Toxicology

Strengthen evidence with forensic toxicology solutions that go further

Redefine limits:
Quantitating and identifying unknowns for Forensic LC-MS analysis

Simultaneous quantitation and discovery (SQUAD) analysis

thermo scientific

Forensic toxicology

In the age of designer drugs and other novel psychoactive substances (NPS), it can be difficult to pinpoint a specific number or type of drug being trafficked and abused globally. Hundreds of fentanyl analogs have been identified, and new designer benzodiazepines have popped up.

But as the saying goes, what was once old is new again. Even drugs like Ketamine seem to periodically make a comeback. To stay on top of the ever-changing landscape of drugs of abuse, the right tools need to be utilized.

From unknown screening to routine quantitative assays, Thermo Fisher Scientific has the innovations and technology to stay one step ahead. We are committed to redefining the standard in drugs of abuse testing.

Analytical toxicology

Small molecule assays

In analytical testing like forensic toxicology, there are two questions being asked: “What is in the sample?” and “How much?” Thermo Scientific™ mass spectrometers offer leading technology for identifying and quantifying complex toxicology samples for both screening and quantitative purposes.

Screening

Screening assays answer the question *What?* and are used to identify and confirm if previously profiled analytes exist in their samples, as well as to detect new compounds which may require identification and characterization.

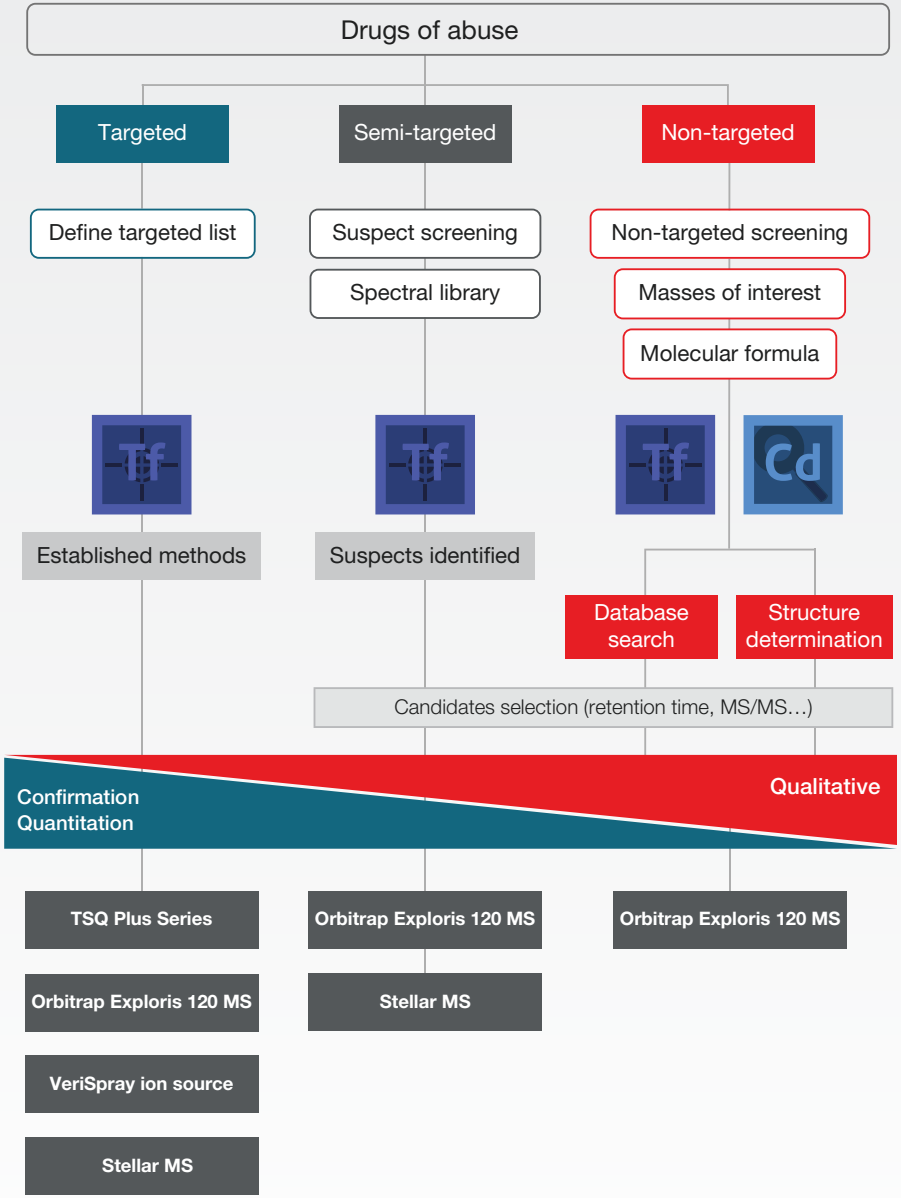
As with profiling, confidence in the quality of data is of utmost importance. Sample complexity can directly impact the certainty of results because the presence of matrix interference can give rise to false positives.

In addition, for many unknowns, reference standards may not be readily available, leaving laboratories without a straightforward way to identify compounds with any confidence.

Targeted quantitation

Targeted quantitation quantifies compounds that have been identified as important. The analytical tool chosen to perform quantitation assays should provide:

- Sensitivity to achieve the limits of detection (LODs) required by regulatory bodies.
- Selectivity and specificity to confidently identify and quantify target analytes in the presence of matrix interferences within complex samples.
- Robustness to minimize system downtime, increasing operational efficiency.



Untargeted toxicology

Powered by Orbitrap mass spectrometry

Thermo Scientific™ Orbitrap™ mass spectrometers offer leading technology for identifying and quantifying complex samples.

Resolution

Orbitrap high-resolution accurate-mass (HRAM) technology resolves analytes of interest from interferences and differentiates ions of interest from interfering ions differing by 5 ppm as low as *m/z* 40. As a result, you substantially reduce the likelihood of false positives and negatives when analyzing complex sample matrices.

