APPLICATION NOTE

Robust LC-MS analysis of pesticides with 1.0 mm i.d. column using the Vanquish Horizon UHPLC system

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Key Words

1.0 mm i.d. column, food safety, micro flow, sensitivity, TSQ Endura Triple Quadrupole MS, Vanquish Horizon UHPLC System

Goal

Demonstrate the performance of the Thermo Scientific™ Vanquish™ Horizon UHPLC system for the LC-MS analysis of pesticides using 1.0 mm i.d. columns for improved sensitivity and reduced solvent usage.

Introduction

UHPLC-MS applications are generally developed with 2.1 mm i.d. columns at relatively high flow rates, in most cases, between 0.3 and 1.0 mL/min. In the last few years, interest in increasing sensitivity and reducing solvent consumption has grown. In this regard, the use of 1.0 mm i.d. columns instead of 2.1 mm i.d. columns presents several advantages. First, microflow UHPLC applications based on 1.0 mm i.d. columns give higher sensitivity when compared to normal flow UHPLC.[1] In addition, it allows the use of less mobile phase and reduces waste, providing a cost savings and a diminished environmental impact of the toxic solvent.

When scaling down a method, the theoretical increase of sensitivity can be calculated as a ratio, in which each of the respective internal diameter values are squared. Thus, scaling down a method from a 2.1 mm i.d. column to a 1.0 mm i.d. column would lead to a sensitivity increase of $2.1^2/1.0^2 = 4.4$ for concentration sensitive detectors, if the same sample amount was loaded onto the column.[2] In many aspects, a mass spectrometric detector with an electrospray ionization (ESI) source behaves as a concentration sensitive detector for LC-MS applications. However, when UHPLC methods with 1.0 mm i.d. columns are coupled to MS detection, the sensitivity increase can be substantially different from the theoretical value.[2] Several aspects, such as the specific physical chemical properties of each compound, ionization efficiency, and emitter ID, can influence the sensitivity. It is evident that this approach is highly attractive for all trace quantitative applications, such as the analysis of pesticide residues in food that must achieve demanding limits of detection (LODs).

Another important aspect that has to be considered for successful applications with 1.0 mm i.d. columns is the performance of the chromatographic system at low flow rates. Pulsation-free pumps at low flow rates are required for optimal gradient performance. At the same time, the system fluidic has to be optimized to minimize the extra column volume and prevent unwanted diffusive phenomenon that can decrease the efficiency of the method and result in peak broadening.

In this study, we test the performance of the Vanquish Horizon UHPLC system coupled to a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer with 1.0 mm i.d. columns for the analysis of pesticides at ppb and sub-ppb levels.

Experimental

Sample preparation

Food samples, such as strawberry and leek, were homogenized (10 g) and weighed into a Thermo Scientific™ HyperSep™ QuEChERS extraction tube (P/N 60105-216). After the addition of 10 mL of acetonitrile, the tube was shaken for 10 min and centrifuged at 5000 rpm for 5 min. Pesticide stock solutions were prepared in water and matrix extracts. Working neat solution and matrix-fortified samples were obtained by dilution in the corresponding solvent or matrix to get the final concentration of 1 or 10 µg/L (1 or 10 ppb).

Instrumentation

- Thermo Scientific Vanquish Horizon UHPLC system including:
  - System Base Vanquish Horizon (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 with Thermo Scientific™ Ion Max NG API source
- Thermo Scientific™ Vanquish™ MS connection kit (P/N 6720.0405)

LC conditions for three methods are listed in Table 1 and the MS conditions are listed in Table 2. Table 3 provides information on capillaries and fittings used to optimize extra-column volume.

Table 1. LC conditions.

