Improving LC/MS Performance with UHPLC Columns

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Abstract

Sub-2-µm and superficially porous columns improve LC/MS productivity by providing more resolution and more analysis speed. To get the most from these column technologies, MS-specific method parameters must be optimized, including mobile phase and data collection rate. Also, the LC system must be optimized for minimal extra-column volume. Pharmaceutical, food and beverage, clinical and toxicology applications will be used to show how more peaks can be resolved and identified per analysis by using UHPLC columns, packed with either totally porous sub-2-µm or superficially porous particles. Furthermore this improved LC/MS separation performance is accomplished in less time with increased sensitivity, as compared to traditional 3.5 and 5-µm particle columns.

Experimental

A variety of Agilent instruments were used, including:

- 1290 Infinity LC System
- 1200 Series Rapid Resolution LC System
- 6410A Triple Quadrupole Mass Spectrometer
- 6460A Triple Quadrupole Mass Spectrometer

The MS was used in a variety of modes (Scan, SIM, MRM) to illustrate detection improvements possible over a range of analytical methods. All connecting capillaries were the shortest possible length with 0.12 mm or 0.075 mm internal diameters to ensure minimal efficiency loss through extra-column volume or sample band broadening.

Numerous Agilent LC columns were also used, including:

- Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm (695775-902)
- Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm (699975-302)
- Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm (695975-302)
- Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7 µm (685775-902)
- RRHD Eclipse Plus C18, 3.0 x 50 mm, 1.8 µm (959757-302)
- RRHD Eclipse Plus C18, 3 x 100 mm, 1.8 µm (959758-302)
- RRHD SB-C18, 2.1 x 100 mm, 1.8 µm (858700-902)
- RRHD HILIC Plus, 2.1 x 50 mm, 1.8 µm (959757-901)
- Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm (959764-902)
- Eclipse Plus C18, 2.1 x 100 mm, 3.5 µm (959793-902)
- Eclipse Plus C18, 3.0 x 100 mm, 3.5 µm (959961-302)
- Eclipse Plus C18, 2.1 x 100 mm, 5 µm (custom)

Conditional peak capacity (n_C) is used to evaluate the various analyses. Peak capacity is the number of peaks that can be theoretically separated over a gradient time:

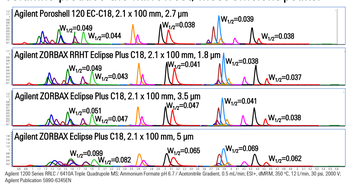
$$n_C = (t_{R,n} - t_{R,1}) / W$$

where: $t_{R,n}$ & $t_{R,1}$ are the retention times of the last and first eluting peaks, and W is the average 4σ peak width

Results and Discussion

Comparison of Particle Sizes with MS Detection

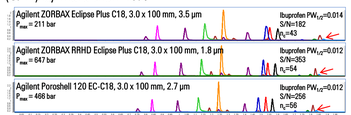
This 25 compound toxicology standard shows similar selectivity across 4 different Agilent particle sizes. The 1.8, 3.5 and 5 µm totally porous particles have identical bonding chemistry, while the superficially porous 2.7 µm column has very similar chemistry. The 5 µm column generates significantly broader peaks resulting in abundant coelution. The 3.5 µm column offers some improvements for peak width, while the 1.8 and 2.7 µm columns produce the narrowest, most efficient peaks.



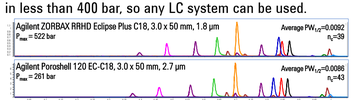
Agilent 1200 Series RRLC / 6410A Triple Quadrupole MS; Ammonium Formate pH 6.7 / Acetonitrile Gradient, 0.5 mL/min; ESI+, dMRM, 350 °C, 12 L/min, 30 psi, 2000 V; Agilent Publication 5990-4346EN

Productivity Improvements with High Efficiency Columns

Fifteen analgesic compounds illustrate the productivity improvements possible with UHPLC columns. While the 3.5 µm column can perform this analysis on a 400 bar instrument, the 2.7 and 1.8 µm columns require 600 bar and >600 bar instruments respectively. However the high efficiency columns result in taller, sharper peaks that increase peak capacity by >20% and improve sensitivity (S/N) by more than 40%.



