All Ions MS/MS: Targeted Screening and Quantitation Using Agilent TOF and Q-TOF LC/MS Systems

Technical Overview

Introduction

All Ions MS/MS is a technique that is available for Agilent high resolution TOF and Q-TOF LC/MS instruments. All Ions MS/MS uses the Agilent proprietary Personal Compound Database and Libraries (PCDLs) to identify compounds with information from their molecular and fragment ions. The PCDLs are content rich and include accurate mass information for thousands of compounds. The incorporation of All Ions MS/MS processing tools into MassHunter means that compound identification is rigorous, and can be easily reviewed. As a result, All Ions MS/MS analysis decreases method setup time, increases throughput, and enables confident, high sensitivity quantitative, and qualitative analysis on a single instrument, in a single analytical run.

Agilent Technologies

Identification by Correlating Precursor to Fragment Ions

In the All Ions MS/MS technique, high resolution accurate mass (HRAM) data is acquired using different conditions: (1) with a low value for the fragmentor voltage or collision energy and (2) one or multiple high energy values. The low energy spectra predominantly show just the molecular (or precursor) ions for the compounds and the high energy spectra provide the precursors plus their fragment ions.

This analytical method is easy to set up using the MassHunter Workstation Acquisition for TOF and Q-TOF (rev. B.05.01 and later). Figure 1 shows a new feature in the Acquisition software, an Experiment # field allows different values of parameters during a time segment. For example, a user can set up Experiment 1 with a collision energy of 0 V, then Experiments 2 and 3 with collision energies of 20 and 40 V.

The result of alternating the fragmentor voltage or collision energy is a data file with a low energy channel that contains predominantly precursor ions and one or several high energy channels that contain precursor and fragment ions. Figure 2A shows a spectrum of simazine with a collision energy of 0 V where the precursor ion at 202.08584 is prominently featured. Figure 2B shows an averaged spectrum of collision energies at both 20 and 40 V which contains the precursor as well as several fragment ions.

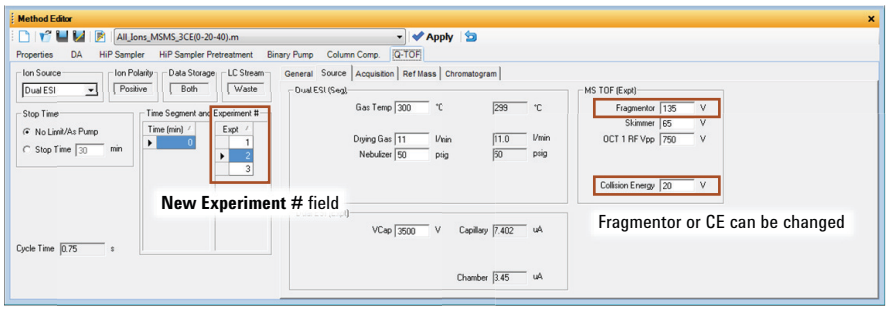


Figure 1. Q-TOF tab in the MassHunter Acquisition Method Editor. Up to four experiments with different values of collision energy (Q-TOF) or Fragmentor voltage (TOF or Q-TOF) can be configured within one time segment.

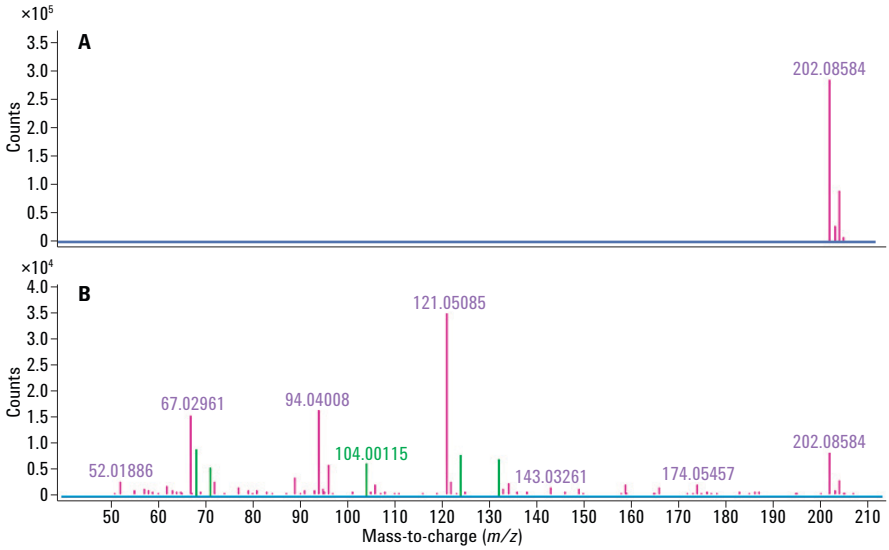


Figure 2. A) The precursor ion and its isotopic cluster for the herbicide simazine are in red. B) Shows an average of spectra acquired at collision energies 20 and 40 V. The precursor at 202.08584 is still present, but the fragment ions have also been acquired.

