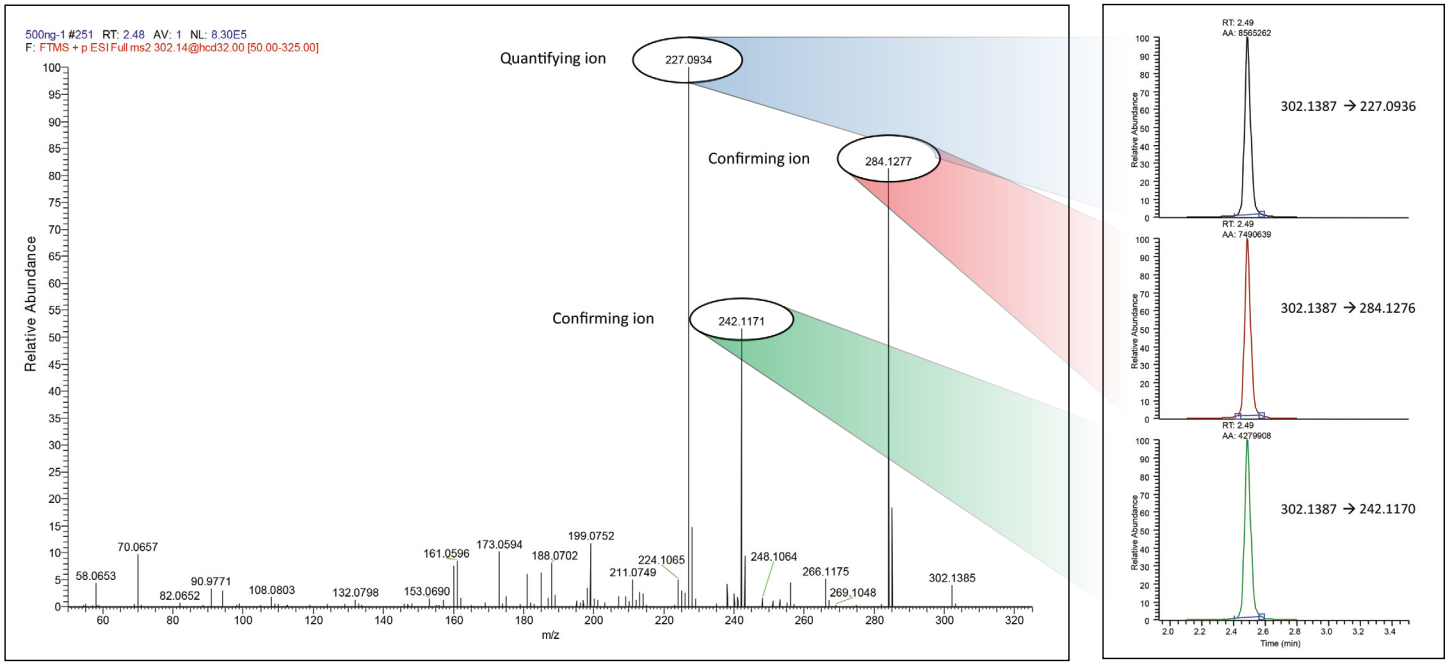



Figure 2. Representative fragmentation spectrum for oxymorphone obtained from a 500 ng/mL calibrator, highlighting the quantifying and confirming ions and showing corresponding chromatograms reconstructed with 5 ppm mass accuracy.

## Results

Limits of quantitation (LOQs) were defined as the lowest concentrations that had back-calculated values within 20%, ion ratios within defined tolerance (tolerance dependent upon actual ratio), and quality controls within 20% RSD as well as meeting the above two requirements. Using these criteria, limits of quantitation for codeine, oxycodone, and oxymorphone were determined to be 2.5 ng/mL. For morphine, hydrocodone, and hydromorphone, the limit was 5 ng/mL. Tables 1 and 2 show the inter- and intra-assay statistics, respectively, for quality controls for all compounds in this method. Limited matrix effects were observed. The average recovery across 58 donor urine samples obtained from a collaborator laboratory ranged from 69% to 81% for the six internal standards evaluated. Figure 3 shows a combined chromatogram for analytes at their respective LOQs, and Figure 4 shows chromatograms for each compound with confirming ion ratio at its LOQ. Figure 5 shows representative calibration curves for all compounds. Figure 6 shows representative chromatograms with ion ratio confirmation for donor samples.

Table 1. Inter-assay precision and bias.

| | Codeine | Hydrocodone | Hydromorphone | Morphine | Oxycodone | Oxymorphone |
|---|---|---|---|---|---|---|
| **5 ng/mL** | | | | | | |
| % RSD | 3.82 | 3.67 | 3.89 | 7.54 | 2.78 | 3.43 |
| % Bias | -4.11 | 7.20 | 5.28 | 0.18 | -6.19 | -2.04 |
| **10 ng/mL** | | | | | | |
| % RSD | 4.13 | 6.06 | 2.79 | 4.35 | 3.35 | 2.78 |
| % Bias | -7.00 | -3.47 | -0.20 | -6.15 | -5.24 | -3.60 |
| **100 ng/mL** | | | | | | |
| % RSD | 3.00 | 6.13 | 2.03 | 2.28 | 2.58 | 1.62 |
| % Bias | 7.43 | 5.51 | -0.16 | 0.98 | 5.34 | 0.26 |
| **1000 ng/mL** | | | | | | |
| % RSD | 3.63 | 4.21 | 1.35 | 2.52 | 1.99 | 2.29 |
| % Bias | 6.07 | 3.64 | 3.53 | 4.67 | 5.44 | 2.86 |

Table 2. Intra-assay precision.

| Maximum %RSD from Three Runs | 5 ng/mL | 10 ng/mL | 100 ng/mL | 1000 ng/mL |
|---|---|---|---|---|
| Codeine | 5.09 | 4.56 | 3.61 | 3.34 |
| Hydrocodone | 3.55 | 5.60 | 6.38 | 4.25 |
| Hydromorphone | 3.16 | 1.87 | 2.41 | 1.90 |
| Morphine | 8.25 | 4.52 | 2.77 | 3.64 |
| Oxycodone | 3.27 | 3.27 | 2.85 | 2.31 |
| Oxymorphone | 4.28 | 3.04 | 1.78 | 2.73 |

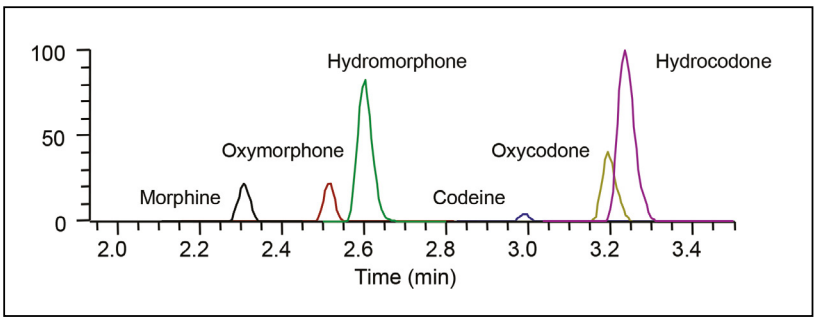
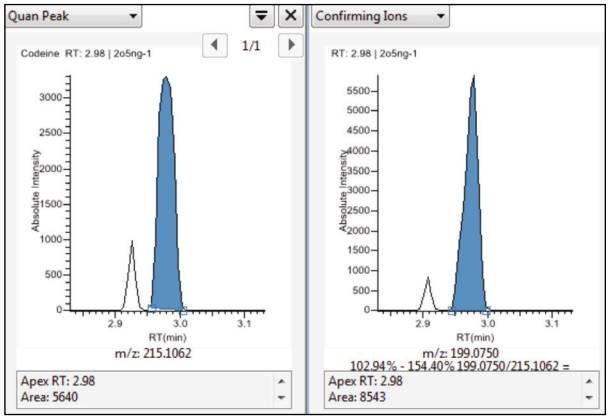


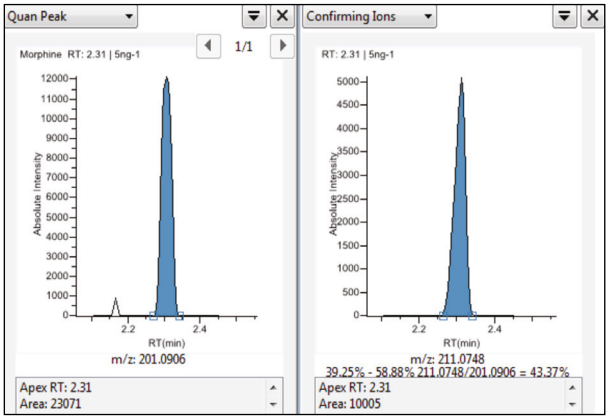

Figure 3. Chromatograms extracted from $MS^2$ spectra obtained from a confirmation PRM experiment for six opiates at their respective LOQs (2.5 ng/mL for codeine, oxycodone, and oxymorphone , and 5 ng/mL for hydrocodone, hydromorphone, and morphine) in hydrolyzed and diluted urine.

Figure 4. Chromatograms showing quantifying and confirming ions with ion ratio at LOQ for each compound in this method.

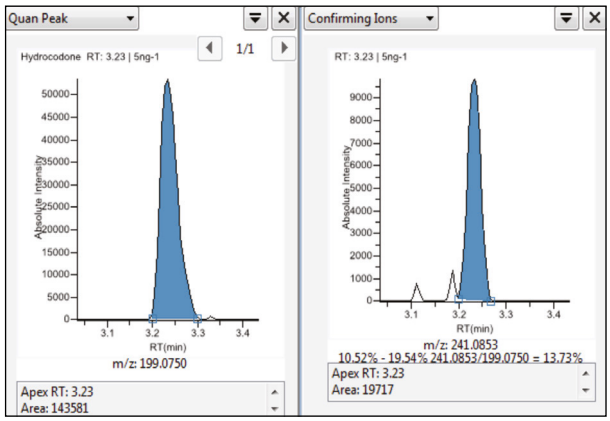
**Codeine LOQ = 2.5 ng/mL**



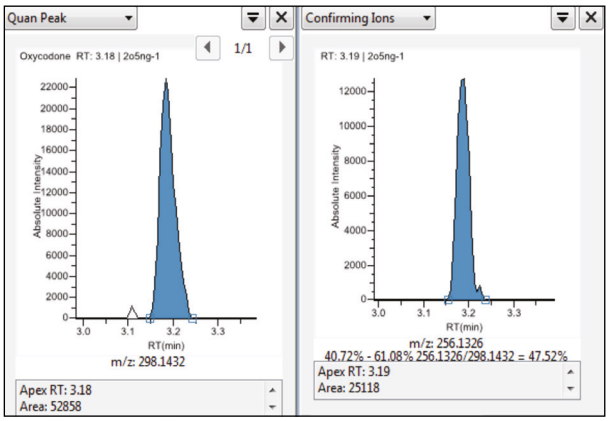

**Morphine LOQ = 5 ng/mL**



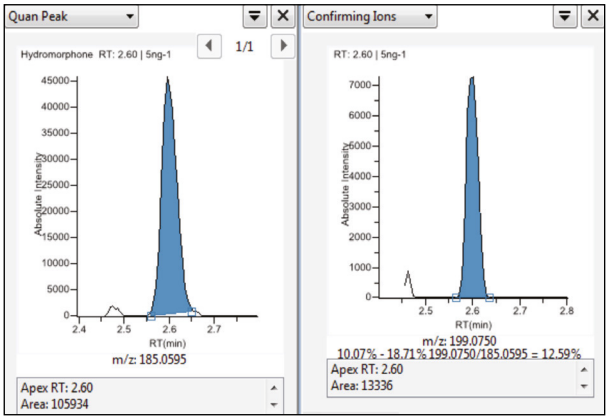

**Hydrocodone LOQ = 5 ng/mL**




**Oxycodone LOQ = 2.5 ng/mL**




**Hydromorphone LOQ = 5 ng/mL**




**Oxymorphone LOQ = 2.5 ng/mL**

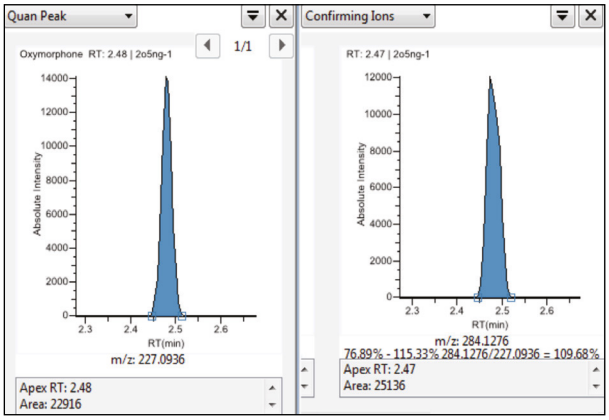
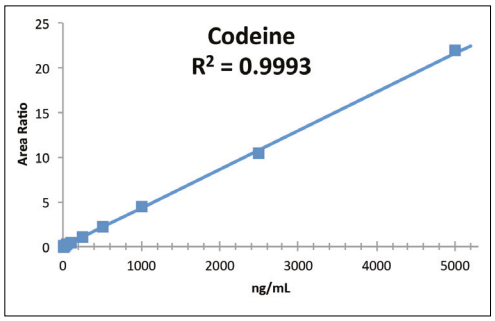
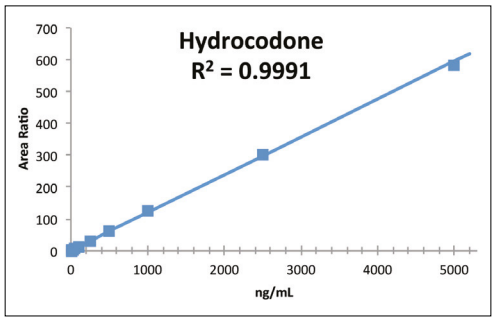


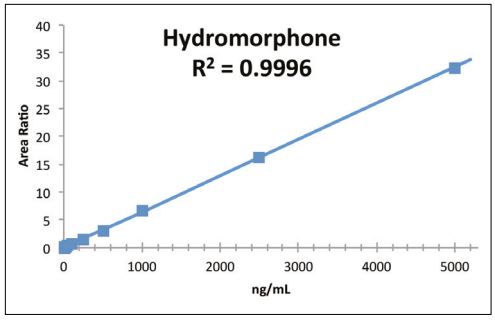
Figure 5. Representative calibration curves from PRM data for six opiates.