Retrospective analysis

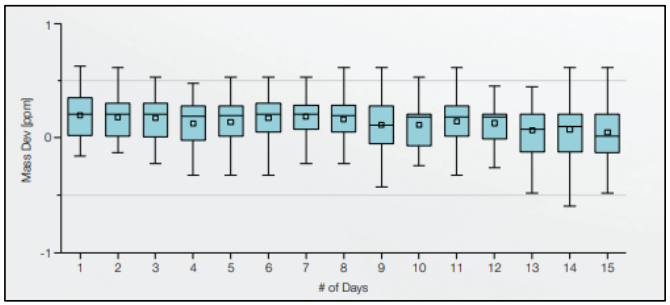
Obtain comprehensive data coverage by capturing all ions in a user defined mass range in the full scan and MS2 with high mass accuracy. Extract desired *m/z* fragment ion values immediately after data acquisition or later using retrospective data analysis.

Speed

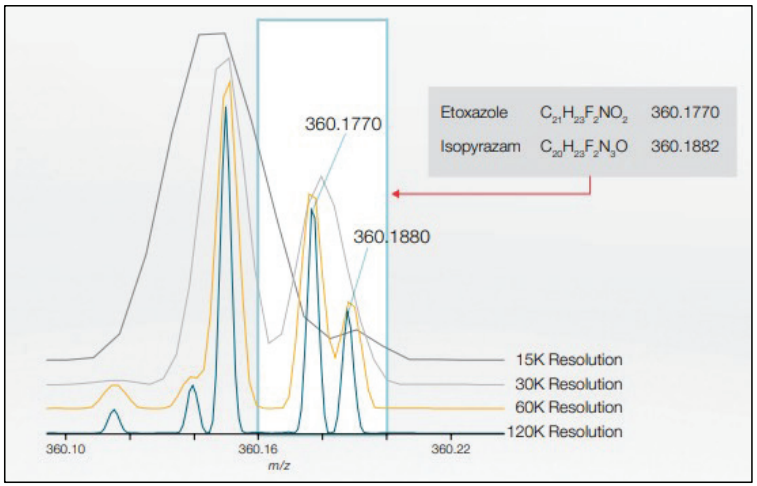
The Thermo Scientific™ Orbitrap Exploris™ 120 triggers even more precursor ions for MS/MS with a scan rate of 22 Hz. Positive and negative polarities can be done in the same run with a single cycle completed in 1.4 Hz.

High mass accuracy

Using the Thermo Scientific™ Easy-IC™ ion source, sub-ppm mass accuracy is achieved for at least 5 days. This automated internal calibrant, fixed between the S-Lens and the Advanced Active Beam Guide, delivers consistent stable mass accuracy until calibration using the Thermo Scientific™ Pierce™ FlexMix™ Calibration solution is performed.



A panel of veterinary drugs with m/z range from 160…900 was analyzed at 100 µg/L over a 15-day period with continual polarity switching, providing exceptional mass accuracy.



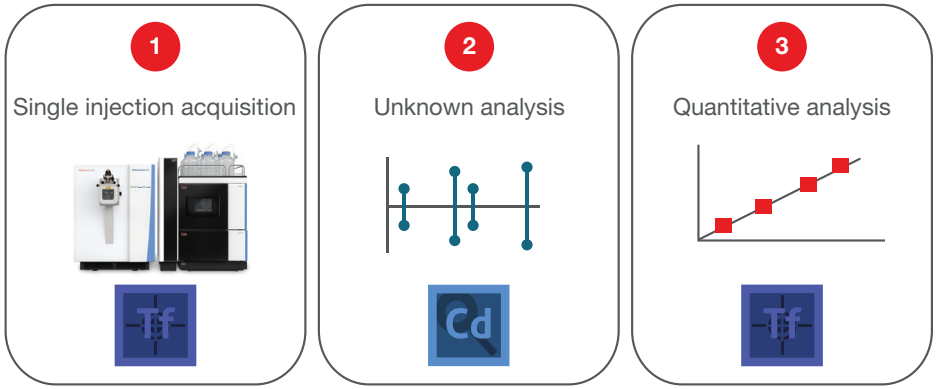
Full-scan mass spectrum of etoxazole and isopyrazam, with the Orbitrap Exploris 120 mass spectrometer at a resolution setting up to 120,000 (FWHM) at *m/z* 200.

SQUAD approach

Complete solution for simultaneous untargeted and targeted workflow

The SQUAD approach involves a single injection on the Orbitrap Exploris 120 MS instrument method and using the raw file simultaneously in two software programs (Thermo Scientific™ Compound Discoverer™ software and Thermo Scientific™ TraceFinder™ software) to both identify unknowns and quantitate knowns. This allows the user to look deeper into the unknown and find new drugs that their targeted-only assays may not be able to identify and also perform quantitation on known targets.

Our untargeted approach uses an Orbitrap Exploris 120 MS with a Full Scan-data dependent MS2 (FS-ddMS2) method, which will perform fragmentation on the highest intensity fragments (Top N=4), seen in the MS Method figure.

