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# Automating component detection of small molecules in complex mixtures using HRAM Q-TOF data

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# Automating component detection of small molecules in complex mixtures using HRAM Q-TOF data

## Overview

- Applying high resolution accurate mass (HRAM) QTOF analysis for suspect screening of small molecules in complex matrices and automating component detection.
- The algorithm automatically locates components that behave as chromatographic features.

## Introduction

To accelerate small molecule component detection in complex mixtures a novel algorithm has been developed for high resolution accurate mass Q-TOF data sets. As with other techniques, the algorithm locates ions that behave as a recognized chromatographic feature (ion intensities rise and fall in abundance in a covariant manner) but differs in its ability to qualify spectral interpretation by identifying isotopes of various charge states in complex interlaced mass spectra. It can also identify and correlate mass spectrometric signals from adducts, neutral losses and alternate charge states to give a simplified "single component" output when multiple ionized species are present. This algorithm was evaluated using a series of HRAM Q-TOF data sets including food safety and toxicology screening.

## Materials and Methods

The algorithm for component detection was applied to complex mixture analysis in food safety and drugs of abuse. Both matrices included a QuEChERS extract and the algorithm was applied to a generic HRAM LC-MS/MS method (Nexera LC and LCMS-9030 QTOF system, Shimadzu Corporation) as shown in figure 1. Components were typically separated using a Restek Raptor ARC18 or Restek Biphenyl column depending on the test panel. The component detection stage requires minimal parameters to function effectively, and definition of typical chromatographic peak width is the only key parameter needed. Two parameters define acceptance criteria for the ion intensity in a mass spectrum and the sum of the ions identified in a component.

# Automating component detection of small molecules in complex mixtures using HRAM Q-TOF data

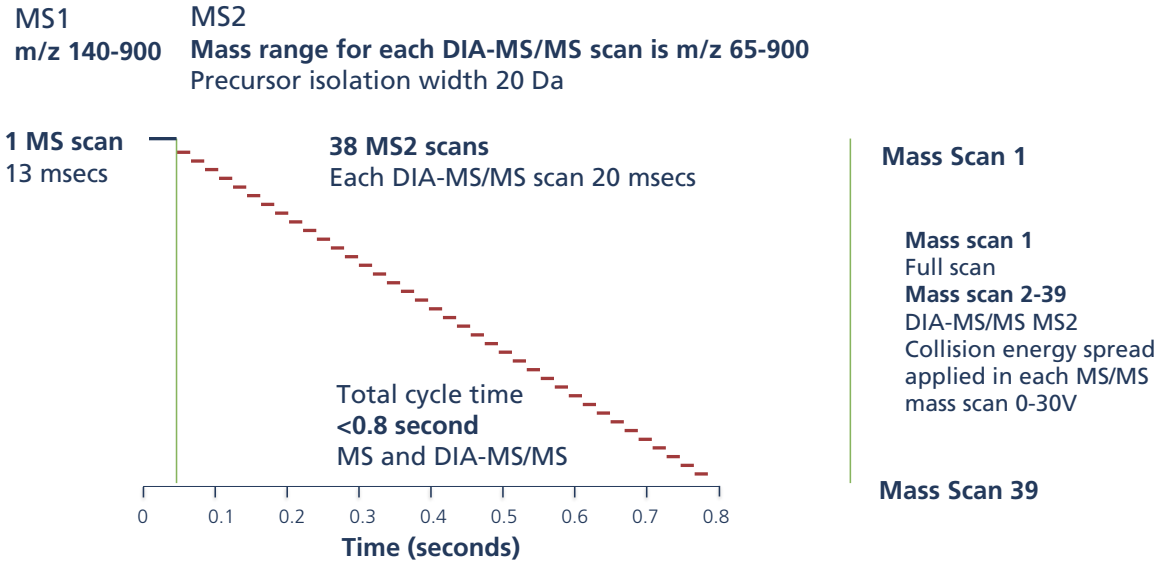


Figure 1. The component detection algorithm was applied to analysis of a complex QuEChERS extract in food safety and drugs of abuse using data acquired by the LCMS-9030 QTOF system. The LC-MS/MS method used in both test panels included full scan MS followed by sequential MS/MS scans. The algorithm was applied to the MS full scan data. (The mass range used for food safety was 140-900 Da and 100-500 Da for drugs of abuse screening).

