Application Note: 437

LC-MS/MS Analysis of Herbicides in Drinking Water at Femtogram Levels Using 20 mL EQuan Direct Injection Techniques

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Key Words

- TSQ Quantum Access
- EQuan System
- Herbicides
- QED
- Water Analysis

Introduction

As concerns grow over the toxic effects of herbicides and other chemicals in our environment, the need to accurately monitor these substances in drinking water and foods becomes even more critical. LC-MS/MS is routinely used by the environmental and food industries to identify and quantify pesticide and herbicide residues. However, this method typically requires extensive offline sample preconcentration methods, which can be expensive and time-consuming, to meet the stringent requirements and low limits of detection set forth by federal and international regulatory authorities. An online preconcentration and cleanup method has been developed that improves both sensitivity and precision and yields unmatched throughput.

The Thermo Scientific EQuan™ system for online sample cleanup and analysis consists of a triple quadrupole mass spectrometer with an electrospray ionization source (ESI), two LC quaternary pumps, an autosampler, and two LC columns having C18 selectivity – one for preconcentration of the sample, the second for analytical separation. A 6-port valve switches between the columns and is controlled by the instrument software. In addition to quantitative information, qualitative full scan product ion spectra are collected in the same analytical run and data file, using a technique called Reverse Energy Ramp (RER). This full scan spectrum provides additional confirmatory information for the compounds being analyzed. The resulting product ion spectra can be library searched for positive identification, or ion ratios can be used to confirm the presence of a particular compound, helping to eliminate "false positive" samples. This method uses drinking water for direct injection onto the loading column, with no sample preparation or offline concentration. This application note provides a comparison of the online sample preconcentration of 1 mL, 5 mL, and 20 mL injections of drinking water samples spiked with herbicide compounds.

Goal

To compare different large volume injections using a loading column and an analytical column with two HPLC pumps.

Experimental Conditions

Sample Preparation

Drinking water containing 0.1% formic acid was spiked with a mixture of the following herbicides: ametryn, atraton, atrazine, prometon, prometryn, propazine, secbumeton, simetryn, simazine, terbuthylazine, and terbutryn (Ultra Scientific, North Kingstown, RI). The concentrations of the herbicides in the spiked water ranged from 0.1 pg/mL to 10 pg/mL. Calibration standards were prepared at the following concentrations: 0.1, 0.5, 1.0, 5.0, and 10.0 pg/mL.

HPLC

Spiked water samples and blank water samples (1 mL, 5 mL, or 20 mL) were injected directly onto a loading column (Thermo Scientific Hypersil GOLD™ 20 mm x 2.1 mm ID, 12 µm) using an HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland). After the sample was completely transferred from the sample loop to the loading column, a 6-port valve was switched to enable the loading column to be back flushed onto the analytical column (Hypersil GOLD 50 mm x 2.1 mm ID, 3 µm), where the compounds were separated prior to introduction into the mass spectrometer. After all of the compounds were eluted, the valve was switched back to the starting position. The loading and analytical columns were cleaned with a high organic phase before being re-equilibrated to their starting conditions (Figure 1a and 1b). Control and timing of the 6-port valve was through the computer data system, LCQUAN™ (Thermo Fisher Scientific, San Jose, CA).