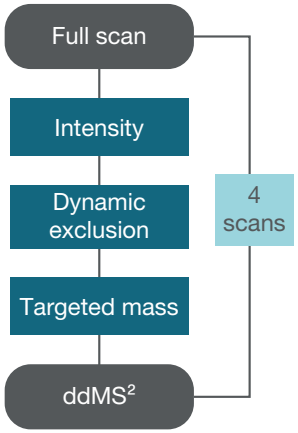


LC method

- Vanquish LC / Phenyl Hexyl column (100 x 2.1 mm, 2.6 µm)
- Mobile Phase A: 2 mM ammonium formate and 0.01% formic acid in water
- Mobile Phase B: 2 mM ammonium formate and 0.01% formic acid in MeOH:ACN (50:50)

MS method

- Ionization mode: HESI, positive/negative
- Spray voltage: 3500 V (+), 2500 V (-) static
- Gas flow (Arb): Sheath @ 55; Aux @ 10; Sweep @ 1
- Ion transfer tube temp: 325°C
- Vaporizer temp: 350°C
- Expected LC peak width (s): 3
- Dynamic exclusion: 1 time, 2 s
- Targeted mass
  - Include targeted mass list
  - "Perform dependent scan on most intense ion if no targets are found"



MS method

SQUAD toxicology LC gradient

Time (min)	Flow rate (mL/min)	%A	%B
0.0	0.5	99	1
1	0.5	99	1
10	0.5	1	99
11.5	0.5	1	99
11.51	0.5	99	1
15.5	0.5	99	1

Compound Discoverer software

Small molecule untargeted data processing

Thermo Scientific™ Compound Discoverer™ software, powered by Thermo Scientific™ mzCloud™ mass spectral library, Thermo Scientific™ mzVault™ mass spectral database, and Thermo Scientific™ mzLogic™ data analysis algorithm, provides an extensive, flexible, and customizable toolkit for processing your untargeted data. Its data visualization capabilities make it easy to understand and review your findings. The software leverages both online and offline library tools for comprehensive data analysis. mzCloud Spectral Library hosts millions of high-quality curated HRAM MSn spectra on tens of thousands of compounds.

Easy, customizable workflows

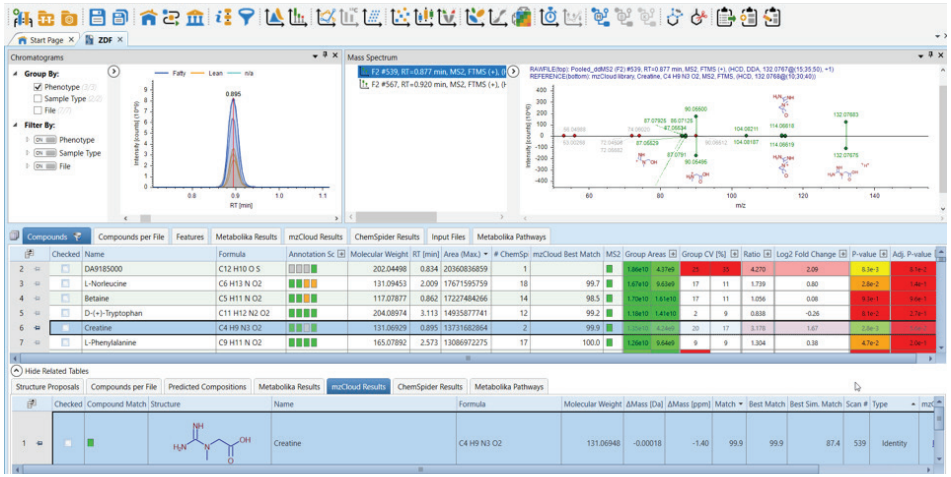
Compound Discoverer software empowers data processing with its customizable workflows. Drag and drop workflow nodes from an extensive list of processing tools into workflow trees that specify your desired data flows. Save your workflow as a template or pick from many pre-made templates to simplify setup.

Powerful features for unknown identification

Compound Discoverer software detects unknown metabolites of drugs of abuse and structurally related designer drugs. Many new drugs contain similar structures, and the Compound Class Scoring Node can be used to score detected compounds against common fragment ions, therefore aiding the ability to find new drugs based upon characteristic fragments. This can help keep you one step ahead on what drugs may be of importance to you!

From untargeted to targeted

This information can be transferred to screening methods to help you keep up with an ever-expanding array of new drugs and their metabolites. Once unknown compounds have been identified using any of the multiple workflows available within Compound Discoverer software, the data can be exported directly to a new or existing mzVault library, or a targeted list that can be used with Thermo Scientific TraceFinder software for screening and quantitation using either quadrupole or high-resolution MS-based techniques.



Compound Discoverer software data view

mzCloud: Compares spectra to mzCloud for common substructure

mzVault: In-house library search, open-access library search, .cdb

mzLogic: Compares spectra to online databases for common substructure performing forward and reverse searches → similarity score

Compound classes: Expected fragment search

Molecular networking: makes connections between MSMS and well-known chemical transformations

Fine isotopic structure: HRAM enabled molecular formula, Sulfur only available by Orbitrap > 100K resolution

Compound Discoverer toxicology workflow

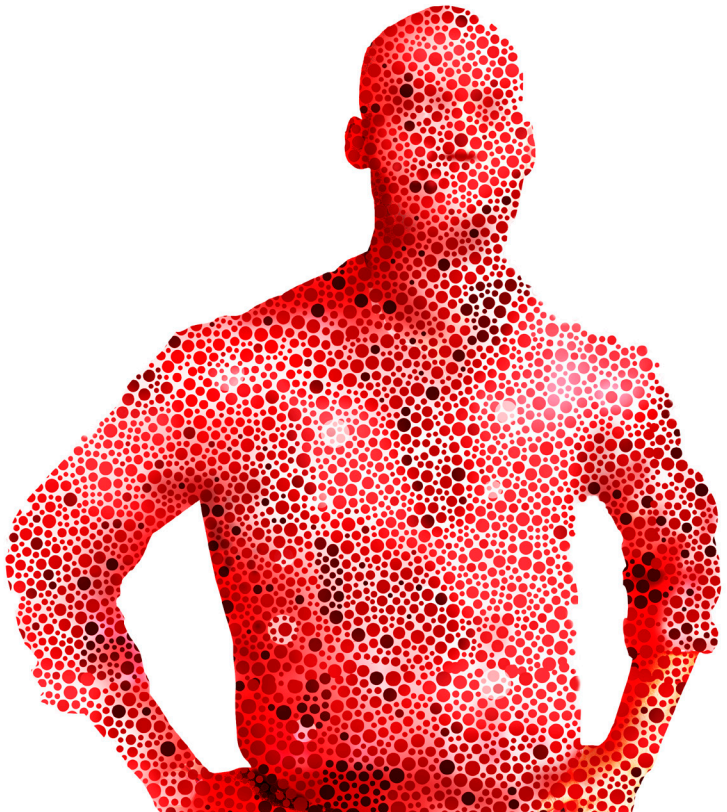
Pre-made workflow template for toxicology applications

