Fast and Ultrafast LC-MS/MS Methods for Robust and Reliable Analysis of Pesticides in Food Using the Vanquish UHPLC System

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Application Note 1138

Key Words
Vanquish UHPLC System, TSQ Endura Triple Quadrupole MS, Retention Time Reproducibility, Timed SRM, High-throughput, Food Safety, Chromeleon 7.2 CDS

Goal
Demonstrate the benefits of method transfer and retention time reproducibility in fast and ultra fast UHPLC separations with timed selected reaction monitoring MS detection for the analysis of pesticides in food matrices.

Introduction
Food safety is an increasing concern that has resulted in stringent pesticide regulation globally and in continuous recalls of food products. Food safety regulations require the screening and the quantitation of a large number of pesticides in food at maximum residue levels generally set in the ppb-ppm range to minimize their possible negative effects on human health. This has prompted the development of generic and reliable analytical multi-residue methods for the analysis of hundreds of pesticides simultaneously.[1] Triple stage quadrupole (TSQ) instruments operating in selected reaction monitoring (SRM) mode are widely used for this purpose and are by far the methodology of choice in routine quantitative analysis. The hyphenation of ultra high performance liquid chromatography (UHPLC) with mass spectrometry brings several advantages to the analyst because it combines high-throughput UHPLC separation with the sensitivity of the mass analyzer. With timed SRM, the compounds are monitored only in a specific time range where they are expected to elute, and not during the full chromatographic run. This brings the benefit of getting increased numbers of scans for each chromatographic peak, thus achieving maximum sensitivity and reproducibility.

Here we present a comparison between fast and ultrafast LC-MS/MS methods in timed SRM mode for the analysis of 250+ pesticides in food extracts. The two methods were compared in terms of analysis time and data quality.

Experimental
Sample Preparation
Three matrices representing soft fruit (strawberry), green vegetable (leek), and cereal grain (wheat flour) were selected for method testing. Each homogenized food sample (10 g) was weighed into a QuEChERS extraction tube (P/N 60105-216). After the addition of 10 mL of acetonitrile (+ 20 mL of water in case of wheat flour), the tube was shaken for 10 min and centrifuged at 5000 rpm for 5 min. Pesticide stock solutions were prepared in acetonitrile and matrix extracts. Working neat solutions and matrix fortified samples were obtained by dilution in the corresponding solvent or matrix to get the final concentration of 5, 10, and 100 µg/L (5–100 ppb).

Instrumentation
- Thermo Scientific™ Vanquish™ UHPLC System including:
  - System Base Vanquish (P/N VH-S01-A)
  - Binary Pump H (P/N VH-P10-A)
  - Split Sampler HT (P/N VH-A10-A)
  - Column Compartment H (P/N VH-C10-A)
- Thermo Scientific™ TSQ Endura™ Triple Quadrupole Mass Spectrometer
- Vanquish MS Connection Kit (P/N 6720.0405)

Thermo SCIENTIFIC

LC Conditions	
Column	Thermo Scientific™ Accucore™ aQ 100 x 2.1 mm, 2.6 µm
Mobile Phase	A) Water/Methanol (98:2, v/v, %) with 5 mM ammonium formate and formic acid 0.1% (v/v, %) B) Water/Methanol (2:98, v/v, %) with 5 mM ammonium formate and formic acid 0.1% (v/v, %)
Temperature	25 °C
Injection Volume	1 µL
15 Min Method	
Gradient	0.00–0.82 min: 0% B; 0.82–7.32 min: 0–70% B; 7.32–9.32 min: 70–100% B; 9.32–12.32 min: 100% B; 12.32–12.42 min: 100–0% B; 12.42–15.00 min: 0% B
Flow Rate	0.300 mL/min
5 Min Method	
Gradient	0.00–0.31 min: 0% B; 0.31–2.44 min: 0–70% B; 2.44–3.11 min: 70–100% B; 3.11–4.11 min: 100% B; 4.11–4.14 min: 100–0% B; 4.14–5.00 min: 0% B
Flow Rate	0.900 mL/min

MS Conditions	
Ionization Conditions	HESI
Polarity	Positive/Negative switching
15 Min Method	
Sheath Gas Flow Rate	40 units
Aux Gas Flow Rate	6 units
Spray Voltage Positive Ion	3,700 V
Spray Voltage Negative Ion	2,500 V
Ion Transfer Tube Temp.	325 °C
Vaporizer Temp.	350 °C
CID Gas	2 mTorr
Cycle Time	0.5 s
5 Min Method	
Sheath Gas Flow Rate	58 units
Aux Gas Flow Rate	15 units
Spray Voltage Positive Ion	3,700 V
Spray Voltage Negative Ion	2,500 V
Ion Transfer Tube Temp.	350 °C
Vaporizer Temp.	400 °C
CID Gas	2 mTorr
Cycle Time	0.34 s

Data Acquisition and Processing

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2 SR2.