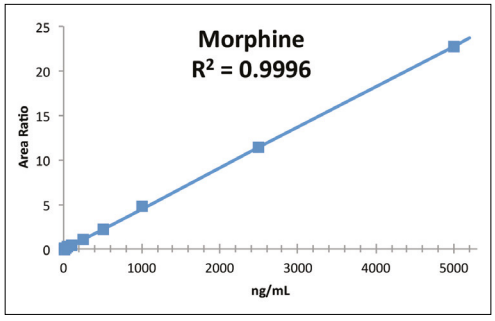

| ng/mL | %Diff |
|---|---|
| 5000 | 1.20 |
| 2500 | -3.69 |
| 1000 | 3.55 |
| 500 | -1.29 |
| 250 | -1.53 |
| 100 | 7.11 |
| 50 | 2.83 |
| 25 | -5.20 |
| 10 | -5.64 |
| 5 | 6.42 |
| 2.5 | -3.76 |



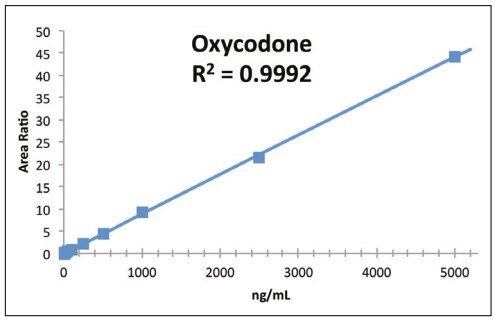

| ng/mL | %Diff |
|---|---|
| 5000 | -2.24 |
| 2500 | 1.63 |
| 1000 | 5.46 |
| 500 | 1.86 |
| 250 | -1.18 |
| 100 | 9.49 |
| 50 | 8.09 |
| 25 | -10.1 |
| 10 | -6.88 |
| 5 | -6.10 |



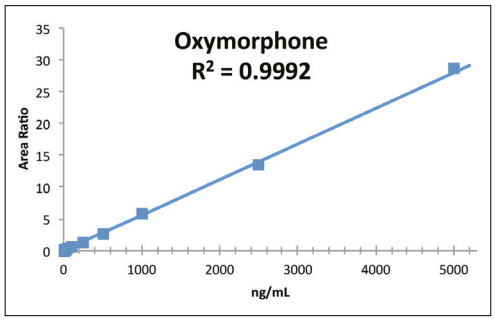

| ng/mL | %Diff |
|---|---|
| 5000 | -0.390 |
| 2500 | 0.580 |
| 1000 | 3.72 |
| 500 | -2.77 |
| 250 | -7.48 |
| 100 | 0.510 |
| 50 | 0.750 |
| 25 | -4.42 |
| 10 | 0.280 |
| 5 | 9.22 |




| ng/mL | %Diff |
|---|---|
| 5000 | -0.140 |
| 2500 | 0.260 |
| 1000 | 4.09 |
| 500 | -5.75 |
| 250 | -4.14 |
| 100 | -1.16 |
| 50 | 3.62 |
| 25 | -7.44 |
| 10 | -6.89 |
| 5 | 5.70 |



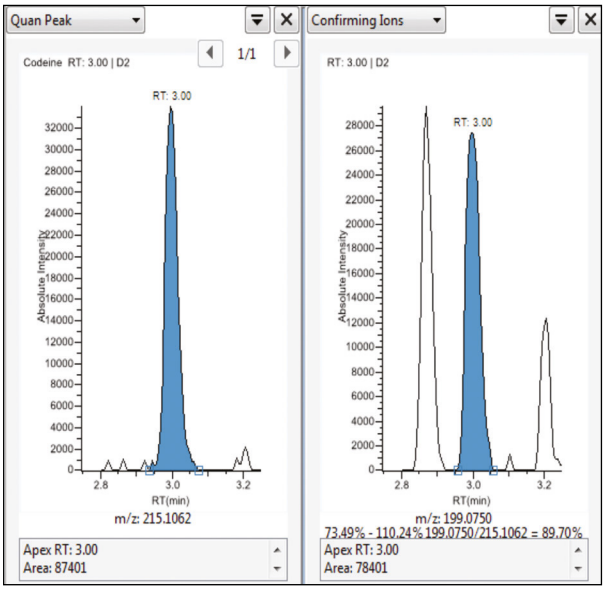

| ng/mL | %Diff |
|---|---|
| 5000 | -0.0200 |
| 2500 | -2.61 |
| 1000 | 6.71 |
| 500 | -0.130 |
| 250 | -2.87 |
| 100 | 4.51 |
| 50 | 6.80 |
| 25 | -0.630 |
| 10 | -4.42 |
| 5 | -7.32 |
| 2.5 | -4.00 |



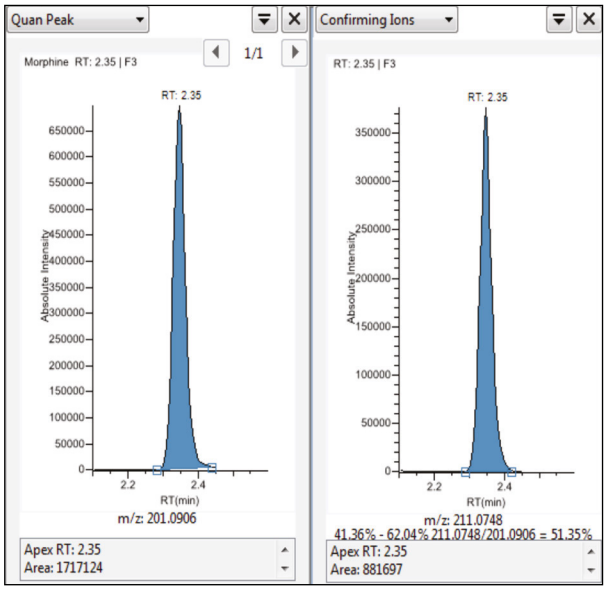

| ng/mL | %Diff |
|---|---|
| 5000 | 1.93 |
| 2500 | -3.57 |
| 1000 | 2.06 |
| 500 | -2.66 |
| 250 | -7.29 |
| 100 | 2.72 |
| 50 | 3.44 |
| 25 | -4.19 |
| 10 | -1.57 |
| 5 | 1.08 |
| 2.5 | 8.04 |

Figure 6. Extracted ion chromatogram from donor sample obtained in confirmation PRM experiment.

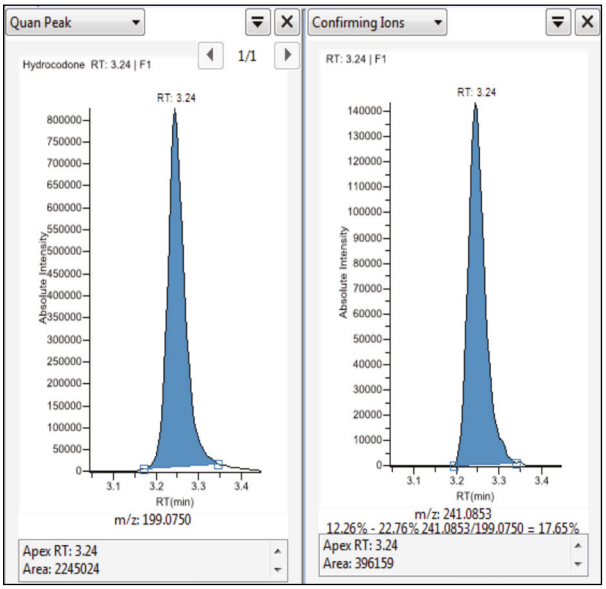
**Codeine in Donor D2, 12.1 ng/mL**



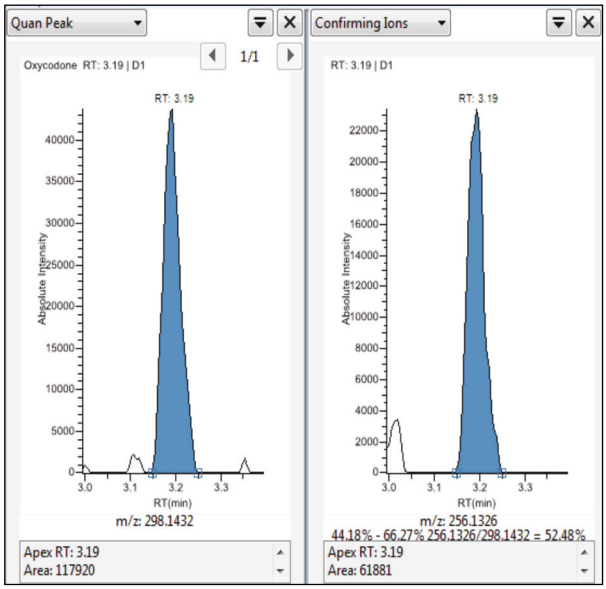

**Morphine in donor F3, 217 ng/mL**



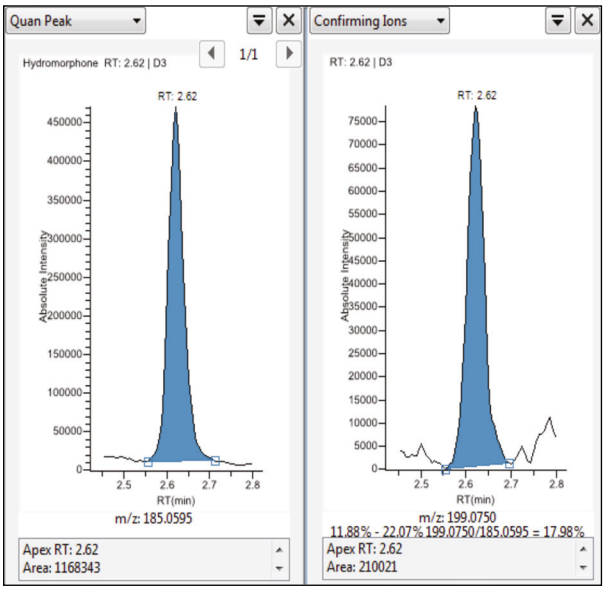

**Hydrocodone in Donor F1, 60.6 ng/mL**



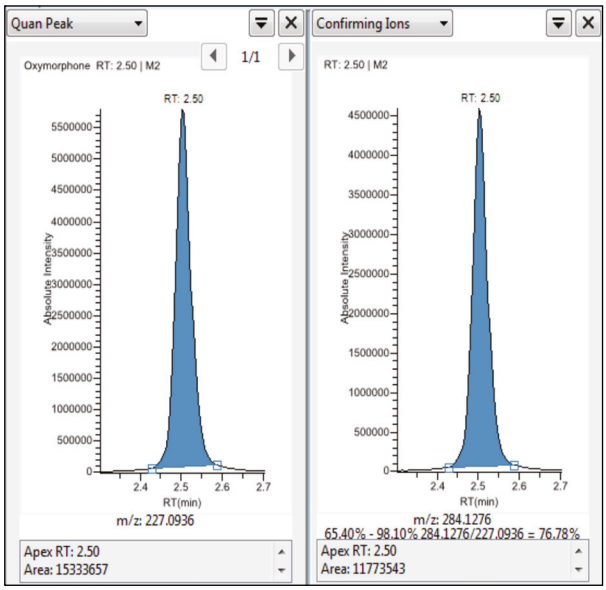

**Oxycodone in Donor D1, 4.38 ng/mL**




**Hydromorphone in Donor D3, 37.1 ng/mL**




**Oxymorphone in Donor M2, 698 ng/mL**



## Conclusion

The Q Exactive Focus MS accurately quantitated all six opiates tested to the low ng/mL level in human urine. This new instrument gives forensic laboratories a single versatile platform capable of both screening large panels[1] and quantitative confirmation of specific panels that provides performance with value.

## References

1. Kozak, M.; Van Natta, K., Thermo Fisher Scientific Application Note 616: Forensic Screening for Drugs in Urine Using High-Resolution MS/MS Spectra and Simplified High-Performance Screening Software, 2014.

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# Uncovering Tracks – Robust, Reproducible Screening Assay for Fentanyls in Urine with LC-HRAM(MS) for clinical research or forensic toxicology

Magnus Olin, Thermo Fisher Scientific, Hägersten, Sweden, Yufang Zheng, Unilabs, Eskilstuna Sweden

## ABSTRACT

**Purpose:** To develop and analytically validate a method for screening of fentanyl and fentanyl analogs in urine for clinical research or forensic toxicology.

**Methods:** The method is based on direct injection of urine into a UHPLC system coupled to a Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap High Resolution Accurate Mass (HRAM) mass spectrometer.

Results: Results were evaluated using Thermo Scientific™ TraceFinder™ software. The method performance was evaluated by testing selectivity, accuracy and precision at the proposed cutoff level, linearity and matrix effects.

## INTRODUCTION

Fentanyl is an opioid used as a pain medication together with other medications for anesthesia. Fentanyl and fentanyl analogues made illegally are also used as recreational drugs. Fentanyl and its analogues are significantly stronger than morphine, with some analogues (carfentanil) exhibiting ~10,000 times higher strength than regular pain medications. The use and abuse of fentanyl and its analogues are also known to cause serious side effects, ranging from respiratory depression to deaths. Problems related to fentanyl and its analogues are still prevalent in many countries according to reports from European Monitoring Centre for Drugs and Drug addiction (EMCDDA ). In 2017, deaths due to overdoses of fentanyl and fentanyl analogues were more common than deaths owing to heroin overdose in Sweden1.This study reports the development and analytical validation of a method for detection of fentanyl and some analogues in urine.

## MATERIALS AND METHODS

The Screening method was based on reversed phase liquid chromatography (LC) coupled to High Resolution Accurate Mass (HRAM) spectrometry for 14 different Fentanyl analogues and metabolites in urine has been developed using a Thermo Scientific™ Ultimate RS3000 UHPLC system and a Thermo Scientific Q Exactive Focus Orbitrap High Resolution Accurate Mass spectrometer operated in data dependent MS/MS (ddMS2) mode. Identification was based on m/z and retention time. Confirmation of the compounds was performed by matching of the MS2 spectrum of the target compound to the recorded library MS2 spectrum. Ion chromatograms were extracted at ± 5 ppm, and quantitation was based on a two-point calibration curve using internal standard calibration. Four different fentanyls labeled with stable isotopes were used as internal standards. The total analysis time was 5 minutes. Sample preparation was performed by dilution and direct injection of urine into LC-HRAM(MS)

### Sample Preparation

Urine was centrifuged at 10000xg for 5 minutes.10 µL urine was diluted with 100 µL Milli-Q® (MilliporeSigma) water containing internal standards. 10 µL was injected into the LC MS System.

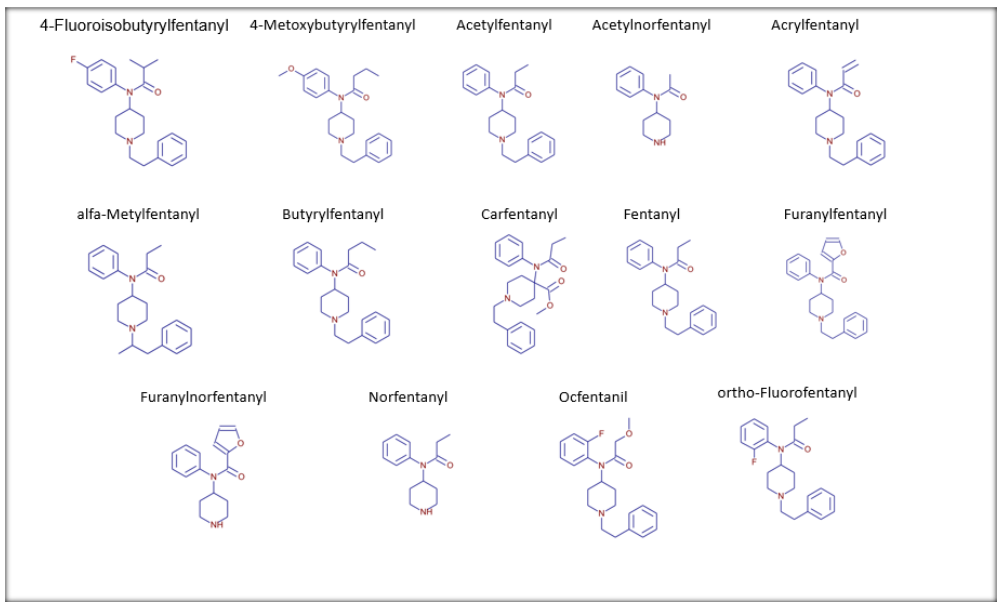
### Test Method(s)

14 different Fentanyls were included in the method. The compounds were chosen from previously reported intoxications. Four stable isotope-labelled internal standards were used for quantitation. Details are given in Table 1.

**Table 1. Compounds, internal standards included in the method and their monoisotopic masses**

| Analytes | Chemical Formula | Monoisotopic mass (M+H) | Internal Standards | Monoisotopic mass (M+H) | LOQ (ng/mL) |
|---|---|---|---|---|---|
| 4-Fluoroisobutyrylfentanyl | C23H29FN2O | 369.2337 | Fentanyl-D5 | 342.2588 | 2 |
| 4-Metoxybutyrylfentanyl | C24H32N2O2 | 381.2537 | Fentanyl-D5 | 342.2588 | 5 |
| Acetylfentanyl | C21H26N2O | 323.2118 | Acetylfentanyl-13C6 | 329.2319 | 1 |
| Acetylnorfentanyl | C13H18N2O | 219.1492 | Noracetylfentanyl-13C6 | 225.1693 | 2 |
| Acrylfentanyl | C22H26N2O | 335.2118 | Fentanyl-D5 | 342.2588 | 1 |
| alfa-Metylfentanyl | C23H30N2O | 351.2431 | Fentanyl-D5 | 342.2588 | 4 |
| Butyrylfentanyl | C23H30N2O | 351.2431 | Fentanyl-D5 | 342.2588 | 2 |
| Carfentanyl | C24H30N2O3 | 395.2329 | Fentanyl-D5 | 342.2588 | 2 |
| Fentanyl | C22H28N2O | 337.2274 | Fentanyl-D5 | 342.2588 | 0.5 |
| Furanylfentanyl | C24H26N2O2 | 375.2067 | Fentanyl-D5 | 342.2588 | 1 |
| Furanylnorfentanyl | C16H18N2O2 | 271.1441 | Norfentanyl-D5 | 238.1962 | 1 |
| Norfentanyl | C14H20N2O | 233.1648 | Norfentanyl-D5 | 238.1962 | 4 |
| Norfuranylfentanyl | C16H18N2O2 | 271.1441 | Norfentanyl-D5 | 238.1962 | 1 |
| Ocfentanil | C22H27FN2O2 | 371.2129 | Acetylfentanyl-13C6 | 238.1962 | 1 |
| ortho-Fluorofentanyl | C22H27FN2O | 355.2180 | Fentanyl-D5 | 342.2588 | 4 |

**Figure 1. Chemical structures of the compounds included in the method**




**LC-Method:**

The LC-system consisted of an Ultimate RS3000 UHPLC High Pressure Gradient pump. The LC-method is described in Table 2. Total cycle time for the method is 5 minutes/sample.

