

# Quantitation Enhanced Data-Dependent (QED) Scanning of Drinking Water Samples Using EQuan for Pesticide Analysis on a Triple Stage Quadrupole

Jonathan Beck, Thermo Fisher Scientific, San Jose, California, USA  
Mihoko Yamaguchi and Kaori Saito, Thermo Electron K.K., Yokohama, Japan

## Overview

In recent years, the use of LC-MS/MS for pesticide analysis has become increasingly popular due to the amenability of electrospray ionization (ESI) for polar compounds. This application note describes the Multi-residue assay of a group of pesticides in drinking water by EQuan. The method utilizes SRM for Quantification, followed by Data Dependent™ QED MS/MS and library searching for the structural confirmation of these analytes.

Traditionally, water samples are extracted in a very time consuming manner, using 1 L of sample that is concentrated 100-1000 times before analysis. This extraction can take several hours or even days of preparation time. Also, the added expense of extraction cartridges leads to more expense per sample for labs. EQuan is a solution that allows for automated online preconcentration of samples without the need for extensive sample preparation or manual intervention.

## Introduction

There is an increasing emphasis for using multiple SRMs (selected reaction monitoring) and the use of Ion Ratio Confirmation (IRC) to positively confirm the presence of banned or controlled substances in samples. For example, the 2002/657/EC European Commission Decision dictates Identification Points (IPs) that must be met for a sample to be deemed “positive.” These criteria can include the number of MS/MS transitions, ion ratios, or the type of mass spectrometer used (high resolution Vs. low resolution).

The Quantitation Enhanced Data-Dependent (QED) scan on a regular triple quadrupole instrument delivers an information rich MS/MS which can be used to confirm the existence of compounds while they are being quantified. When using QED, a “full scan MS/MS” mass spectrum is obtained by Data Dependent scanning for confirmatory analysis during the single reaction monitoring experiment (SRM), which is used for routine quantitation. Once a particular SRM transition reaches a “user set” intensity threshold, the instrument automatically triggers QED, using an innovative new technique called Reversed Energy Ramp (RER) which produces the high sensitivity product ion spectrum. The RER function linearly ramps the collision energy from a high to low value, while scanning Q3. The RER scan generates a highly sensitive, fragment-rich MS/MS spectrum that can be used to positively confirm the existence of a compound.

Many pesticide samples are regulated at a very low level (ppt, or ng/L levels), and in order to detect compounds at these low levels, time consuming extraction and concentration of samples is required before analysis. EQuan utilizes two LC pumps, a large volume auto-sampler, two HPLC columns, and a TSQ Quantum Mass Spectrometer (Thermo Scientific) to reduce sample preparation time and to analyze the samples at the concentration levels that are required.

## Experimental Conditions

**Samples**—Drinking water was spiked with tricyclazole (0.8), carbaryl (0.5), carbofuran (0.05), asulam (2.0), diruon (0.2), siduron (3.0), daimuron (8.0), carpropamid (0.4), thiodicarb (0.8), azoxystrobin (5.0), flazasulfuron (0.3), bensulfuron methyl (4.0), and halosulfon methyl (3.0) at concentration levels from 0.5 ppt (pg/mL) to 1000 ppt. These compounds are all regulated by the Japanese Ministry of Health Labour and Welfare. The reporting level for each compound, as set by the Japanese Ministry of Health, Labour, and Welfare, in ppb (µg/L) is given in parenthesis after each compound.

**HPLC Conditions**—Two pump systems were used, a Finnigan Surveyor L-Pump for loading the 1 mL sample onto the loading column (Hypersil GOLD™ 20×2.1 mm 12µ), and a Surveyor™ MS Pump Plus for eluting the compounds off of the loading column and separation on the analytical column (Hypersil GOLD 50×2.1 mm 3µ). The mobile phase for both pumps was Water with 0.1% Formic Acid (A), and Acetonitrile with 0.1% Formic Acid (B). For the loading L Pump, the gradient used is shown in Table 1, and the gradient used for the analysis pump is shown in Table 2. A divert valve on the mass spectrometer is programmed by the data system to control the loading and elution of the two LC columns. In this experiment, the valve is in the load position from 0 to 1.5 minutes to allow for the entire 1 mL sample to collect on the loading column before switching to the analysis position until all of the analytes are eluted, 12.5 minutes in this case. After switching back to the loading position, the loading column can be rinsed and re-equilibrated by the loading L pump. A schematic of the EQuan system is shown in Figure 1.