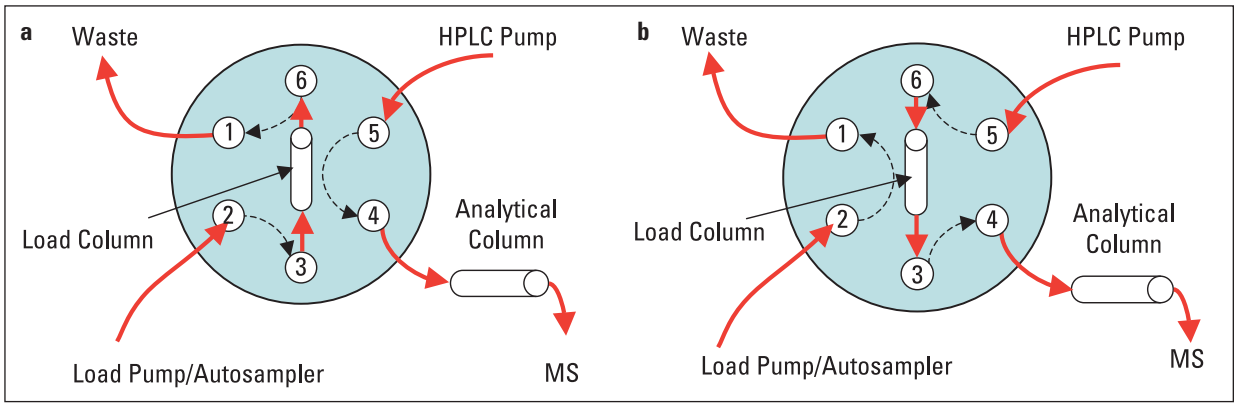

Figure 1: a) 6-port valve in position 1 (load position), for loading the sample onto the loading column. b) 6-port valve in position 2 (inject position), for eluting the compounds trapped on the loading column onto the analytical column.

Slightly different LC programs were used in each method, depending on the volume of the sample injected. The loading pump flow rates ranged from 1 mL/min for 1 mL samples to 5 mL/min for 20 mL samples. This allowed the run times at the higher injection volumes to be shortened because the time to transfer the sample from the sample loop to the loading column depends on the flow rate. The same LC program was used for the analytical column.

Two HPLC pumps were used for the analysis: one for transferring the sample from the injection loop to the loading column, and one for back flushing the compounds off of the loading column and separating them on the analytical column. The loading pump was a Surveyor Plus™ LC pump (Thermo Fisher Scientific, San Jose, CA) and the analytical pump was a U-HPLC Accela™ pump (Thermo Fisher Scientific, San Jose, CA).

The HTC autosampler was equipped with a 5 mL syringe. To accommodate larger injection volumes (> 5 mL), a CTC™ macro sequence was programmed to allow for multiple syringe fills and deliveries to the sample loop from a 10 mL vial. For 20 mL samples, two 10 mL vials were used and the macro allowed sampling from adjacent vials filled with the same sample. The macro is shown in Figure 2. Because this multi-sampling scheme can be quite time consuming, the ability to perform "look-ahead" injections allows for significant time savings. The loop can be switched to an offline position during a run, and subsequent samples can be prepared and injected while a sample is being run.

