

Testing LC-MS System Robustness with Automated Sample Cleanup Using Red Wine as a Matrix

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Key Words

- Finnigan TSQ Quantum Ultra™
- EQuan™ system
- Hypersil GOLD™ columns
- Pesticides

Introduction

Achieving low limits of detection (LODs) of pesticides, antibiotics and veterinary residues in food residues and drinking water is of paramount importance in order to monitor the regulatory levels as stated by the US, Japanese and EU directives. These substances pose a significant health threat and therefore, need to be accurately detected at the lowest levels, typically at part per trillion (ppt). Traditionally, LC-MS/MS has been used by the environmental and food industries for the identification and quantitation of these residues. However, this methodology typically requires extensive offline sample preparation, which can be time consuming and expensive.

Thermo Electron's EQuan environmental quantitation system consists of a TSQ Quantum™ series mass spectrometer, two Surveyor™ HPLC pumps with a preconcentration column, an analytical column, and a CTC autosampler. The unique capabilities of EQuan for online preconcentration and cleanup of samples result in improved sensitivity and precision, as well as unmatched throughput.

In previous experiments, using the EQuan system for online sample preconcentration and detection of pesticides in ground water yielded lower limits of detection compared to standard injection techniques. See Table 1.

Typically, when red wine is analyzed using LC-MS/MS, some form of sample preparation and/or extraction is necessary prior to injection. In this application note, the EQuan system was tested for robustness using a matrix of neat red wine spiked with a mixture of pesticides using large volume (1000 µL) injections.

Goal

To test the robustness of an LC-MS system for an automated online preconcentration system using a dirty matrix.

Experimental Conditions

Sample Preparation

Red Burgundy wine was spiked with a mixture of nine herbicides and six fungicides at a level of 500 pg/mL (500 ppt). The following herbicides were analyzed: atrazine, cyanazine, simazine, propazine, trietazine, metazachlor, propachlor, pendimethalin, and propyzamide. The following fungicides were analyzed: flutriafol, triadimefon, epoxiconazole, flusilazole, tebuconazole, and propiconazole. No other sample treatment was performed prior to injection.

HPLC

HPLC analysis was performed using an HTC PAL™ Autosampler (CTC Analytics, Zwingen, Switzerland) with two LC quaternary pumps and two LC columns, the first for preconcentration of the sample and the second for the analytical analysis. A sample of 1000 µL of the spiked neat wine was injected directly onto the Hypersil GOLD 20 × 2.1 mm, 12 µm loading column (Thermo, Bellefonte, PA) in a high aqueous mobile phase (see Figure 1a). After 1 minute, a six-port valve on the mass spectrometer was switched by LCQUAN™ 2.5 instrument control software (Thermo, San Jose, CA). This enabled the load column to be back flushed onto the analytical column (Hypersil

	1 mL Injection Area	100 µL Injection Area	Gain Factor	1 mL Injection Area	100 µL Injection Area	Gain Factor	1 mL Injection Area	100 µL Injection Area	Gain Factor	1 mL Injection Area	100 µL Injection Area	Gain Factor
	Propham			Isoproturon			Diuron			Linuron		
1 ppt				5.53E+04	NA		1.97E+04	1.73E+03	11			
5 ppt	2.17E+04			3.35E+05	3.17E+04	11	4.15E+04	5.65E+03	7	6.96E+03		
10 ppt	2.71E+04			6.68E+05	4.90E+04	14	8.25E+04	1.18E+04	7	1.99E+04		
50 ppt	5.09E+04			3.33E+06	2.82E+05	12	4.47E+05	3.72E+04	12	5.91E+04	7.98E+03	7
100 ppt	6.51E+04			6.54E+06	5.24E+05	12	8.83E+05	7.60E+04	12	1.34E+05	2.50E+04	5
500 ppt	2.47E+05	3.00E+04	8	3.11E+07	2.60E+06	12	4.65E+06	3.80E+05	12	7.36E+05	1.28E+05	6
1000 ppt	5.29E+05	5.69E+04	9	5.81E+07	5.23E+06	11	9.39E+06	7.63E+05	12	1.43E+06	2.47E+05	6
5000 ppt	2.59E+06	2.82E+05	9	2.58E+08	2.44E+07	11	4.95E+07	3.68E+06	13	9.49E+06	1.25E+06	8

