Toxicology

Frequently asked questions

Tox Explorer Collection for HRAM and Triple Quadrupole mass spectrometry

An all-in-one LC-MS/MS solution for increased confidence in toxicology

What is the Thermo Scientific™ Tox Explorer™ Collection?

A robust LC-MS/MS workflow for toxicology analysis, comprised of:

- Reliable liquid chromatography and mass spectrometry (LC-MS/MS) instruments.
- An extensive library of drug compounds and their metabolites.
- HPLC columns and proven methods that can enable every toxicology laboratory—for every analyte, matrix type, choice of mass spectrometer, and user expertise level.
- Accuracy and reliability, backed by comprehensive training and support.

On which MS platform is the Tox Explorer Collection available?

The first platform featured the Thermo Scientific™ Q Exactive™ Plus hybrid-quadrupole Orbitrap™ mass spectrometer. Now, the Tox Explorer Collection is available on the latest MS platforms—the Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer and the Thermo Scientific™ TSQ Quantis™ Plus mass spectrometer combined with the Thermo Scientific™ Vanquish™ Flex UHPLC system (Figures 1 and 2).

Figure 1. Orbitrap Exploris 120 mass spectrometer coupled with Vanquish Flex UHPLC system

Figure 2. TSQ Quantis Plus mass spectrometer coupled with Vanquish Flex UHPLC system