Parameter	Setting		
	Method A	Method B	Method C
Column:	Thermo Scientific™ Acclaim™ PepMap™ RSLC 150 x 1.0 mm, 2.0 µm	Thermo Scientific™ Hypersil GOLD™ 100 x 2.1 mm, 1.9 µm	Thermo Scientific Hypersil GOLD 100 x 1.0 mm, 1.9 µm
Mobile Phases:	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:	60 °C, Forced Air Mode	40 °C, Still Air Mode	40 °C, Still Air Mode
Injection Volume:	0.25–1 µL	2 µL	2 µL
Gradient:	0.00–0.10 min: 20% B; 0.10–0.67 min: 20–60% B; 0.67–3.00 min: 60–100% B; 3.00–5.00 min: 100% B; 5.00–5.17 min: 100–20% B; 5.17–6.00 min: 20% B	0.0–4.0 min: 20–95% B; 4.0–4.2 min: 95% B; 4.2–4.6 min: 95-20% B; 4.6–7.0 min: 20% B	0.0–4.0 min: 20–95% B; 4.0–4.2 min: 95% B; 4.2–4.6 min: 95-20% B; 4.6–7.0 min: 20% B
Flow Rate:	0.200 mL/min	0.450 mL/min	0.100 mL/min

Table 2. MS conditions.

Parameter	Setting		
	Method A	Method B	Method C
Ionization Conditions:	HESI	HESI	HESI
Polarity:	Positive/Negative switching	Positive	Positive
Acquisition Mode:	SRM	SRM	SRM
Sheath Gas Flow Rate:	25 units	40 units	20 units
Aux Gas Flow Rate:	9 units	20 units	7 units
Spray Voltage Positive Ion:	3500 V	3200 V	3200 V
Spray Voltage Negative Ion:	2500 V	-	-
Ion Transfer Tube Temp.:	300 °C	350 °C	350 °C
Vaporizer Temp.:	300 °C	400 °C	200 °C
CID Gas:	2 mTorr	2 mTorr	2 mTorr
Cycle Time:	0.52 s	0.05 s	0.05 s

Table 3. Optimized extra-column volume.

Product		Part Number
Capillary from the Column to the Grounding Union	350 mm x 50 µm i.d.; Thermo Scientific™ nanoViper™ capillary	6041.5540
Grounding Union	Zero dead-volume Thermo Scientific™ Viper™ union	6040.2304
Grounding Union-Needle Insert	150 mm x 50 µm i.d.; nanoViper capillary	6041.5124
Needle Insert	Needle insert, electrospray, low flow	80000-60152

Data acquisition and processing

Data were acquired with Thermo Scientific™ SII for Xcalibur™ software 1.2 and processed with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software 7.2 SR4.

Results and discussion

Optimization of the system configuration

To reduce the extra-column volume, all the fluidic path form the outlet of the column to the mass spectrometer was optimized using fused silica capillaries with 50 µm inner diameter that are smaller than the standard configuration. A needle insert for low flow that has a smaller inner diameter was used to reduce post column peak broadening.[3]

Analytical figures of merit for Vanquish Horizon UHPLC system with 1.0 mm i.d. column

A mixture containing 255 pesticides was analyzed with the Vanquish Horizon UHPLC system coupled to a TSQ Endura triple quadrupole mass spectrometer with a 1.0 mm i.d. column and extra-column volume optimized as described above (Figure 1), according to method A. The maximum system pressure was 1030 bar. The analysis was performed at four calibration levels, acquiring six replicates for each calibration level. In detail, a 5 ppb solution was analyzed at four different injection volumes (0.05, 0.1, 0.2, and 1 µL volume injections, corresponding respectively to 0.25, 0.5, 1, and 5 pg on column, or alternately to 0.25, 0.5, 1, and 5 ppb concentration).

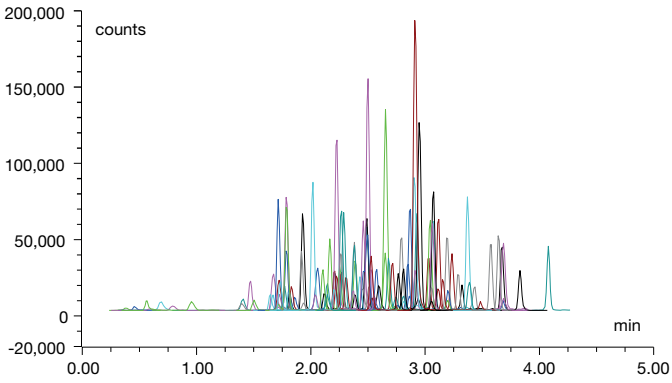

Figure 1. Extracted ion chromatograms of 1 ppb calibration standard mixture containing 255 pesticides separated on 1 mm i.d. column.