## Key Words

- TSQ Quantum™
- EQuan
- LC-MS/MS
- Pesticides
- QED
- RER
- Water Analysis

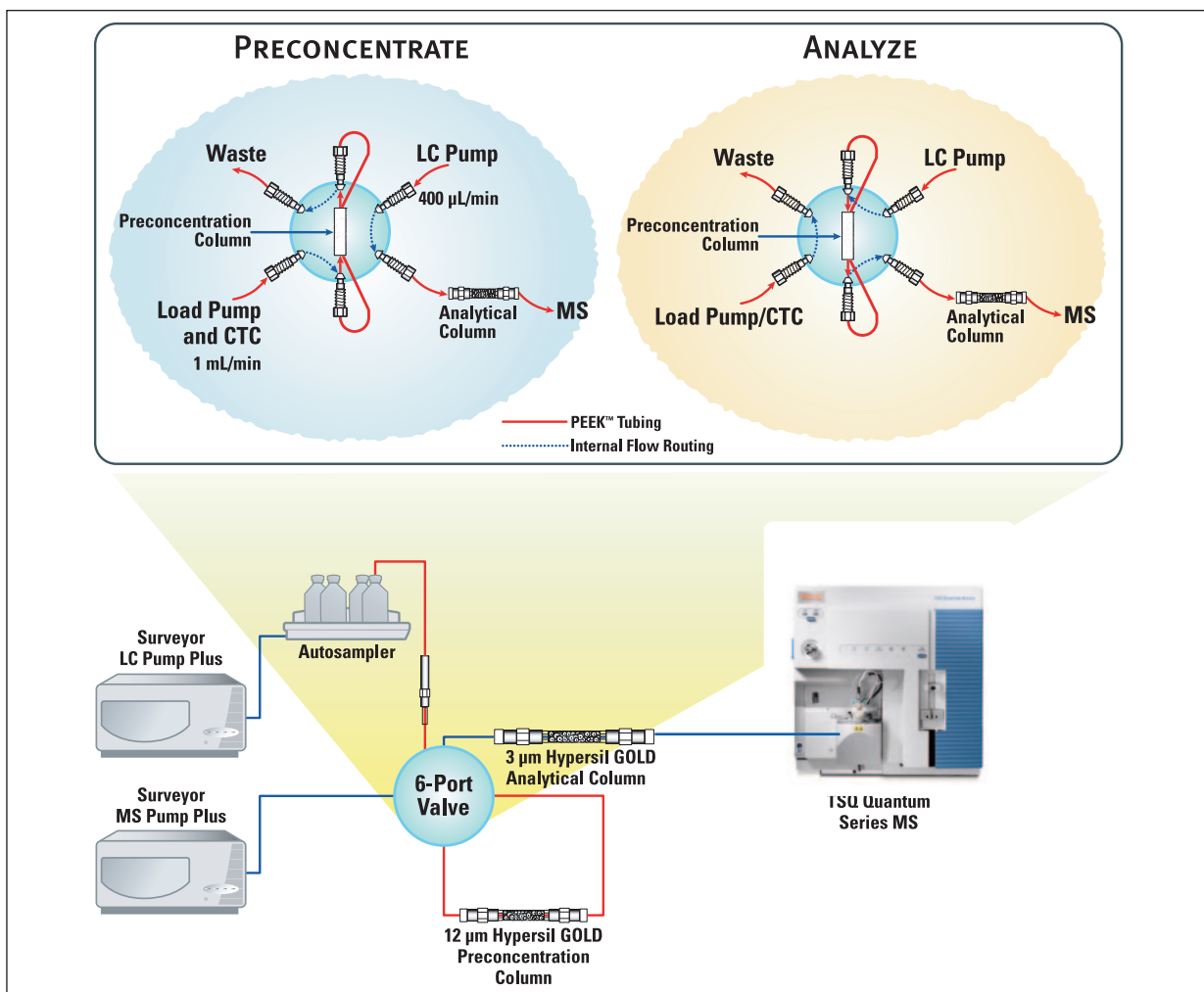


Figure 1: Diagram showing the EQuan setup used in this experiment.