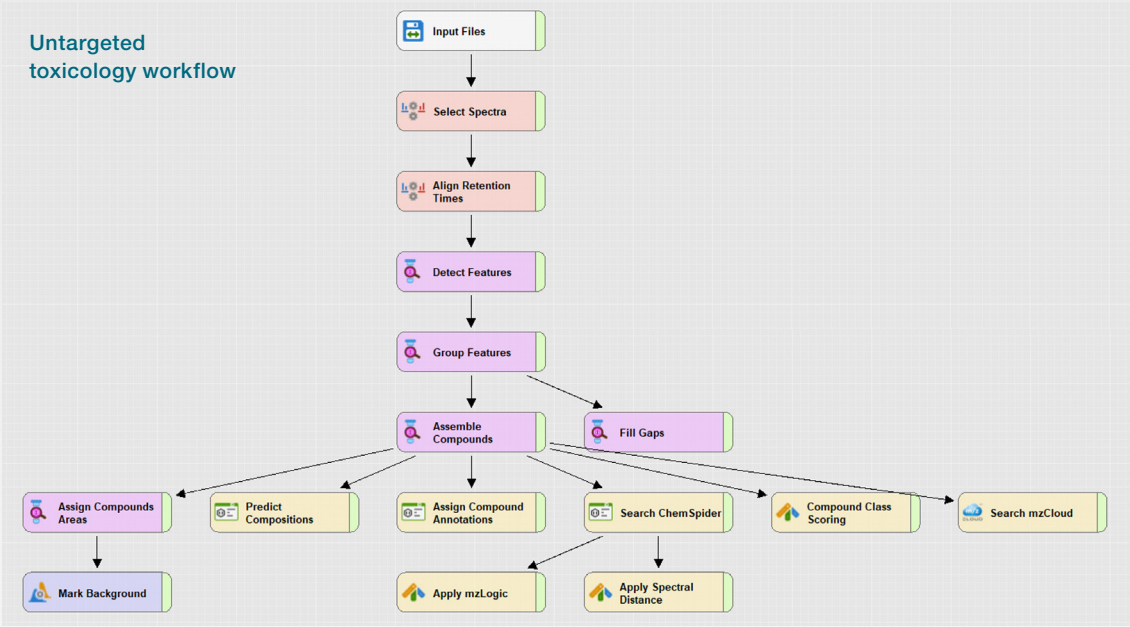
Compound Discoverer workflow

The workflow editor includes pre-built templates containing nodes with flexible data processing parameters and smart drag-and-drop functionality. Design and save advanced built-for-purpose custom workflows using the extensive capabilities to streamline your data processing.

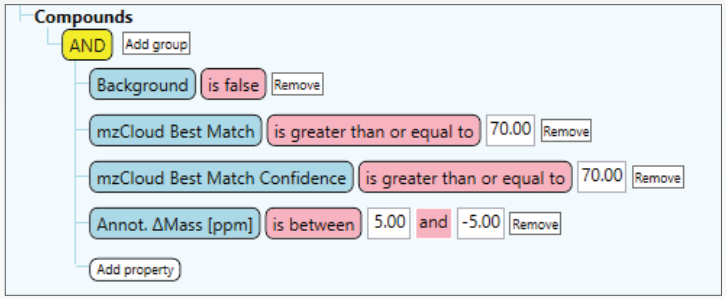
Customizable filtering tool

Compound Discoverer software has customizable data visualization with the filtering tool which allows you to narrow in on compounds of interest.





Compound Discoverer workflows reduce processing clicks needed to transform mass spectral data into results.