The All Ions MS/MS technique works in the MassHunter Qualitative Analysis Software by correlating the elution profile of the precursor ion in the low energy channel to those of the fragments generated under higher energy conditions. For example, Figure 3 shows the precursor (in red) and a fragment at *m/z* 132.03221 with qualifying coelution (in green). This is achieved by obtaining all the fragment information from existing compounds in the Agilent PCDL, which contains MS/MS spectra acquired at several different collision energies[1]. In addition, PCDLs also contain compound information including the name, formula, accurate mass, structure, and database identifiers such as the CAS number. The PCDL is used to select potential fragment ions which are then analyzed for coelution with the precursor ion.

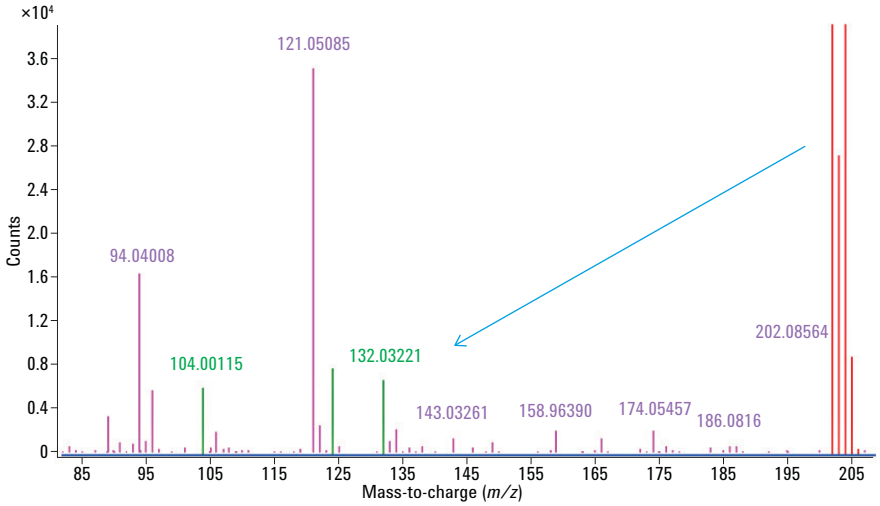
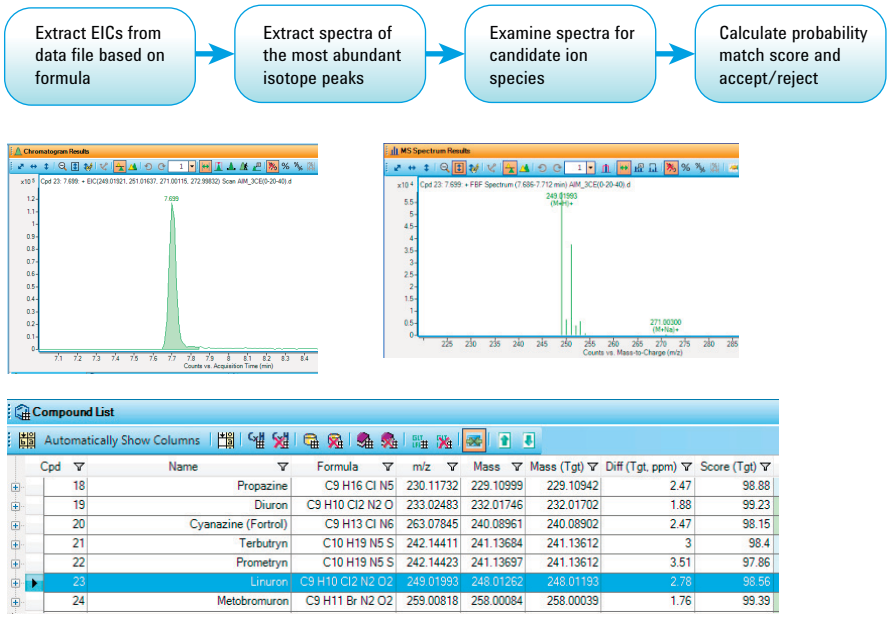


Figure 3. The precursor ion for simazine 202.08564 *m/z* (in red) is correlated to its fragment ion 132.03221 *m/z* (in green) using the All Ions MS/MS technique.

