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1.0 mm i.d. Columns Packed with 1.7 µm Particles Improve LC/ESI-MS Limits of Detection

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Overview

Many LC/ESI-MS assays use 2 mm i.d. columns packed with 5 µm particles. This situation is a result of the widespread availability of these columns, the compatibility of the optimal flow rates of these columns with electrospray ionization interfaces, and the ability of modern HPLC instrumentation to run these columns with minimal loss in performance due to extra-column bandspreading. Utilizing 1.7 µm particles and 1 mm i.d. columns should lead to an improvement in sensitivity leading to lower limits of detection. These improvements are dependant on having an LC system that operates at higher pressures and has minimal system volume.

Methods

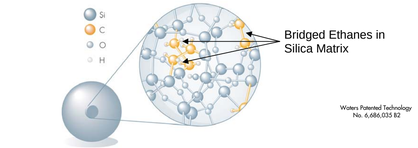
LC/ESI-MS sensitivity of a traditional HPLC and UPLC™ (Ultra Performance LC™) were compared using a 2.1 mm i.d. column packed with 5 µm bridged ethyl-siloxane hybrid (BEH) particles to demonstrate the benefit of using a low dispersion LC as an MS inlet in order to gain sensitivity.

LC/ESI-MS sensitivity was further compared on the UPLC™ using 2.1 mm i.d. columns packed with 5 µm and 1.7 µm BEH particles to establish the benefit of using sub-2 µm particles for improving sensitivity when performing LC/MS^n.

Additionally, a 1 mm i.d. column packed with 1.7 µm BEH particles was used to demonstrate improved limits of detection compared to traditional 2.1 mm i.d. columns packed with 5 µm particles.

A New Generation of Hybrid Packings

Optimal linear velocities of sub-2 µm particles require operations at higher pressures. Inorganic-organic hybrid materials have demonstrated increased mechanical stability compared to traditional silica based materials*[1]. A 1.7 µm bridged ethyl-siloxane hybrid (BEH) particle was designed to meet the demands of operation at pressures as high as 15,000 PSI, as well as mobile phase pH in the range of 1—12.



Waters Patented Technology
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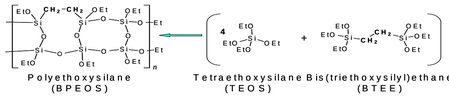


Polyethoxysilane (BPEOS) | Tetraethoxysilane (TEOS) | Bis(triethoxysilyl)ethane (BTEE)

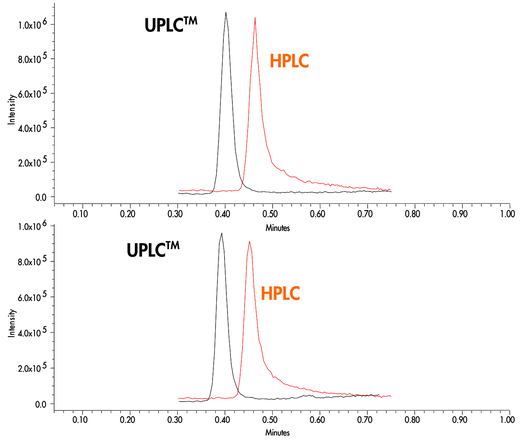
Utilizing a Low Dispersion UPLC™ System with Conventional 5 µm Particles as a Mass Spectrometer Inlet

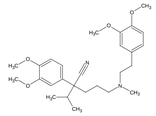
Chromatographic Conditions:
Column: BEH C_{18} 2.1 x 50 mm, 5.0 µm
Mobile Phase A: 0.1% NH_4OH in H_2O
Mobile Phase B: 0.1% NH_4OH in MeOH
Flow Rate: 0.6 mL/min
Gradient:

Time (min)	Profile %A	%B
0	20	80
0.75	5	95

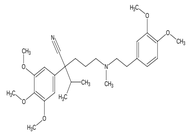
Injection Volume: 20 µL (HPLC); 15 µL (UPLC™)
Sample Diluent: 70:30 MeOH: H_2O with 0.1% NH_4OH
Sample Concentration: 0.5 ng/mL
Temperature: 38 °C
Instrument:
HPLC: Alliance® HT System, 2795 Separations Module with Waters ZQ™
UPLC™: ACQUITY UPLC™ with Waters ZQ™

