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Robust LC-MS Analysis of Pesticides with 1.0 mm i.d. Columns Using State of the Art UHPLC Instrumentation

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ABSTRACT

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 frequently developed on 2.1 mm i.d. columns at fairly high flow rates in the range of 0.3-1.0 mL/min. Lower flow rates promise better sensitivity and lower detection limits in mass spectrometry due to easier solvent removal and better ionization efficiency. Simply reducing the flow rate on the same column geometry would worsen the separation efficiency and reduce both chromatographic resolution and sensitivity. The use of 1.0 mm i.d. columns instead of 2.1 mm i.d. columns offers the advantage that the same LC performance can be generated at substantially lower flow rates, additionally reducing mobile phase consumption and hazardous waste production. On top, micro-flow UHPLC applications based on 1.0 mm I.D. columns will show a higher sensitivity compared to normal flow UHPLC, assuming that the injected analyte mass stays the same.[1]

When scaling down a method this theoretical increase of sensitivity follows the ratio of the squares of the internal column diameters. Thus, scaling a method down from 2.1 mm I.D. to 1.0 mm I.D. would lead to a sensitivity increase of $2.1^2/1.0^2 = 4.4$ for concentration-sensitive detectors, which mostly applies to mass spectrometry with electrospray ionization (ESI) sources. However, if UHPLC methods with 1.0 mm I.D. columns are coupled to MS detection, the sensitivity increase can deviate substantially from the theoretical value due to the specific physical and chemical properties of the analytes influencing ionization efficiency, or mismatch of fluidic dimensions such as the emitter ID.[2] Next to the MS, also the fluidic design and the instrument performance of the UHPLC system need to keep pace with the requirements claimed by the use of 1.0 mm ID columns.

In this study, we evaluate the compatibility of the latest state of the art in UHPLC instrumentation and Triple Quadrupole Mass Spectrometry with 1.0 mm I.D. columns for the trace-level analysis of pesticides at (sub-)ppb level.

MATERIALS AND METHODS

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)
- Vanquish™ MS connection kit (P/N 6720.0405)

Thermo Scientific™ TSQ Endura™ Triple Quadrupole mass spectrometer with Thermo Scientific™ Ion Max NG API source

LC and MS conditions
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 experimental 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
Inj. 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 experimental 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 Pos Ion	3500 V	3200 V	3200 V
Spray Voltage Neg 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	Prototype	-
Grounding union-Needle Insert	150 mm x 50 µm ID; 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

Optimization of the system configuration
To reduce the extra-column volume, all of the fluidic paths from the outlet of the column to the mass spectrometer were optimized using fused silica capillaries with 50 µm inner diameter that are smaller than the standard configuration. A needle insert for low flow having 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.

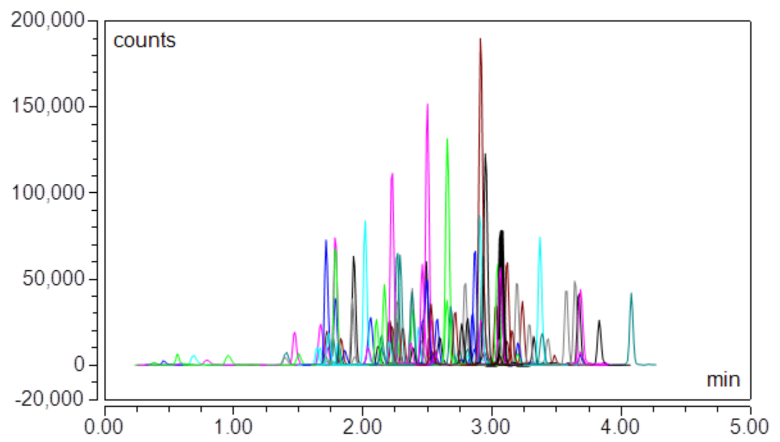
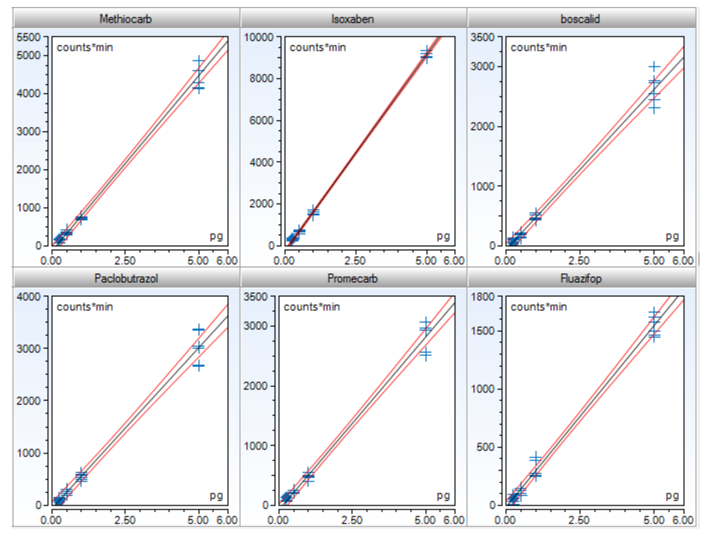
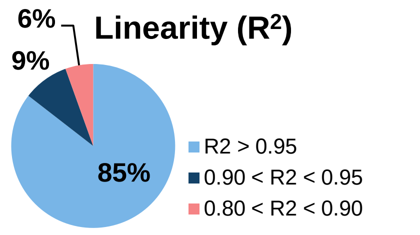


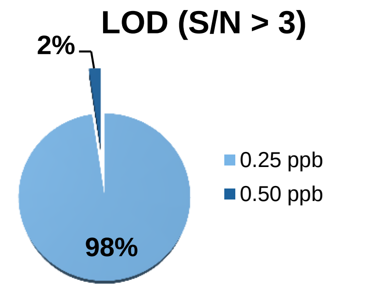
Figure 2. Calibration plots for six generic pesticides.