Mobile Phase A: Water + 0.1%NH4OH

Mobile Phase B: Methanol

Column: WATERS® ACQUITY BEH 2.1x100 mm (WATERS)

Temperature: 60 ° C

Injection Volume: 10 µL

**Table 2. LC method**

| Time (min) | Flow rate (mL/min) | %A | %B |
|---|---|---|---|
| 0 | 0.6 | 95 | 5 |
| 1.3 | 0.6 | 95 | 5 |
| 1.31 | 0.4 | 50 | 50 |
| 2.6 | 0.4 | 20 | 80 |
| 2.8 | 0.4 | 15 | 85 |
| 2.9 | 0.4 | 10 | 90 |
| 3 | 0.4 | 10 | 90 |
| 3.01 | 0.6 | 0 | 100 |
| 4 | 0.6 | 0 | 100 |
| 4.1 | 0.6 | 95 | 5 |
| 4.5 | 0.6 | 95 | 5 |

**MS-Method:**

A Q Exactive Focus Orbitrap High Resolution Accurate Mass mass spectrometer was operated in Full Scan/data dependent MS/MS (ddMS2) in confirmation mode (i.e. an inclusion list is used). The ion source settings are presented in Table 3, and the method settings are presented in Table 4.

**Table 3. Ion Source Parameters**

| Tune File | |
|---|---|
| Spray Voltage | 3500 |
| Capillary Temperature | 300 |
| Sheath Gas | 75 |
| Aux Gas | 12.5 |
| Sweep Gas | 2 |
| Probe Heater Temp. | 450 |
| S-Lens RF Level: | 90 |

**Table 4. Mass spec method**

| | |
|---|---|
| **General** | |
| Polarity | positive |
| dd-MS² | Confirmation |
| In-source CID | – |
| **Full MS** | |
| Scan range | 215 to 400 m/z |
| Resolution | 70,000 |
| # Scan ranges | 1 |
| AGC target | 1e6 |
| Maximum IT | auto |
| Microscans | 1 |
| Spectrum data type | Profile |
| **dd-MS² Confirmation** | |
| Apex trigger | – |
| Resolution | 17,500 |
| Isolation window | 1.5 m/z |
| Isolation offset | – |
| (N)CE / stepped (N)CE | ce: 30 |
| Fixed first mass | – |
| Default charge state | 1 |
| AGC target | 5e4 |
| Maximum IT | auto |
| Loop count | 2 |
| Minimum AGC target | 1.00 |
| Intensity threshold | auto |
| Dynamic exclusion | 1.0 s |
| Spectrum data type | Profile |

Identification was based on *m/z* and retention time. Confirmation of the compounds was performed by matching of the MS2 spectrum of the target compound to the recorded library MS2 spectrum. Ion chromatograms were extracted at ± 5 ppm, and quantitation was based on a two-point calibration curve using internal standard calibration. Quantitation was performed to determine if detected concentrations were above the cutoff value of the method.

For validation, test samples were prepared by spiking blank human urine with known amounts of test compounds. Certified test compounds were obtained from Cerrilliant®, Chiron AS and Cayman Chemical.

TraceFinder software was used for data evaluation.

## RESULTS

### Results from Method Characterization

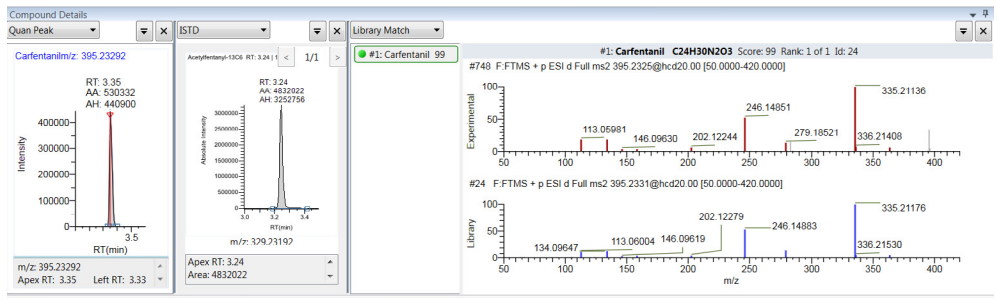
The method developed in this study was tested and analytically validated by monitoring accuracy of identification, linearity, matrix effects, accuracy and Coefficient of Variation (%CV) at the lower limit of quantitation. All compounds present in the spiked matrix were detected and confirmed at 0.5 – 5 ng/mL. The accuracy (bias%) and %CV was within ±15% for all compounds. The accuracy and precision (%CV) at the lower limit of quantitation is presented in table 5.

**Table 5. Accuracy and precision at the cutoff level**

| | 4-Fluoroisobutyryl fentanyl | 4-Metoxybutyryl fentanyl | Acetylfentanyl | Acetylnorfentanyl | Acrylfentanyl | alfa-Metylfentanyl | Butyrylfentanyl |
|---|---|---|---|---|---|---|---|
| Nominal conc (ng/mL) | 2 | 5 | 1 | 2 | 1 | 4 | 2 |
| Mean | 2.15 | 4.93 | 0.81 | 2.29 | 0.96 | 4.00 | 2.00 |
| Accuray% | 107.6 | 98.6 | 80.9 | 114.5 | 95.6 | 99.9 | 99.9 |
| CV% | 5.5 | 13.4 | 4.0 | 1.8 | 8.0 | 7.7 | 7.7 |
| | **Carfentanil** | **Fentanyl** | **Furanylfentanyl** | **Furanylnorfentanyl** | **Norfentanyl** | **Ocfentanil** | **ortho-Fluorofentanyl** |
| Nominal conc (ng/mL) | 0.5 | 0.5 | 1 | 1 | 4 | 1 | 4 |
| Mean | 0.51 | 0.49 | 1.00 | 1.03 | 3.80 | 0.96 | 4.24 |
| Accuray% | 102.4 | 98.4 | 99.9 | 103.2 | 94.9 | 95.9 | 106.0 |
| CV% | 5.0 | 4.0 | 7.7 | 6.0 | 3.0 | 2.5 | 4.5 |

The identity of all compounds was confirmed by the accurate mass (±5 ppm), the retention time (± 0.25 min) and matching against a spectral library (reversed search) recorded using pure standards. An example is presented in figure 2.

**Figure 2. Example of confirmation: Carfentanil at 0.5 ng/mL**




The identity of Carfentanil was confirmed by the exact mass ±5ppm, the retention time and matching against a library spectrum. Regular

The linearity of the method was good at 50-1000 ng/mL for all compounds. Most of the compounds also showed linearity from 0.5-10 ng/mL. The Lower Limit of quantitation was set with regards to this.

The stability of all compounds in urine was good at room temperature (nominally 22 ° C), in refrigerator (nominally 4° C) and in refrigeration (nominally -18° C).

## DISCUSSION

There is a steady increase in use and demand of methods for known/unknown screening and untargeted/targeted quantitation of analytes in complex biological matrices. Methods based on Thermo Scientific Q Exactive are suitable for this task since data can be collected in untargeted mode, which makes gives the possibility for retrospective interrogation of data. The instrument is sensitive enough for quantitation, even at the low levels required for Fentanyls, and it can provide HRAM spectra that together with retention time and accurate mass.

## CONCLUSIONS

In this study, we report a robust, reliable, and reproducible method for screening of Fentanyls in Urine for clinical research or forensic toxicology.

## REFERENCES

1. Swedish National Threat Assessment on fentanyl analogues and other synthetic opioids, Swedish National Board of Forensics, The Swedish Police Authority, National Operations Department, A503.217/2017, October 2019

## TRADEMARKS/LICENSING



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# Forensic Screening for Drugs in Urine Using High-Resolution MS/MS Spectra and Simplified High-Performance Screening Software

Marta Kozak, Kristine Van Natta, Thermo Fisher Scientific, San Jose, CA

## Key Words

Q Exactive Focus, ToxFinder, forensic toxicology, screening, drugs of abuse

## Goal

To evaluate the performance of the Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap mass spectrometer as an LC-MS/MS screening platform for forensic detection and quantitation of very large numbers of drugs in human urine.

## Introduction

Forensic toxicologists need an economical solution to screen for a virtually unlimited number of compounds in urine. Here we present a method for screening using a dilute-and-shoot approach with the Q Exactive Focus mass spectrometer and Thermo Scientific™ ToxFinder™ software.

## Experimental Methods

### Sample Preparation

Samples were processed by simple dilution. Briefly, an aliquot of centrifuged urine was spiked with stable-isotope-labeled internal standard and diluted 30-fold before an aliquot was analyzed by gradient HPLC on a Q Exactive Focus mass spectrometer. No hydrolysis was performed. The internal standard used was tolbutamide-$d_9$. This compound was used for its versatility because it ionizes in both positive and negative mode. Limits of detection were determined by spiking compounds of interest into pooled blank urine in the range of 1 to 500 ng/mL.

### Liquid Chromatography

Gradient elution was performed using a Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC with OAS autosampler (Figure 1). Mobile phases consisted of 10 mM ammonium formate in water and methanol (Fisher Chemical brand) for mobile phases A and B, respectively. The column used was a Thermo Scientific™ Accucore™ PFP column, 2.6 µm, 100 x 2.1 mm fused core (PN 17426-102130). The gradient was run at a flow rate of 0.45 mL/min from 5 to 100% mobile phase B over 6 minutes followed by a column wash and re-equilibration to starting conditions. The total run time was 10 minutes.

Figure 1. Q Exactive Focus MS with UltiMate 3000 RSLC pump and UltiMate 3000 OAS autosampler.

### Mass Spectrometry

Compounds were detected on a Q Exactive Focus hybrid quadrupole-Orbitrap mass spectrometer equipped with a Thermo Scientific™ Ion Max source and heated electrospray ionization (HESI II) sprayer. Data were acquired in full-scan data-dependent $MS^2$ ($ddMS^2$) mode. In this mode, both positive and negative high-resolution, full-scan data at resolution of 70k were collected, then $MS^2$ spectra at a resolution of 17.5k were triggered for compounds entered in the inclusion list (Figure 2). Figure 3 shows an example of the data acquired with this method.


Figure 2. Diagram of data-dependent $MS^2$ method for detection and quantitation of drugs in urine.

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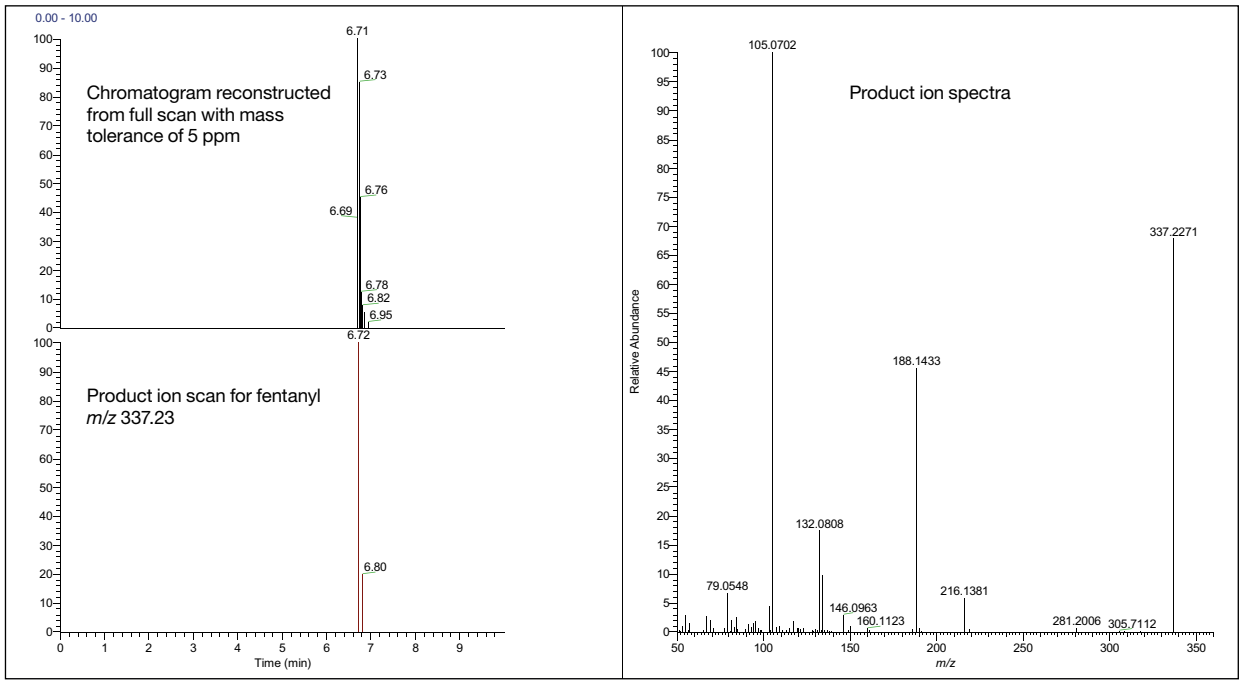


Figure 3. Fentanyl data, showing full scan at 5 ppm, product ion scan, and product ion spectra.

## Method Evaluation

Three hundred compounds, both positively and negatively ionizing, from different classes including therapeutic drugs, drugs of abuse, and environmental toxins, were selected for evaluation. Spiking solutions of 8–10 compounds each were prepared and used to make test mixes at concentration of 500, 100, 50, 10, 5, and 1 ng/mL in pooled donor urine.

Test mixes were processed using the previously described sample preparation procedure and then analyzed. Additionally, positive donor samples from a collaborator laboratory were processed and analyzed.

## Data Analysis

Data was processed using ToxFinder software. ToxFinder software uses a database that contains compound-related information and tolerances for identification. It also utilizes proprietary spectral libraries including forensic toxicology libraries containing drugs of abuse, therapeutic drugs, and environmental toxins, and food safety and environmental libraries containing pesticides, mycotoxins, veterinary drugs, and PFCs. Other important features include semi-quantitation, relative retention time calculation, a custom reporting package, and easy output for importation into LIMS systems.

The ToxFinder software database and libraries are combined into a processing method (Figure 4). After a method is created, the analyst imports a sample list and processes the data. Results can be printed immediately or reviewed before printing (Figure 5).

In this note, the primary method identified compounds based on retention time, accurate *m/z*, and spectral library matching. The LOD/cut-off for each compound was determined to be the lowest spiked concentration in which peaks were identified by the ToxFinder software. If even greater identification confidence is required, isotopic pattern matching can also be added to the method parameters. Table 1 shows the parameters used in each method.

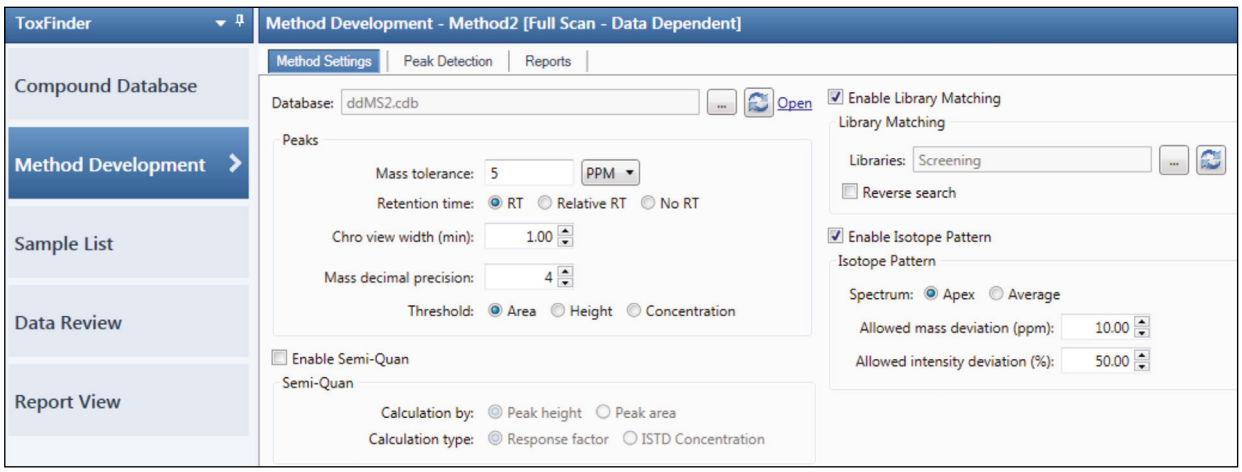


Figure 4. Example of a ToxFinder method using library matching (Method #2).