Results and Discussion

Method Transfer from an UltiMate 3000 RSLC System to Vanquish UHPLC System

An already existing Thermo Scientific LC-MS method developed with the Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system for the quantitative analysis of more than 250 pesticides in food extracts was transferred to the Vanquish UHPLC system. On the basis of the estimated differences in the gradient delay volumes of the two LC systems, the method for the Vanquish UHPLC system was corrected with the addition of an initial isocratic step of 0.32 min. This gradient delay volume between two systems was due not only to the modules but also to the specific configurations used in these applications, such as solvent mixer and capillaries. Alternatively, the Vanquish UHPLC system features various convenient fluidics adjustments to reflect the original extra-column and delay volumes. In this case the LC gradient requires no changes. Retention time shift between the two LC systems was on average less than 5 s. The negligible difference between calculated and experimentally observed shift of retention times was extremely important to avoid time consuming correction of narrow SRM scan windows of 30 s (Figure 1). Therefore, the SRM MS method was not further optimized.

Method Transfer from Fast to Ultrafast Separation with Vanquish UHPLC System

The 15 min method was shortened to 5 min with an increase of the sample throughput of 300%. The flow rate was increased from 0.3 mL/min to 0.9 mL/min, and the gradient slope was adjusted accordingly, as shown in Figure 2. The maximum system pressure was 360 bar for the 15 min method and 1010 bar for the 5 min method.

Taking benefit from the significant reduction of the peak widths in UHPLC mode with the ultrafast separation, the timed SRM scan window was decreased from 30 s to 9 s. The TSQ Endura MS has a 500 SRM/s data acquisition rate capability[2] and, a decrease of the SRM scan window gave the possibility to decrease the cycle time to 0.34 s. This allowed acquisition of 10–15 data points across the LC peak, which is optimal for accurate quantitation.

The Vanquish UHPLC system showed an outstanding retention time precision from run-to-run and from sample-to-sample that was the key factor for the development of the ultrafast UHPLC-MS method with very narrow SRM scan window (Figure 3).[3,4] The run-to-run retention time repeatability was evaluated by seven consecutive injections for 50 compounds detected in all three matrices at 5 ppb level and reveled SD below 0.30 s. The matrix-to-matrix retention time reproducibility was evaluated for the same compounds at 5 ppb level in the three matrices and revealed SD below 0.15 s (Figure 4). LC-MS analysis with ultranarrow SRM scan windows are possible only in combination with LC systems that can ensure high retention time precision because each minimal retention time shift would lead the LC peaks outside the SRM scan window compromising significantly data quality with an increased number of false negatives.