The validation and robustness of the method was evaluated based on the following parameters:

Linearity – Linearity of the system response was evaluated plotting the compound areas against the nominal concentration. Calibration plots showed excellent linearity (R^2 > 0.95) for most of the compounds (Figure 2).

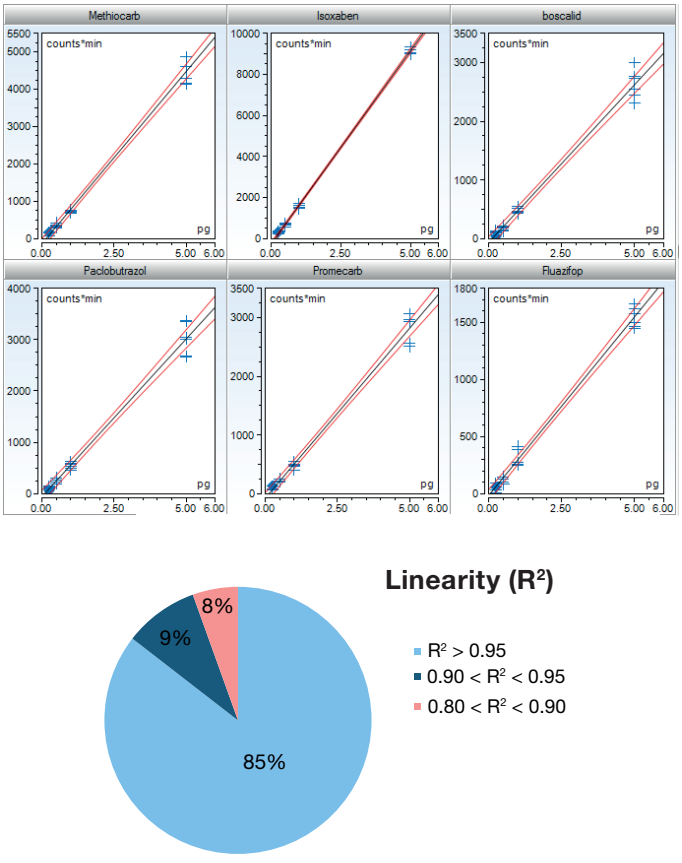

Figure 2. Calibration plots for six generic pesticides. Linearity, measured as squared correlation coefficient (R^2), showed R^2 > 0.95 for most of the compounds. Results are presented as percentage of the compounds with R^2 in the ranges described in the legend.

LOD – Limits of detection (LODs) were calculated based on the signal-to-noise ratio (S/N). Almost all the compounds showed S/N > 3 at the lowest calibration level of 0.25 ppb (Figure 3). These data highlight the excellent sensitivity of the method.

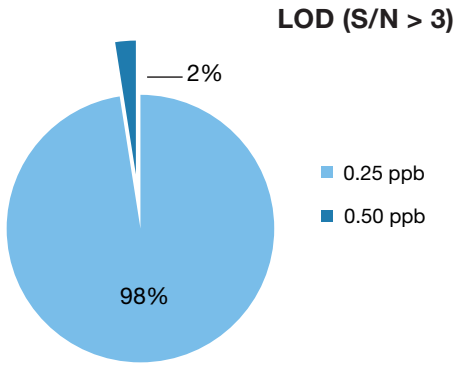

Figure 3. Limits of detection (LODs) based on the signal-to-noise ratio (S/N) showed S/N > 3 for almost all the compound at the lowest calibration level of 0.25 ppb. Results are presented as a percentage of the compounds with S/N >3 at the concentration level described in legend.

Area precision – Area precision was evaluated at 0.50 ppb level and calculated as RSD% of six replicates. More than half of the compounds were detected at this low concentration with good area RSD below 25% (Figure 4). At 1 ppb level, almost 80% of the compounds were detected with area RSD < 25%.