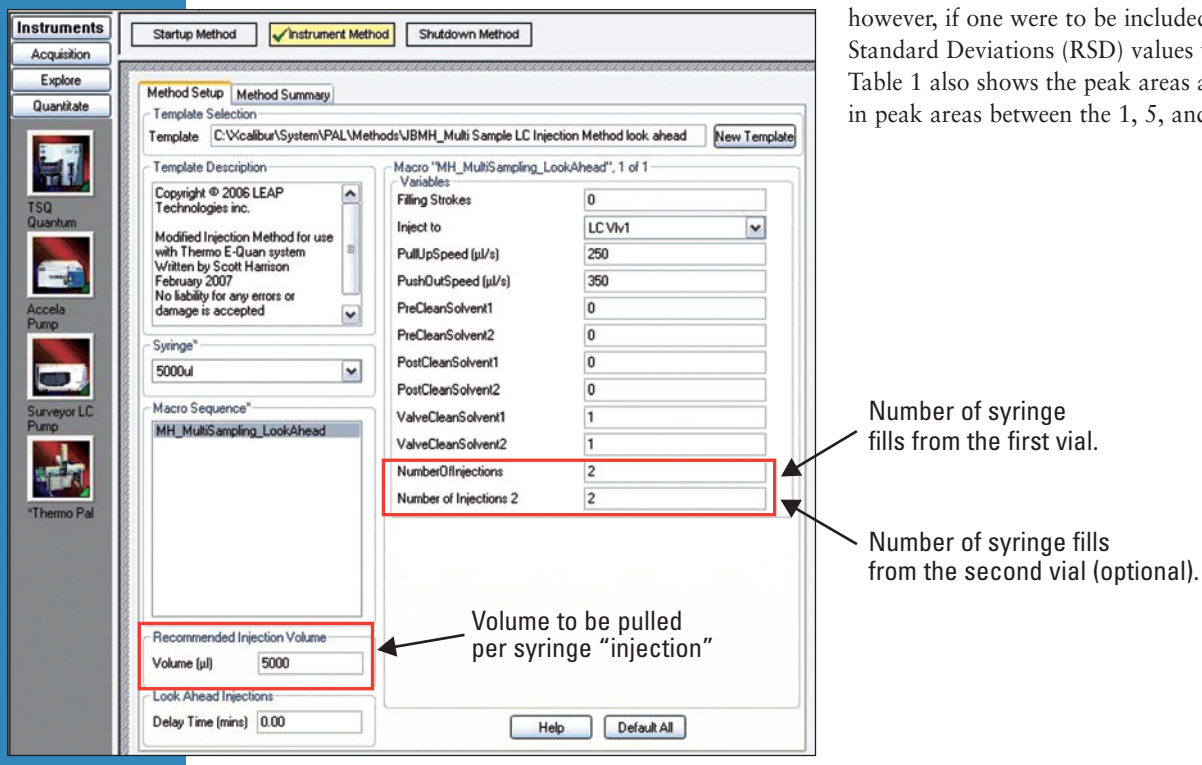

Figure 2: The method setup screen for the CTC Autosampler, showing the capability to perform multiple injections from the same vial. The red box highlights the parameters used to control the number of syringe fills from two consecutive vials. In this example, a total of 20 mL will be injected.

MS

MS analysis was carried out on a TSQ Quantum Access™ triple stage quadrupole mass spectrometer with an electrospray ionization (ESI) source (Thermo Scientific, San Jose, CA). The MS conditions were as follows:

Ion Source Polarity:	Positive ion mode
Spray Voltage:	4000 V
Ion Transfer Tube Temperature:	300 °C
Sheath Gas Pressure:	30 arbitrary units
Auxiliary Gas Pressure:	5 arbitrary units
Collision Gas (Ar):	1.5 mTorr
Q1/Q3 Peak Resolution:	0.7 Da
Scan Width:	0.002 Da

Quantitative and qualitative data were collected in the same run and data file.

Results and Discussion

Chromatograms of the herbicide simazine at three different injection volumes are shown in Figure 3. A very small peak can be seen for the 1 mL injection volume; however, the integration is not shown in the chromatogram. Injections at higher volumes show superior signal-to-noise ratios and intensity, which allow for analysis of very low concentration samples (pg/mL and sub pg/mL). To test the reproducibility of the multiple syringe fill method with a 20 mL loop, eight replicate injections were performed using the 1 pg/mL calibration standard. The results of this study are shown in Table 1. No internal standard was used in this analysis; however, if one were to be included, the % Relative Standard Deviations (RSD) values would likely improve. Table 1 also shows the peak areas and calculated difference in peak areas between the 1, 5, and 20 mL injections.

Compound	Area, 1 mL	Area, 5 mL	Area, 20 mL	Factor 1 mL to 5 mL	Factor 5 mL to 20 mL	%RSD (n = 8)
Atraton	ND	1.16E+07	5.42E+07	N/A	4.69	11.15
Simetryn	ND	4.27E+06	1.94E+07	N/A	4.56	8.93
Prometon/Secbumeton	3.26E+06	1.07E+07	4.80E+07	3.30	4.47	9.89
Ametryn	4.34E+06	1.42E+07	5.99E+07	3.27	4.22	11.59
Simazine	3.18E+05	1.28E+06	5.70E+06	4.03	4.44	5.32
Prometryn/Terbutryn	6.19E+06	1.89E+07	7.61E+07	3.05	4.02	3.99
Atrazine	1.26E+06	4.45E+06	1.55E+07	3.53	3.49	4.97

Table 1: Reproducibility for 20 mL injections (n = 8) at a 1 pg/mL concentration level, without an internal standard.