Time	%A	%B	Flow Rate (mL/min)
0	95	5	1.0
1.5	95	5	1.0
2	95	5	0.0
12.5	95	5	0.0
12.6	5	95	1.0
14.5	5	95	1.0
14.6	95	5	1.0
17	95	5	1.0

Table 1: Gradient program for the loading pump. The flow is turned off from 2 to 12.5 minutes to conserve mobile phase, and the column is rinsed from 12.6 to 14.5 minutes with a high organic phase, before re-equilibrating to starting conditions.

Time	%A	%B
0.00	95	5
1.50	95	5
10.0	0	100
12.0	0	100
12.1	95	5
17.0	95	5

Table 2: Gradient program for the analysis pump. The flow rate for this analysis is 0.2 mL/min.

#### MS Conditions

TSQ Quantum Discovery™

Ion source and polarity: ESI, Positive ion mode

Spray Voltage: 4500 V

Sheath Gas: 45 units (N<sub>2</sub>)

Auxiliary Gas: Not Used

Transfer Tube Temperature: 330°C

Collision Gap Pressure: 1.0 units (Ar)

#### MS Scan Functions

Two different scan functions, a SRM (selected reaction monitoring) followed by a data dependent QED scan function were selected in the method. The SRM transitions can be seen in Figure 2a, and the QED scan function can be seen in Figure 2b.

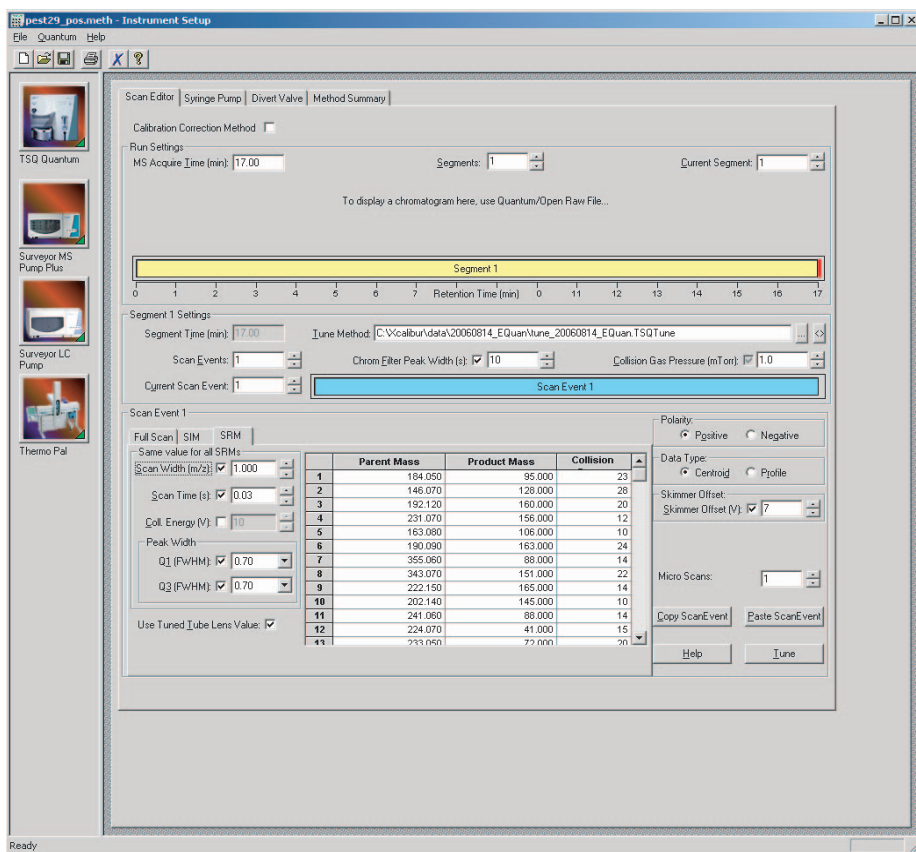


Figure 2a: SRM transitions monitored in the experiment.