Mass Spectrometer Conditions (ESI+):
Capillary (kV): 3.0
Cone (V): 35 (MV), 30 (V)
Extractor: 3 V
RF Lens: 0.5 V
Source Temperature (°C): 150
Desolvation Temperature (°C): 350
Cone Gas Flow (L/Hr): 60
Desolvation Gas Flow (L/Hr): 500
SIR m/z: 455.45 verapamil (V); 485.45 methoxyverapamil (MV)
Dwell Time: 50 milliseconds
Interchannel Delay: 50 milliseconds
Interscan Delay: 50 milliseconds





Verapamil



Methoxyverapamil

An improvement in peak shape is observed due to the lower dispersion volume of the ACQUITY UPLC™ system. Although the true benefits of UPLC™ cannot be reached unless sub–2 µm particles are used, the ACQUITY UPLC™ system can be used as a low dispersion LC for conventional particles. Greater sensitivity can be achieved due to the low dispersion characteristics of the UPLC™ system. A further improvement in sensitivity can be observed by utilizing a smaller particle size.

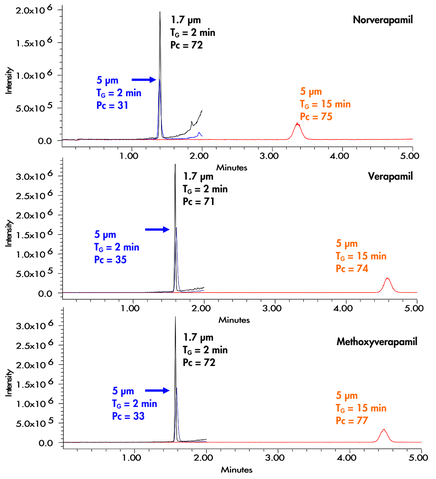
Improved ESI-MS Response Utilizing 2.1 mm i.d. Columns Packed with 1.7 µm Particles

Chromatographic Conditions:
Column: ACQUITY UPLC™ BEH C_{18} 2.1 x 50 mm, 1.7 µm, BEH C_{18} 2.1 x 50 mm, 5.0 µm
Mobile Phase A: 0.1% NH_4OH in H_2O
Mobile Phase B: 0.1% NH_4OH in MeOH
Flow Rate: 0.5 mL/min
Gradient:

Time (min)	Profile %A	%B
0	50	50
15/2.0	5	95

Injection Volume: 20 µL
Sample Diluent: 70:30 MeOH: H_2O with 0.1% NH_4OH
Sample Concentration: 1.0 ng/mL
Temperature: 38 °C
Instrument: ACQUITY UPLC™ with Waters ZQ™

Mass Spectrometer Conditions (ESI+):
Capillary (kV): 3.0
Cone (V): 35 (NV, MV), 30 (V)
Extractor: 3 V
RF Lens: 0.5 V
Source Temperature (°C): 150
Desolvation Temperature (°C): 350
Cone Gas Flow (L/Hr): 60
Desolvation Gas Flow (L/Hr): 500
SIR m/z: 441.4 norverapamil (NV); 455.45 verapamil (V); 485.45 methoxyverapamil (MV)
Dwell Time: 50 milliseconds
Interchannel Delay: 50 milliseconds
Interscan Delay: 50 milliseconds



The efficiency of a gradient separation is measured in terms of peak capacity (Pc). In order to achieve equivalent peak capacity on both the ***1.7 µm*** and ***5 µm*** columns, a gradient duration (T_G) of **2 *minutes*** and ***15 minutes*** must be used, respectively. A 5X improvement in sensitivity can be observed on the 1.7 µm column at the same peak capacity and within 8X faster run times. If the 5 µm column is run with the same gradient duration time as the 1.7 µm column, a 2X improvement in sensitivity can be observed on the 1.7 µm column