Table 1: Calculations demonstrating the gain in peak areas due to larger injection volumes in ground water samples

GOLD 50×2.1 mm, 3 μm), where the compounds were separated prior to introduction into the mass spectrometer (see Figure 1b). After all of the compounds were eluted from the analytical column, the 6-port valve was switched back to the starting position, and the loading and analytical columns were cleaned with a high organic phase before being re-equilibrated to their starting conditions. The total run time for each analysis was 22 minutes. The mobile phases for the analysis were water and methanol, both with 0.1% formic acid.

MS

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

- Electrospray ionization: Positive
- Spray voltage: 3.0 kV
- Ion transfer tube temperature: 350 °C
- Sheath gas pressure: 45 arbitrary units
- Auxiliary gas pressure: 5 arbitrary units
- Ion sweep gas pressure: 3 arbitrary units
- Collision gas (Ar): 1.0 mTorr
- Q1/Q3 peak resolution: 0.7 Da
- Scan width: 0.002 Da

The source of the mass spectrometer was adjusted so that the ESI probe was off axis to prevent contamination of the ion transfer tube. The position of the probe was set so that the main spray pattern of the electrospray hit the Ion Sweep™ cone below the center line and off to the left by about 0.5 cm. The probe depth was set to position “C” on the electrospray probe. An ion sweep gas of three arbitrary units was set to prevent any large droplets from entering the ion transfer tube of the mass spectrometer.

Results and Discussion

The back pressure of the loading column and the analytical column were monitored over the course of the wine injections to determine if the columns were becoming clogged with any particulates from the wine. Over 600 injections, the back pressure on the 12 μm loading column remained at approximately 20 bar under the starting conditions of the analytical run, while the back pressure on the 3 μm analytical column remained at approximately 72 bar.

The resulting spray pattern of the electrospray can be seen in Figure 2. A thick deposit of red wine residue is clearly visible from just below the center of the sweep cone to the outside radius. The red wine spray can also be seen on the inside of the electrospray housing in Figure 3.