Find by Formula

The All Ions MS/MS technique (Figure 4) is an extension of Agilent's unique Find by Formula (FBF) algorithm in MassHunter Qualitative Analysis software. FBF starts with the formula of a compound (for example, from the PCDL) and calculates the monoisotopic mass and isotope pattern. It then pulls out Extracted Ion Chromatograms (EICs) from the data file based on the most abundant isotopes for each selected charge carrier, and extracts the averaged spectra from the top 50% of the integrated peaks. Thus, if the compound is present in the data file, it should generate chromatographic peaks for each of its major molecular ions.



Cpd	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score (Tgt)
18	Propazine	C9 H16 Cl N5	230.11732	229.10999	229.10942	2.47	98.88
19	Diuron	C9 H10 Cl2 N2 O	233.02483	232.01746	232.01702	1.88	99.23
20	Cyanazine (Fortrol)	C9 H13 Cl N6	263.07845	240.08961	240.08902	2.47	98.15
21	Terbutryn	C10 H19 N5 S	242.14411	241.13684	241.13612	3	98.4
22	Prometryn	C10 H19 N5 S	242.14423	241.13697	241.13612	3.51	97.86
23	Linuron	C9 H10 Cl2 N2 O2	249.01993	248.01262	248.01193	2.78	98.56
24	Metobromuron	C9 H11 Br N2 O2	259.00818	258.00084	258.00039	1.76	99.39

Figure 4. FBF uses a formula to calculate the monoisotopic mass and its isotopes for all selected charge carriers, extracting EICs, and then extracting peak spectra for chromatographic peaks. The peak spectra are then searched for ions associated with each compound and a probability match score is assigned. Compounds that exceed a user set threshold are labeled as identified in MassHunter Qualitative Analysis.

The extracted mass spectra from the peak are examined for candidate ion species based on the compound's formula. A probability match score is calculated for each species based on its mass, isotopic abundance and spacing (Figure 5). If a compound passes the match score filter, it is labeled as identified. FBF is fast and can examine a data file against an accurate mass database (CSV, PCD or PCDL) with thousands of formulas in a few minutes.

Fragment Confirmation

In the All Ions MS/MS technique, the FBF algorithm is enhanced with the additional step of fragment confirmation using one or several high energy channels. If the compound has at least one MS/MS spectrum in the PCDL, the most abundant product ions in the library spectra are extracted as EICs from the respective high energy channel. For example, Figure 6A displays the MS/MS library spectrum for simazine at 20 V collision energy from the Pesticide PCDL. Figure 6B shows a high energy spectrum from a sample that contains simazine. Using the PCDL, the software was able to locate fragments including those at *m/z* 104.00 and 132.03 despite much more abundant extraneous ions at *m/z* 94.04 and 121.05.

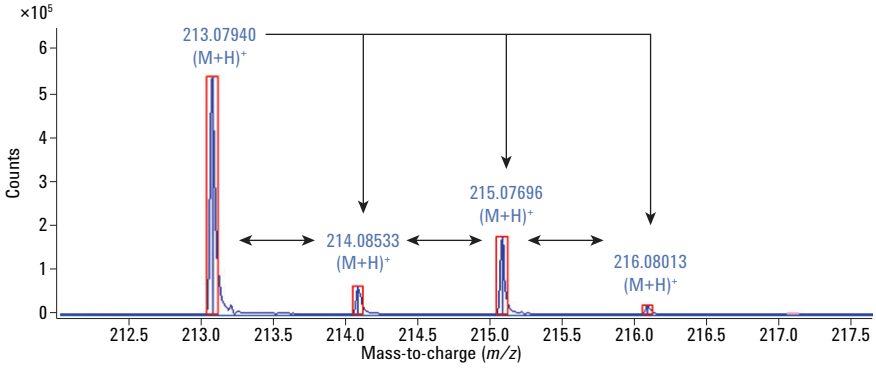


Figure 5. Identification of a compound using FBF makes use of three characteristics of the precursor ion's isotopic cluster: the accurate mass of each isotope, its abundance (denoted by the red boxes), and its spacing relative to the other isotopes.