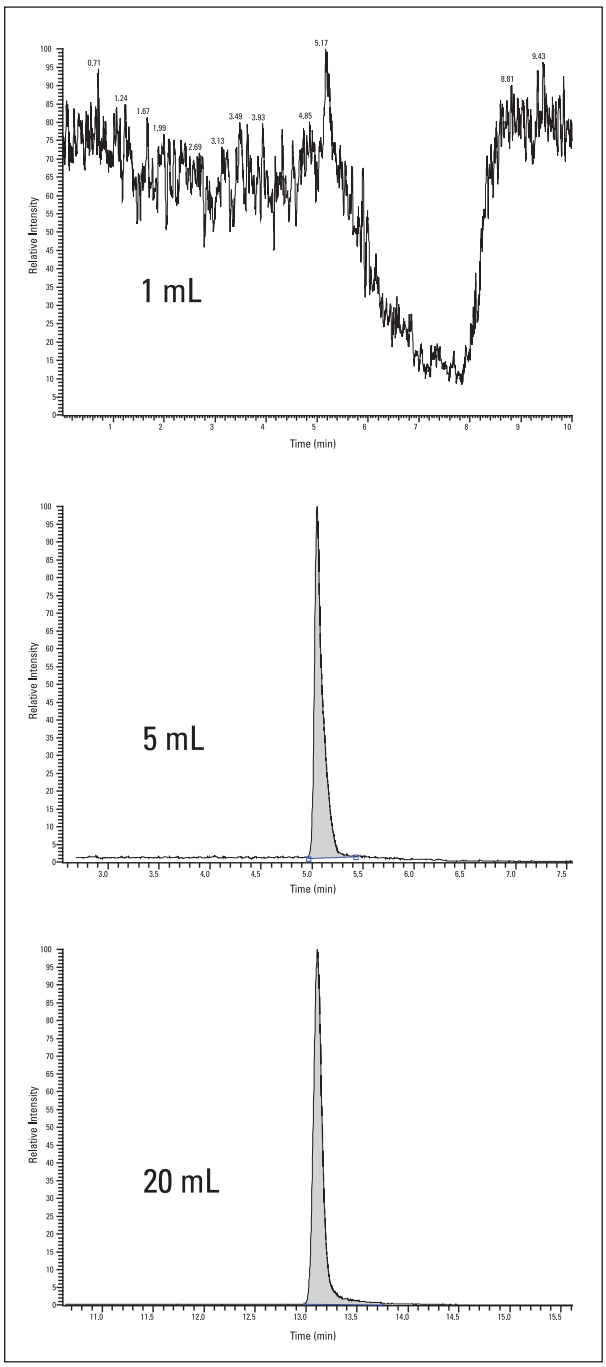

Figure 3: Chromatograms showing the injection of simazine with 1, 5, and 20 mL injection volumes. The concentration of simazine is 1 pg/mL for all three injections.

In addition to quantitative data, qualitative data was collected for each analyte using Quantitation-Enhanced Data-Dependent MS/MS (QED-MS/MS) scanning with the Reverse Energy Ramp (RER) scan function. The reverse energy ramp allows the collision energy in Q2 to be ramped from a high energy to a lower energy as Q3 is scanning the product ions from Q2 from low mass to high mass. This provides a rich product ion spectrum that can be used for library searching or ion ratio calculations to help eliminate "false positive" results. The RER provides a much "richer" product ion when compared to a Q3 product ion scan collected with a static collision energy. For this experiment, the collision energy for the RER was set to 25 eV and the ramp value was set to 20 eV. This results in a ramp from 45 eV at the low mass range of Q3. As Q3 scans to higher masses, the collision energy in Q2 is ramped lower and ends at a collision energy of 25 eV. Figure 4 shows the full scan Q3 spectrum that was collected during the analytical run for the calibration standard at a level of 1 pg/mL. It also shows a ramp illustrating the collision energy ramp applied to Q2.