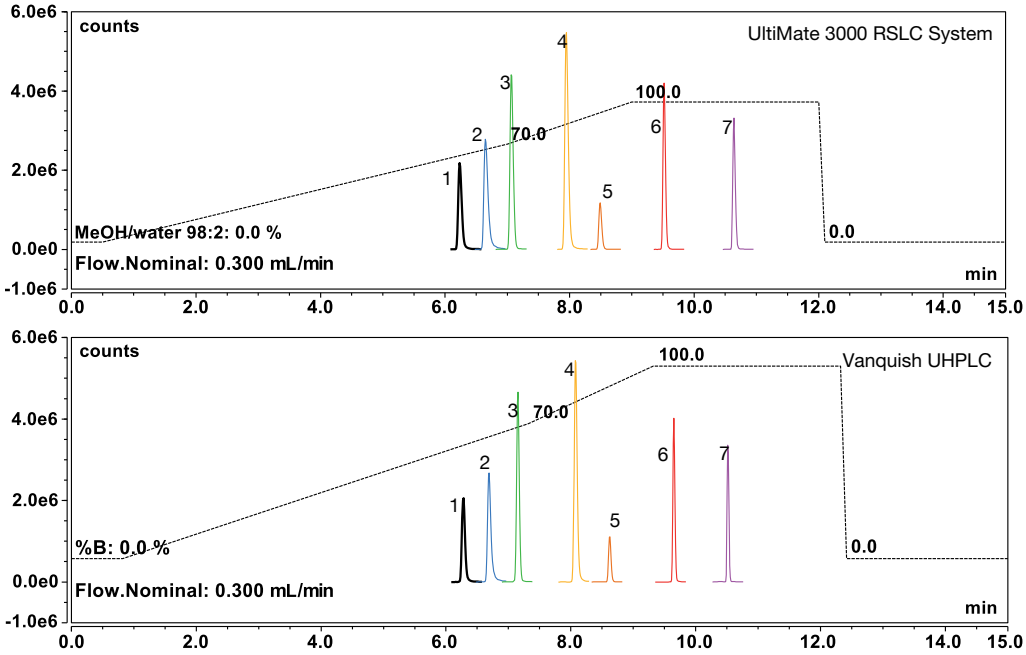
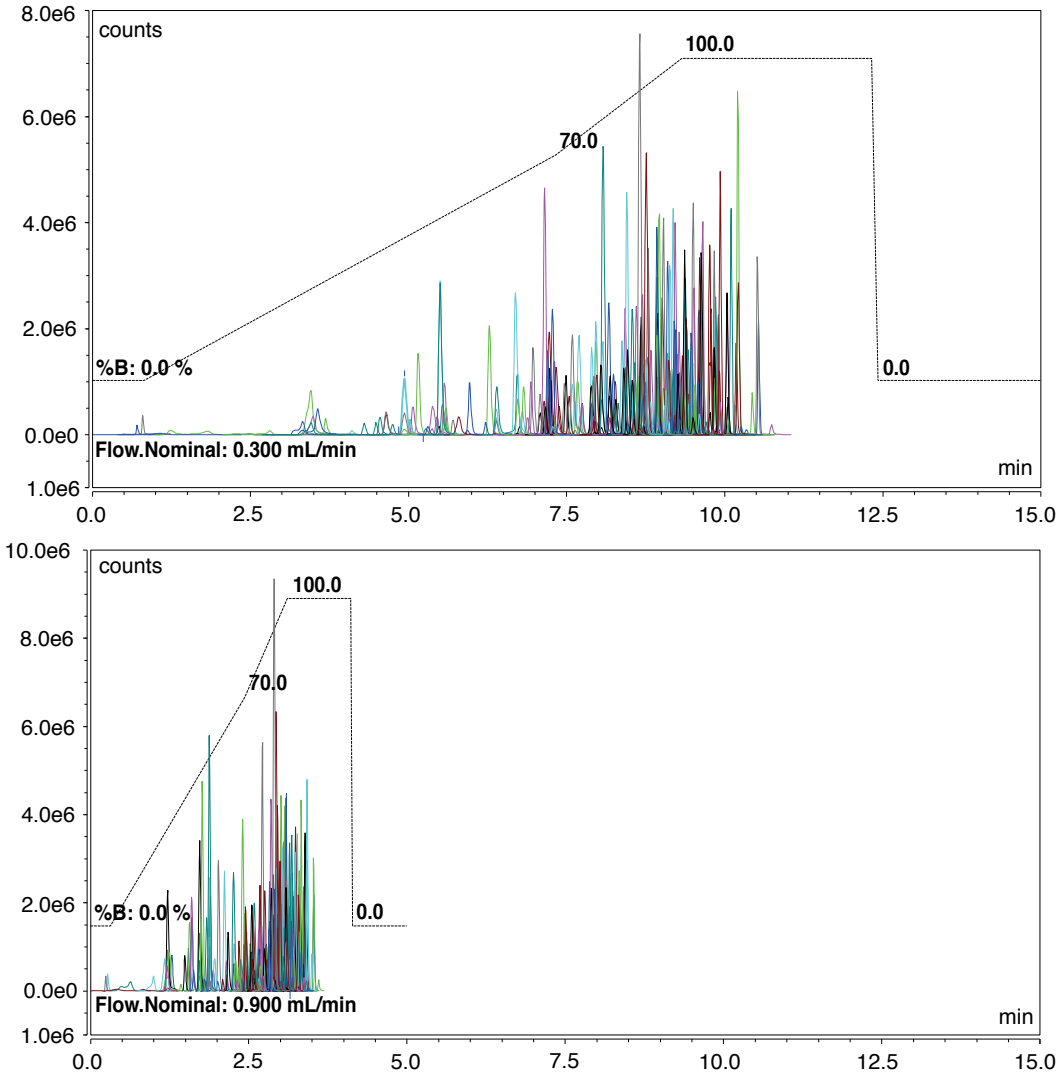


Figure 1. Comparison of extracted ion chromatograms of seven pesticides in strawberry extracts analyzed with an UltiMate 3000 RSLC system and a Vanquish UHPLC system after method transfer.