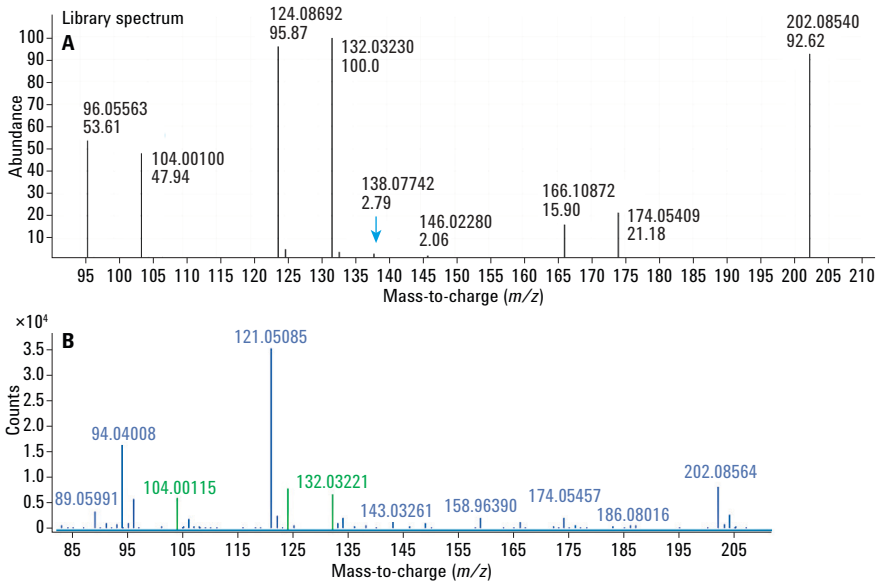


Figure 6. A) Shows an MS/MS library spectrum for simazine acquired at a 20 V collision energy. It shows the product ions for the compound including *m/z* 104.00 and 132.03. These ions are visible (in green) in the bottom spectrum (B) of the high energy channel despite more abundant ions at *m/z* 94.04 and 121.05.

The fragments from MS/MS spectra in the PCDL are extracted as EICs and overlaid with the precursor ion EIC. If no MS/MS spectra are available for a compound in the PCDL, then the most abundant fragments in the average of all the high energy channel spectra are extracted as EICs and overlaid. These overlaid EIC are evaluated using an unique coelution score parameter. The coelution score is derived from a technique which is similar to Peak Purity[2] used in UV chromatography, in that the software calculates a number based on abundance, peak shape (symmetry), peak width, and retention time. The normalized ratio of the fragment ions to the precursor ion intensity are plotted over the retention time and made available to the user for inspection in a Coelution Plot. Figure 7A shows the overlaid EICs for simazine while Figure 7B shows a Coelution Plot with all ions at the same apex (5.571 minutes).

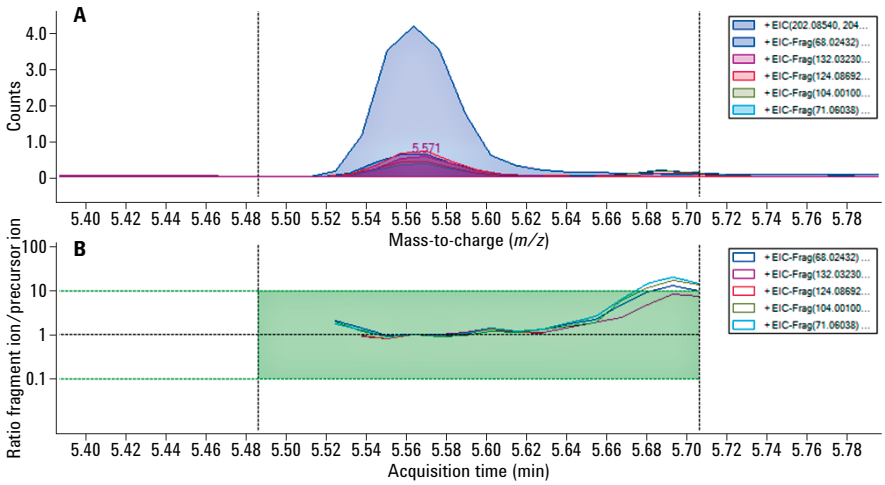


Figure 7. A) Shows EICs for simazine, with the precursor ion in blue and its fragment ions in other colors. All ions have the same chromatographic apex. B) Shows the Coelution Plot for simazine, which plots the ratio of the normalized fragment ion intensity over the precursor ion intensity over the retention time. All fragment ions exhibit ratios of approximately 1 across the middle of the precursor peak indicating strong coelution. This provided confirmation for the identification of simazine in the sample.