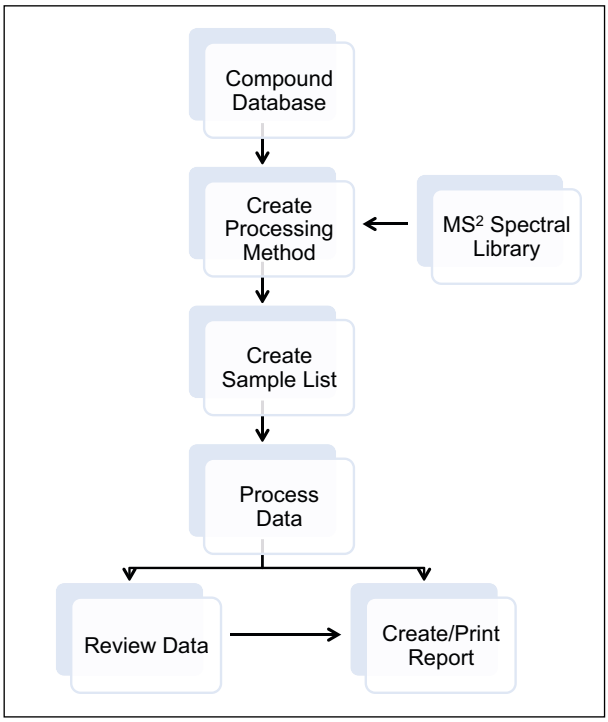


Figure 5. ToxFinder software workflow.

Table 1. ToxFinder method parameters used for compound identification.

| Method 1 | Method 2 |
|---|---|
| Accurate *m/z* | Accurate *m/z* |
| Retention time | Retention time |
| Library search | Library search |
| — | Isotopic pattern |

## Results

Using the primary data processing method, the vast majority of compounds analyzed had detection limits at or below 10 ng/mL. Figure 6 shows the number of compounds detected at each concentration, and Table 2 shows the limits of detection for all compounds. When the additional requirement of isotopic pattern matching was employed, the limits of detection were slightly higher. This is to be expected because of the naturally lower abundance of isotopic ions. Figure 7 shows the ToxFinder software Data Review page with precursor scan, library search results matching, and isotopic pattern comparison results for oxycodone. Complete results for this method are again shown in Figure 6 and Table 2.

Figure 8 shows reconstructed chromatograms for compounds detected in a donor urine sample. Figure 9 shows the ToxFinder results for cyclobenzaprine from the same samples.

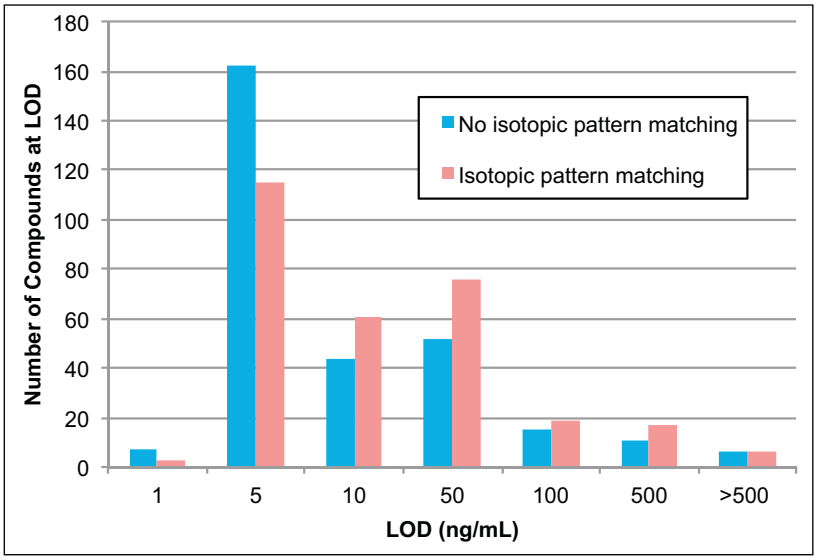


Figure 6. Number of compounds at each limit of detection using ToxFinder methods with and without isotopic pattern matching.

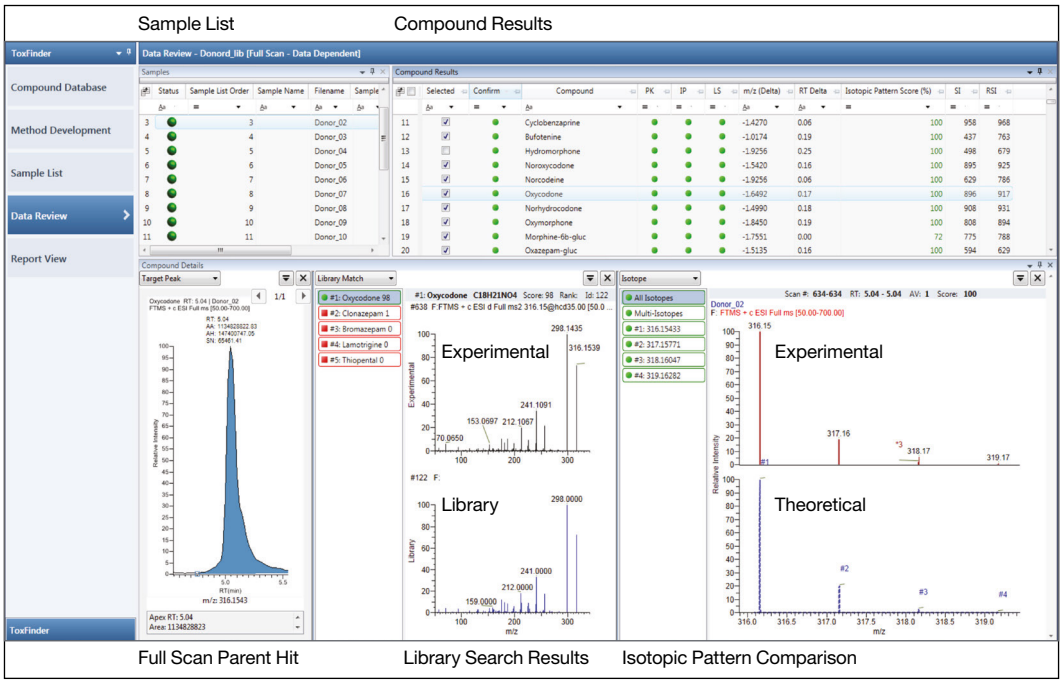


Figure 7. ToxFinder software data review page.

Table 2. Limits of detection for compound, with and without isotopic pattern matching.

| Analyte | LOD (ng/mL) | |
|---|---|---|
| | No Isotopic Pattern Matching | Isotopic Pattern Matching |
| 1-(3-Chlorophenyl)-Piperizine | 5 | 5 |
| 1,1-Dimethylbiguanide | 50 | 50 |
| 2-Bromo-Alpha-Ergocryptine | 10 | 50 |
| 4bromo-2,5dimethoxyphenethylamine | 5 | 10 |
| 6-Acetylcodeine | 5 | 5 |
| 6-Acetylmorphine | 50 | 5 |
| 7-Amino-Flunitrazepam | 10 | 10 |
| Acebutolol | 5 | 5 |
| Acetaminophen | 5 | 5 |
| Albuterol | 5 | 10 |
| Allobarbital | 100 | 500 |
| Alprazolam | 5 | 5 |
| Alprenolol | 50 | 50 |
| Aminorex | 5 | 5 |
| Amitriptyline | 1 | 5 |
| Amobarbital | 100 | 500 |
| Amoxapine | 5 | 10 |
| Amphetamine | 5 | 10 |
| AnhydroecgonineMethylEster | 1 | 5 |
| Antipyrine | 5 | 5 |
| Apomorphine | 500 | 500 |
| Aprobarbital | 100 | 500 |
| Astemizole | 50 | 500 |
| Atenolol | 5 | 5 |
| Atropine | 5 | 5 |
| Barbital | >500 | >500 |
| BDB | 5 | 5 |
| Benzocaine | 10 | 10 |

| Analyte | LOD (ng/mL) | |
|---|---|---|
| | No Isotopic Pattern Matching | Isotopic Pattern Matching |
| Benzoylecgonine | 5 | 5 |
| Benzylpiperazine | 5 | 10 |
| Betamethasone | 50 | 50 |
| Betaxolol | 5 | 10 |
| Bisoprolol | 10 | 10 |
| Bromazepam | 50 | 50 |
| Brompheniramine | 5 | 10 |
| Bufotenine | 10 | 50 |
| Bupivocaine | 5 | 5 |
| Buprenorphine | 5 | 10 |
| Buprenorphine-glucuronide | >500 | >500 |
| Buspirone | 1 | 1 |
| Butabarbital | 100 | 500 |
| Butorphanol | 5 | 5 |
| Caffeine | 5 | 5 |
| Carbamazepine | 5 | 5 |
| Carbinoxamine | 5 | 5 |
| Chloroquine | 500 | 500 |
| Chlorothiazide | 50 | 50 |
| Chlorpromazine | 10 | 50 |
| Chlorprothixene | 10 | 50 |
| Cimetidine | 10 | 100 |
| Cinnarizine | 100 | 100 |
| Ciprofloxacin | 100 | 100 |
| Cisapride | 5 | 50 |
| Citalopram | 5 | 5 |
| Clozapine | 5 | 10 |
| Clenbuterol | 10 | 50 |

| Analyte | LOD (ng/mL) | |
|---|---|---|
| | No Isotopic Pattern Matching | Isotopic Pattern Matching |
| Clobazam | 5 | 5 |
| Clomipramine | 5 | 10 |
| Clonazepam | 10 | 10 |
| Clozapine N-Oxide | 50 | 50 |
| Cocaethylene | 5 | 5 |
| Cocaine | 5 | 5 |
| Codeine | 5 | 5 |
| Codeine-glucuronide | >500 | >500 |
| Coumetetrayl | 5 | 5 |
| Cyclobenzaprine | 5 | 5 |
| Desalkylflurazepam | 10 | 10 |
| Desipramine | 5 | 5 |
| Desmethyl-citalopram | 5 | 5 |
| Desmethyl-clomipramine | 5 | 5 |
| Desmethyldoxepin | 5 | 5 |
| Desmethyl-flunitrazepam | 10 | 50 |
| Desmethyl-selegiline | 5 | 5 |
| Dexamethasone | 50 | 50 |
| Dextromethorphan | 5 | 5 |
| Diazepam | 5 | 10 |
| Diclofenac | 10 | 50 |
| Dihydrocodeine | 5 | 5 |
| Disopyramide | 1 | 5 |
| Dothiepin | 10 | 50 |
| Doxepin | 5 | 5 |
| Doxylamine | 5 | 5 |
| EcgonineMethylEster | 5 | 5 |
| EDDP | 5 | 5 |
| EMDP | 10 | 10 |
| Enalapril | 10 | 10 |
| Ephedrine | 10 | 10 |
| Estazolam | 5 | 5 |
| Ethylamphetamine | 5 | 5 |
| Etomidate | 5 | 5 |
| Fendiline | 10 | 10 |
| Fenoprofen | 500 | 500 |
| Fentanyl | 1 | 1 |
| Flecainide | 5 | 5 |
| Flumethasone | 50 | 50 |
| Flunitrazepam | 5 | 5 |
| Flunixin | 5 | 5 |
| Fluoxetine | 10 | 10 |
| Fluphenazine | 50 | 50 |
| Flurazepam | 5 | 5 |
| Flurbiprofen | >500 | >500 |
| Fluvoxamine | 5 | 10 |
| Furosemide | 100 | 100 |
| Gabapentin | 10 | 50 |
| Glafenine | 10 | 10 |
| Gliclazide | 5 | 50 |

| Analyte | LOD (ng/mL) | |
|---|---|---|
| | No Isotopic Pattern Matching | Isotopic Pattern Matching |
| Glimepiride | 50 | 100 |
| Glipizide | 50 | 100 |
| Glutethimide | 50 | 50 |
| Glyburide | 50 | 100 |
| Haloperidol | 5 | 50 |
| Heroin | 500 | 500 |
| Hexobarbital | 500 | 500 |
| HMMA | 50 | 50 |
| Hydrocodone | 10 | 10 |
| Hydromorphone | 5 | 10 |
| Hydroxy-Benzoylecgonine | 5 | 5 |
| Hydroxy-Ethyl-Flurazepam | 50 | 50 |
| Hydroxy-Midazolam | 50 | 50 |
| Hydroxy-Nordiazepam | 5 | 5 |
| Hydroxy-Triazolam | 50 | 50 |
| Hydroxyzine | 50 | 50 |
| Ibogaine | 5 | 5 |
| Imipramine | 5 | 5 |
| Indomethacin | 1 | 5 |
| Isocaffeine | 5 | 5 |
| Isoproterenol | 500 | 500 |
| Ketamine | 5 | 10 |
| Ketoconazole | 10 | 50 |
| Ketoprofen | 10 | 10 |
| Ketorolac | 5 | 50 |
| Labetalol | 50 | 50 |
| Lamotrigine | 5 | 5 |
| Levotiracetam | 50 | 50 |
| Lidocaine | 5 | 5 |
| Loratadine | 50 | 50 |
| Lorazepam | 10 | 50 |
| Lorazepam-glucuronide | >500 | >500 |
| Lormetazepam | 5 | 50 |
| LSD | 5 | 5 |
| Malathion | 50 | 100 |
| Maprotiline | 5 | 5 |
| MBDB | 5 | 5 |
| MDA | 5 | 5 |
| MDEA | 5 | 5 |
| MDMA | 5 | 5 |
| MeclofenamicAcid | 500 | 500 |
| Meperidine | 5 | 5 |
| Mepivacaine | 5 | 5 |
| Meprobamate | 50 | 50 |
| Mescaline | 10 | 50 |
| Mesoridazine | 5 | 50 |
| Metaproterenol | 10 | 50 |
| Methadone | 5 | 5 |
| Methamphetamine | 5 | 10 |
| Methaqualone | 5 | 5 |

| Analyte | LOD (ng/mL) | |
|---|---|---|
| | No Isotopic Pattern Matching | Isotopic Pattern Matching |
| **Methohexital** | 500 | 500 |
| **Methotrexate** | 50 | 50 |
| **Methoxyverapamil** | 5 | 5 |
| **Methylphenydate** | 5 | 5 |
| **Methyprylon** | 5 | 5 |
| **Metoclopramide** | 5 | 5 |
| **Metronidazole** | 5 | 50 |
| **Mexiletine** | 5 | 5 |
| **Mianserin** | 5 | 5 |
| **Miconazole** | 500 | 500 |
| **Midazolam** | 5 | 5 |
| **Mirtazapine** | 5 | 5 |
| **Molsidomine** | 5 | 10 |
| **Morphine** | 1 | 1 |
| **Morphine-3-beta-glucuronide** | 100 | 500 |
| **Morphine-6-beta-glucuronide** | >500 | >500 |
| **Nabumetone** | 100 | 100 |
| **N-Acetylprocainamide** | 5 | 10 |
| **Nalbuphine** | 5 | 5 |
| **Nalorphine** | 5 | 10 |
| **Naloxone** | 5 | 5 |
| **Naltrexol** | 5 | 5 |
| **Naltrexone** | 5 | 5 |
| **Naproxen** | 10 | 50 |
| **N-desmethyl-cis-tramadol** | 50 | 50 |
| **N-Desmethylselegiline** | 10 | 10 |
| **N-Desmethyltrimipramine** | 5 | 5 |
| **Nicardipine** | 50 | 50 |
| **Nifedipine** | 50 | 50 |
| **Nimodipine** | 50 | 50 |
| **Nitrazepam** | 5 | 5 |
| **Nitrendipine** | 50 | 50 |
| **Nizatidine** | 50 | 50 |
| **Norbenzoylecgonine** | 5 | 10 |
| **Norbuprenorphine** | 10 | 50 |
| **Norcocaethylene** | 10 | 10 |
| **Norcocaine** | 5 | 5 |
| **Norcodeine** | 10 | 10 |
| **Nordiazepam** | 5 | 5 |
| **Nordoxepin** | 5 | 5 |
| **Norfentanyl** | 5 | 5 |
| **Norfluoxetine** | 50 | 50 |
| **Norhydrocodone** | 10 | 10 |
| **Norketamine** | 5 | 10 |
| **Nor-LSD** | 50 | 50 |
| **Normeperidine** | 5 | 50 |
| **Normorphine** | 10 | 50 |
| **Noroxycodone** | 5 | 10 |
| **Noroxymorphone** | 50 | 50 |
| **Norpropoxyphene** | 50 | 50 |
| **Nortriptyline** | 5 | 5 |