Fast Gradient
15 min run time
0.3 mL/min
360 bar
SRM-MS
Scan win. 30 s
Cycle Time 0.5

Ultrafast Gradient
5 min run time
0.9 mL/min
1010 bar
SRM-MS
Scan win. 9 s
Cycle Time 0.34

Figure 2. Extracted ion chromatograms of pesticides in strawberry matrix extract applying a gradient length of 15 and 5 min. Other conditions are described in figure.

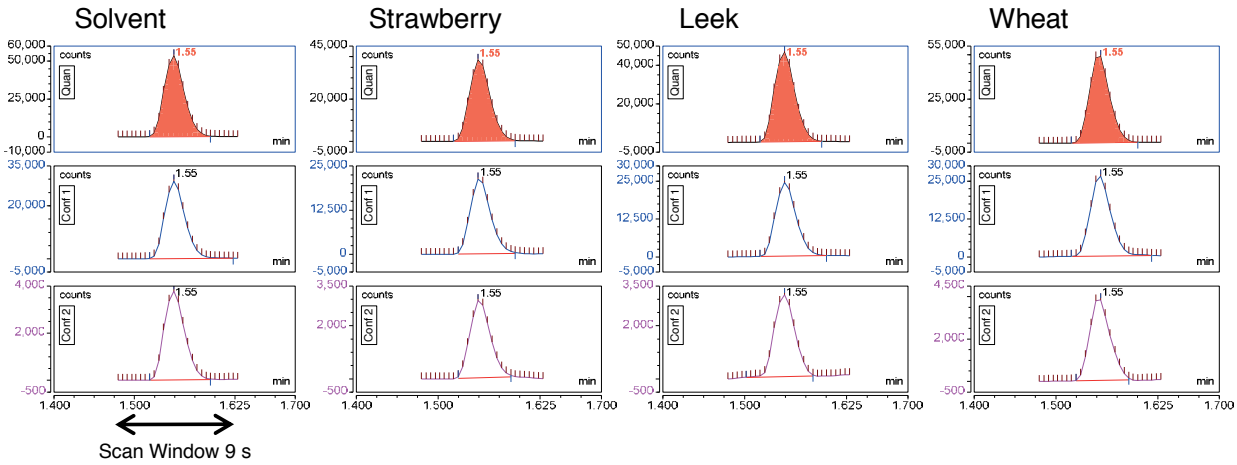


Figure 3. Demeton-S-methyl sulfone at 10 µg/kg in solvent and food matrix extracts acquired with fast cycle time and scan window of 9 s. More than 10 data points are acquired for both quantitation and confirmation ions.