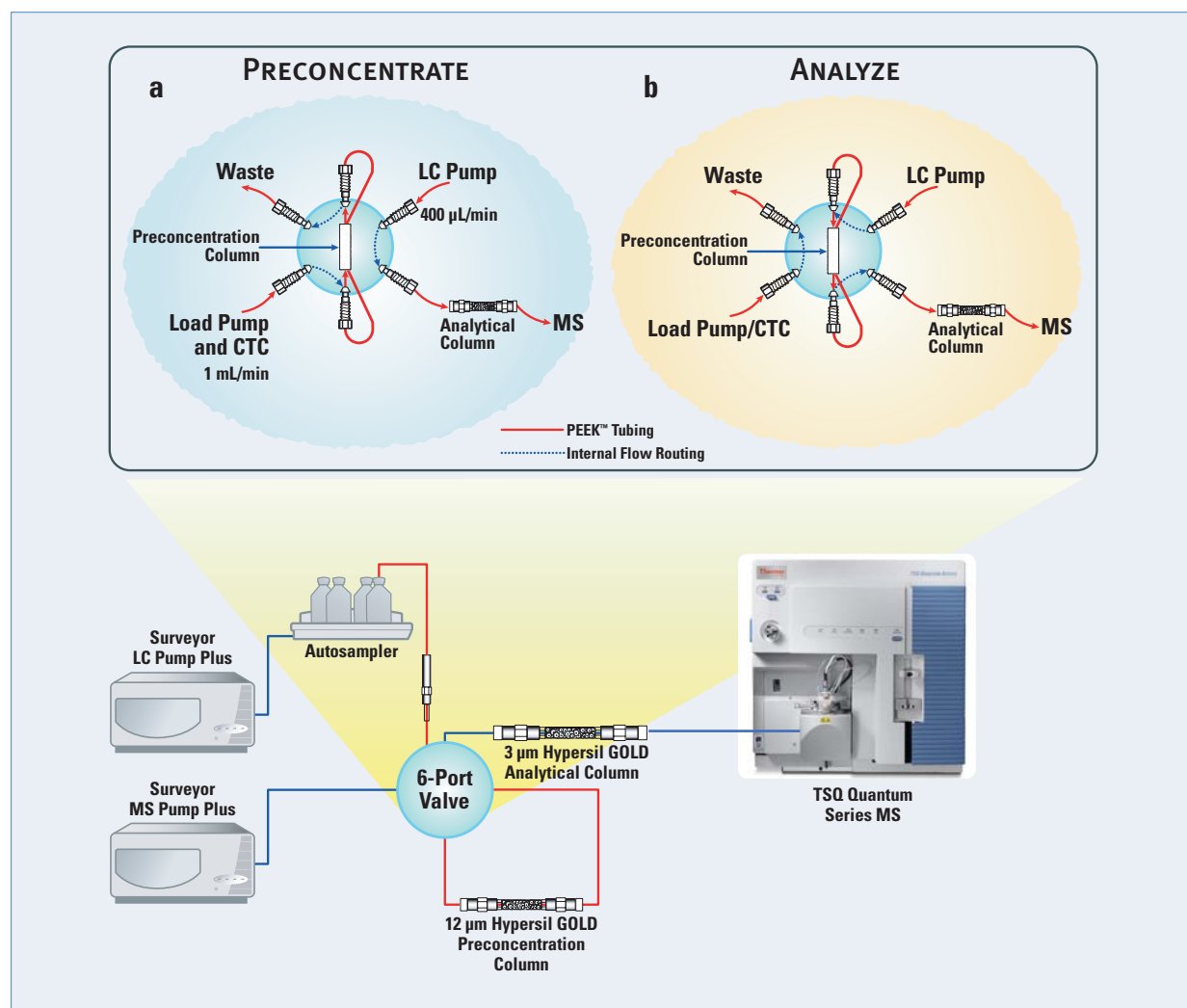


Figure 1: The schematic of the EQuan system used for this assay

In the picture, the drain is dark purple in color, illustrating that the main excess spray of the red wine was directed to the bottom of the ion source and away from the main orifice of the mass spectrometer. Additionally, the ESI probe can be adjusted to be closer to the ion transfer tube, which increases robustness by allowing less side scatter from the electrospray beam, thus focusing the main spray pattern lower on the ion sweep cap.

The reproducibility of the method is shown in Figure 4. The graph plots the peak area for metazachlor for 164 injections of red wine. The first four injections were

excluded from the %RSD calculation. Because the loading column was new at the beginning of the runs, several injections were required to condition the column before a stable peak area was achieved. A representative chromatogram is shown in Figure 5.

As shown in Figure 6, after several hundred injections of the spiked red wine matrix, no degradation in column performance or source robustness was observed. In total, over 600 injections were made on the system with no loss in column performance.



Figure 2: Ion sweep cap after several hundred injections, showing contamination from red wine



Figure 3: Electrospray ionization source with the electrospray probe removed, showing the main spray pattern directed towards the drain