| Analyte | LOD (ng/mL) | |
|---|---|---|
| | No Isotopic Pattern Matching | Isotopic Pattern Matching |
| **Noscapine** | 10 | 10 |
| **O-demethyl-cis-tramadol** | 10 | 10 |
| **O-desmethyl-venlafaxine** | 50 | 50 |
| **Ondansetron** | 5 | 5 |
| **Opipramol** | 5 | 50 |
| **Oxazepam** | 5 | 5 |
| **Oxazepam-glucuronide** | >500 | >500 |
| **Oxcarbazepine** | 50 | 50 |
| **Oxycodone** | 5 | 10 |
| **Oxymorphone** | 5 | 10 |
| **Papaverine** | 5 | 5 |
| **Paraxanthine** | 5 | 10 |
| **Paroxetine** | 10 | 10 |
| **Pentazocine** | 5 | 10 |
| **Pentobarbital** | 50 | 100 |
| **Perphenazine** | 50 | 100 |
| **Phenobarbital** | 50 | 100 |
| **Phenolphthalein** | 50 | 50 |
| **Phentermine** | 10 | 10 |
| **Phenylpropanolamine** | 10 | 50 |
| **Phenyltoloxamine** | 5 | 5 |
| **Phenytoin** | 500 | 500 |
| **Physostigmine** | 5 | 5 |
| **Pindolol** | 5 | 5 |
| **Piroxicam** | 100 | 100 |
| **PMA** | 5 | 50 |
| **PMMA** | 5 | 5 |
| **Prazosin** | 5 | 5 |
| **Prilocaine** | 5 | 5 |
| **Primidone** | 50 | 50 |
| **Procainamide** | 5 | 5 |
| **Procaine** | 5 | 5 |
| **Promazine** | 5 | 10 |
| **Promethazine** | 50 | 50 |
| **Prometryn** | 100 | 100 |
| **Propafenone** | 5 | 10 |
| **Propoxyphene** | 10 | 10 |
| **Propranolol** | 5 | 5 |
| **Protriptyline** | 5 | 5 |
| **Pseudoephedrine** | 5 | 5 |
| **Pyrilamine** | 5 | 5 |
| **Quetiapine** | 5 | 50 |
| **Quinidine** | 5 | 5 |
| **Quinine** | 1 | 1 |
| **Ranitidine** | 50 | 50 |
| **Risperidone** | 5 | 5 |
| **Ritalinic Acid** | 5 | 10 |
| **Scopolamine** | 5 | 10 |
| **Secobarbital** | 100 | 500 |
| **Selegiline** | 50 | 50 |
| **Sertraline** | 10 | 50 |

| Analyte | LOD (ng/mL) | |
|---|---|---|
| | No Isotopic Pattern Matching | Isotopic Pattern Matching |
| **Sotalol** | 5 | 5 |
| **Spironolactone** | 50 | 50 |
| **Strychnine** | 10 | 10 |
| **Sufentanil** | 50 | 50 |
| **Sulindac** | 10 | 50 |
| **Sulpiride** | 5 | 5 |
| **Tamoxifen** | 100 | 100 |
| **Tapentadol** | 5 | 5 |
| **Telmisartan** | 5 | 10 |
| **Temazepam** | 50 | 50 |
| **Tenoxicam** | 5 | 50 |
| **Terbutaline** | 5 | 10 |
| **Terfenadine** | 50 | 10050 |
| **Tetracaine** | 50 | 5 |
| **Theophylline** | 5 | 100 |
| **Thiopental** | 100 | 100 |
| **Thioridazine** | 100 | 50 |
| **Thiothixene** | 5 | 100 |
| **Tiagabine** | 5 | 5 |
| **Tiapride** | 5 | 5 |

| Analyte | LOD (ng/mL) | |
|---|---|---|
| | No Isotopic Pattern Matching | Isotopic Pattern Matching |
| **Timolol** | 5 | 5 |
| **Tolmetin** | 5 | 10 |
| **Topiramate** | 5 | 5 |
| **Tramadol** | 5 | 10 |
| **Trazodone** | 10 | 10 |
| **Triazolam** | 5 | 10 |
| **Trifluoperazine** | 10 | 50 |
| **Trimethoprim** | 5 | 5 |
| **Trimipramine** | 5 | 5 |
| **Triprolidine** | 5 | 5 |
| **Venlafaxine** | 5 | 105 |
| **Verapamil** | 5 | 5 |
| **Vincamine** | 5 | 5 |
| **Warfarin** | 5 | 5 |
| **Zaleplon** | 5 | 5 |
| **Zimelidine** | 5 | 10 |
| **Zolpidem** | 5 | 5 |
| **Zolpidem phenyl-4-COOH** | 10 | 10 |
| **Zonisamide** | 5 | 10 |
| **Zopiclone** | 500 | 500 |

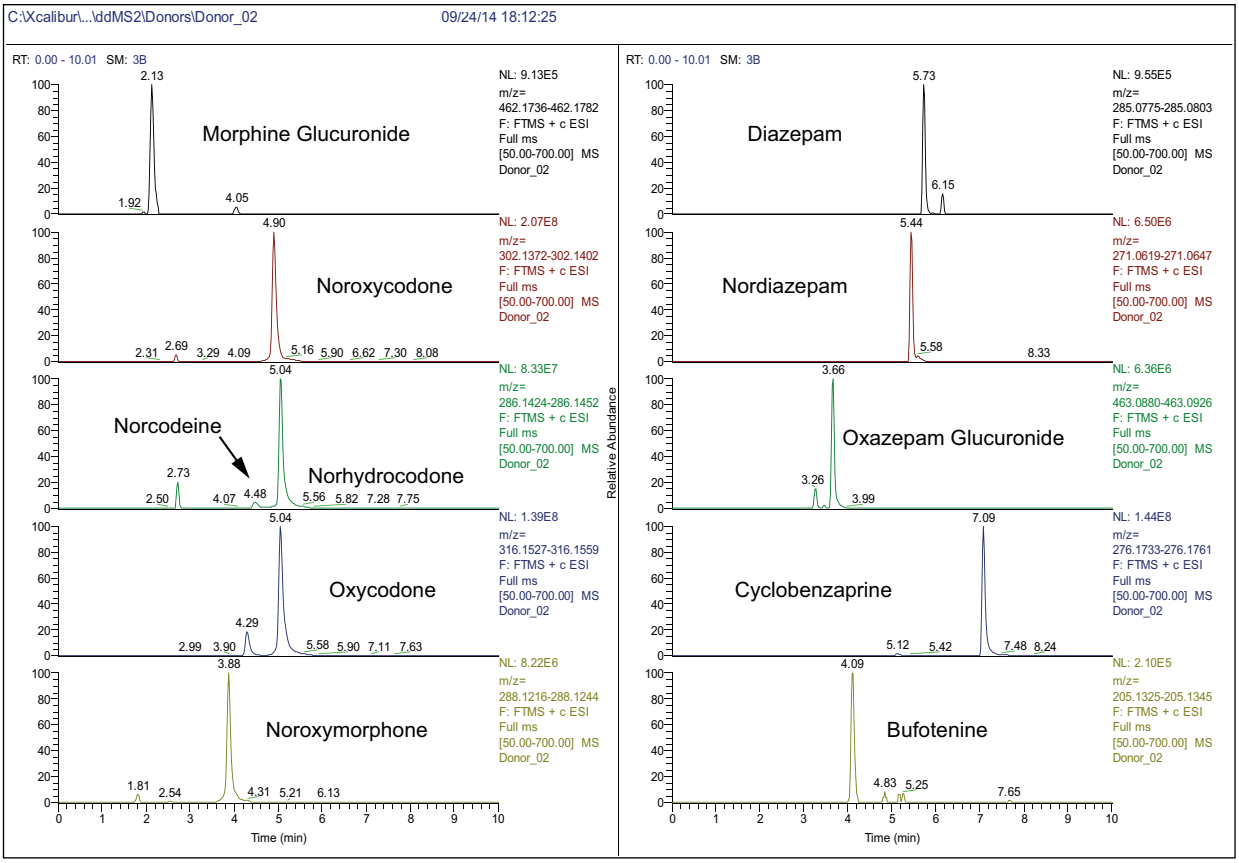


Figure 8. Donor #2 urine analysis results - identified compounds.

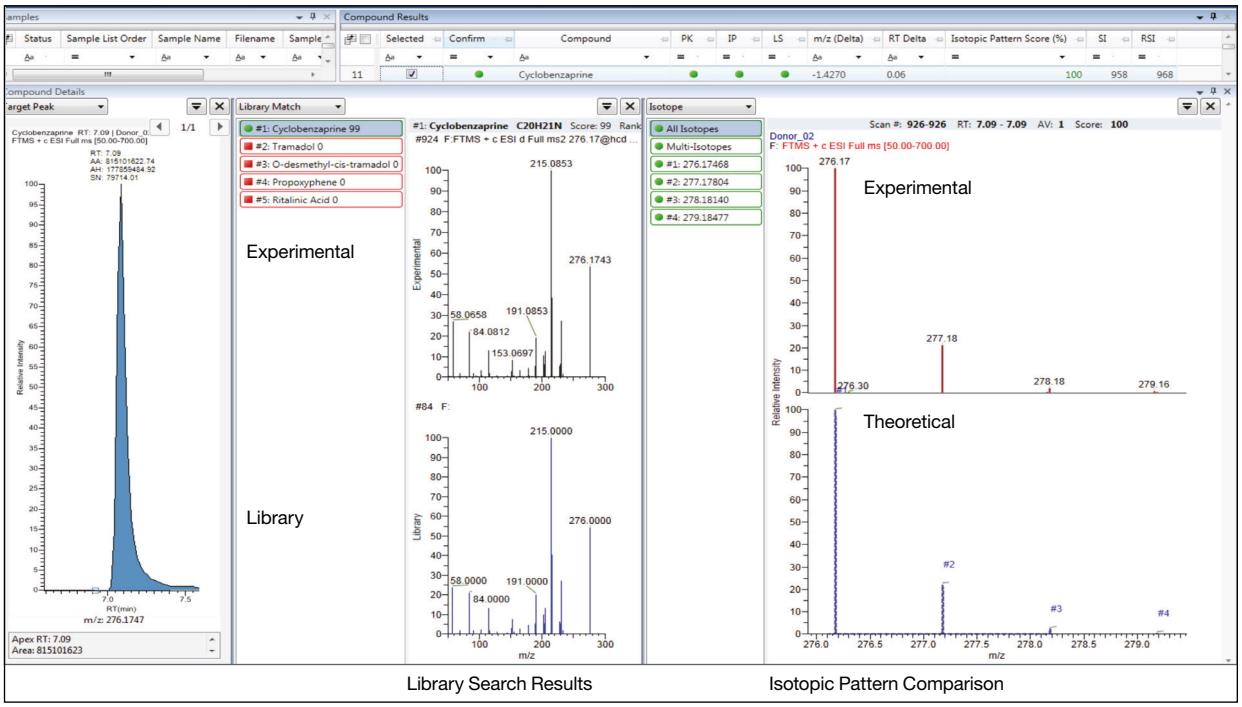


Figure 9. Cyclobenzaprine identified in donor sample.

## Conclusion

- A urine screening method for about 300 compounds, both positively and negatively ionizing, including drugs of abuse and environmental toxins, was successfully evaluated.
- Collected data demonstrated good method sensitivity and specificity in diluted urine samples.
- ToxFinder software's simple user interface enabled quick method development and rapid data review.
- The Q Exactive Focus mass spectrometer and ToxFinder software together provided high confidence in data output by combining the power of an Orbitrap mass analyzer with the comprehensive identification software workflow.

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# Targeted Forensic Screening and Semi-quantitation of Drugs in Plasma using High-resolution Accurate-mass Detection and On-line Sample Preparation

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## ABSTRACT

**Purpose:** The aim of this work was to generate a large forensic screening panel in a short chromatographic run. Then, the method was tested to combine the screening capabilities of a Thermo Scientific™ Q Exactive™ Focus mass spectrometer to the quantitation of 41 drugs in plasma matrix for a partial analytical validation of the screening method.

**Methods:** Two different analytical methods were used, one based on HPLC with a run time of 15.5 minutes, and the other based on on-line extraction using Thermo Scientific™ TurboFlow™ technology prior to HPLC separation, with a runtime of 16.75 minutes. For the generation of the spectral library and compound database, 1513 standard solution were injected with the both methods to obtain retention times and MS/MS spectra. The limit of detection (LOD), the limit of quantification (LOQ) and the limit of identification (LOI) were determined for 41 compounds in spiked plasma with the on-line extraction approach.

**Results:** A compound database and a spectral library for the screening of 1513 compounds were implemented on a Thermo Scientific™ Transcend™ II TLX-1 system coupled to a Q Exactive Focus Orbitrap high-resolution, accurate-mass spectrometer. A partial analytical method validation was performed in plasma. The compounds can be used as a basis for the method validation since they cover different drug classes, retention times and polarities.

## INTRODUCTION

In forensic toxicology, it is of high importance to be able to screen a large panel of compounds on a single injection of sample for further confirmation by more specific methods. Methods developed for this purpose need to use a low volume of sample and to include the capability of monitoring a very large panel of compounds; it is also desirable to reduce the runtime of these methods to increase throughput. The development of a spectral library and compound database for the screening and semi-quantitation of more than 1500 compounds in plasma samples, but which is applicable to other biological matrices, is reported. For each compound, the database includes the exact mass, chemical formula, retention time, and exact masses of main fragments.

**Figure 1. System configuration used for this work consisting on a Transcend II TLX1 system coupled to a Q Exactive Focus mass spectrometer.**

For a quantitation method, analytical validation is generally based on the evaluation of the LOQ and the intra-day and inter-day accuracy and precision. The approach is difficult to apply in this case considering the large number of compounds in the panel. This would suggest the preparation, injection, acquisition, and processing of data for more than 1400 compounds. Moreover, there are no official guidelines regarding the analytical validation of a screening method. A possible solution consists of selecting some compounds that are representative of different drug groups that appear in the complete retention time window of the chromatographic run, and that can present different polarities.

## MATERIALS AND METHODS

### Sample Preparation

Standard solutions for library generation were prepared in groups of 20 compounds at a concentration of 0.1 µg/mL in methanol/water 30:70 v/v solution.
Calibrators were prepared by spiking the compounds into blank plasma matrix from Innovative Research (Le Perray-en-Yvelines, France). Sample preparation previous to injection consisted of the precipitation of proteins as follows: 25 µL of a solution containing isotopically labeled internal standards (2 mg/L amphetamine-d5, 1 mg/L THC-COOH-d3, 5 mg/L haloperidol-d4, prazepam-d5 and morphine-d3, and 0.2 mg/L trimipramine-d3 in methanol) and 100 µL of acetonitrile were added to 100 µL of calibrator. After vortex mixing, the calibrators were centrifuged and the supernatant was transferred to a vial for sample injection.

### Liquid Chromatography

The system used for this method was a Transcend II TLX1 system. This system is presented in Figure 1. The system used allows for the use of either an HPLC-only method or an HPLC method combined with on-line extraction of the sample. Both methods are reported in table 1 and Table 2 accordingly.

**Table 1. Gradient conditions for the HPLC screening method**

| Step | Time (min) | Duration (s) | Loading pump | | | | | Tee | Loop | Eluting pump | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Flow | Grad | %A | %B | %C | | | Flow | Grad | %A | %B |
| 1 | 0 | 60 | 0 | Step | 100 | - | - | - | Out | 0.5 | Step | 99 | 1 |
| 2 | 1 | 540 | 0 | Step | 100 | - | - | - | Out | 0.5 | Ramp | 1 | 99 |
| 3 | 10 | 90 | 0 | Step | 100 | - | - | - | Out | 0.5 | Step | 1 | 99 |
| 4 | 11.5 | 240 | 0 | Step | 100 | - | - | - | Out | 0.5 | Step | 99 | 1 |

**Table 2. Gradient conditions for the TurboFlow extraction coupled to HPLC separation screening method**

| Step | Time (min) | Duration (s) | Loading pump | | | | | Tee | Loop | Eluting pump | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Flow | Grad | %A | %B | %C | | | Flow | Grad | %A | %B |
| 1 | 0 | 20 | 2 | Step | 100 | - | - | - | Out | 0.5 | Step | 99 | 1 |
| 2 | 0.3 | 5 | 0.5 | Step | 100 | - | - | - | In | 0.5 | Step | 99 | 1 |
| 3 | 0.4 | 60 | 0.5 | Step | 99 | 1 | - | T | In | 0.05 | Step | 99 | 1 |
| 4 | 1.4 | 540 | 0.5 | Ramp | 1 | 99 | - | T | In | 0.05 | Ramp | 1 | 99 |
| 5 | 10.4 | 90 | 0.5 | Step | 1 | 99 | - | T | In | 0.05 | Step | 1 | 99 |
| 6 | 11.9 | 10 | 1 | Step | - | - | 100 | - | In | 0.5 | Step | - | 100 |
| 7 | 12.1 | 10 | 1 | Step | 100 | - | - | - | In | 0.5 | Step | - | 100 |
| 8 | 12.2 | 10 | 1 | Step | - | - | 100 | - | In | 0.5 | Step | - | 100 |
| 9 | 12.4 | 10 | 1 | Step | 100 | - | - | - | In | 0.5 | Step | - | 100 |
| 10 | 12.6 | 10 | 1 | Step | - | - | 100 | - | In | 0.5 | Step | - | 100 |
| 11 | 12.8 | 60 | 0.3 | Step | 100 | - | - | T | In | 0.05 | Step | 99 | 1 |
| 12 | 13.8 | 180 | 1 | Step | 100 | - | - | - | In | 0.5 | Step | 99 | 1 |

### Mass Spectrometry

Data were acquired on a Q Exactive Focus Orbitrap mass spectrometer. The detection was performed by Full Scan acquisition in data dependent acquisition with an inclusion list. Full Scan data were acquired in both positive and negative mode with a resolution of 35,000 FWHM at *m/z* 200, and the $MS^2$ spectra for confirmation were acquired with a resolution of 17,500 FWHM at *m/z* 200. The experiment schematics are presented in Figure 2.