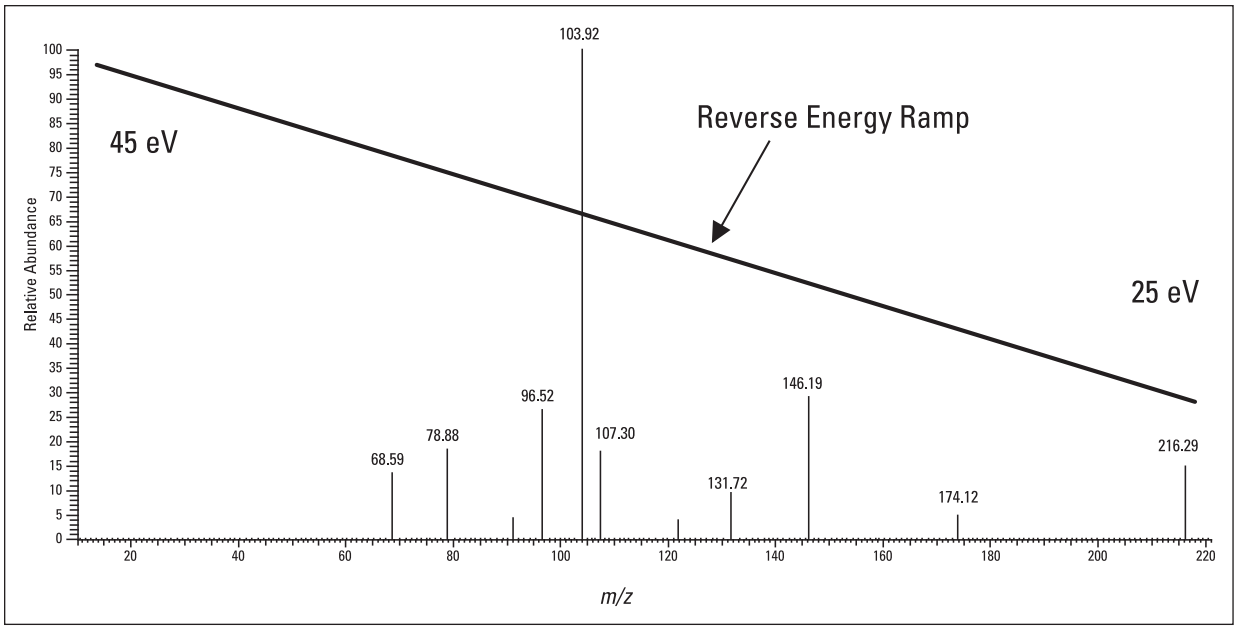

Figure 4. QED-MS/MS Q3 spectrum for a 1pg/mL injection of atrazine. The collision energy was 25 eV and the ramp was 20 eV.

Conclusion

Using a preconcentration column in tandem with an analytical HPLC column allowed for the quantitation of a triazine herbicide mixture over the concentration range 0.1 – 10.0 pg/mL. Direct 20 mL injections were performed with the two HPLC columns. The large injection volume capabilities of the EQuan system eliminated the need for laborious and expensive offline preconcentration using solid phase extraction. Injection volumes ranging from 1 mL to 20 mL are possible using this configuration, thus offering flexibility for laboratories based on their sensitivity and reporting requirements.

Acknowledgements

We would like to thank Scott Harrison from LEAP Technologies (Carrboro, NC) for assistance with the CTC macro for multiple syringe fills and Mark Harrison from Thermo Fisher Scientific (Hemel-Hempsted, UK) for further assistance with the macro and for technical discussions.