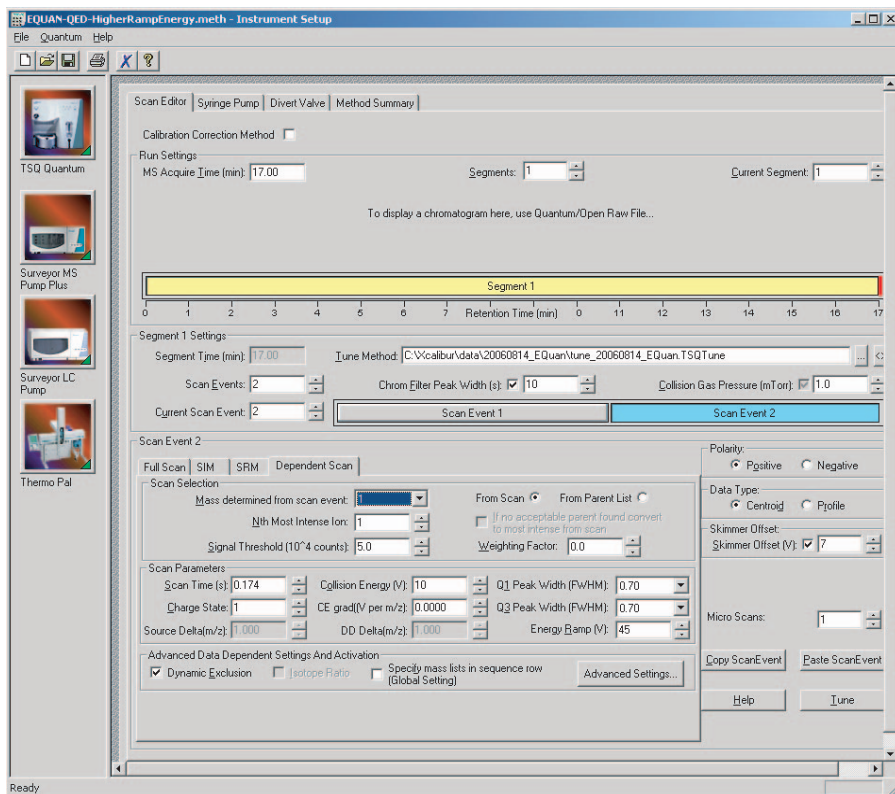


Figure 2b: QED scan function. A RER from 55 to 10 eV triggered by a SRM transition greater than  $5.0 \times 10^4$  counts. Dynamic exclusion was used to allow only one QED spectrum to be collected for each SRM.

## Results and Discussion

Figures 3 and 4 show the chromatogram of the 10 ppt standard from 5 to 11.5 minutes for all of the analytes listed in the Experimental Conditions section. This level is five times lower than the lowest MRL for the mixture of compounds (Carbofuran, 50 ppt). Asulam, the peak show-

ing the lowest S/N in at this concentration level is 200 times lower than the MRL. Excellent linearity is obtained for all of the compounds over the concentration range 1 ppt to 500 or 1000 ppt at the high end. Figure 5 shows the calibration curve for Asulam. Table 3 summarizes the calibration data for each compound, individually.