# Results

## Food safety screening
## HRAM DIA-MS/MS data acquisition

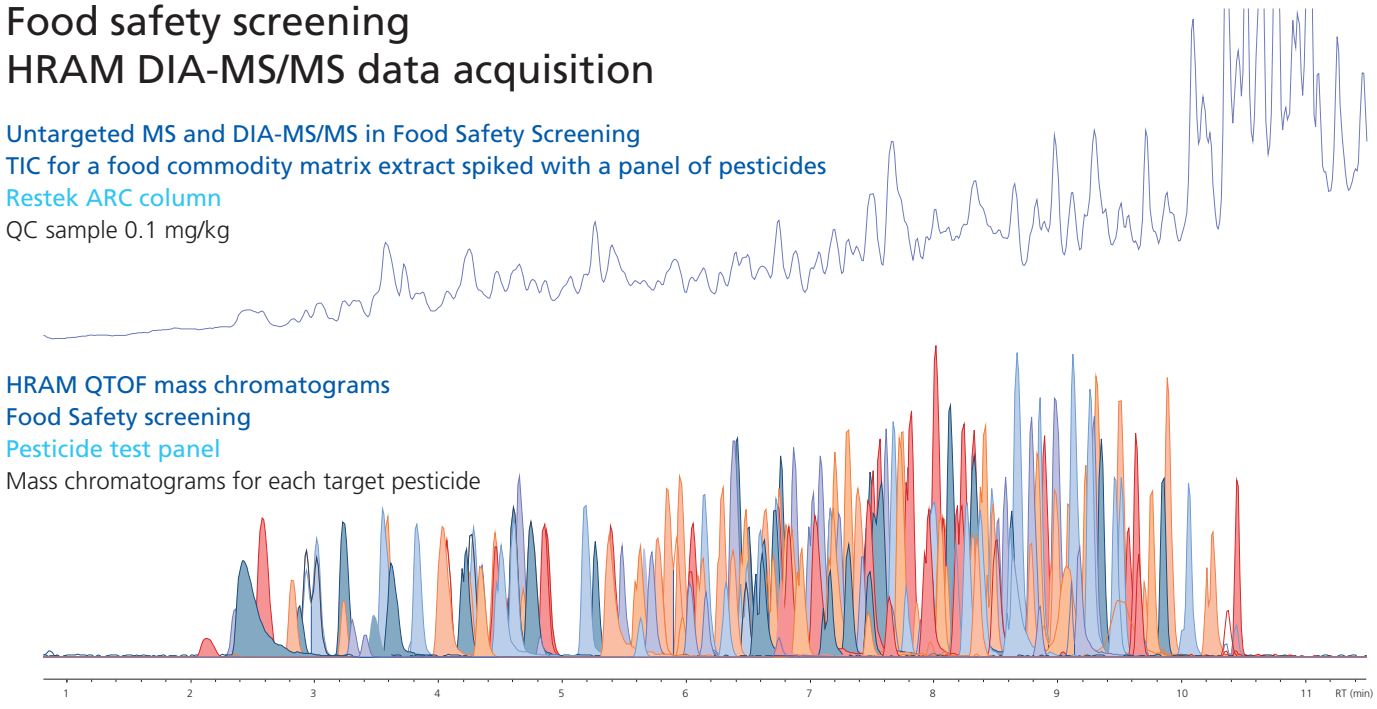


Figure 2. The component detection algorithm was applied to analysis of a complex QuEChERS extract of an apple matrix spiked with 212 pesticides at a concentration of 0.1 mg/kg corresponding the QC concentration. Default parameters for threshold and a peak width (FWHM of 0.05 minute) were applied and the algorithm detected 3100 grouped components (including adducts, neutral losses and alternate charge states) in the extract.