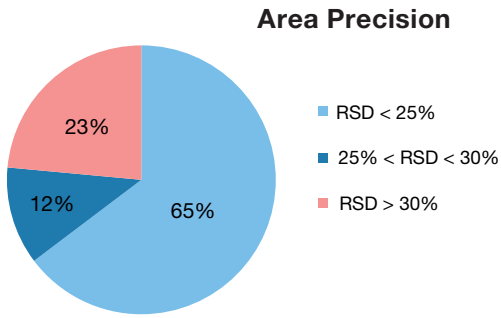

Figure 4. Area precision (RSD% of six replicates) calculated at 0.50 ppb level. Results are presented as a percentage of the compounds with area precision in the ranges described in the legend.

Retention time precision – The system provided excellent retention time precision over all the gradient with retention time SD < 0.01 min (= 0.6 s) (Figure 5). Retention time precision is of utmost importance in target SRM quantitation experiments, giving the confidence that the peaks of interest run after run will not shift out of the acquisition window.

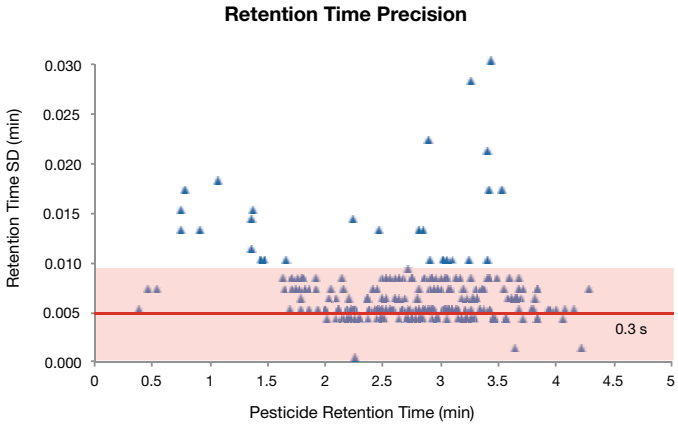

Figure 5. Retention time precision evaluated as retention time SD of six replicates at 0.50 ppb level.

Peak width – The peak width was measured at half maximum (full peak width at half maximum, FWHM). The system provided very narrow peaks with FWHM below 2 seconds for the majority of the compounds (Figure 6). Narrow peaks bring the benefit of having less compound co-elution in short gradient methods, with consequently less matrix effect for a better sensitivity. In these experiments 10–15 data points were acquired per peak.

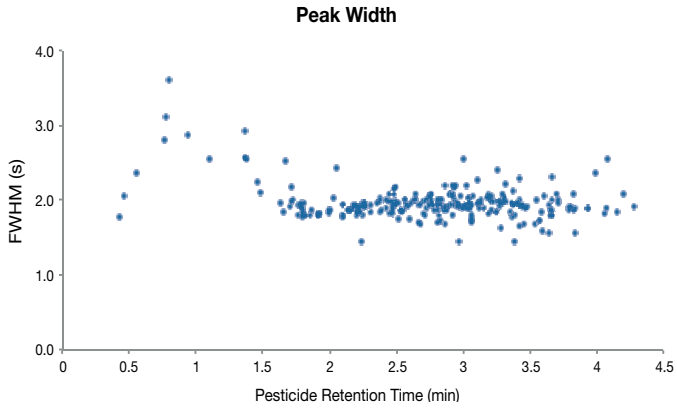

Figure 6. FWHM of pesticides at 0.50 ppb level. Values displayed are average of six replicates.

Comparison of multi-pesticide methods with 1.0 and 2.1 mm i.d. columns

Method A, based on the use of a 1.0 mm i.d. column operated at a flow rate of 200 µL/min, was compared with a previously developed method based on a 2.1 mm i.d. column operated at flow rate of 900 µL/min (linear velocity approximately 6 mm/s). The two methods have the same linear velocity and were applied for the analysis of 98 pesticides in strawberry matrix at 1 ppb level. (For more information on the method based on 2.1 mm i.d. column, please refer to Application Note 1138.[4]) The data showed that the sensitivity achieved with the 1.0 mm i.d. column, although it is compound dependent, is around two times higher in comparison with the 2.1 mm i.d. column (Figure 7).