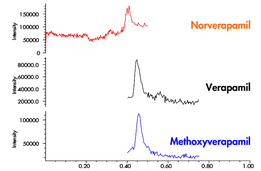
5 µm
PSI_{MAX} = 2,300

1.7 µm
PSI_{MAX} = 11,100

Several factors contribute to the improved response of these analytes at low ng/mL concentrations on a single quadrupole mass spectrometer. The largest contributing factors being the particle size and mobile phase composition. As the particle size is decreased, the peak width decreases and the peak height increases, thus yielding higher analyte response. Additionally, the use of basic pH under ESI$^+$ conditions has demonstrated a significant signal enhancement effect compared to equivalent concentrations of acidic mobile phases for a majority of basic analytes*[2]. The high organic content of the mobile phase also contributes to enhanced analyte response allowing for efficient mobile phase desolvation and compound ionization. All of these factors allow for lower limits of detection compared to traditional HPLC analysis.

Single Quadrupole Linearity and Limits of Detection at Sub-ng/mL Analyte Concentrations

An ACQUITY UPLC™ BEH C_{18}, 1.0 x 50 mm, 1.7 µm column was used to demonstrate the low limits of detection achievable when using 1.0 mm i.d. columns packed with 1.7 µm particles. Verapamil and related compounds were analyzed over the working range of 0.01—10.0 ng/mL, using linear regression to determine the Limits of Detection (LOD) and Limits of Quantitation (LOQ) . Limits of detection were determined to be 0.025 ng/mL for V, NV and MV based on a signal-to-noise ratio of 3:1. Limits of quantitation were determined to be 0.05 ng/mL for V, NV and MV based on a signal-to-noise ratio of 10:1. Six replicates were performed to yield correlation coefficients of 0.9993, 0.9962 and 0.9997 for verapamil, norverapamil and methoxyverapamil, respectively. Linearity of the mass spectrometer over 3 orders of magnitude for each individual standard is shown in Table 1.



An extremely low **LOD of 0.025 ng/mL** for V, NV and MV is achievable on a single quadrupole mass spectrometer by the utilization of 1.0 mm i.d. columns packed with 1.7 µm particles. Typical values using single quadrupole MS under single ion recording mode are 10 ng/mL (±10X).

Table I
Precision and Accuracy of Calibration Standards (N = 6)

Verapamil (ng/mL)										
Nominal concentration	0.01	0.025	0.05	0.10	0.25	0.50	1.00	2.50	5.00	10.00
Calculated mean conc.	0.01	0.024	0.05	0.10	0.25	0.53	1.09	2.58	5.09	9.70
RSD (%)	0.00	6.15	0.00	0.00	0.00	4.00	5.84	2.19	1.25	2.19
Norverapamil (ng/mL)										
Nominal concentration	0.01	0.025	0.05	0.10	0.25	0.50	1.00	2.50	5.00	10.00
Calculated mean conc.	0.01	0.025	0.04	0.09	0.26	0.53	0.99	2.59	5.43	9.55
RSD (%)	0.00	2.95	17.68	7.86	2.72	4.00	0.71	2.46	5.60	3.33
Methoxyverapamil (ng/mL)										
Nominal concentration	0.01	0.025	0.05	0.10	0.25	0.50	1.00	2.50	5.00	10.00
Calculated mean conc.	0.01	0.024	0.05	0.10	0.24	0.51	1.07	2.60	5.01	9.82
RSD (%)	0.00	2.95	0.00	0.00	2.95	1.39	4.63	2.72	0.14	1.30

Conclusions

Improved LC/ESI-MS sensitivity can be observed when utilizing a UPLC™ as an MS inlet with traditional 5 µm particles. Sensitivity can be further improved upon when using 1.7 µm particles yielding a 5X improvement in sensitivity at the same peak capacity and is achieved 8X faster compared to traditional 5 µm particles. Additionally, the utilization of 1.0 mm i.d. columns packed with 1.7 µm particles yields significant improvements in the capabilities of reaching extremely low limits of detection (0.025 ng/mL).

1. Wyndham, K.D., O' Gara, J.E., Walter, T.H., Glose, K.H., Lawrence, N.L., Alden, B.A., Izzo, G.S., Hudalla, C.J. and Iraneta, P.C. Anal. Chem. 2003, 75, 6781-6788
2. Mallet, C.R., Lu, Z. and Mazzeo, J.R., Rapid Commun. Mass Spectrom. 2004; 18: 49-58