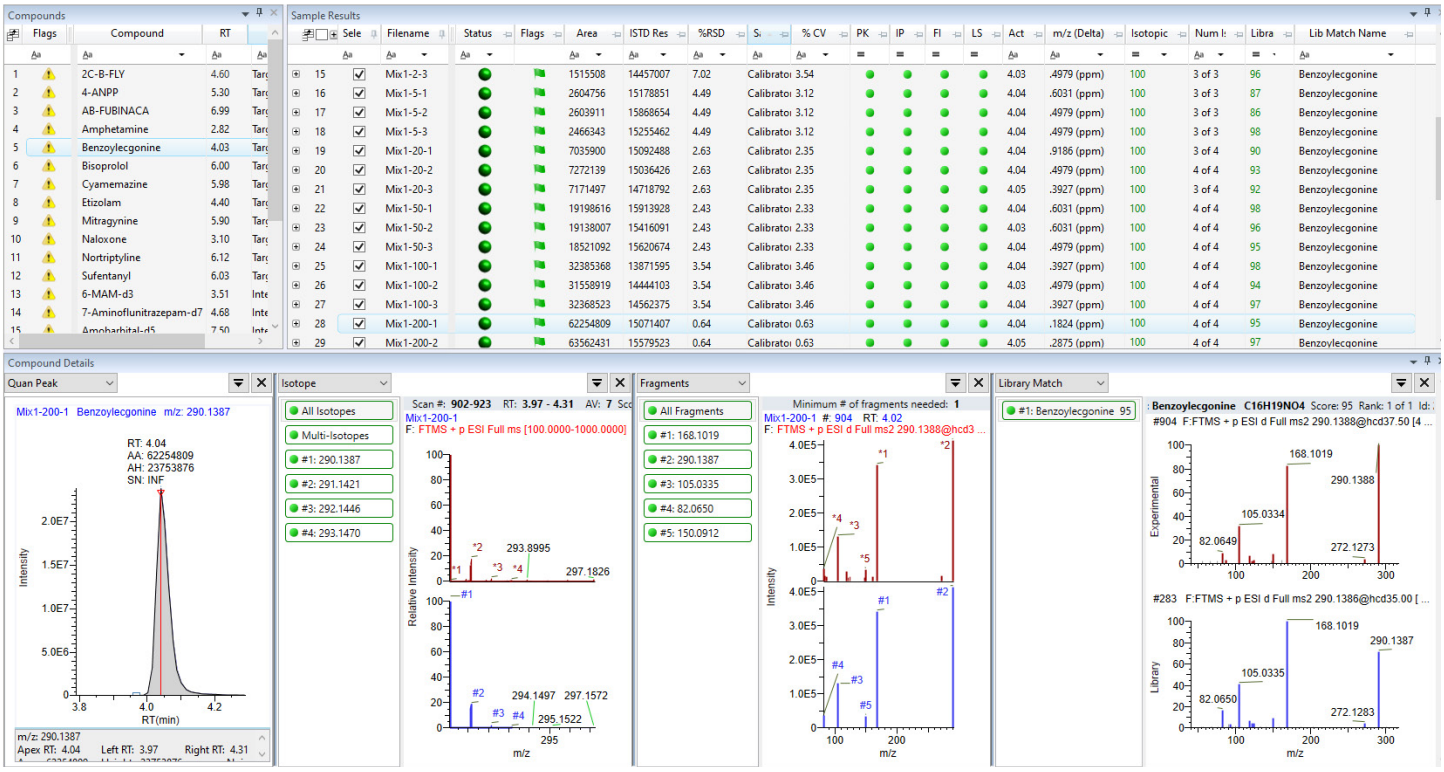


Figure 3. Data review of targeted screening using Thermo Scientific™ TraceFinder™ 5.1 software

What laboratories will this workflow serve?

The Tox Explorer Collection workflow provides forensic toxicology, clinical research toxicology, sports anti-doping, and reference laboratories with confident, comprehensive, and concise toxicology screening, identification, and confirmation.

Will I receive training and support for the Tox Explorer Collection?

Yes. Included in the Tox Explorer Collection bundle are the instrument and enterprise services installation (with an additional day included), along with two days of on-site or virtual application-focused training provided by our Center of Excellence.

What Is Included in the Tox Explorer Collection?

The UHPLC and MS systems are included in the Orbitrap Exploris 120 mass spectrometer high resolution accurate mass (HRAM) or TSQ Quantis Plus (triple quadrupole) mass spectrometer bundle options which also includes method, data package, and training. Your sales representative will discuss available options if you already own Thermo Scientific instrumentation and software.

Is the method optimized and can it be adjusted?

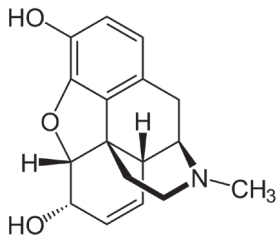
The method contains a proven LC gradient that has been standardized by many laboratories around the world, including several reference laboratories where the users are focused on assays such as: sports anti-doping, clinical, and forensics toxicology. The method can be adjusted. However, keep in mind any changes in chromatography will cause a shift in the retention times in the compound database which would require re-establishing a vast number of retention times in the method. The current chromatographic run length allows for adequate chromatographic separation of the current set of compounds, as well as addition of more compounds in the future. You are free to modify this method as desired, but please note that you will be responsible for determining and updating the database with the new retention times.

Can I use different column chemistries?

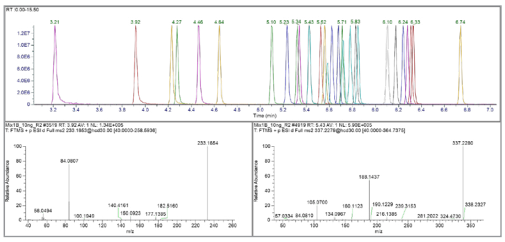
Other column chemistries would affect and change the retention times, so we recommend staying with the Thermo Scientific™ Accucore™ phenyl hexyl column provided. This column is best suited for analyzing a wide range of compounds. (Part No: 179-26-102130)

Can sample preparation be modified?

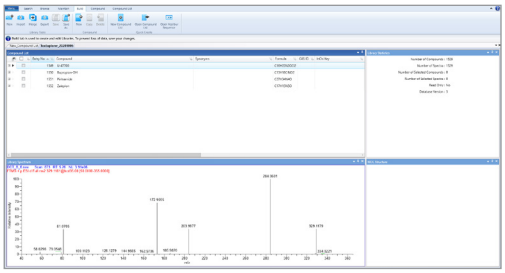
The Tox Explorer Collection focuses on standardized conditions for LC-MS, consisting of separation and detection parameters with corresponding compound database or library and retention times. You may choose to use the sample preparation approach you desire.