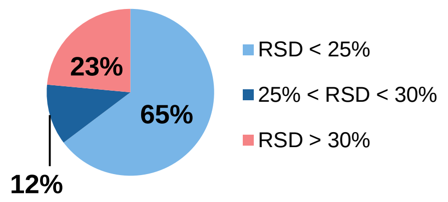
Linearity – Linearity of the system response was evaluated plotting the compound areas against the nominal concentration. Calibration plots showed excellent linearity ($R^2 > 0.95$) for most of the compounds (Figure 2).

Figure 3. Limits of detection (LODs) based on the signal-to-noise ratio (S/N).



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 4. Area precision.



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 5. Retention time precision at 0.50 ppb level.

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.

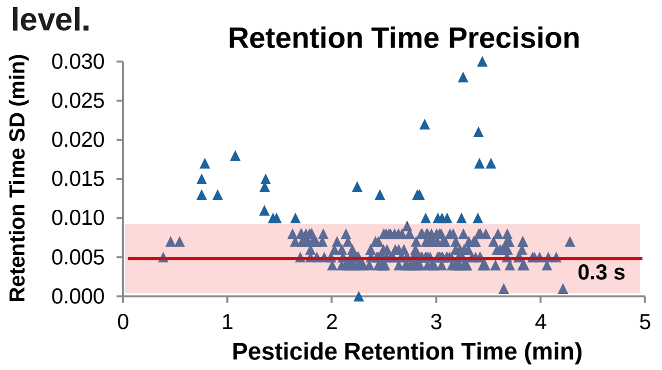
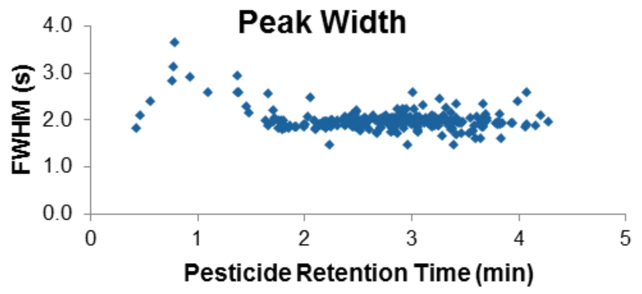
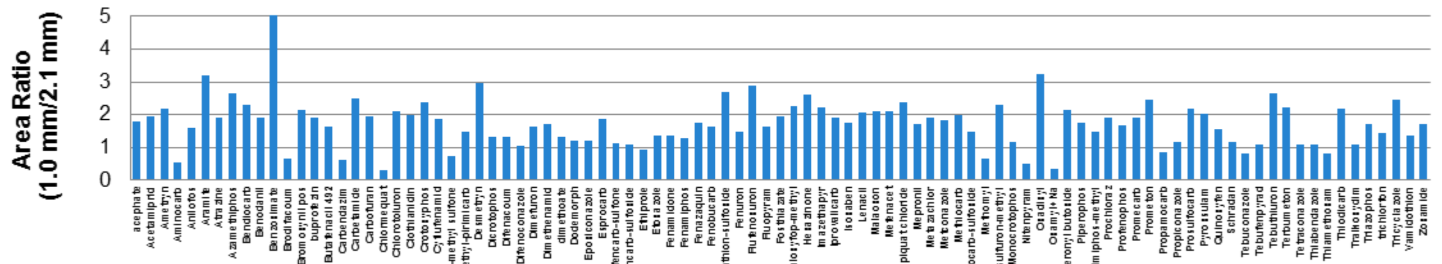


Figure 6. FWHM of pesticides at 0.50 ppb level.



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.

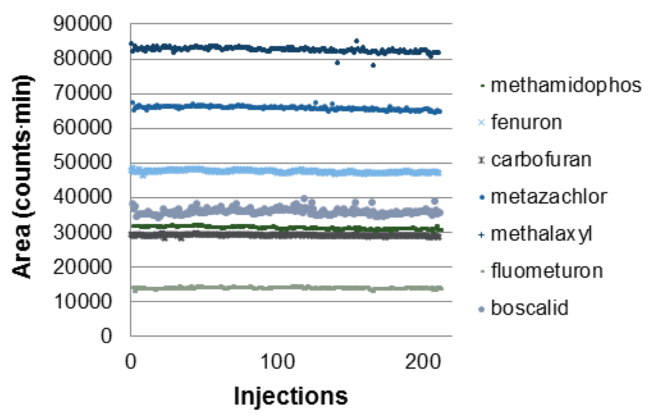
Figure 7. Analysis of 98 pesticides spiked in strawberry matrix at 1 ppb level. Comparison between the analysis performed with 1.0 mm i.d. column and with a 2.1 mm i.d. column.



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 and 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 a 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 refer to Application Note 1138).[4] The sensitivity achieved with the 1.0 mm i.d. column is around two times higher in comparison with the 2.1 mm i.d. column (Figure 7).

System performance at 100 µL/min
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).

Figure 8. Peak area reproducibility of seven pesticides spiked in leek extract.



Long-term robustness
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 8).

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%. 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.

CONCLUSIONS

This work 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).

REFERENCES

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