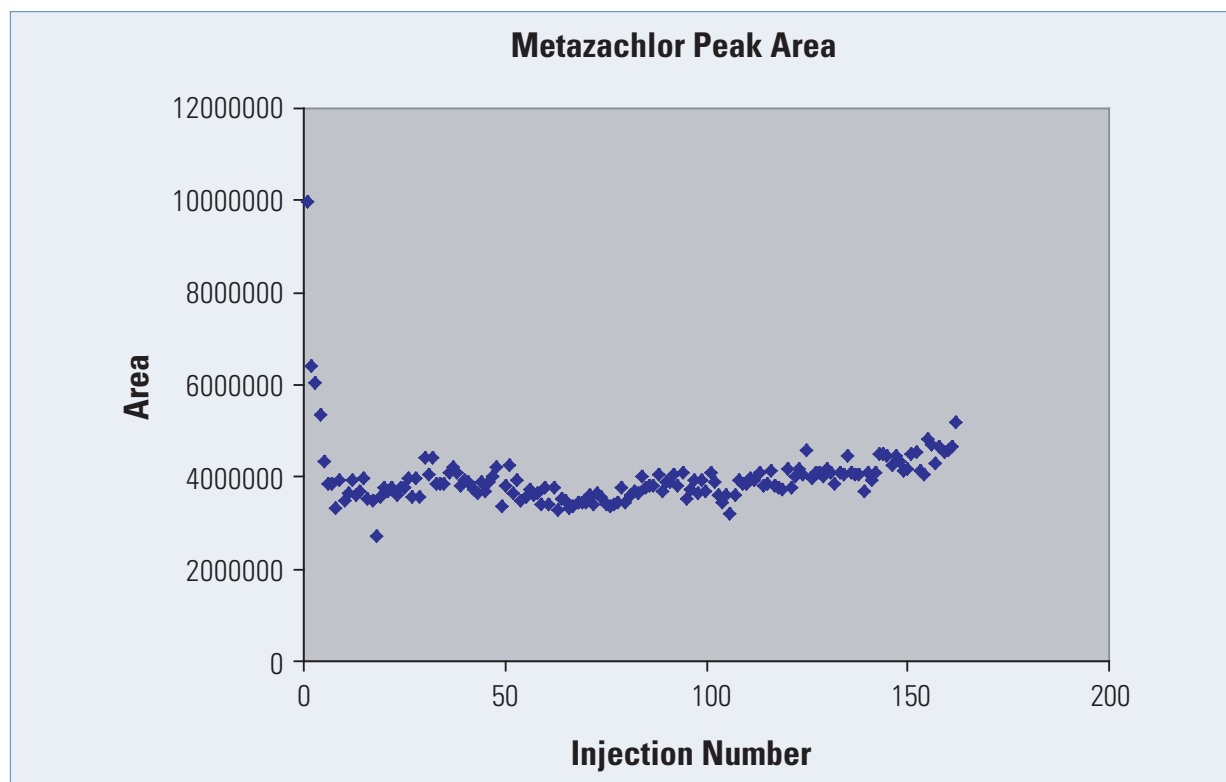


Figure 4: Scatter plot of the peak area for 164 injections (1000 µL) of metazachlor spiked in red wine. The %RSD is 9% when the first four points are excluded.