Compound Discoverer data filtering

AcquireX software

Intelligent data acquisition

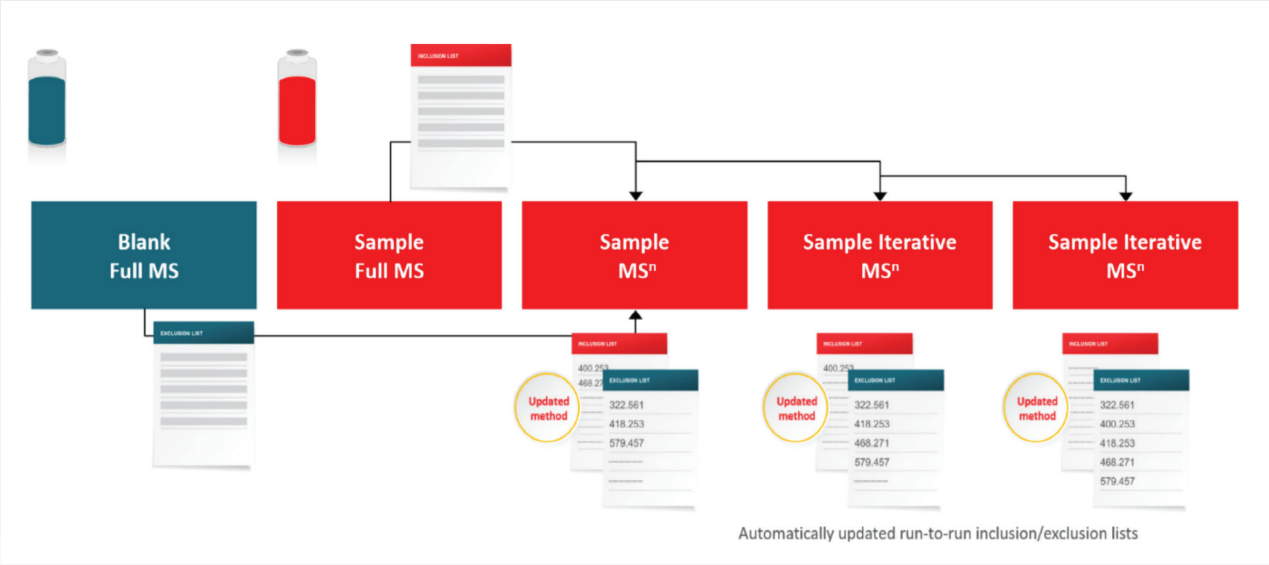
A deeper dive into the unknown

Thermo Scientific™ AcquireX™ software allows you to exclude any background and focus only on the unique analytes of each unknown. The software creates iterative data acquisition from automatically generated inclusion lists and provides the ability to collect very low intensity ions as well as perform retrospective analysis. Using DDA with the AcquireX data acquisition workflow improves data quality and creates a significant increase in the number of compounds with MS/MS spectra, resulting in improved mzLogic ranking and higher mzCloud similarity scores, ultimately providing higher overall confidence in compound identification.

Benefits

- Enables specialized workflows for unknowns
- Retrospective analysis on pooled samples
- Not missing the MS2 that matter
- High quality, low abundant MS2 collected

AcquireX: Comprehensive MS^n analysis of all of the features in the sample



AcquireX acquisition workflow

TraceFinder software

Complete solution for small molecule targeted data processing

Designed for fast and flexible compound screening and quantitation, Thermo Scientific™ TraceFinder™ software provides unique features to support a wide range of applications. Built with flexible method templates, comprehensive compound database, and access to extensive spectral fragmentation libraries, TraceFinder software allows operators of all experience levels to confidently drive laboratory productivity.

Ease of use

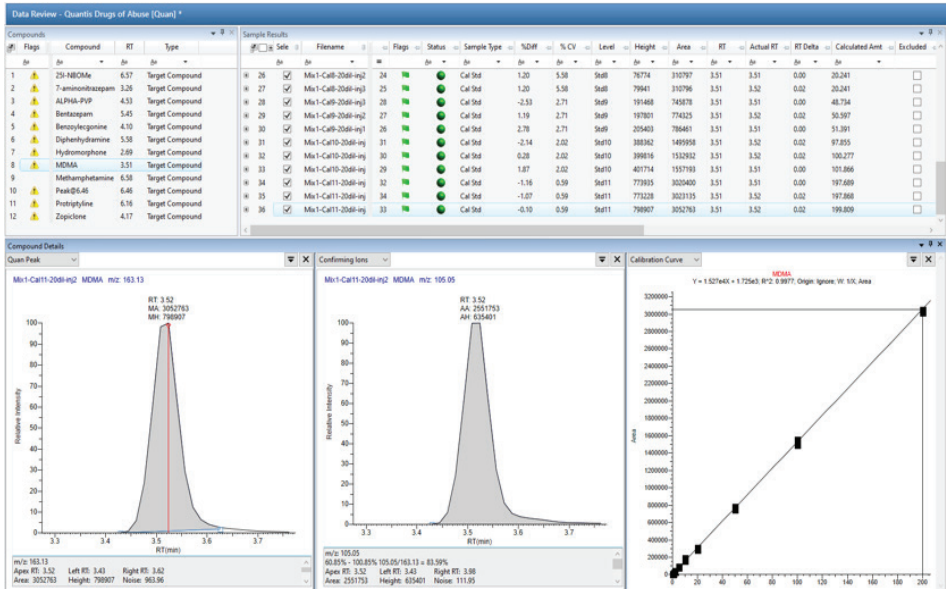
TraceFinder software simplifies routine quantitation with a 4-click acquisition, saved templates, and reports. The auditing/administration tool provides labs with users with varying access to parameters and development features. With the use of a single channel LC, intelligent sequencing can be employed to reinject samples and blanks.

One software platform

Method development, acquisition, and processing is all on the same software providing efficiency. Users can also choose from three different types of methods: routine quantitation, targeted screening, and unknown screening. For more advanced searching/features when analyzing unknowns, Compound Discoverer software is used. For confirmation, there are three identification tools that are used: Library Search (compares experimental data to mzVault library), Isotopic Pattern (compares isotopic pattern observed to expected based on empirical formula), and Fragment Match (compares experimental mass of the fragment ions to expected).

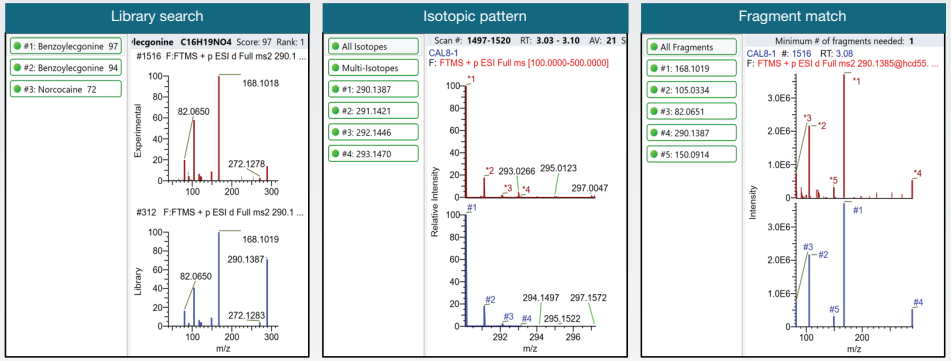
Customization

TraceFinder software has many customization features to make your data analysis process easier including report template creation, sample and compound flagging, and window view setup.



TraceFinder data processing compound view with target peak, ISTD peak, and calibration curve.