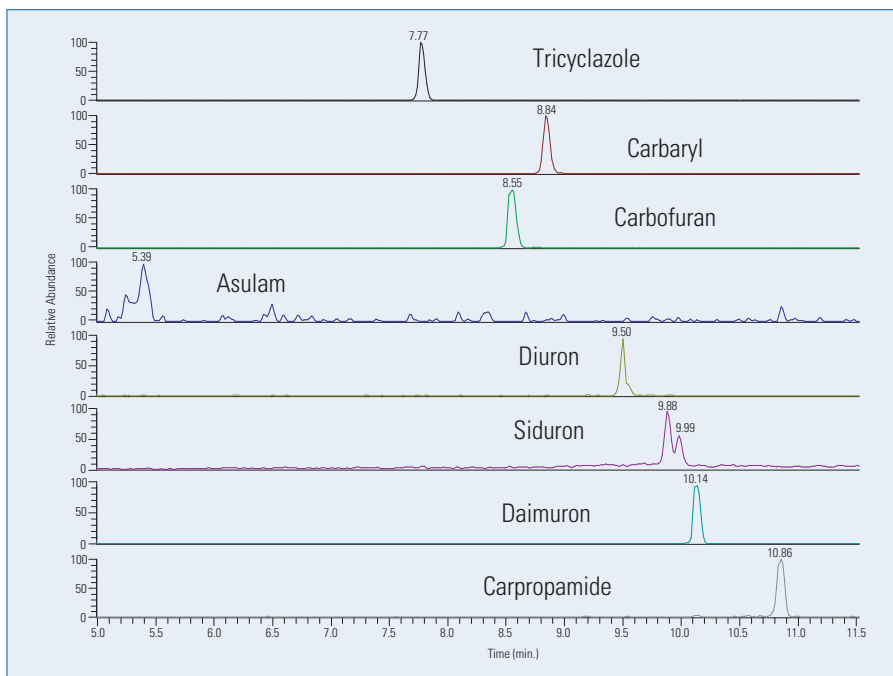


Figure 3: A chromatogram at a concentration level of 10 ppt for the first eight compounds (in order of increasing precursor ion mass) analyzed in the mixture, from 5 to 11.5 minutes.

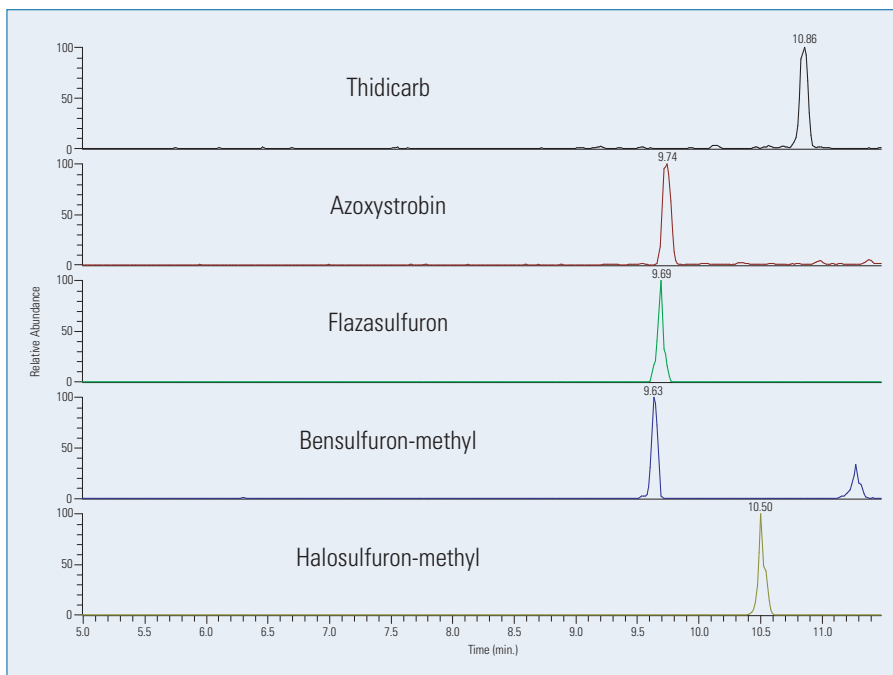


Figure 4: A chromatogram at a concentration level of 10 ppt for the last five compounds (in order of increasing precursor ion mass) analyzed in the mixture, from 5 to 11.5 minutes.

While excellent linearity and quantitative results were gathered using SRM transitions, additional QED data were collected during each run for each compound. An example of a QED full scan MS/MS spectrum is shown in Figure 6 for the compound Carbofuran. This QED scan function fragmented the precursor ion  $m/z$  222 for Carbofuran over a reverse energy ramp of 10 to 55 eV, as specified in the second scan function (Figure 2b).