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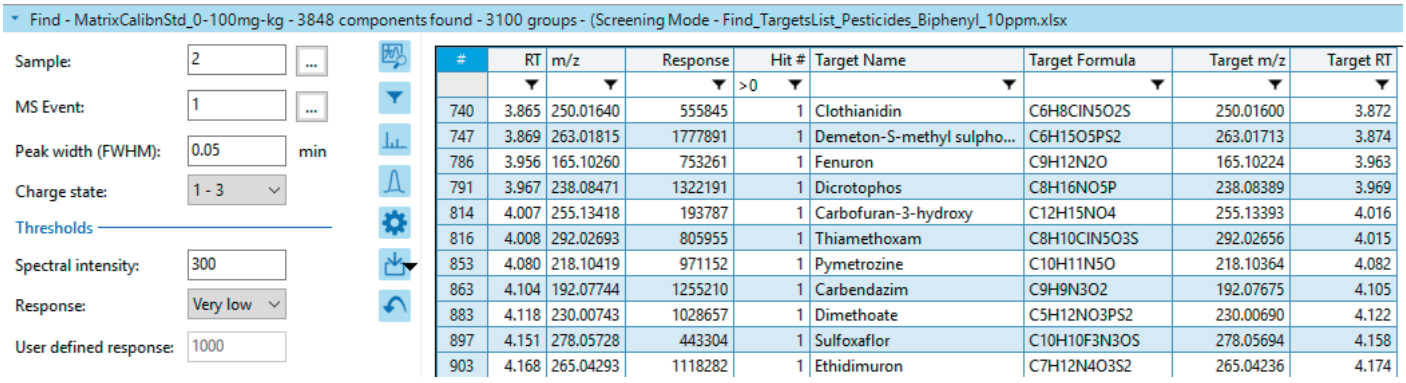


| # | RT | m/z | Response | Hit # | Target Name | Target Formula | Target m/z | Target RT |
|---|---|---|---|---|---|---|---|---|
| | | | | >0 | | | | |
| 740 | 3.865 | 250.01640 | 555845 | 1 | Clothianidin | C6H8ClN5O2S | 250.01600 | 3.872 |
| 747 | 3.869 | 263.01815 | 1777891 | 1 | Demeton-S-methyl sulpho... | C6H15O5PS2 | 263.01713 | 3.874 |
| 786 | 3.956 | 165.10260 | 753261 | 1 | Fenuron | C9H12N2O | 165.10224 | 3.963 |
| 791 | 3.967 | 238.08471 | 1322191 | 1 | Dicrotophos | C8H16NO5P | 238.08389 | 3.969 |
| 814 | 4.007 | 255.13418 | 193787 | 1 | Carbofuran-3-hydroxy | C12H15NO4 | 255.13393 | 4.016 |
| 816 | 4.008 | 292.02693 | 805955 | 1 | Thiamethoxam | C8H10ClN5O3S | 292.02656 | 4.015 |
| 853 | 4.080 | 218.10419 | 971152 | 1 | Pymetrozine | C10H11N5O | 218.10364 | 4.082 |
| 863 | 4.104 | 192.07744 | 1255210 | 1 | Carbendazim | C9H9N3O2 | 192.07675 | 4.105 |
| 883 | 4.118 | 230.00743 | 1028657 | 1 | Dimethoate | C5H12NO3PS2 | 230.00690 | 4.122 |
| 897 | 4.151 | 278.05728 | 443304 | 1 | Sulfoxaflor | C10H10F3N3OS | 278.05694 | 4.158 |
| 903 | 4.168 | 265.04293 | 1118282 | 1 | Ethidimuron | C7H12N4O3S2 | 265.04236 | 4.174 |

## Data workflow

### Acquire data using MS and DIA-MS/MS method (Figure 1)

MS 140-900 Da (40 msecs; mass range m/z 140-900) followed by 38 DIA-MS/MS sequential mass scans (each DIA-MS/MS mass scan 20 msecs; MS/MS mass range m/z 65-900; precursor ion isolation 20Da);

### Open the component detection research application

### Select default intensity threshold and chromatographic peak width

### Run the component detection algorithm for the QC sample

Using a low response threshold a high number of components were detected and grouped together (this will detect all components including matrix derived ions from the QuEChERS extract).

### Search for a target list of pesticides

### Open an Excel spreadsheet with a target list of pesticides (the search criteria include name, formula, accurate mass and if available the retention time).

From the list of targets submitted in the suspect screening search, 207 components were matched by accurate mass (within 5 ppm; below m/z 200 the mass tolerance was set to <1 mDa) and retention time (within 0.25 minute). 5 compounds were not detected based upon default chromatographic peak width and signal to noise setting.