Users can set up parameters for the All Ions MS/MS technique in a new tab in the FBF area of MassHunter Qualitative Analysis called Fragment Confirmation (Figure 8). The tab allows the user to specify whether to use a spectral library or average fragment spectrum, and how many of the most abundant ions to extract. Limits can also be set for fragment ion EICs based on retention time difference, minimum signal-to-noise (S/N) ratio, and coelution score.

Once FBF has been run with fragment confirmation, users can view results for each compound in the Compound Identification Results pane (Figure 9). The name of the compound, its chemical formula, and probability match score from FBF is found at the top. The individual fragment ions used for the identification are listed below with their coelution score, collision energy (CE), and whether the fragment is qualified or not. If a fragment is not qualified, a reason is given, for example, Low S/N ratio.

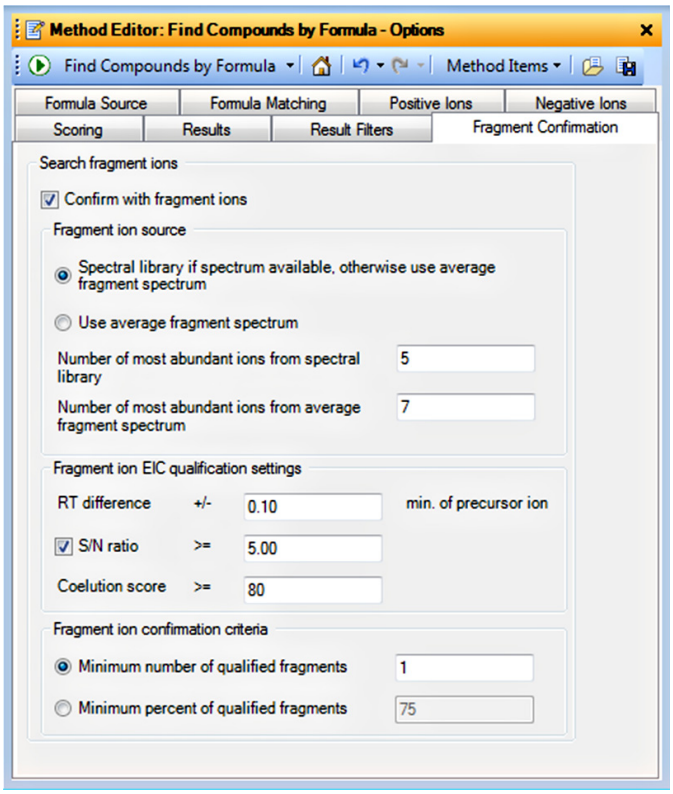
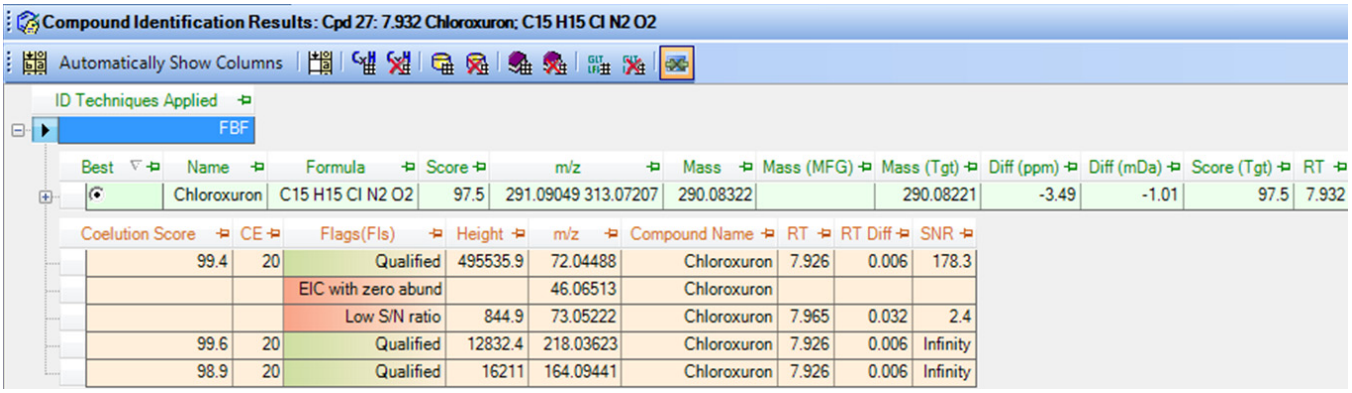


Figure 8. Select the **Fragment Confirmation** tab from the FBF area in MassHunter Qualitative Analysis. Click **Confirm with fragment ions** to modify FBF to perform the All Ions MS/MS technique. Users can decide whether to confirm with a spectral library (PCDL) or to use the most abundant ions from the average fragment spectrum. They can set the number of ions to use and set limits on qualifying the EICs, as well as set the minimum qualified fragments to confirm a compound's identity.