HRAM confirmation features



SQUAD case study

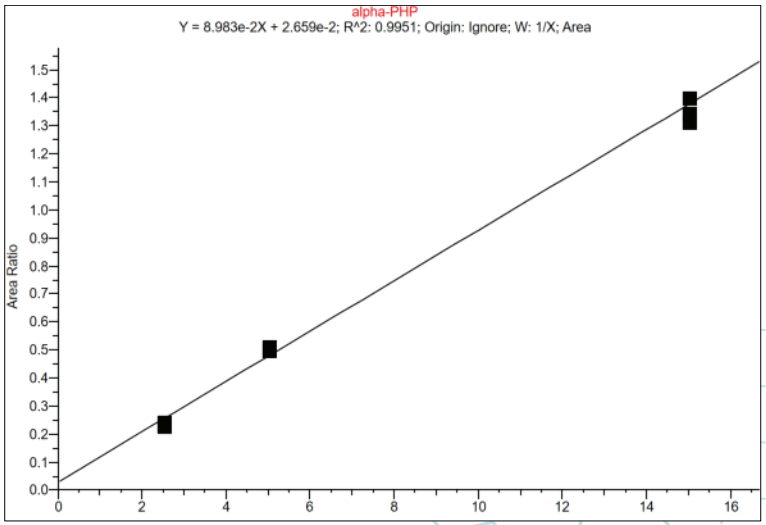
Using the workflow to quantitate known analytes

Quantitative results

After injecting NPS calibration curves and the three case study samples, compounds were quantitated. Six NPS's were identified and their concentrations in each sample are depicted in the heat map. The identified compound concentrations ranged from sub 50 ng/mL to approximately 9,000 ng/mL.

Heat map of calculated concentrations (ng/mL) for several NPS compounds detected from the three samples.

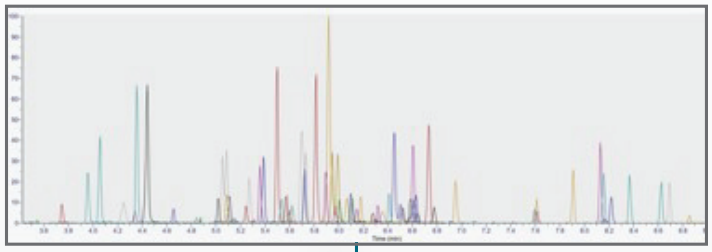
	Sample 1	Sample 2	Sample 3
4-Fluorofentanyl	106.2	116.7	0.0
α-Pyrrolidinohexanophenone (α-PHP)	41.0	0.0	0.0
Fentanyl	179.9	159.1	212.6
N,N-Dimethylpentylone	2376.8	0.0	4835.3
Norfentanyl	2828.6	1409.0	699.6
Pentylone	1774.6	0.0	8555.7



Calibration curve of α-PHP which is used to quantitate the unknown samples.

Assay overview

Calibration curve of NPS compounds



Injected samples

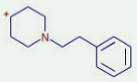
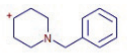
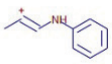
or

1 Quantitate unknown samples

2 Analyze untargeted compounds

SQUAD case study

Using the workflow to identify unknowns

	m/z	Structure	Formula	Charge
1	84.08078		C5 H10 N	1
2	91.05423		C7 H7	1
3	188.14338		C13 H18 N	1
4	174.12773		C12 H16 N	1
5	177.13863		C11 H17 N2	1
6	132.08078		C9 H10 N	1
7	105.06988		C8 H9	1
8	82.06513		C5 H8 N	1

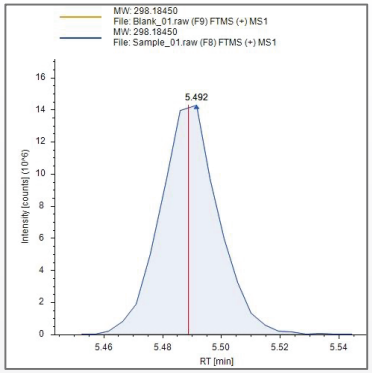
Class coverage for common fentanyl fragments

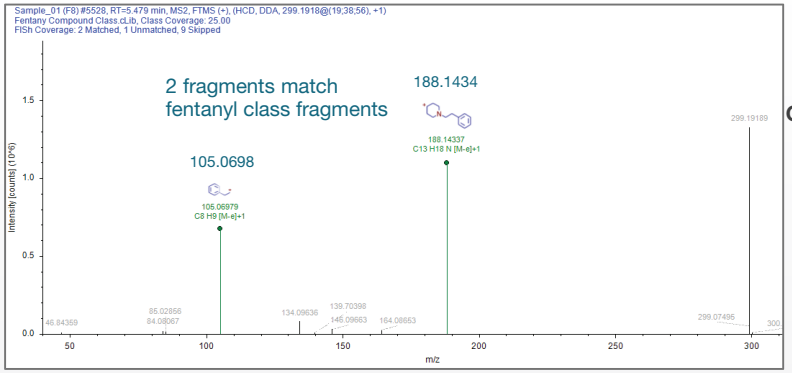
Compound class coverage

Compound Class libraries can easily be created in the software and used during data analysis to compare common class fragments to your data. Here is an example Compound Class library with eight common fentanyl analogue fragments. The software will make note of any fragments that appear in the spectra with a green spectrum line. Additionally in the results table, the Class Coverage score will indicate the number of Compound Class fragments found for each compound. This is useful for drug class identification.