# Automating component detection of small molecules in complex mixtures using HRAM Q-TOF data

## Toxicological screening
## HRAM DIA-MS/MS acquisition

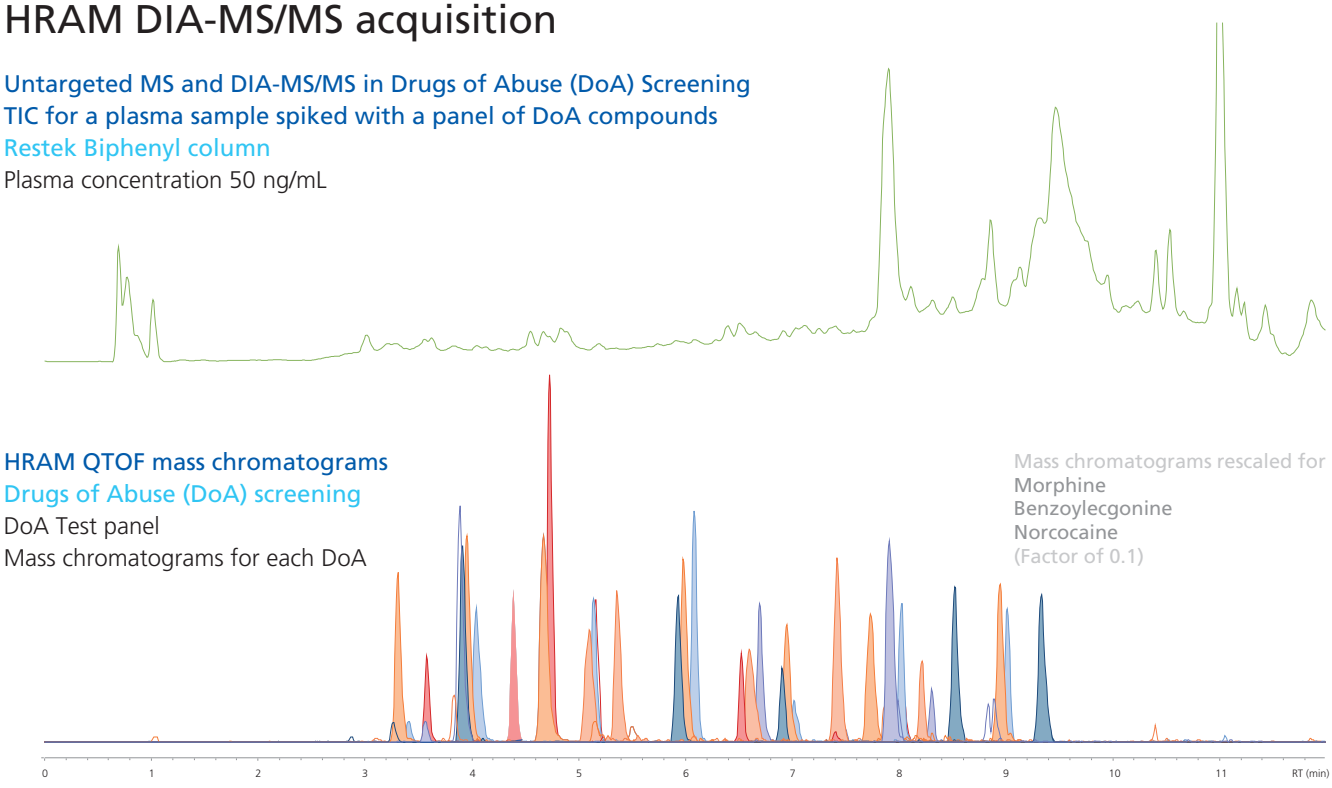


Figure 3. TIC and extracted mass chromatograms for a panel of 45 drugs of abuse targets spiked at 50 ng/mL into a human plasma extract. To find a list of DoA targets, the component detection algorithm was used to deconvolute the data file using default search parameters.