Best	Name	Formula	Score	m/z	Mass	Mass (MFG)	Mass (Tgt)	Diff (ppm)	Diff (mDa)	Score (Tgt)	RT
◉	Chloroxuron	C15 H15 Cl N2 O2	97.5	291.09049 313.07207	290.08322		290.08221	-3.49	-1.01	97.5	7.932

Coelution Score	CE	Flags(FIs)	Height	m/z	Compound Name	RT	RT Diff	SNR
99.4	20	Qualified	495535.9	72.04488	Chloroxuron	7.926	0.006	178.3
		EIC with zero abund		46.06513	Chloroxuron			
		Low S/N ratio	844.9	73.05222	Chloroxuron	7.965	0.032	2.4
99.6	20	Qualified	12832.4	218.03623	Chloroxuron	7.926	0.006	Infinity
98.9	20	Qualified	16211	164.09441	Chloroxuron	7.926	0.006	Infinity

Figure 9. Shows the Compound Identification Results for Chloroxuron. The first line of the table indicates the best compound match found with its name, formula, and probability matching score from FBF. The lower table shows that five fragment ions were evaluated with their coelution scores, collision energies, and whether they were qualified or not. Three fragment ions were qualified while two were rejected with a reason.

Viewing Compound Details

It is possible to inspect All Ions MS/MS results quickly in the new Compound Details View (Figure 10). Users can scroll through all compounds in the screen and efficiently view the library match result, both MS and fragment spectra, overlaid EICs and Coelution Plots.

At this point, the user will have developed a qualitative method for the identification of compounds using the All Ions MS/MS technique. Users can continue to analyze samples without having to access the PCDL's MS/MS spectra since the information about the fragment ions is saved with the MassHunter Qualitative Analysis method. New compounds can easily be added to the screening through addition to the PCDL followed by data reprocessing. This important capability means that subsequent data re-interrogation is simple and available without sample re-analysis.