Using the built in environmental compound library available for the TSQ Quantum that includes over 1000 compounds, Carbofuran was the #1 hit in the list of

possible compounds (Figure 7). This feature allows for additional positive confirmation data for compounds that are analyzed, providing the required number of IPs needed for the positive presence of a compound. Library searching for all of the compounds in the experimental mixture yielded matches for either the first or second compound in the list of possible compounds. Furthermore, the library searches of the QED scans for the two compounds Diuron and Siduron, which both have precursor masses of  $m/z$  233, correctly identified each unique compound, based on differences in their QED spectra.

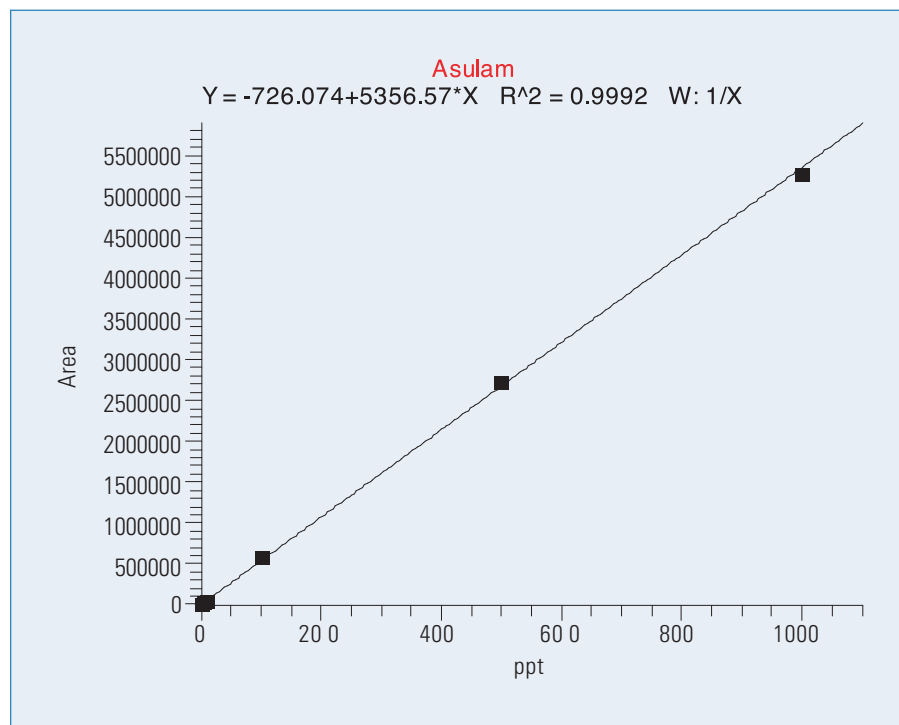


Figure 5: Calibration curve for the analyte Asulam from 1 to 1000 ppt.

Compound	Retention Time (min)	High Concentration	Equation
Tricyclazole	7.7	500 ppt	$y = 21149 + 112678x$ $R^2 = 0.9993$
Carbaryl	8.7	500 ppt	$y = 36518 + 78565x$ $R^2 = 0.9964$
Carbofuran	8.5	500 ppt	$y = 54509 + 290697x$ $R^2 = 0.9977$
Asulam	5.3	1000 ppt	$y = -726 + 5356x$ $R^2 = 0.9992$
Diuron	9.4	500 ppt	$y = 758 + 32087x$ $R^2 = 0.9988$
Siduron	9.8	500 ppt	$y = 51461 + 88505x$ $R^2 = 0.9994$
Daimuron	10.0	500 ppt	$y = 144173 + 285515x$ $R^2 = 0.9963$
Carpropamide	10.8	500 ppt	$y = 10377 + 37079x$ $R^2 = 0.9999$
Thidicarb	8.8	500 ppt	$y = 16505 + 35334x$ $R^2 = 0.9959$
Azoxystrobin	9.6	1000 ppt	$y = 45456 + 198901x$ $R^2 = 0.9978$
Flazasulfuron	9.6	1000 ppt	$y = -3499 + 86802x$ $R^2 = 0.9989$
Bensulfuron-methyl	9.5	1000 ppt	$y = -2657 + 65708x$ $R^2 = 0.9945$
Halosulfuron-methyl	10.4	500 ppt	$y = 1944 + 40565x$ $R^2 = 0.9981$

Table 3: Results of pesticide calibration curves. All curves were a linear curve fit with a weighting factor of  $1/x$ .