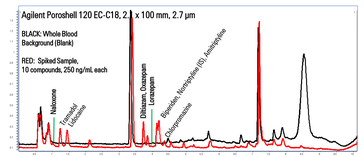
Because the UHPLC columns outperform the 3.5 µm column by so much, shorter columns can be used. Here, 50 mm UHPLC columns achieve similar peak capacity to a 100 mm 3.5 µm column (above) in half the time. Pressure is noteworthy, as the Poroshell analysis is accomplished in less than 400 bar, so any LC system can be used.



Agilent 1200 Infinity LC / 6410A Triple Quadrupole MS; Formic Acid pH 2.8 / Acetonitrile Gradient, 1 mL/min; ESI+, Scan 100-400, 350 °C, 12 L/min, 50 psi, 3500 V; Agilent Publication 5990-8429EN

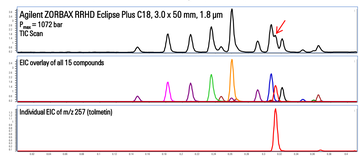
Results and Discussion

MS Resolving Power Combined with UHPLC Columns for Complex Samples



Agilent 1200 Series RRLC / 6460A Triple Quadrupole MS; Ammonium Acetate pH 5 / Acetonitrile Gradient, 0.4 mL/min; ESI+, Scan 100-800, 400 °C, 12 L/min, 40 psi, 3500 V; Agilent Publication 5990-8623EN

A practical example of what higher peak capacity can do is illustrated above with whole blood extracted by QuEChERS. Here, an overlay of a blank whole blood extract is shown with a spiked whole blood extract. Because the 2.7 µm Poroshell 120 column is highly efficient, producing sharp peaks, it consequently also generates higher peak capacity. The UHPLC column separates 10 analytes from background peaks in the same matrix. Occasionally coeluting peaks can suppress the signal of one or both of the compounds, therefore the best protection against this ion suppression is to separate all peaks chromatographically.



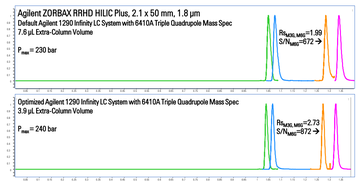
Agilent 1290 Infinity LC / 6410A Triple Quadrupole MS; Formic Acid pH 2.8 / Acetonitrile Gradient, 2.75 mL/min; ESI+, Scan 100-400, 350 °C, 12 L/min, 50 psi, 3500 V; Agilent Publication 5990-8428EN

The ultra-fast analgesics separation shown above demonstrates adequate scanning speed by the MS to detect 15 sharp peaks in 0.4 minutes. The total ion chromatogram shows coeluting peaks around tolmetin. Because each compound is identified by a unique mass, extracted ion chromatograms allow isolation of one peak for simple integration and reproducible quantitation.

It should be noted that the 2.75 mL/min flow rate is for example only, to demonstrate adequate detector speed for the narrow peaks generated by UHPLC columns under extreme conditions. Flow rates beyond 1 mL/min are not recommended for a 6410A MS. When increased throughput is needed, the Agilent Jet Stream Thermal Gradient Focusing Technology with ESI source can be used to extend the flow rate range beyond 2 mL/min without negatively impacting the analysis.

Results and Discussion

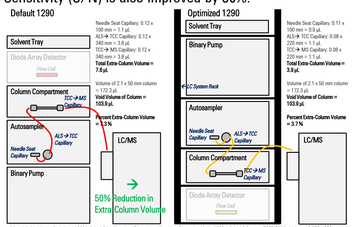
Benefits of Ultra-Low Dispersion with MS Detection and Isobaric Compounds



Agilent 1290 Infinity LC / 6410A Triple Quadrupole MS; Acetonitrile / Ammonium Formate pH 3.2 Gradient, 0.4 mL/min; ESI+, dMRM, 350 °C, 11 L/min, 40 psi, 2000 V; Agilent Publication 5990-8502EN