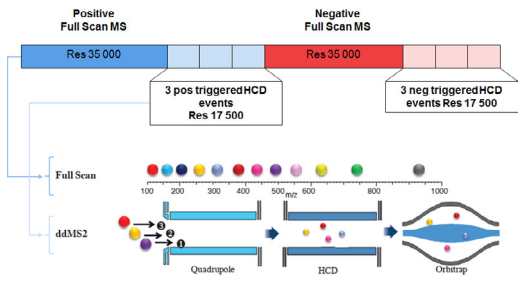


**Figure 2. Full Scan data dependent acquisition schematics**

### Data Analysis

Data were acquired and analysed with Thermo Scientific™ TraceFinder™ 4.1 software. TraceFinder software uses a database that contains compound-related information for identification and confirmation. It also uses proprietary $MS^2$ spectral libraries containing the spectra of the 1513 compounds tested. The spectra generated for this application were imported into a Thermo Scientific™ mzVault™ library. mzVault library is a new library search algorithm from mzCloud for improved library matching. mzCloud is a high resolution accurate masses fragmentation library available through the site: www.mzCloud.org. It contains spectral information on multi-energy, multi-level and multi-fragment techniques.

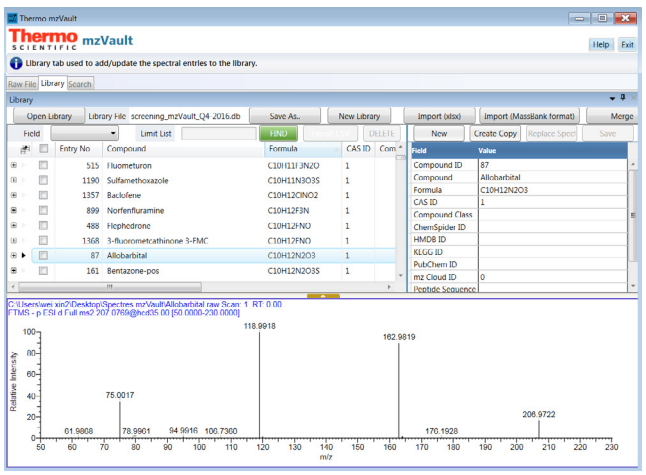


**Figure 3. View of the screening mzVault spectral library**

## RESULTS

A database containing compound related information was created for both methods, one using HPLC-only and one using TurboFlow technology on-line extraction on a Transcend II TLX1 system. For the development of the database, concentrated solutions were used. 1433 out of 1513 injected solutions were detected in both approaches. The somewhat lower number for the TurboFlow approach is due to poor retention of some of the analytes in the extraction columns. An example of the review of the data oriented to a screening approach in TraceFinder 4.1 software is presented in Figure 4.

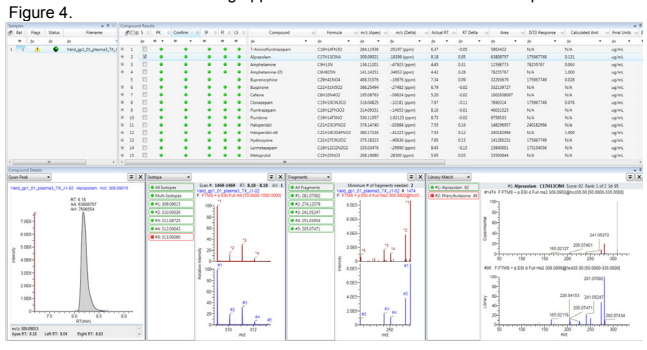

**Figure 4. View of the screening data review in TraceFinder 4.1 software**

The analytical method was then partially validated. To this end, 41 compounds were selected from the panel, covering different compound classes, retention times and polarities. The 41 compounds used for this stage were divided in three groups for the preparation of the calibrators according to the levels of concentration of the analytes to be assessed in plasma samples. Calibrators had concentrations going from 0.1 ng/mL to 250ng/mL for compounds on group A, and from 10ng/mL to 5000ng/mL for groups B and C. TraceFinder 4.1 software has the possibility to perform in the same batch a screening workflow with identification and confirmation of compounds, and to obtain a quantitative result based calibration curves. The quantitation data review is presented in Figure 5.

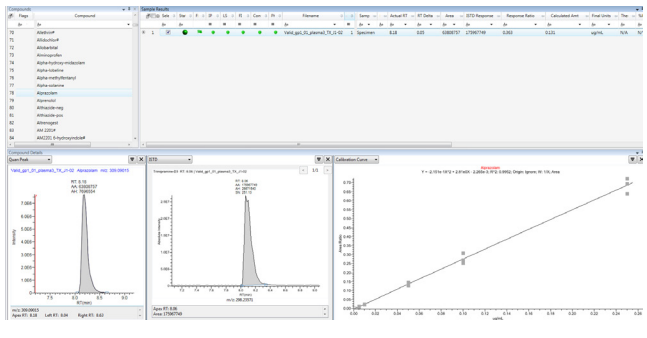

**Figure 5. View of the quantitative data review in TraceFinder 4.1 software**

For the TurboFlow approach, the limit of quantitation (LOQ), the limit of detection (LOD) and the limit of identification (LOI) were determined for spiked plasma samples. The LOD was obtained as the lowest concentration for which a peak is still observed for 3 different plasma matrices tested. The LOQ was obtained as the lowest concentration for which a quantitation has an accuracy with a bias inferior to 20% and a %RSD inferior as well to 20% for 3 repeated injections in three different plasma matrices. The bias determination was based on the calibration curves generated from 0.1 to 250ng/mL for group A compounds, and from 10ng/mL to 5000ng/mL for groups B and C. Finally, the LOI was determined as the lowest concentration for which a compound can be identified based on the following conditions: m/z of the parent (< 5 ppm), isotopic pattern match, fragment ion presence, and $MS^2$ spectra matching. The corresponding results are presented in Table 3.

**Table 3. LOD, LOQ and LOI obtained for 41 compounds with the TurboFlow method**

| Group | Compound | LOD (ng/mL) | LOQ (ng/mL) | LOI (ng/mL) |
|---|---|---|---|---|
| A | Alprazolam | 5 | 50 | 50 |
| | Amphetamine | 50 | 50 | 100 |
| | Buprenorphine | 5 | 5 | 50 |
| | Buspirone | 10 | 10 | 10 |
| | Clonazepam | 10 | 50 | 100 |
| | Flunitrazepam | 5 | 50 | 50 |
| | Haloperidol | 1 | 1 | 50 |
| | Hydroxyzine | 1 | 5 | 10 |
| | Lormetazepam | 10 | 10 | 100 |
| | Mianserine | 0.5 | 0.5 | 5 |
| | Morphine | 50 | 100 | 250 |
| | Olanzapine | 5 | 50 | 50 |
| | Prazepam | 5 | 5 | 50 |
| | Zopiclone | 50 | 50 | 100 |
| B | Amoxapine | 50 | 100 | 100 |
| | Chlordiazepoxide | 50 | 100 | 100 |
| | Chlorpromazine | 50 | 500 | 500 |
| | Doxepine | 50 | 50 | 50 |
| | EDDP | 50 | 100 | 100 |
| | Estazolam | 50 | 100 | 100 |
| | Fluoxetine | 50 | 1000 | 1000 |
| | Norclobazam | 100 | 1000 | 1000 |
| | Nordiazepam | 50 | 100 | 100 |
| | Nortriptyline | 50 | 100 | 100 |
| | Temazepam | 50 | 500 | 500 |
| | Amitriptyline | 10 | 50 | 50 |
| C | Bisoprolol | 10 | 50 | 50 |
| | Clobazam | 10 | 10 | 50 |
| | Clomipramine | 10 | 50 | 50 |
| | Clozapine | 10 | 10 | 50 |
| | Codeine | 10 | 10 | 50 |
| | Cyamemazine | 10 | 10 | 50 |
| | Desipramine | 10 | 10 | 10 |
| | Doxylamine | 10 | 50 | 50 |
| | Fluvoxamine | 50 | 50 | 50 |
| | Imipramine | 10 | 50 | 50 |
| | Levomepromazine | 10 | 50 | 50 |
| | Metformin | 50 | 250 | 500 |
| | Methadone | 10 | 50 | 50 |
| | Tramadol | 10 | 50 | 50 |
| | Trimipramine | 10 | 50 | 50 |

## CONCLUSIONS

- A compound database and a spectral library for the **forensic** screening of 1513 compounds were implemented on a Transcend II TLX-1 system coupled to a Q Exactive Focus Orbitrap high resolution accurate mass spectrometer.
- The panel includes compounds **of interest in forensic toxicology** both positively and negatively ionized such as drugs of abuse and metabolites, antidepressants, beta-blockers, antibiotics, pesticides and other classes.
- This opens possibilities to increase even more the screening panel to new substances.
- The drug screening method presented in this work covers a large panel of compounds with a short run time of 15.5 minutes and an option for an on-line extraction approach of only 16.75 minutes.
- Analytical validation for the TurboFlow method was performed on 41 compounds spiked in plasma matrix.
- The screening and quantitation workflows can be used both at the same time within an acquired batch in TraceFinder 4.1 software

## REFERENCES

1. Orbitrap technology for comprehensive metabolite-based liquid chromatographic-high resolution-tandem mass spectrometric urine drug screening - exemplified for cardiovascular drugs - Helfer A.G., Michely J.A., Weber A.A., Meyer M.R., Maurer H.H. – Anal Chim Acta, 891 (2015), 221-233.
2. LC-HR-MS/MS standard urine screening approach: Pros and cons of automated on-line extraction by turbulent flow chromatography versus dilute-and-shoot and comparison with established urine precipitation - Helfer A.G., Michely J.A., Weber A.A., Meyer M.R., Maurer H.H. – J Chromatogr B, 1043 (2017), 138-149.

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## TRADEMARKS/LICENSING



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# Selecting the best Q Exactive Orbitrap mass spectrometer scan mode for your application

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**Keywords**
Q Exactive hybrid quadrupole-Orbitrap series, data acquisition mode, scan mode, method transfer

## Introduction

Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap™ series mass spectrometers offer analysts a range of scan modes that provide functionality comparable to the modes provided by conventional tandem quadrupole mass spectrometers: the product ion, fragment ion, and data-dependent neutral loss trigger scan modes. Although the Q Exactive series mass spectrometers provide the analyst with a toolbox similar to that of triple quadrupole (QQQ) instruments, key differences exist between the two technologies. Most importantly, the Orbitrap analyzer of the Q Exactive series mass spectrometer is a trapping, not a scanning, device. Certain Q Exactive series mass spectrometer data acquisition modes may resemble conventional QQQ scan modes, but there are differences in their selectivity and sensitivity characteristics, as well as the number of data points acquired per unit time. This white paper describes each of the various scan modes, and the key differences between trapping and scanning devices. Method development guidance, including consideration of intrinsic dependencies such as resolution and scan speed, sensitivity, and selectivity, is also provided.[1]

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## General principles and considerations

As shown in Figure 1, the Q Exactive series mass spectrometer includes a quadrupole mass filter (blue) for either broad or narrow mass range transmission, a C-Trap (green) for ion storage, a higher-energy collision dissociation cell (HCD) for fragmentation (red), and the Thermo Scientific™ Orbitrap™ mass analyzer (purple) for ion detection and mass analysis. The importance of each of these parts is explained briefly below. More in-depth explanation of the instrument and its hardware features is provided in the reference publications.[2–6]

**Figure 1. Schematic of the Q Exactive series mass spectrometer.** Key parts of the instrument include the quadrupole (blue), C-Trap (green), higher-energy collision dissociation cell (HCD) (red), and the Orbitrap mass analyzer (purple).

The isolating quadrupole (Q) in front of the C-Trap is used as a mass filter for either wide (e.g. 10 to 100 Da isolation) or narrow (e.g., 0.4 to 2 Da isolation) mass range selection. High-end instruments, including the Thermo Scientific™ Q Exactive™ Plus hybrid quadrupole-Orbitrap™ mass spectrometer, Thermo Scientific™ Q Exactive™ HF hybrid quadrupole-Orbitrap™ mass spectrometer and Thermo Scientific™ Q Exactive™ HF-X hybrid quadrupole-Orbitrap™ mass spectrometer, utilize a segmented isolating quadrupole that provides sharper isolation and improved transmission compared to the Q Exactive and Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ mass spectrometers, which feature a hyperbolic quadrupole. Notably, the quadrupole is not used for scanning as it is in conventional tandem quadrupole mass spectrometers. Instead it is used purely for isolation. Additionally, mass spectrometer selectivity is obtained with narrower isolation, and $MS^2$ selectivity is gained with enhanced fragment resolution.

The C-Trap is a radio frequency (RF)-based ion collecting and cooling device. To avoid overfilling and space-charge effects, the C-Trap is controlled by automatic gain control (AGC) and the C-Trap Charge detector (CTCD) behind the HCD cell. The C-Trap directs ions either to the higher-energy collisional dissociation cell (HCD) for ion fragmentation, or to the Orbitrap mass analyzer for detection.

The HCD cell is used to fragment ions passed through the isolation quadrupole. Fragmentation with residual nitrogen gas occurs when ions are accelerated from the C-Trap into the HCD cell. Locating the HCD cell behind the C-Trap provides unique advantages, including parallelization and multiplexing. User-defined collision energies allow spectral multiplexing for up to 10 injections, which is not possible using quadrupole-time-of-flight (Q-TOF) instruments.

The Orbitrap mass analyzer acts as both an analyzer and a detector. Ions inside the Orbitrap mass analyzer undergo an extensive number of oscillations, producing ion separation and generates a time-varying signal, referred to as a transient, from which the mass spectrum may be derived. The length of a transient affects the mass resolving power but does not influence the measurement sensitivity. With Q-TOF instruments, sensitivity is reduced with higher resolving power. High-field (HF) Orbitrap mass analyzers are deployed on both the Q Exactive HF and Q Exactive HF-X mass spectrometers, allowing scan speeds of up to 40 Hz for MS and MS/MS scans.*

*Please note, the Orbitrap mass spectrometer is not a true scanning device, but it produces HRMS spectra like that obtainable when employing a quadrupole. Hence for convenience reasons, the word “scan” is used throughout the white paper.

Both the Q Exactive series mass spectrometers and QQQ instruments perform tandem MS. However, the technologies differ in how product ions are measured and, most significantly, the resulting mass resolution. Compared to QQQ technology, Q Exactive series instruments provide a much higher mass resolving power for product ion detection and full mass range scan analysis.