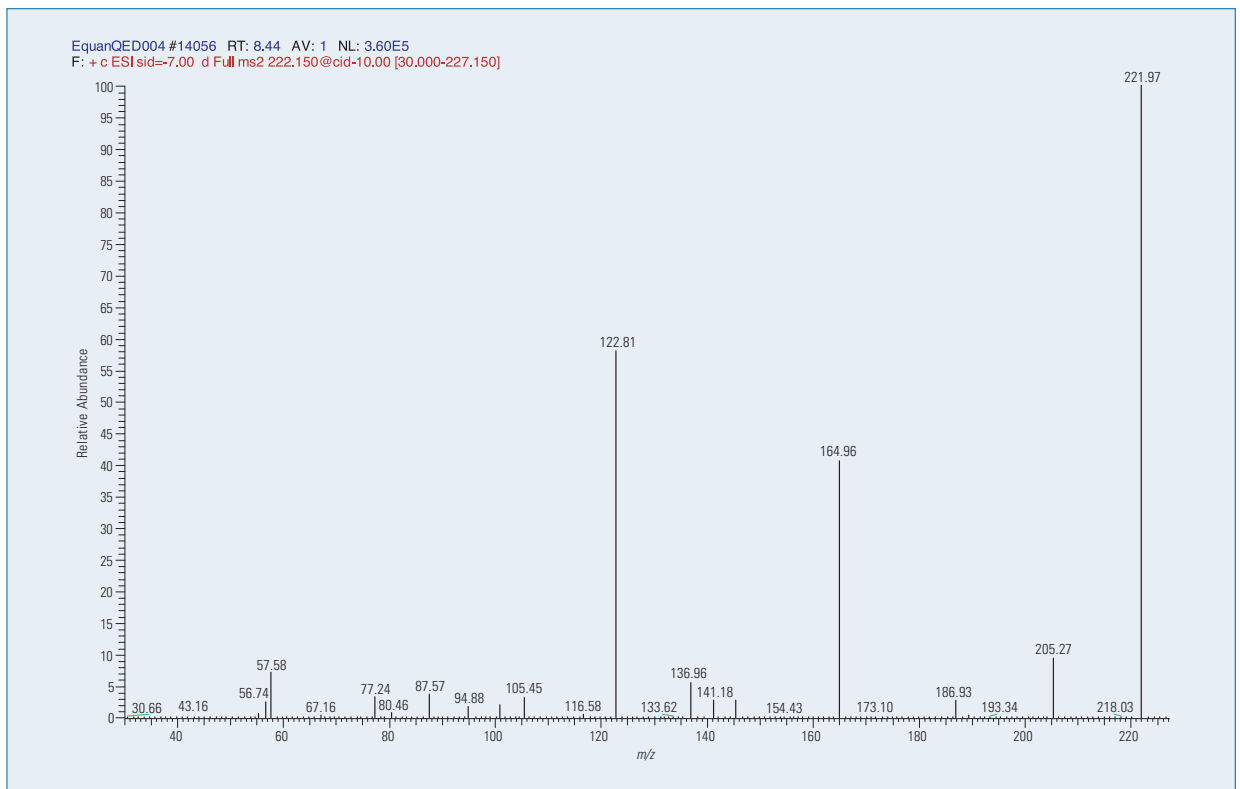


Figure 6: QED spectrum of Carbofuran at the 5ppt calibration level. Searching against the standard library available on the TSQ Quantum instrument platform yields a positive confirmation.