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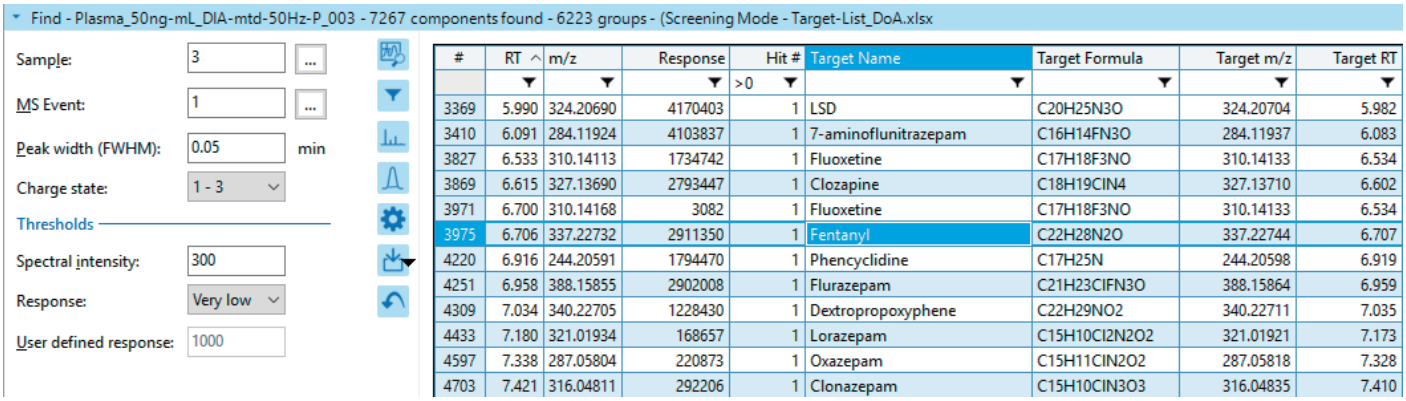


| # | RT | m/z | Response | Hit # | Target Name | Target Formula | Target m/z | Target RT |
|---|---|---|---|---|---|---|---|---|
| | | | | >0 | | | | |
| 3369 | 5.990 | 324.20690 | 4170403 | 1 | LSD | C20H25N3O | 324.20704 | 5.982 |
| 3410 | 6.091 | 284.11924 | 4103837 | 1 | 7-aminoflunitrazepam | C16H14FN3O | 284.11937 | 6.083 |
| 3827 | 6.533 | 310.14113 | 1734742 | 1 | Fluoxetine | C17H18F3NO | 310.14133 | 6.534 |
| 3869 | 6.615 | 327.13690 | 2793447 | 1 | Clozapine | C18H19ClN4 | 327.13710 | 6.602 |
| 3971 | 6.700 | 310.14168 | 3082 | 1 | Fluoxetine | C17H18F3NO | 310.14133 | 6.534 |
| 3975 | 6.706 | 337.22732 | 2911350 | 1 | Fentanyl | C22H28N2O | 337.22744 | 6.707 |
| 4220 | 6.916 | 244.20591 | 1794470 | 1 | Phencyclidine | C17H25N | 244.20598 | 6.919 |
| 4251 | 6.958 | 388.15855 | 2902008 | 1 | Flurazepam | C21H23ClFN3O | 388.15864 | 6.959 |
| 4309 | 7.034 | 340.22705 | 1228430 | 1 | Dextropropoxyphene | C22H29NO2 | 340.22711 | 7.035 |
| 4433 | 7.180 | 321.01934 | 168657 | 1 | Lorazepam | C15H10Cl2N2O2 | 321.01921 | 7.173 |
| 4597 | 7.338 | 287.05804 | 220873 | 1 | Oxazepam | C15H11ClN2O2 | 287.05818 | 7.328 |
| 4703 | 7.421 | 316.04811 | 292206 | 1 | Clonazepam | C15H10ClN3O3 | 316.04835 | 7.410 |

## Data workflow

### Acquire data using a MS and DIA-MS/MS method (as in Figure 1; changes applied to the mass range)

MS 100-500 Da (20 msecs; mass range m/z 100-500) followed by 19 DIA-MS/MS sequential mass scans (each DIA-MS/MS mass scan 20 msecs; MS/MS mass range m/z 40-500; precursor ion isolation 20Da);

### Open the component detection research application

### Select default intensity threshold and chromatographic peak width

### Run the component detection algorithm for the 50 ng/mL spiked plasma sample

The plasma sample was prepared as a protein crash (using acetonitrile) with QuEChERS clean-up. The component detection algorithm found 6223 grouped compounds.

### Apply a target list of DoA; the forensic toxicology screening library of 1278 compounds was filtered to search for classes of drugs of abuse (the library was filtered to 153 targets)

### Open an Excel spreadsheet with a target list of drugs of abuse (the search criteria include name, formula, accurate mass and if available the retention time).