Another difference between technologies is that the second high-resolution mass spectrometer (HRMS) dimension does not continuously measure ions. Instead, it processes pulsed ion beams in a sequential manner. Here, the Q Exactive mass spectrometer technology is similar to Q-TOF technology in that both measure sequential ion pulses. The Q Exactive series mass spectrometer however provides a significantly greater mass resolving power (up to 280,000 (FWHM) at *m/z* 200 on the Q Exactive Plus mass spectrometer) and does not require spectral averaging for high-confidence data generation. The Q Exactive HF mass spectrometer can be used to monitor six data points at a resolution setting of 60 k (FWHM) at *m/z* 200, or 12 at resolution setting of 30 k (FWHM) at *m/z* 200, which is sufficient for both screening and quantitation with ultra-high performance liquid chromatography (UHPLC). For further information, refer to Thermo Fisher Scientific Technical Note 64287.[7]

## Scan modes: technical principles

Figures 2 through 10 illustrate how the scan modes function, showing the flow path of the ions within the Q Exactive series mass spectrometer, from left to right (x-axis). The y-axis shows the time sequence of individual experiments. The quadrupole is represented as a long bar (rectangle). The length of the bar represents the possible scan range. The shaded sections within the bar indicate the user-defined mass range (*m/z* value) of the ion that was selected or isolated by the quadrupole. The height of the bar corresponds to the typical ion injection time into the C-Trap for a particular scan mode. The height of the Orbitrap mass analyzer corresponds to the required transient measurement time for the selected mass resolution. Unfragmented ions are shown in blue


**Magic triangle: Sensitivity – Selectivity – Scan Speed**

and fragmented ions (e.g., fragmentation induced in the HCD cell) are shown in red. Solid arrows present the physical flow of ions, and dashed arrows indicate logical connections, such as when an accurate mass listed in an inclusion list was detected, leading to the triggering collection of a selected ion monitoring (SIM) or MS/MS spectra in the next experiment. Note that the C-Trap is shown twice in each ion flow path. Although the Q Exactive series mass spectrometer contains only one C-Trap, ions can be forwarded from the C-Trap to the HCD and back into the C-Trap. The second C-Trapping event is depicted as a dashed square in the sequence of events.

## Full MS

The full MS mode uses a full MS scan without HCD fragmentation (Figure 2) and is often used for both applied and research applications.

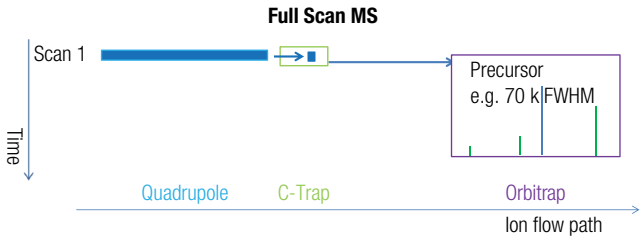


**Figure 2. Full Scan MS without HCD fragmentation**

A mass range can be defined that ensures the recording of all ions with an *m/z* value located within it with HRMS (MS1) spectral information subsequently obtained. The quadrupole in front of the Orbitrap mass analyzer is operated as a wide-pass mass filter, preventing filling the C-Trap with ions outside the defined mass range. This approach permits the detection of all compounds ionized in the electrospray (ESI) ion source with *m/z* values in the defined mass range. Because the full scan range is covered, the user can extract any desired *m/z* value after data acquisition has been completed. The Full MS mode is the most frequently used mode since it contains comprehensive information and provides good quantitative data. However, there are other modes available that provide superior sensitivity or selectivity.

## Full MS/AIF

The Full MS/AIF mode uses a full MS scan (without HCD fragmentation), followed by an all ion fragmentation (AIF) scan (with a fragmentation energy applied) (Figure 3). Only ions of the second scan event enter the HCD cell; ions of the first scan event do not. This mode is most often used in small molecule applications.

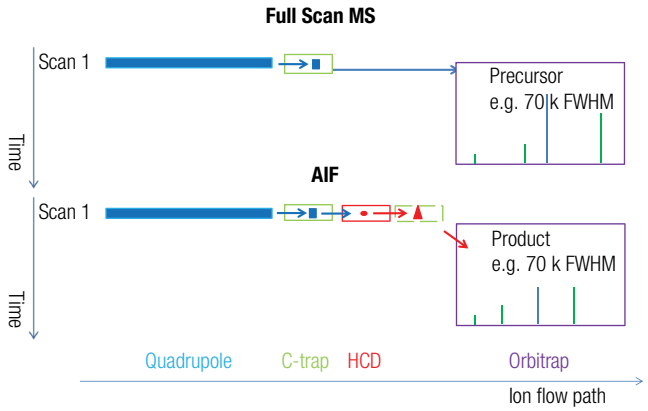


**Figure 3. Full Scan MS followed by an AIF scan**

In the Full MS/AIF mode, the instrument continuously acquires two different full MS scans. The ions intended for the first full MS scan are directly injected from the C-Trap into the Orbitrap mass analyzer central electrode. The ions intended for the second AIF scan are transferred as a whole into the HCD cell, where they are fragmented. All resulting product ions and remaining precursor ions are returned to the C-Trap, and then injected into the Orbitrap mass analyzer central electrode. The first scan consists of precursor ions, while the second scan contains non-precursor selected product ions that are the result of all the incoming precursor ions (AIF). Alternatively, the user can induce fragmentation within the ESI source interface instead of the HCD cell.

## Targeted-SIM (t-SIM)

The t-SIM mode acquires SIM scans based on the specified inclusion list (Figure 4), which is mandatory and always activated. This mode provides ultimate sensitivity and is often used in small molecule applications.

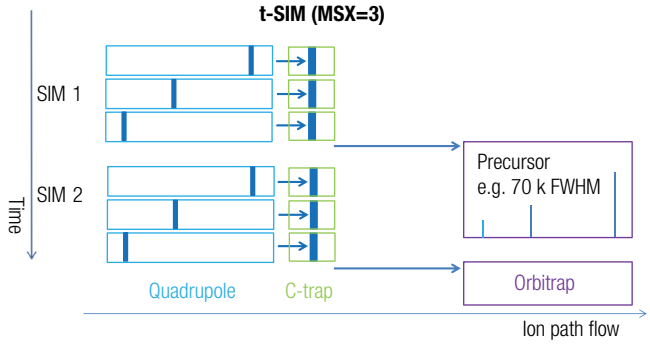


**Figure 4. t-SIM with multiplexed SIM (MSX = 3)**

The SIM mode monitors a narrow user-defined mass range (as narrow as 0.4 Da). Figure 4 also shows multiplexed SIM. Mass isolation is performed by the quadrupole, where its geometry permits very sharp mass cut offs. The Orbitrap mass analyzer central electrode resolves ions located within the narrow mass range specified. t-SIM is not more selective than full MS, but it does provide enhanced detection sensitivity. Detection sensitivity is affected by the number of ions (charges) injected into the Orbitrap mass analyzer central electrode. The number of ions is limited by the capacity of the C-Trap, which currently accommodates a maximum of 3,000,000 charges. However, the ESI interface produces many more ions than can normally be accommodated during a single measurement cycle. Therefore, usually only a small fraction (time segment) of the continuously entering ion beam is actually sampled by the C-Trap. Compared to the Full MS mode, using the quadrupole as a mass isolation device (SIM mode) greatly reduces the number of ions transmitted. Accordingly, a much longer segment of the continuously entering ion beam can be collected (long injection time) within the C-Trap before the target capacity (i.e., 3,000,000 charge) is exceeded. This concept is represented by the increased height of the quadrupole rectangles shown in Figure 4. The higher number of target ions sent to the Orbitrap mass analyzer central electrode results in significantly higher sensitivity.

## PRM or Targeted-MS$^2$ (t-MS$^2$)

The Parallel Reaction Monitoring (PRM) mode acquires MS/MS scans based on the specified inclusion list (Figure 5). The mandatory inclusion list is processed from the first to the last row without automated sorting. This mode is most often used for small molecule applications involving analyses of complex sample matrices when ultimate selectivity is required.

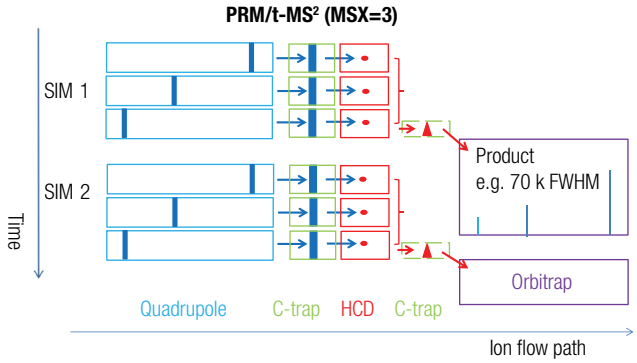


**Figure 5. Parallel Reaction Monitoring (PRM) scan or t-MS$^2$ (targeted MS/MS) mode with use of multiplexed t-MS2 (MSX = 3)**

In this mode, the quadrupole is used as a mass filter to isolate precursor ions. Only ions belonging to a narrow user-defined mass range (e.g., 1 Da) enter the C-Trap. The ions are then are transmitted to the HCD cell, fragmented, and returned to the C-Trap, where they are subsequently injected into the Orbitrap mass analyzer. The PRM mode corresponds to a QQQ instrument product ion scan. However, unlike QQQ-based product ion scans, a PRM scan produces significantly higher selectivity and sensitivity. Precursor selection combined with high-resolution product ion scanning provides enhanced selectivity. High sensitivity results from the long ion injection times (represented by the increased height of the quadrupole rectangles shown in Figure 5). Operating the quadrupole in SIM mode reduces the ion beam abundance. As a result, the ion bean can be collected for a much longer period without causing trap overfilling. However, the instrument only collects spectral information related to one particular precursor *m/z* value from the user-defined target list. Figure 5 also illustrates multiplexed PRM.

## Full MS/dd-MS² (Top N)

The Full MS/dd-MS² mode acquires a full MS scan (without HCD fragmentation) followed by a set of data-dependent (dd) scans with fragmentation energy applied (Figure 6). Ions of the second scan event enter the HCD collision cell; ions of the first do not. This mode is most often used in proteomic applications for peptide and protein identification.

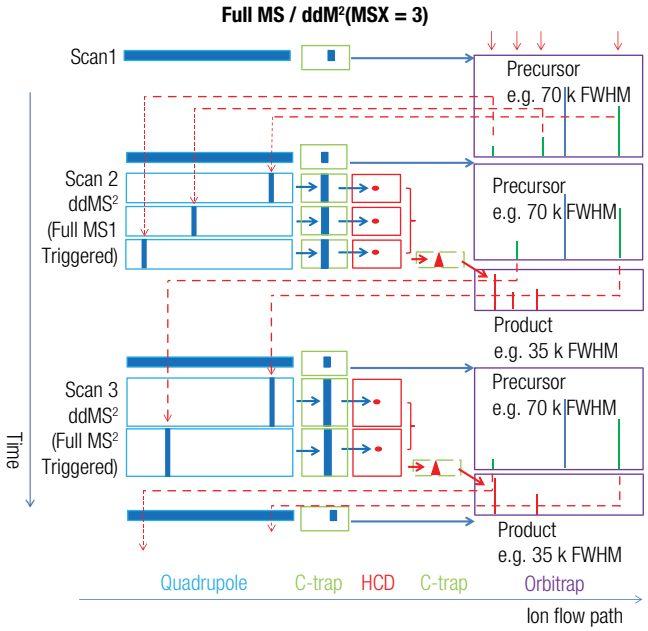


**Figure 6. Full MS/dd-MS2: data-dependent acquisition based on survey scans.** MSX = 3 multiplexing permits collection of three triggered precursor ions. Please see page 8 (Combining different scan modes) to better understand this figure.

In this mode, the instrument measures one full MS scan (known as a survey scan) and then analyzes the HRMS spectral information obtained based on the user-defined target list. Upon finding the presence of a mass peak corresponding to the accurate mass in the target list, precursor isolation is initiated in the quadrupole. Masses of the target ions are shown as small vertical arrows on the top right of Figure 6. Shown by the dashed arrows, collection of ions corresponding to these masses is triggered. The unit-mass resolved ions are precursor ions selected by the quadrupole and then later fragmented in the HCD cell. The resulting product ions are injected into the Orbitrap mass analyzer central electrode, where they are measured. A variety of parameters (e.g., mass window, ion abundance required for triggering, and maximum number of events) can be defined to control the number of triggering events. The inclusion and exclusion lists can contain a large number of potential target and non-target ions, respectively. However, triggering initiates precursor selection in a sequential mode, which in turn determines the total cycle time. The time interval between the two full scans will be prolonged when several consecutive triggering events are initiated. The selection of ions to trigger is based on the observed ion abundance, and the user can define the maximum number of ions to trigger after one survey scan. This parameter is called "Top N." Multiplexing permits the collection of more than one targeted precursor ion species in one dd-MS² experiment (Figure 6).

## Targeted SIM/dd-MS²

The Targeted SIM/dd-MS2 mode acquires SIM scans based on the inclusion list, which is mandatory and always activated (Figure 7). This mode is most often used for small molecule applications.

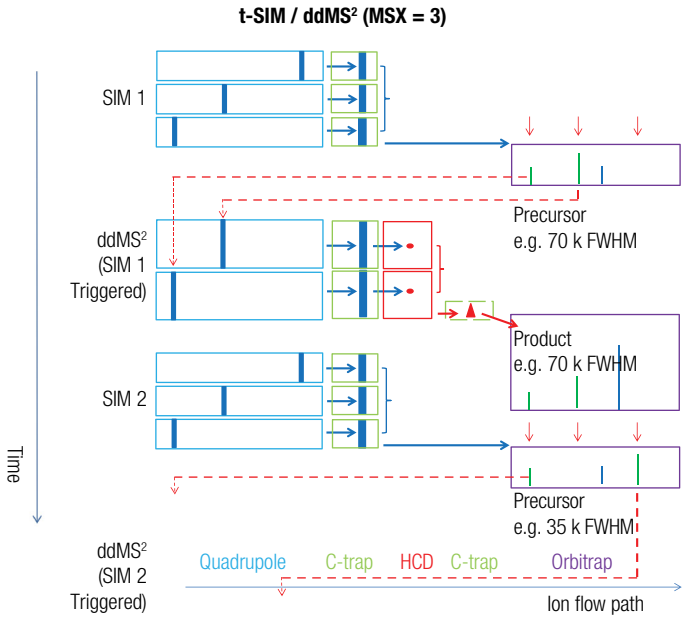


**Figure 7. t-SIM /dd-MS2: data-dependent acquisition based on a survey SIM.** The MSX = 3 multiplexing mode permits the collection of two triggered precursor ions.

The Targeted SIM/dd-MS² mode is closely related to the full MS/dd-MS² mode. However, in the Targeted SIM/dd-MS² mode, the quadrupole is operated in the SIM mode (survey scan), allowing only a narrow mass range to enter the C-Trap. A SIM scan, instead of a full scan, serves as the survey scan. The resulting reduction of ion abundances permits use of long ion collection times. Triggering of product ion spectra is obtained at lower analyte concentrations. Furthermore, the spectra

observed show high ion abundances. Because the t-MS² mode usually provides more comprehensive information, the Targeted SIM/dd-MS² mode has limited application. Figure 7 shows operation of the instrument in the multiplexing mode (MSX=3), where it simultaneously looks for the presence of the three different analytes.

## Full MS/AIF NL/dd-MS²

The Full MS/AIF NL/dd-MS² mode acquires a full MS scan followed by an AIF-scan (Figure 8). When the Q Exactive series mass spectrometer recognizes the user-defined *m/z* loss between two signals in the scan events, it automatically performs a data-dependent MS/MS scan on the precursor ion. This mode is predominantly used in research applications.

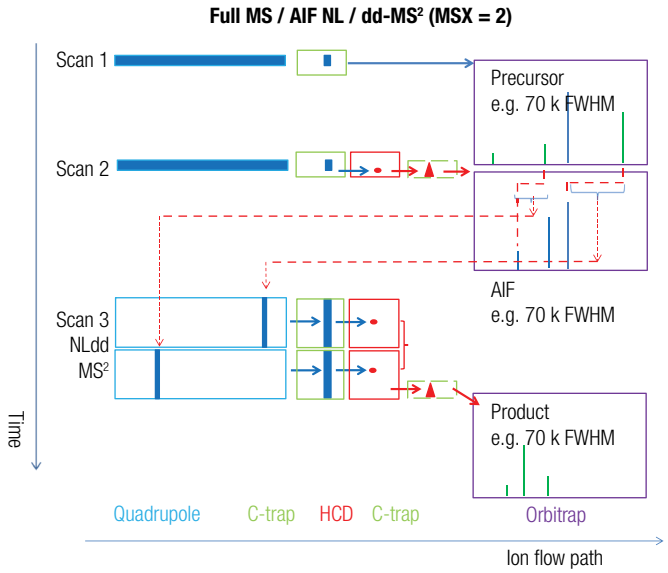


**Figure 8. Full MS/AIF NL/dd-MS²: neutral loss scan based on two survey scans trigger an MS² scan as soon as a user-defined neutral loss is observed.** MSX = 2 multiplexing permits collection of two triggered precursor ions.