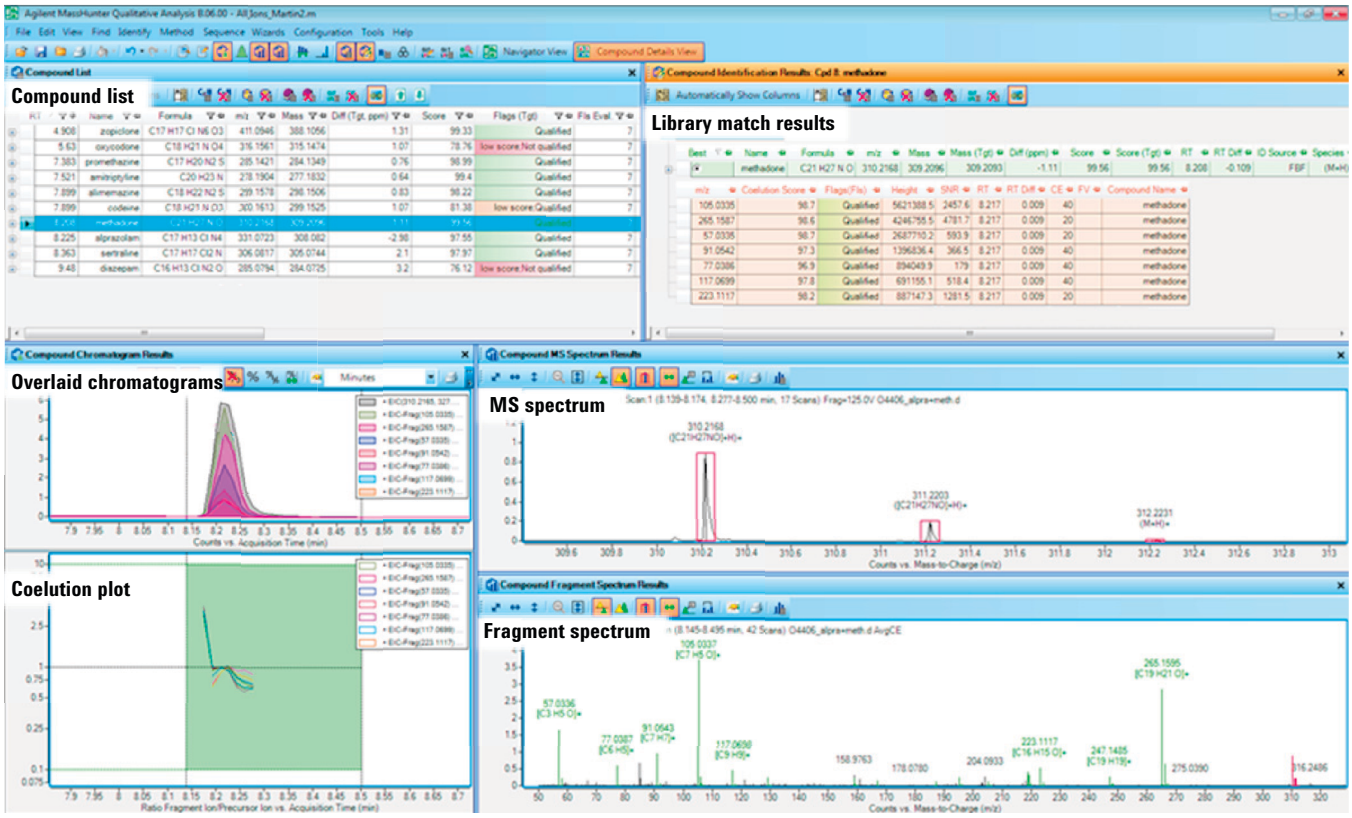
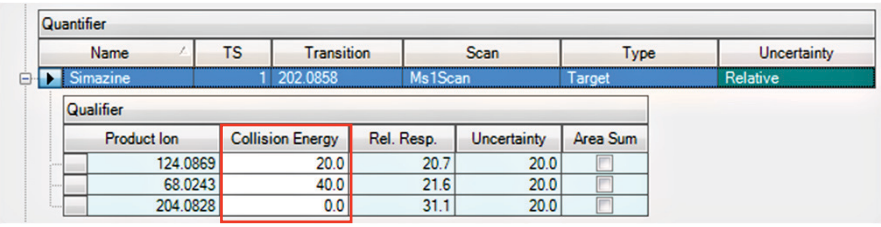


Figure 10. By selecting **Compound Details View** in MassHunter Qualitative Analysis, users are able to quickly and effectively review the All Ions MS/MS results. Starting at the top of the Compound List, they can move from compound to compound to confirm results and review details for rejected compounds.

Quantitative Analysis

Once the review in Compound Details View is complete, the data can be exported to MassHunter Quantitative Analysis Software using a Compound Exchange Format (CEF) file. The CEF file contains information necessary to set up a quantitative method: compound name, retention time, precursor ion, fragment ions (to create qualifiers), collision energy, and relative abundances. This significantly speeds up the creation of a quantitative data processing method. The MassHunter Quantitative Analysis Software automatically selects the major precursor and fragment ions with a relative abundance above 10% for each compound, saving tedious manual processing. Fragment ions with different collision energies or fragmentor voltages can be selected and used by the software (Figure 11). Optionally, one of the isotopes of the precursor ion from the low energy channel can be used as a qualifier as well, see the product ion at *m/z* 204.0828 in Figure 11.

The Quantitative Analysis software extracts chromatograms for the quantifier (target), qualifier ions, and isotopic cluster of the molecular ion. The isotope pattern can be confirmed by viewing an overlay with the theoretical pattern (Figure 12).



Quantifier

Name	TS	Transition	Scan	Type	Uncertainty
Simazine	1	202.0858	Ms1Scan	Target	Relative

Qualifier

Product Ion	Collision Energy	Rel. Resp.	Uncertainty	Area Sum
124.0869	20.0	20.7	20.0	
68.0243	40.0	21.6	20.0	
204.0828	0.0	31.1	20.0	

Figure 11. Method setup in Agilent MassHunter Quantitative Analysis. Compound details are imported with its name, precursor ion, product ions, and relative response ratio. Note that the collision energies for each of the qualifiers are different, giving users more flexibility for the ions used. The ion at *m/z* 204.0828 with 0 V collision energy is an isotope of the precursor ion and is used as a qualifier for further confirmation.