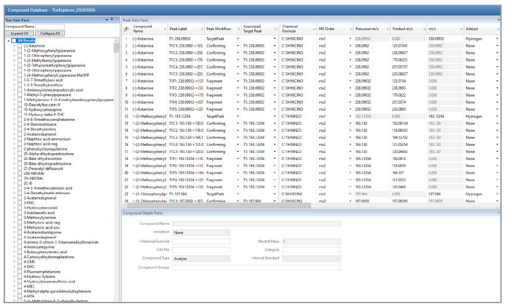
Known data: *m/z* and empirical formula to calculate isotope pattern



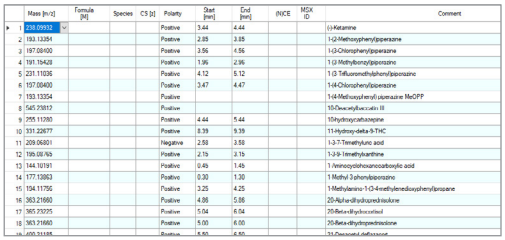
Run standard and obtain RT and MS 2 spectrum (includes fragments)



Import MS spectrum in mzVault



Export to compound database



Export compound database to inclusion list

Figure 4. Workflow for adding compounds to the database

Can I add my own compounds to the library?

Absolutely. Compounds can be added from external libraries or standard optimization. See figure 4 for step-by-step instructions.

Does the Tox Explorer Collection work with Chromeleon CDS software?

Methods are provided for Thermo Scientific™ TraceFinder™ software processing. Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software currently cannot perform screening of MS/MS HRAM data. Until this feature is available, Chromeleon CDS software cannot be used.

Is the Tox Explorer Collection library regularly updated with new compounds?

- The Tox Explorer Collection compound database and spectral library will be updated periodically and will be made available for download with the compatible versions of TraceFinder software. There is no additional cost for the updated library once the Tox Explorer Collection has been purchased.
- Users can add their own spectra to the existing library or create their own library.
  - The customer will be responsible for adding the retention times of the new analytes that are added to the database.
- When purchasing TraceFinder software, Thermo Scientific™ mzCloud™ mass spectral libraries that are routinely updated can be downloaded in Thermo Scientific™ mzVault™ mass library format. These analytes, as well as other analytes, can be added directly to the compound database in TraceFinder software.
  - Remember to update the new analyte's retention time.
  - For triple quadrupoles, there is a feature for database integration accessing over 19,000 curated selected reaction monitoring (SRM) transitions for direct insertion into new or existing instrument methods.

Can the Tox Explorer Collection be used for unknown or discovery-type analyses? Can it be used with Compound Discoverer software?

The Tox Explorer Collection is a targeted screening method, however the method can be altered to acquire unknown data which can be processed using Thermo Scientific™ Compound Discoverer™ software. Additional support beyond that which comes with the Tox Explorer Collection may be needed for such a method, and it is not guaranteed that the instrument will detect and find all unknown compounds.

Is ion chromatography and gas chromatography part of the Tox Explorer Collection for Orbitrap platforms?

Currently, no. The compound database can be used for exact mass, isotopes, and fragment ions, but retention times would have to be re-established. The SRM compound database that contains transitions can be used, but retention times will have to be re-established based on separation technique.

How is mzCloud mass spectral library being used with this workflow?

Currently, there is no direct route from mzCloud library to TraceFinder software as in Compound Discoverer software. A library from mzCloud library can be downloaded for use in TraceFinder software, but if the analytes are not in the database, retention times need to be assigned.

Can multi-channel HPLC be used with the Tox Explorer Collection?

Retention times were acquired on a single channel Vanquish Flex UHPLC system. Moving to a multi-channel system will alter the retention times, however, it is a feasible option.

How many compounds can I quantitate on a triple quadrupole instrument?

Currently 101 analytes with 5 internal standards were tested and quantified, and the Tox Explorer TSQ compound database contains over 1,200 compounds with SRM transitions.

For best sensitivity, it is recommended to limit the number of SRM transitions to maintain an adequate dwell time for each transition.

How many compounds can it quantitate on an HRAM instrument? Will SIM or PRM work?