Using a filtered library of drugs of abuse compounds all 45 targets were matched by accurate mass (within 5 ppm; below m/z 200 the mass tolerance was set to <1 mDa) and retention time (within 0.2 minute of the target list entries in the spreadsheet data base).

# Suspect screening in complex mixtures

Using a series of HRAM Q-TOF data sets for food safety and drugs of abuse screening, the component detection algorithm was used to locate covariant ions using a two-step process, 'search' followed by 'validation'.

In the first step, a set of processed mass spectral data is created by using a 'moving FWHM window' based on the user defined chromatographic FWHM value. This creates a set of combined scans over the whole chromatogram. Masses from each combined scan are then correlated with equivalent masses from adjacent combined mass spectra to link data across the chromatographic space. A peak identification method is applied to locate the apex of each proposed chromatographic component using the spatially linked masses. A background ion detection algorithm is then applied to the chromatographic data so that only chromatographically relevant components reported further. In parallel to locating covariant ions, a spectral interpretation process is applied where isotopic clusters are found. Finally, the search process reports a list of proposed monoisotopic masses and approximate peak apex times.

In the second step, the algorithm further validates the results of the search process to accurately locate the chromatographic component in the raw data. Spectra are combined over the identified chromatographic peak, and then subject to spectral interpretation, to give the most accurate interpretation of isotopic distribution for each component. The validation process improves the accuracy of the component detection process and eliminates false positive reporting. Finally, specific species such as adducts, neutral losses and alternate charge states are identified and correlated so that the monoisotopic mass of the protonated species is reported as the component mass.

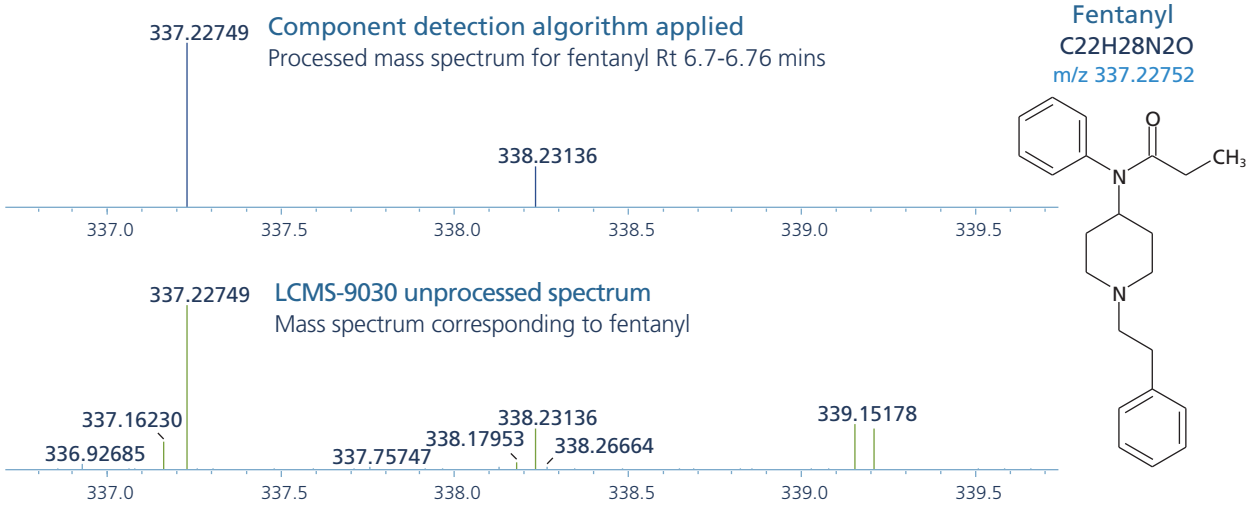


Figure 4. Mass spectrum for fentanyl processed by the component detection algorithm and the corresponding unprocessed mass spectrum. The component detection algorithm is designed to report a simplified "single component" output when multiple ionized species are present and the most accurate interpretation of isotopic distribution for each component.

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## Conclusions

- A novel component detection algorithm has been applied to accelerate small molecule detection in complex mixtures for high resolution accurate mass Q-TOF data sets.
- Using existing HRAM MS and DIA-MS/MS methods, accurate masses of the ions detected and identified by the component detection algorithm were compared to the exact masses of compounds in a pesticide database and in a forensic toxicology database that was filtered for drugs of abuse targets.
- The algorithm can be applied to targeted and untargeted analysis of complex mixtures.

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