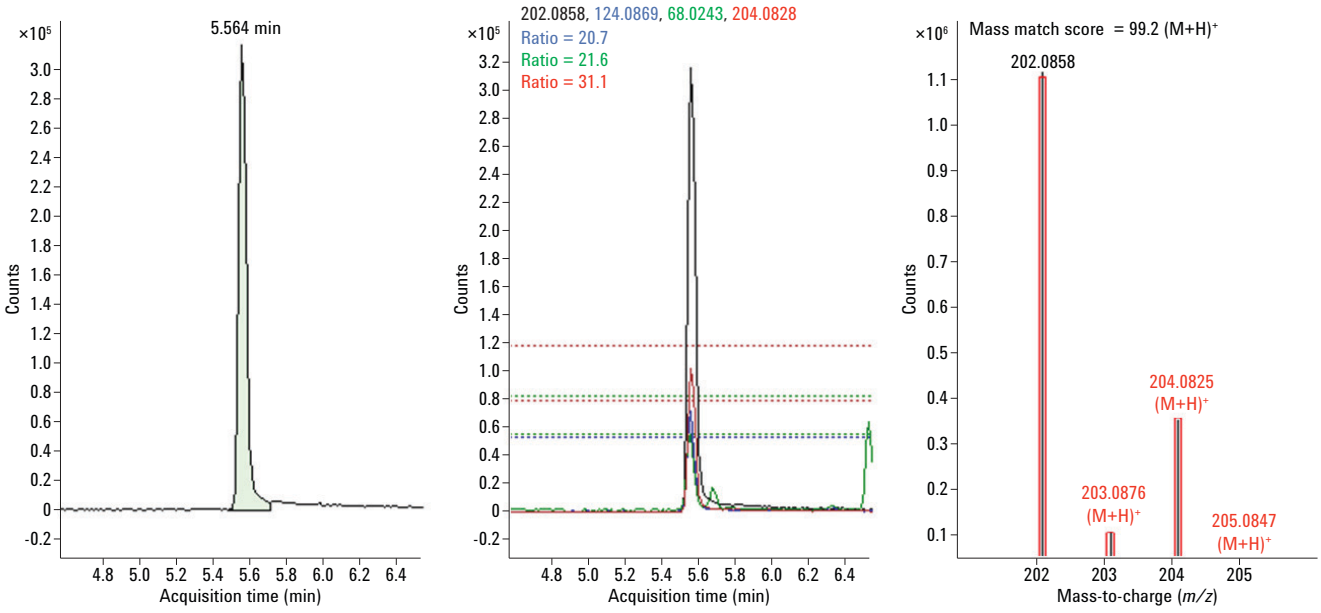


Figure 12. Extracted ion chromatograms of the quantifier (target) ion, qualifiers and the isotopic cluster of the molecular ion. The theoretical isotope abundance pattern is denoted by the red boxes.

Conclusions

All Ions MS/MS is a technique that increases sample throughput, provides both qualitative and quantitative information, and minimizes the cost of analyses. This workflow is much faster than setting up quantitative analyses with triple quadrupole LC/MS instruments which requires many hours of entering compound information and choosing high abundance qualifier ions. Since the data acquired with the All Ions MS/MS technique is full scan accurate mass data, it can be re-interrogated for additional compounds simply by adding them to the method, no re-acquisition is required. With All Ions MS/MS, users can confidently carry out both targeted qualitative screening and rapid quantitative analysis using a single instrument, in a single injection.

References

1. MassHunter Personal Compound Database and Library Manager Quick Start Guide, Publication number G3336-90014, February **2011**.

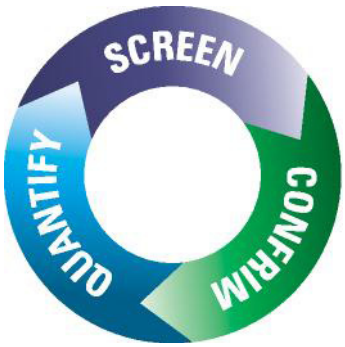
2. Hans-Jürgen P. Sievert, Anton C.J.H. Drouen, "Spectral matching and peak purity in Diode Array Detection in High Performance Liquid Chromatography", New York: Marcel Dekker, **1993**.

For More Information

- 5991-2295EN - Application Note on All Ions MS/MS with Pesticides

- 5991-2319EN - Application Note on All Ions MS/MS with Forensics

- All Ions MS/MS Workflow Guide



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