The Full MS/AIF NL/dd-MS² mode resembles the constant neutral loss scan capability of QQQ technology. However, neutral losses are mathematically calculated, rather than physically detected and resolved. Q Exactive series instruments operate in the full MS/AIF mode and constantly check for an ion pair with the mass difference (neutral loss) corresponding to the user-defined neutral loss list. Triggering product of ion spectra is initiated when an observed precursor ion in the full MS scan and another observed product ion in the AIF scan (within the same cycle) differ by an *m/z* value in the user-defined neutral loss mass list.

## Data independent acquisition (DIA)

The data-independent acquisition (DIA) mode covers the scan range using targeted HCD events (Figure 9). The inclusion list specifies the centred *m/z* value and the static isolation window determines the isolation precursor *m/z* range. The mandatory inclusion list is processed from the first to the last row without automated sorting. This mode is used in both applied and research applications.

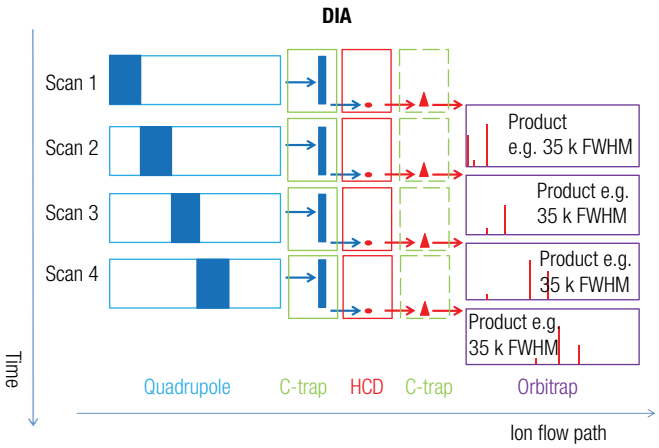


**Figure 9. DIA (data independent acquisition).** The entire scan range is sliced into consecutive sub-scans. After fragmentation, MS² results are recorded.

In DIA mode, the user enables a selective and sensitive method for a global view of MS² data for the user-defined mass range. The quadrupole isolates a relatively narrow mass range (e.g., 20 Da), which is advanced to the collision cell where an all ion fragmentation is performed. The entire ion cloud is injected into the Orbitrap mass analyzer central electrode, which monitors the entire MS² mass range. In the following scan, the quadrupole shifts the isolation mass range upwards (e.g., by 20 Da). All isolated ions are again fragmented and analyzed by the Orbitrap mass analyzer. Continuous upward shifting of the isolation mass range continues until the entire selected mass range is covered. The fragment ion spectra obtained are from a wider user-defined mass range, instead of isolated unit mass precursors.

## Combining different scan modes

By cycling modes (loop count), users can define the number of repetitions of the corresponding scan event before continuing to the next scan event or experiment cycle. This is most often used for $MS^2$ acquisition in research applications.

All the modes described here can be combined and processed in sequence. Care must be taken to obtain sufficient data points across fast eluting chromatographic peaks when combining experiments. The general rule is to have five or more scans across an LC peak for screening, and 10 or more scans for quantitation. Simply adding the individual Orbitrap mass analyzer measurement times may result in a slightly longer observed cycle rate than calculated. There are various reasons for this. One is the need for the AGC of the Orbitrap mass analyzer to produce a periodic "pre-scan." The pre-scan is required to evaluate current ion abundance. There are also minor time variations related to the scan range chosen. In addition, a data-dependent scan can only be triggered after a survey scan has been measured, undergone Fourier Transformation, and interpreted by the software. Thus, the detection of an ion in a survey scan cannot directly lead to the triggering of a t-MS2 scan. A "positive" survey scan is followed by another survey scan, and only then by the t-$MS^2$ scan.

## Multiplexing (MSX)

In spectral multiplexing, multiple preselected precursors are collected in the C-Trap, with the option of fragmentation, followed by simultaneous detection in the Orbitrap mass analyzer (Figure 10). This capability increases the duty cycle and shortens the analysis.

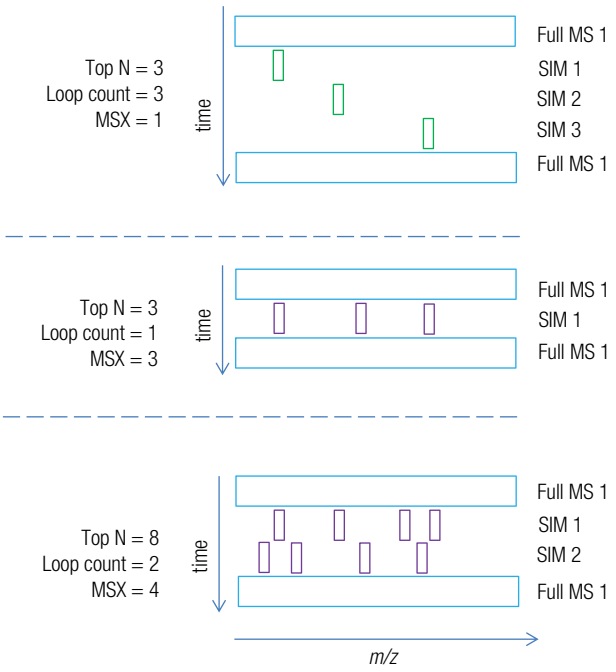


**Figure 10. Time sequence of sequential (Top N) and "parallel" MSX or multiplexed measurements.** The width of the boxes indicates the quadrupole selected mass range(s).

Targeted-$MS^2$ or targeted-SIM can be processed in sequence. The quadrupole isolates a particular precursor ion mass, sends the unit mass-resolved *m/z* ion package to the C-Trap, and from there it is accelerated into the HCD cell. Fragmentation at a given collision energy occurs when ions enter the HCD (t-$MS^2$ mode). The resulting ions are stored in the HCD cell, and a second set of precursor-selected ions are isolated in the quadrupole, accelerated, fragmented at another user-defined collision energy, and then transferred into the HCD cell. This process can be repeated for up to 10 different *m/z* values (multiple fills with targeted compounds). All the fragmented ions derived from the various precursors are stored in the HCD cell. After completion of the collection of all the precursor fragments, they are returned in a single ion cloud into the C-Trap. The C-Trap then focuses and injects the entire set of ions into the Orbitrap mass analyzer.

The user can define a different collision energy for every selected precursor ion, ensuring that the optimum collision energy is applied to each analyte. The resulting product ion spectrum consists of all the product ions derived from all the unit-mass-isolated precursor ions. Though a particular product ion cannot be directly linked to a specific precursor ion, this mode of operation has several advantages. Unit mass isolation of precursor ions reduces the ion abundance, thus permitting longer ion injection times without overloading the C-Trap and ultimately higher sensitivity. Some selectivity is lost by accumulating different precursor ions into one product ion scan, yet this loss of selectivity is more than compensated for by the high resolution of the product ion scan. Analytes that produce identical product ions should not be placed into the same MSX function. The following formula links Top N, loop count and MSX:

$$Top\ N = loop\ count \times MSX$$

### Positive/negative switching

Positive/negative switching toggles between positive and negative ion polarity for maximum compound coverage in a single injection. This feature is frequently used in applied markets.

Q Exactive series mass spectrometers allow continuous positive/negative switching with a resolution of 35,000 FWHM within 1 s. Unlike some Q-TOF instruments, Q Exactive series mass spectrometers do not lose mass calibration during polarity switching and switching can be performed on the LC time scale.

## Method development recommendations

To facilitate the development of analytical methods that take full advantage of the various scan modes described here, the authors provide the following recommendations. These recommendations apply to analytical methods intended to detect, quantify, and confirm analytes in complex matrices. Generally, QQQ instruments provide the best results (sensitivity and selectivity) when operated in selected reaction monitoring (SRM) mode, while Q Exactive series instruments provide the most comprehensive data, in terms of ions and compounds monitored, when operated in full MS mode. Therefore, with Q Exactive series mass spectrometers, method development typically employs the full MS mode, and, in many cases, there is no reason to use any other mode. QQQ-trained users should avoid the impulse to look for MS transitions (targeted mode) when using Q Exactive series instruments. Instead they should give their instrument the chance to detect and quantify the unfragmented analyte with sufficient sensitivity and selectivity in full MS mode.

When developing methods, selection of mass range is important. A narrow mass range increases sensitivity because lower ion abundances allow longer ion injection times. In our laboratory, the quadrupole is used more frequently as a wide pass filter to exclude ions resulting from the mobile phase and column bleed than as a mass isolation device that captures precursor ions. Single-stage Orbitrap mass analyzers are not designed to perform this function (the octapole is for ion transmission, not the quadrupole). Consequently, single-stage Orbitrap mass analyzer systems, like the Thermo Scientific™ Exactive™ Plus Orbitrap mass spectrometer, are not equally suited to applications involving very complex matrices. It is a good habit to look at the ion injection time across the entire chromatographic time range where the analytes of interest elute. Sections with very short ion injection times are not ideal due to low sensitivity in the matrix and may require definition of a narrower scan range or targeted experiments.

When the application requires unequivocal confirmation of a tentatively found residue in addition to screening and quantification, data-dependent modes of operation (e.g., full MS/dd-MS$^2$) are recommended. However, unequivocal confirmation may also be achieved by monitoring an AIF trace.[8] Quantification should be based on the full-scan trace. Using data-dependent techniques requires optimization to set the appropriate triggering levels. It is beyond the scope of this white paper to discuss this optimization process in detail. Nonetheless, standard settings are for protein and peptide applications and they need to be turned off when using data-dependent techniques for small molecule applications. If the user has access to suitable quantification software, such as Thermo Scientific™ TraceFinder™ data processing software, DIA methods present an attractive solution.

There may be situations where sensitivity or selectivity for some analytes is insufficient. Often, it is only one or a small set, of analytes that cause sensitivity problems. In this case, it may be helpful to quantify the "non-problem" analytes using the full MS mode and then add additional experiments to address the problematic compounds. If a lack of sensitivity is a concern, using a SIM experiment may be the answer. If sensitivity and selectivity must be improved, the authors recommend using the PRM mode. The PRM mode generally provides sensitivity rivaling high-end QQQ instruments operating in the SRM mode. Furthermore, the PRM mode provides selectivity surpassing that of a unit-mass-resolving QQQ instrument. If a number of problematic compounds must be analyzed, retention time-dependent experiments can help.

For all modes, it is recommended that the user optimize the precursor RF entrance lens in front of Q1, because in-source fragmentation might occur, particularly for fragile compounds.

Multiplexing is a way to combine the best modes to address an application, and can reduce cycle time, while still providing reliable confirmatory data.

Table 1 summarizes the scan modes available on each of the Q Exactive series mass spectrometers.

## Conclusion

The scan modes available on Q Exactive series mass spectrometers permit transfer of almost any QQQ- or Q-TOF-based method to an Orbitrap mass analyzer environment, with comparable, or even enhanced sensitivity, selectivity, and dynamic range. Depending on the particular problem presented by an analytical method, different scan functions and experiments can be used. For best results, methods should be migrated in a way that fully utilizes the capabilities of Orbitrap mass analyzer technology. In particular, the quadrupole is best used as either a wide-mass-range cut-off filter or a unit-mass-isolation device.

The Full Scan MS mode is always a good starting point for most applications. The SIM and PRM modes are best deployed when ultimate sensitivity and selectivity are required, respectively. Top N methods often are used for proteomics applications. DIA has the advantage of being able to fragment "everything" for both applied and research applications.

Real-world examples of use of Q Exactive series mass spectrometers for small and large molecule quantitation and confirmation by full MS, PRM, and SIM, are available in AI-64645, a summary of over 50 peer-reviewed publications in the fields of food safety testing, environmental contaminants, clinical research, pharmaceutical discovery, and omics.[9]

**Table 1. Availability of scan modes provided by each of the Q Exactive series mass spectrometers models**

| Scan Function per Instrument | Full MS | Full MS/AIF | Targeted-SIM | PRM | Full MS/dd-MS² (Top N) | Targeted SIM/dd-MS² | Full MS/AIF NL/dd-MS² | DIA | Multiplexing (MSX) |
|---|---|---|---|---|---|---|---|---|---|
| Q Exactive Focus | Yes | *Yes* | Yes | Yes | Yes (Top 3) | No | No | Yes (vDIA)* | Yes (SIM only) |
| Q Exactive | Yes | *Yes* | Yes | Yes | Yes (Top 10) | Yes | Yes | Yes | Yes (SIM & PRM) |
| Q Exactive Plus | Yes | *Yes* | Yes | Yes | Yes (Top 10) | Yes | Yes | Yes | Yes (SIM & PRM) |
| Q Exactive HF | Yes | *Yes* | Yes | Yes | Yes (Top 20) | Yes | Yes | Yes | Yes (SIM & PRM) |
| Q Exactive HF-X | Yes | *Yes* | Yes | Yes | Yes (Top 40) | Yes | Yes | Yes | Yes (SIM & PRM) |

*vDIA method is not available in the United States of America

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## References


1. Bromirski, M. First choice in high resolution mass spectrometry with Orbitrap mass analyzer technology for screening, confirmation and quantitative analyses; Thermo Fisher Scientific White Paper 65146; February 2018.
2. A. Makarov, E. Denisov, A. Kholomeev, W. Balschun, O. Lange, K. Strupat, S. Horning, Performance evaluation of a hybrid linear ion trap/Orbitrap mass spectrometer. *Anal. Chem.* **2006,** *78,* 2113
3. Hu, Q.; Noll, R. J.; Li, H.; Makarov, A.; Hardman, M.; Cooks R. G. The Orbitrap: a new mass spectrometer. *J. of Mass Spectrom.* **2005,** *40,* 430.
4. Perry, R. H.; Cooks, R. G.; Noll, R. J. Orbitrap mass spectrometry: Instrumentation, ion motion and applications. *Mass Spectrom. Rev.* **2008,** *27,* 661.
5. Hu, Q.; Cooks, R. G.; Noll, R. J. Phase-enhanced selective ion ejection in an Orbitrap mass spectrometer. *Am. Soc. Mass Spectrom.* **2007,** *18,* 980.
6. Zubarev, R.; Makarov, A. Orbitrap mass spectrometry. *Anal. Chem.* **2013,** *85,* 5288.
7. Strupid, K.; Scheibner, O.; Bromirski, M. High-Resolution, Accurate-Mass Orbitrap Mass Spectrometry – Definitions, Opportunities, and Advantages; Thermo Fisher Scientific Technical Note 64287; December 2016.
8. Kaufmann, A.; Butcher, P.; Maden, K.; Walker, S.; Widmer, M. Reliability of veterinary drug residue confirmation: High resolution mass spectrometry versus tandem mass spectrometry. *Anal. Chim. Acta.* **2015,** *856,* 54.
9. Thermo Fisher Scientific AI65645. Orbitrap quantitation: lab of the future.


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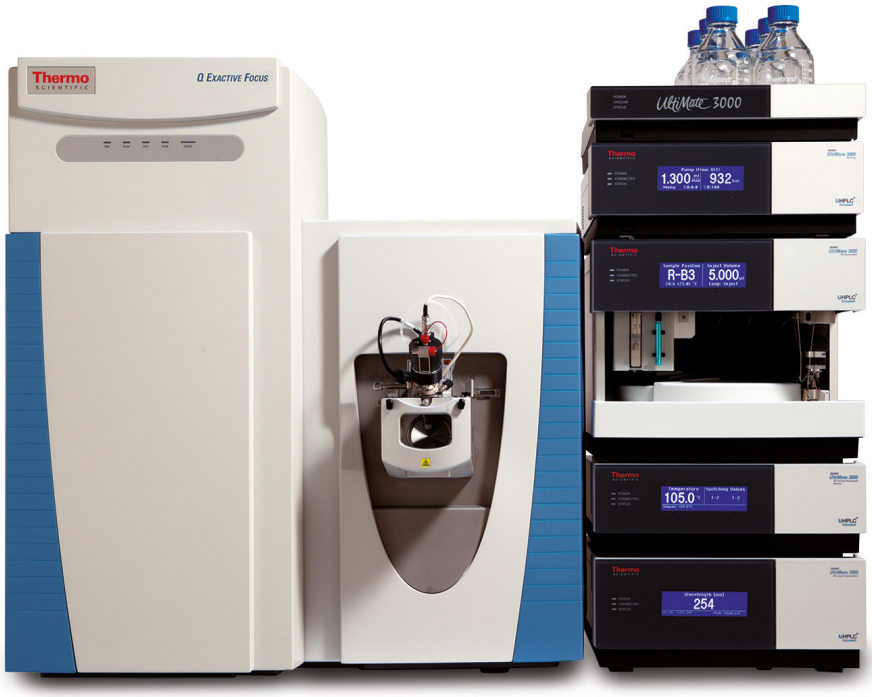


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