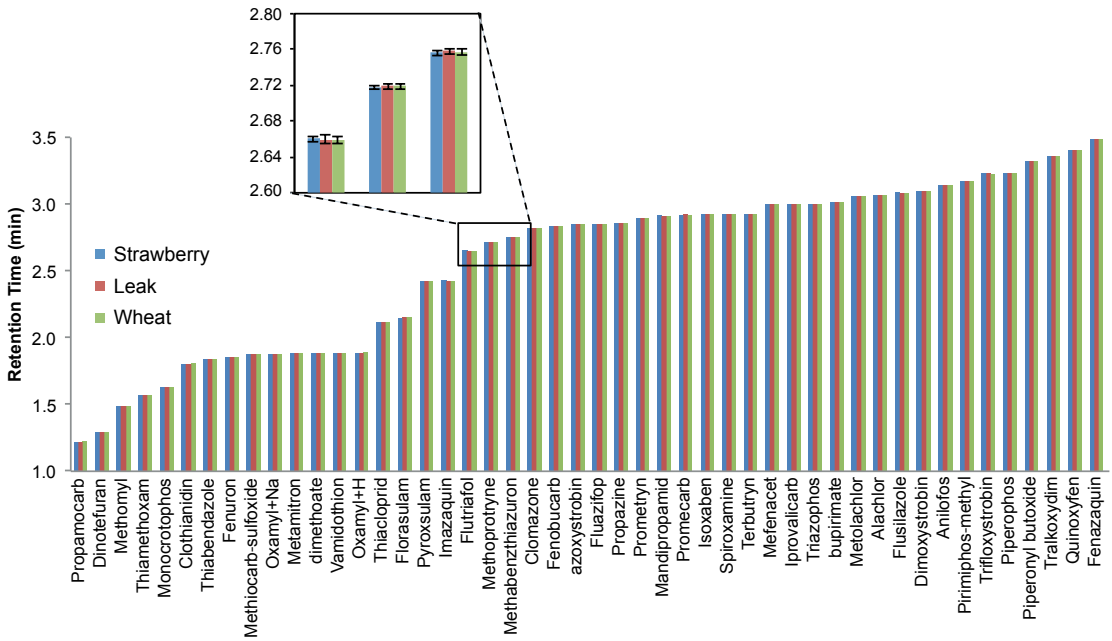


Figure 4. Retention times for 50 compounds at 5 ppb level in strawberry, leek, and wheat extracts. For each compound in each matrix is displayed the average of seven consecutive injections (±SD<0.30 s, see inset).

Method Validation

The 15 min and 5 min LC-MS methods were validated based on the following criteria: 1) accuracy, estimated at the 3 different levels in the 3 different matrices, 2) limits of quantification (LOQs), based on RSD ≤ 15% and ion ratios, 3) repeatability (%), based on RSDs%, 4) linearity measured as squared correlation coefficient.

Pesticide residues were considered reliably measured if they passed all the following evaluation criteria:

- Accuracy 80–120%
- RSDs ≤ 15%
- Ion Ratio tolerance ± 30% rel., ion co-elution 0.010 min

The results obtained with the 5 min LC-MS method in terms of accuracy, LOQs, and repeatability were compared with the 15 min method. As shown in Figure 5, the UHPLC method provided similar results saving 67% of analysis time.

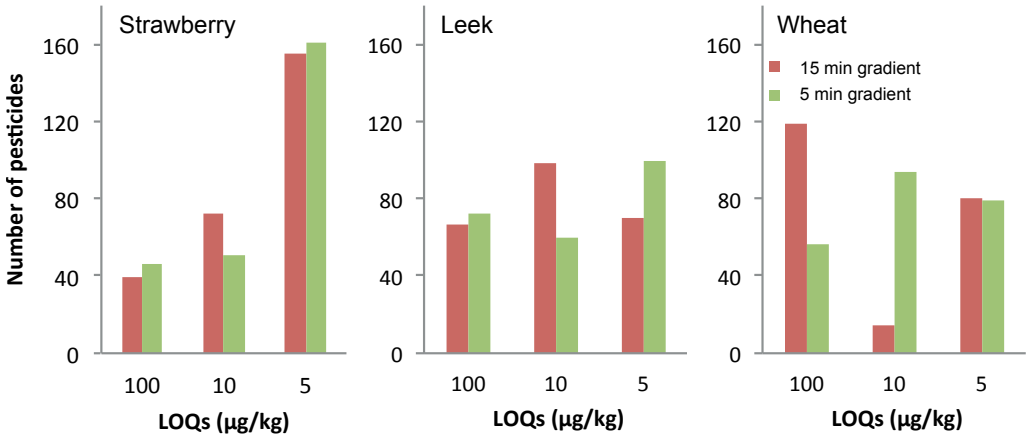


Figure 5. The 15 min and 5 min LC-MS method provide comparable data quality in all three food matrices.

Conclusion

This application note compared fast and ultrafast UHPLC-MS/MS analysis for the quantitation of 250+ pesticides in food extracts. The results showed that:

- Fast UHPLC separations in combination with ultranarrow timed SRM scan windows allowed maintaining the number of monitored transitions without compromising data quality.
- Outstanding retention time stability achieved with the Vanquish UHPLC system is the key factor for fast timed SRM MS analysis with a high number of data points across the peak.
- Ultrafast UHPLC separation resulted in saving 67% of analysis time and an increase of the sample throughput of 300% without losing information.

References

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