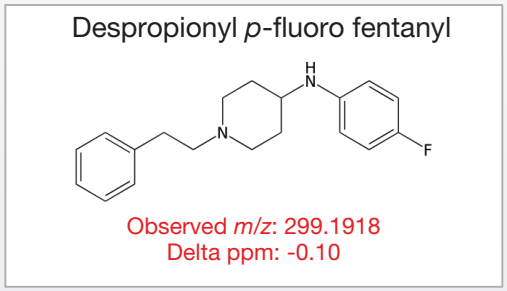
Identification of despropionyl p-fluoro fentanyl

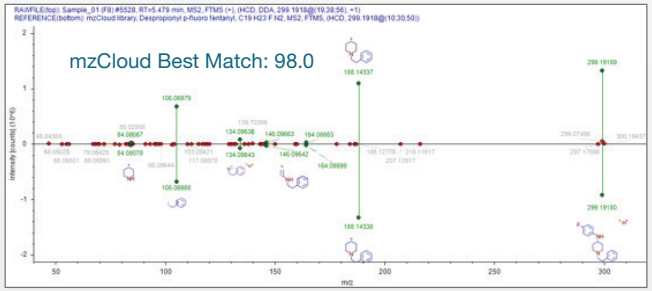
The SQUAD workflow/method includes over 210 fentanyl analogues in the mzCloud and mzVault libraries. With two fentanyl fragments detected in the MS2 scan and a 98% mzCloud best match score, the metabolite of para-fluoroisobutyryl fentanyl (FIBF) and an impurity and precursor in the synthesis of *para*-fluorofentanyl was identified as despropionyl *p*-fluoro fentanyl.





Compound classes





mzCloud

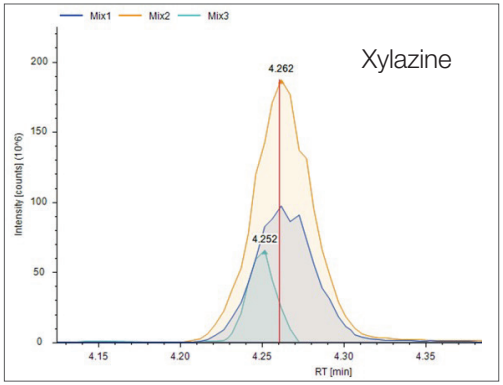
Compound Discoverer data for identified compound despropionyl *p*-fluoro fentanyl including chromatogram, fragmentation spectrum, compound structure, and mirror plot with mzCloud data

SQUAD case study

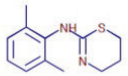
Using the workflow to identify unknowns

Identification of xylazine

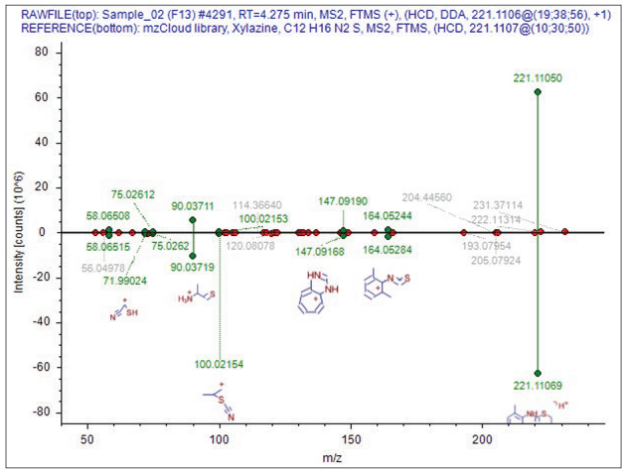
In these case study samples, xylazine at varying intensities was found. With a mzCloud match score of 99.5, this compound was confirmed to be xylazine.



Chromatograms of three samples showing relative intensities of xylazine

Compound Match	Structure	Name	Formula	Molecular Weight	ΔMass [ppm]	Match	Best Match	mzCloud ID	Compound Class
■		Xylazine	C12 H16 N2 S	220.10342	-0.56	99.5	99.5	Reference-4884	Therapeutics/Prescription Drugs; Drugs of Abuse/Illegal Drugs; Sports Doping Drugs

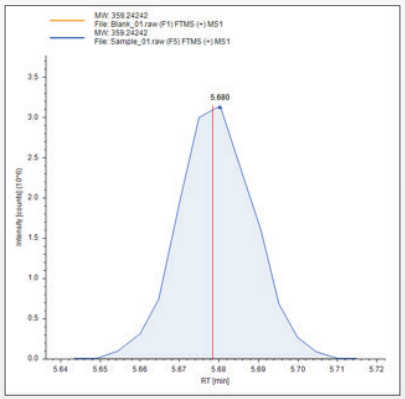
mzCloud results showing structure, name, molecular weight, mass accuracy, and mzCloud match scoring

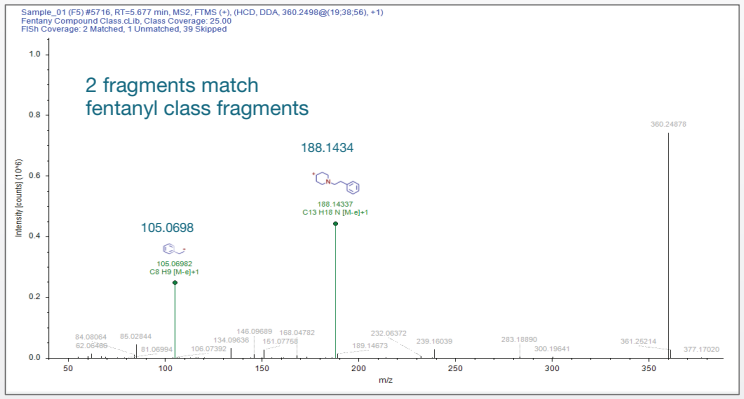


Mirror plot between case study sample and mzCloud spectra identifying xylazine

Unidentified compound

An unidentified compound at *m/z* 360.2478 was found with no library matches. With a retention time similar to fentanyl analogues and two fentanyl fragments detected in the MS2 scan, this might be a suspected fentanyl analyte. Forensic researchers now have the right combination of data and software to accurately interpret complex, unknown samples and uncover the truth.