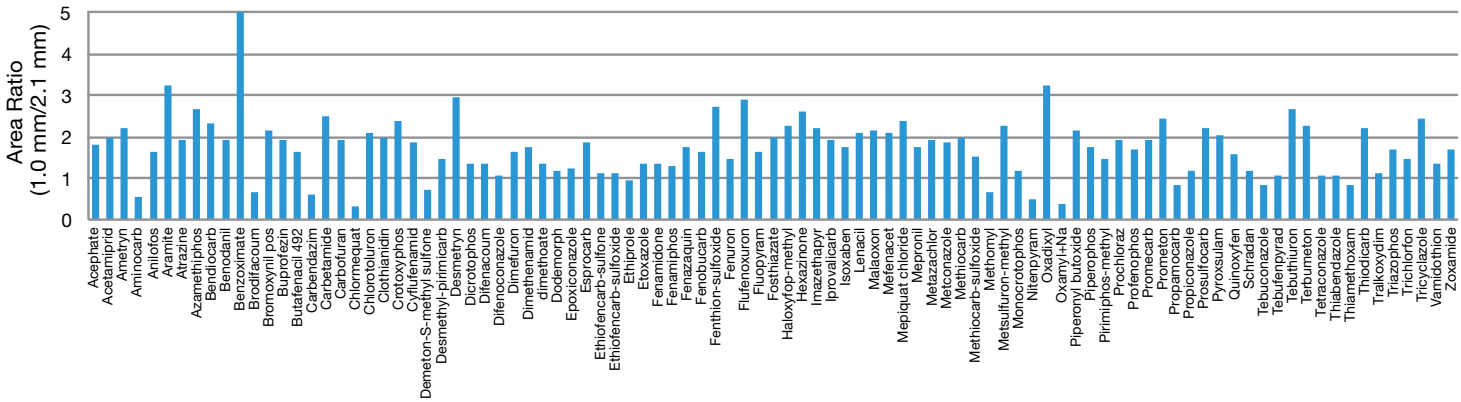

Figure 7. Analysis of 98 pesticides spiked in strawberry matrix at 1 ppb level. Comparison between the analysis performed with 1.0 mm i.d. column operated at flow rate of 200 µL/min and with a 2.1 mm i.d. column at 900 µL/min. For each compound, the area value was the average of six replicates with area RSD < 20%.

System performance at 100 µL/min

To evaluate the performance of the Vanquish Horizon UHPLC system at a lower flow rate, a mixture of seven pesticides at 1 ppb level was analyzed in gradient mode with a 2.1 mm i.d. column operated at flow rate of 450 µL/min (method B). The method was then transferred to a 1.0 mm i.d. column operated at flow rate of 100 µL/min to keep constant the linear velocity at approximately 3 mm/s (method C).

Area and retention time precision

In both cases, the Vanquish Horizon UHPLC system provides very good retention time precision and generally RSD is below 0.1% (Figure 8). The results obtained at flow rates as low as 100 µL/min were comparable to those collected at higher flow rates of 450 µL/min, highlighting the great performance of the pump even at a lower flow rate. Similarly, comparable area reproducibility was observed with the two methods (Figure 9).

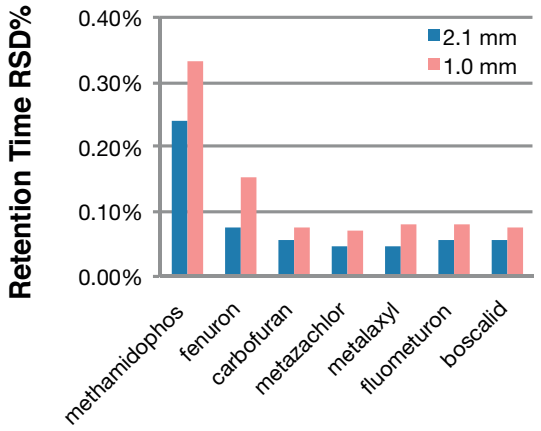

Figure 8. Retention time precision (RSD%) for seven pesticides at 1 ppb level analyzed in gradient mode with 2.1 mm i.d. column operated at flow rate of 450 µL/min and 1.0 mm i.d. column operated at flow rate of 100 µL/min.