- Currently 101 compounds were tested and quantified on concentrations of 0.1 to 1000 ng/mL, and the Tox Explorer Collection HRAM library contains over 1,800 compounds.
- Quantitation was performed using the precursor ion *m/z*. Using this approach, any number of compounds could be quantified with this method. Limits of detection will vary from compound to compound, and the user will be responsible for validation of the method.
- SIM and PRM would work with the same inclusion list, but a different processing method for TraceFinder software would be needed. SIM and PRM data acquisition methods are more time-intensive and will result in fewer scans across a peak.

Can we run the Tox Explorer Collection on versions of TraceFinder software prior to version 5.1?

No, the library format will only will work with TraceFinder 5.1 software or higher.

Can All Ion Fragmentation (AIF) be used with this method?

This could be used, however, it would be limited if the concentration of the analyte was low and if many analytes eluted at the same time. Also, the AIF fragmentation spectrum will not give as good a match to the single-compound library spectrum.

What kits are compatible with the Tox Explorer Collection?

There are currently no kits that are recommended for use with the Tox Explorer Collection.

How many injections can be performed with the Tox Explorer Collection?

Thousands. The UHPLC column can be the limiting factor. Depending on your sample preparation type, we suggest switching out to a clean transfer tube at around 500 injections to maintain sensitivity.

Why are there more compounds available on the Orbitrap Exploris 120 mass spectrometer versus the TSQ Quantis Plus mass spectrometer options?

All analyte information was collected from standards but not all SRMs were established for the triple quadrupole method.

What if my targeted list of analytes is only 100, why do I need a library with over a 1,000 compounds?

The large list of SRM transitions/mass spectral library enables you to customize the target list to suit your needs. It is possible to easily reduce the analyte list down to just your 100 compounds of interest.

What compound classes does the Tox Explorer Collection cover?

Therapeutic/prescription drugs, sports doping, drugs of abuse, pesticides, endogenous metabolites, natural toxins, industrial chemicals, excipients/additives/colorants, steroids/vitamins/hormones, extractables/leachable, PCPs/cosmetics, textile chemicals, illegal additives, perfluoroinated hydrocarbons, counterfeit drugs, and fentanyls

Are there regional differences for regulations regarding quantitation?

Yes, please work with your regional account managers to understand requirements needed for your specific toxicology analysis. A list of 3rd party consultants can also be provided.

Do you have a list available that I can review to see if my compound of interest is in the library?

Yes, please reach out to your local Thermo Fisher Scientific representative for more information.

What is the sample preparation method used?

Guidelines for urine, blood, and plasma are addressed in the technical guide that comes with the Tox Explorer Collection. Some of the techniques included are dilute and shoot, enzymatic hydrolysis, or protein precipitation. Support for more extensive sample preparation such as liquid-liquid extraction or solid phase extraction can be supported, but the customer is responsible for all validation.

Can instruments other than the Vanquish Flex UHPLC system coupled with the TSQ Quantis Plus mass spectrometer, the Q Exactive Plus hybrid quadrupole Orbitrap mass spectrometer, or the Orbitrap Exploris 120 mass spectrometer be used with the Tox Explorer Collection?

Other mass spectrometers are amenable but will need support regarding different RF lens values. Retention times were created using the Vanquish Flex UHPLC system. The customer would be responsible for re-establishing and verifying retention times with an alternate LC system.

If I already have the LC-MS/MS instrumentation needed for the Tox Explorer Collection, can I just purchase the consumables and library?

As long as the instruments are the Vanquish Flex UHPLC system coupled with the Q Exactive Plus hybrid-quadrupole Orbitrap mass spectrometer, the Orbitrap Exploris 120 mass spectrometer, or the TSQ Quantis Plus mass spectrometer, the consumables, LC tubing, and library can be purchased separately. The library can be purchased for other LC-MS/MS instruments, but retention times would need to be re-established.

Do I need internal standards for all the compounds I'm analyzing?

This question will need to align with regional regulations and what experiments need to be performed (i.e., screening or quantitation).

Related documents of interest

Current Guidelines for Drugs of Abuse

National Forensic Laboratory Information System (NFLIS): data collection effort of drug chemistry results from local, State and Federal forensic laboratories

LC-MS/MS toxicology workflow on the new Orbitrap Exploris 120 mass spectrometer for screening, quantitation, and confirmation of drugs

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

Learn more at **thermofisher.com/toxexplorer**