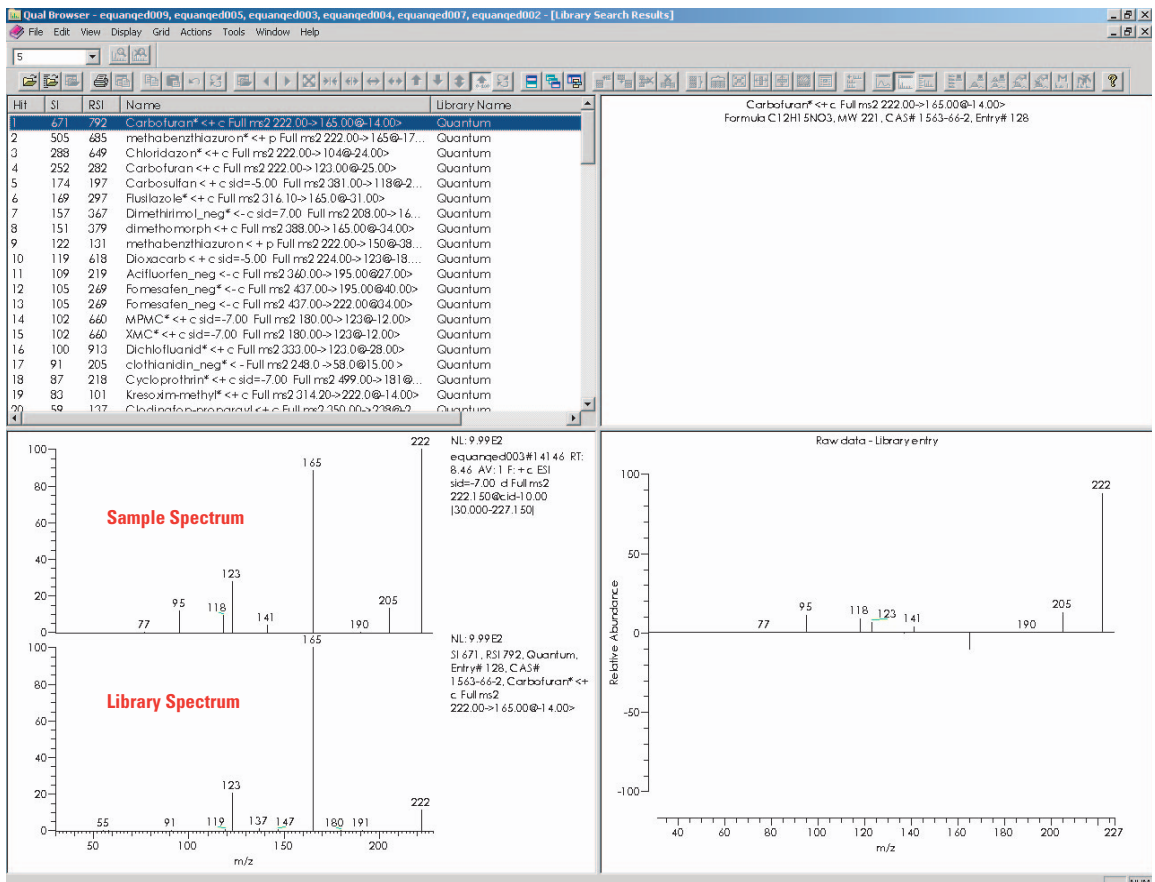


Figure 7: Library search result for the QED spectrum generated at the 5 ppt calibration level. Carbofuran, highlighted in blue is the #1 hit in the list of possible compounds.

## Conclusions

The Quantitation Enhanced Data Dependent scan function, standard on all TSQ Quantum mass spectrometers, allows the user to obtain confirmatory data following quantitative analysis. This is of particular significance when analyzing environmental pollutants in water samples. EQuan, with its large injection volume, allows for significant time savings over traditional SPE concentration methods, and allows for detection and quantitation of compounds at levels well below the regulatory requirements.

Thermo Scientific's built in library of over 1000 compounds in the industry standard NIST format can help users to positively identify compounds based on EU regulations. Additionally, users have the ability to add to or replace the spectra in the library to increase their positive hit probabilities when searching the library.

## References

The 2002/657/EC European Commission Decision can be found on the World Wide Web at:

[http://ec.europa.eu/food/food/chemicalsafety/residues/lab\\_analysis\\_en.htm](http://ec.europa.eu/food/food/chemicalsafety/residues/lab_analysis_en.htm)

The Japanese Ministry of Health, Labour, and Welfare can be found on the World Wide Web at:

<http://www.mhlw.go.jp/index.html> (Japanese)

<http://www.mhlw.go.jp/english/index.html> (English)

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