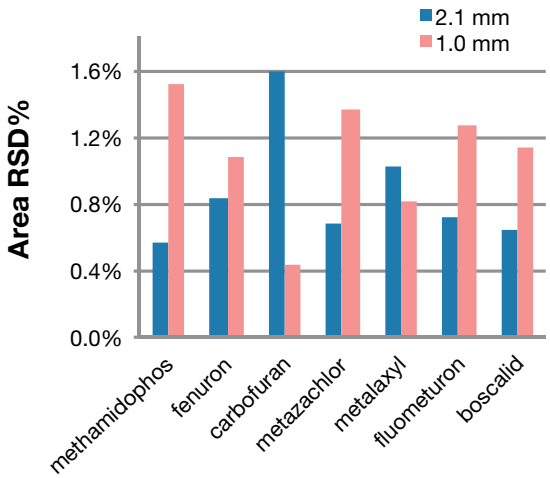

Figure 9. Area precision (RSD%) for seven pesticides at 1 ppb level analyzed in gradient mode with 2.1 mm i.d. column operated at flow rate of 450 µL/min and 1.0 mm i.d. column operated at flow rate of 100 µL/min.

Long-term robustness

The robustness of the method at 100 µL/min was evaluated. For this purpose, the system was stressed by consecutive injections of leek extracts for two days (over 200 injections). The area response showed excellent stability for all the duration of the analysis, with area RSD below 2.5% (Figure 10). The system with the 1.0 mm i.d. column operated at flow rate of 100 µL/min is robust and capable of long, demanding analyses.

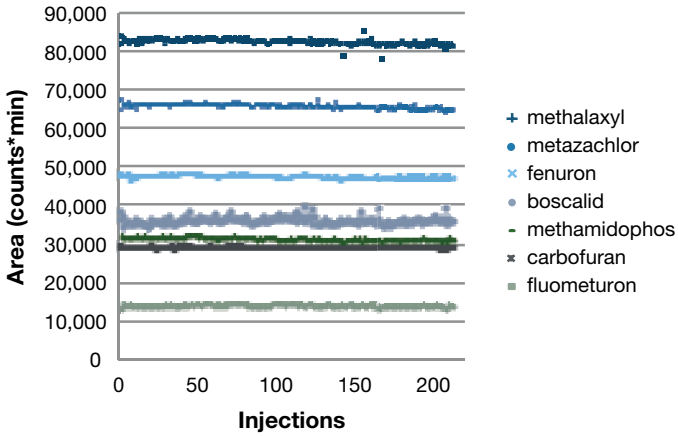

Figure 10. Peak area reproducibility of seven pesticides spiked at 10 ppb level in leek extract analyzed with 1.0 mm i.d. column operated at flow rate of 100 µL/min.

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Conclusion

This application note highlights the excellent performance of Vanquish Horizon UHPLC system with 1.0 mm i.d. columns for robust and sensitive UHPLC-MS/MS quantifications. The results showed the following:

- The pump of the Vanquish Horizon UHPLC system delivers excellent performance using 2.1 mm, as well as 1.0 mm, i.d. columns.
- The wide flow-pressure footprint of the Vanquish Horizon UHPLC allows it to operate 1 mm i.d. columns at high linear velocity/flow rate and achieve high-throughput separations of complex samples.
- The use of 1.0 mm i.d. columns increases MS sensitivity for pesticides analysis, allowing the detection of compounds at sub-ppb level.
- UHPLC methods at low flow rates are fast and robust, and at the same time allow considerable solvent saving (around four times less solvent is required for operating 1.0 mm i.d. columns in comparison with 2.1 mm i.d. columns).

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