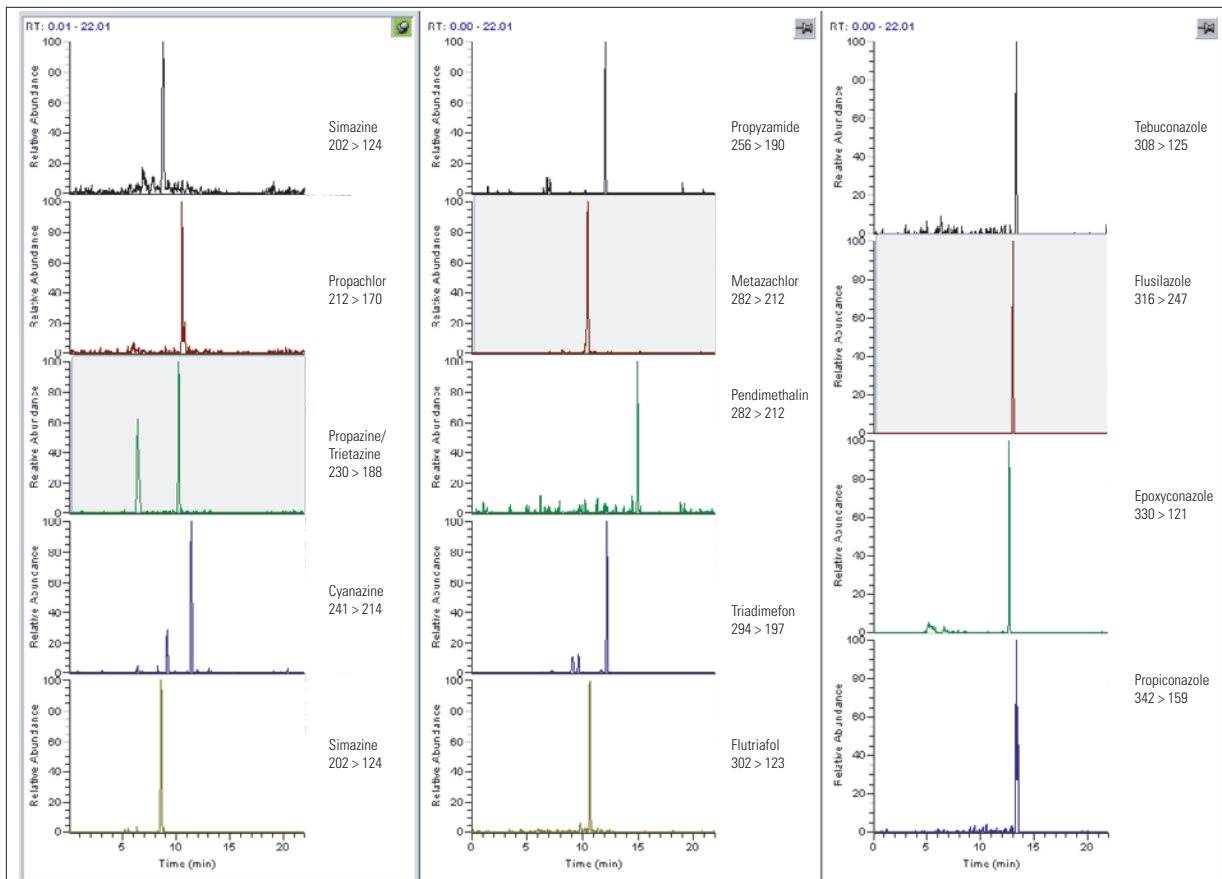


Figure 5: Example chromatograms for a 1000 µL injection of spiked red wine

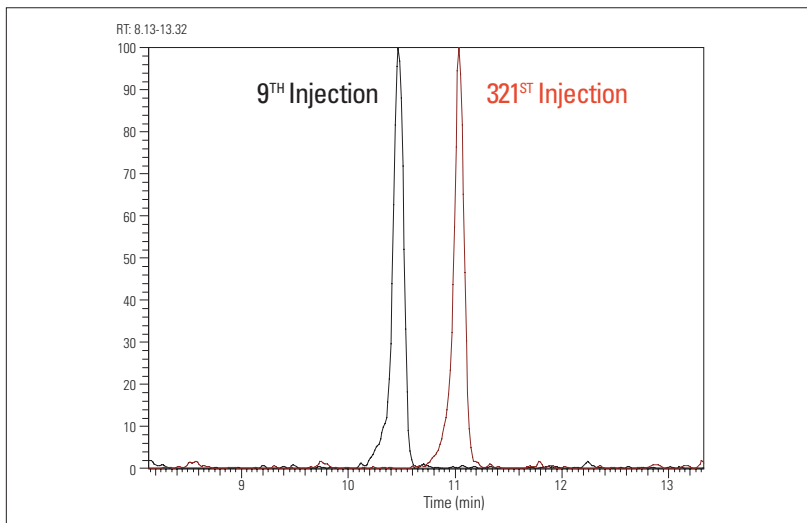


Figure 6: Different injections of metazachlor (retention times have been offset for greater visibility)

Conclusion

This application note demonstrates the robustness of the TSQ Quantum Ultra triple quadrupole mass spectrometer and an online extraction and preconcentration method. The described sample cleanup technique improves signal-to-noise ratios by a factor of 10 to 100 (based on injection volume) for low concentration samples in red wine matrices. Preliminary results using onion and tobacco matrices have yielded similar results in terms of column performance and mass spectrometer robustness. Further studies will be conducted in other matrices, as well as with other pesticides, herbicides, and insecticides.

References

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