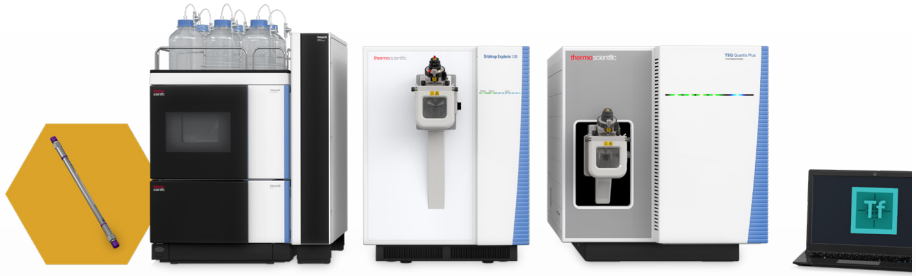




Compound classes

A comprehensive portfolio

Our complete Thermo Scientific Forensics LC-MS lineup



Thermo Scientific™ Tox Explorer™ Collection

The Thermo Scientific Tox Explorer Collection is an all-in-one LC-MS/MS solution that provides confident answers to scientists in forensic toxicology, clinical research toxicology, employee drug testing, and sports anti-doping. It is available in HRAM and QQQ MS platforms

Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer

The Thermo Scientific Orbitrap Exploris 120 mass spectrometer sets the new standard in instrument productivity and ruggedness, delivering high confidence results for a broad range of toxicology compounds.

Thermo Scientific™ VeriSpray™ PaperSpray ion source

Improve turnaround time, reduce cost per test and minimize sample preparation with the Thermo Scientific VeriSpray PaperSpray ion source, a fully automated, high-throughput, direct ionization technique used with our newest Thermo Scientific TSQ triple quadrupole mass spectrometers.

Thermo Scientific™ Stellar™ mass spectrometer

The Thermo Scientific Stellar mass spectrometer provides higher sensitivity with its CID and HCD fragmentation, greater specificity through MSn confirmation, and increased throughput with our fastest mass spectrometer yet for unrivaled productivity.

Thermo Scientific™ TSQ Altis™ Plus triple quadrupole mass spectrometer

With superior acquisition speeds, enhanced sensitivity, and exceptional robustness, the TSQ Altis Plus mass spectrometer delivers accuracy and precision for low-level compound detection and quantitation in complex matrices.

A comprehensive portfolio

Our complete Thermo Scientific Forensics LC-MS lineup

Thermo Scientific™ Vanquish™ Flex UHPLC system

Obtain new benchmarks in accuracy, precision and sensitivity with the Thermo Scientific Vanquish Flex UHPLC systems. Providing biocompatibility with a state-of-the-art binary high-pressure solvent blending and design focused on robustness and reliability.

Thermo Scientific™ Transcend™ VLX-2 UHPLC system

Improve your LC-MS throughput and get a faster return on investment. The Thermo Scientific Transcend VLX-2 system offers a new level of simplicity and cost-effectiveness with multichannel technology and increased sample capacity with the Thermo Scientific Vanquish Charger module for a total capacity of up to 9000 samples, while the barcode reader simultaneously manages the complete inventory.

Thermo Scientific™ Transcend™ TLX UHPLC system

Enable fast cleanup of complex matrices for accelerated online sample preparation, lower your laboratory costs, and improve data quality using Thermo Scientific Transcend TLX systems with TurboFlow™ technology. Use a combination of chromatographic techniques for online sample cleanup to improve LC-MS detection, reduce manual work, and provide higher productivity while the systems' additional channel capabilities improve throughput by up to 400%.

Thermo Scientific™ TraceFinder™ software

Thermo Scientific TraceFinder software is a method building, acquisition, and analysis software all in one that allows for quantitative and qualitative data analysis.

Thermo Scientific™ Compound Discoverer™ software

Thermo Scientific Compound Discoverer software is a complete solution for small molecule unknown data processing and powered by mzCloud, mzVault, and mzLogic.

Thermo Scientific™ AcquireX™ software

Thermo Scientific AcquireX software allows for a deeper dive into unknown analysis with automatic background exclusion and iterative data acquisition.

mzCloud™

mzCloud is a freely searchable web-based collection of spectra, spectral trees, structures, fragments, precursor ions, chromatographic data and compound related references.

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Our complete Thermo Scientific Forensics LC-MS lineup

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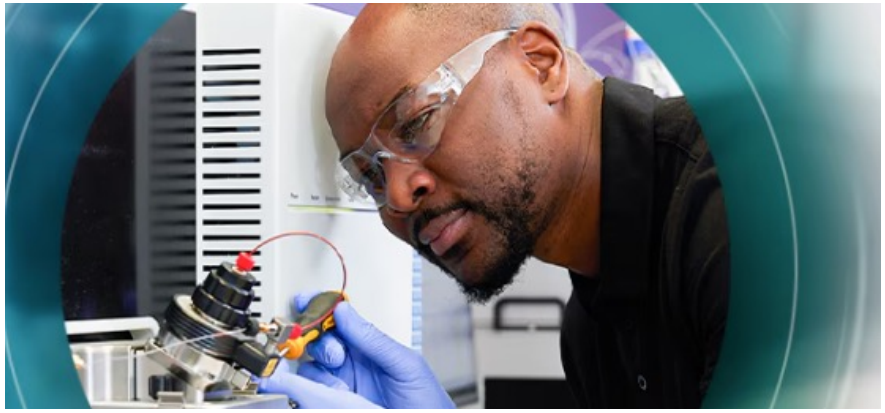

Strengthen evidence with forensic toxicology solutions that go further

Accurate identification and precise quantitation of known and new substances can make or break cases. So Thermo Fisher Scientific is advancing forensic toxicology to a higher level of confidence with a comprehensive portfolio of toxicology solutions based on LC-MS technology that builds greater reliability into the entire toxicology workflow.

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