Compound Name	Precursor Ion	Product Ion	Fragmentor	Collision Energy
Normorphine	272	152	170	65
Normorphine	272	165	170	35
Morphine	286	152	170	65
Morphine	286	165	170	35
M6G	462	286	170	30
M6G	462	201	170	45
M3G	462	286	170	30
M3G	462	201	170	45

The LC/MS/MS separation of morphine and its metabolites on a 1.8 µm HILIC Plus column shown above demonstrates the importance of optimizing performance of a UHPLC column, by minimizing system dispersion with LC/MS analyses. While the MS can typically isolate coeluting peaks, in this case M6G and M3G have the same ion transitions and need to be baseline separated chromatographically. Reducing the extra-column volume by 50% improves the resolution of these two peaks by 37%. Sensitivity (S/N) is also improved by 30%.



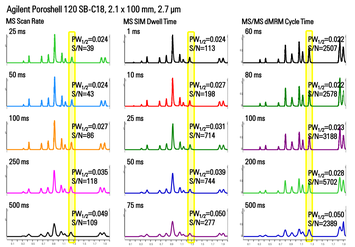
Materials Needed to Optimize a 1290 Infinity LC for Ultra-Low Dispersion: Ultra-Low Dispersion Kit for 1290 Infinity LC System (5067-5188), includes: 0.075 x 220 mm capillary, 0.075 x 340 mm capillary, 1.0 µL heat exchanger, 0.075 mm id needle seat; Ultra-Low Dispersion Max Light Cartridge Flow Cell (G4212-60038)for UV analyzer; Agilent LC System Rack, 5067-5726; Agilent Publication 5990-8502EN

The illustration above details how the UHPLC system was optimized for minimal extra-column volume. All standard 0.12 mm id capillaries were replaced with 0.075 mm id capillaries, and the system was rearranged to ensure the shortest possible connections. The result was 50% less extra-column volume.

Results and Discussion

Optimizing MS Scan Rates to Balance Sensitivity and Peak Capacity

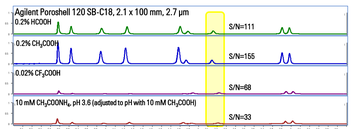
Using the MS detector in various modes, the effect of data collection rate is compared, showing its effect on analytical performance. In all cases the MS has more than enough speed for data collection, to the point where negative effects are apparent with faster rates. The fastest rates generate the narrowest peaks, however they also generate more baseline noise, thus reducing the sensitivity. The optimal data collection rate for a given analysis should be a balance between peak width and sensitivity as determined by the analytical requirements.



Agilent 1290 Infinity LC / 6410A Triple Quadrupole MS; Acetic Acid pH 3.8 / Acetonitrile Gradient, 1 mL/min; ESI+, Scan/SIM/dMRM, 350 °C, 10 L/min, 50 psi, 3500 V; Agilent Publication 5990-7624EN

Impact of Mobile Phase on MS Sensitivity

MS-friendly, volatile, mobile phases are screened for use with the tea catechin analysis below. Selectivity and peak shape remain constant, so the optimal mobile phase was selected based on signal strength of the analytes. Ammonuim acetate buffer and trifluoroacetic acid suppress the signal substantially. While, acetic acid produces slightly more intense peaks than formic acid. Comparing the most sensitive mobile phase to the least shows that five times the sensitivity can be achieved by choosing the appropriate mobile phase for an analysis.



Agilent 1200 Series RRLC / 6410A Triple Quadrupole MS; Acidified Water / Acetonitrile Gradient, 0.7 mL/min; ESI-, SIM, 350 °C, 10 L/min, 50 psi, 3500 V; Agilent Publication 5990-7624EN

Conclusions

- **High Efficiency UHPLC columns, including sub-2-µm and superficially porous 2-3 µm, enhance LC/MS performance over traditional 3.5 and 5 µm columns**
- **The Agilent family of LC columns offers similar bonding chemistry in many particle sizes for easy method scalability and transferability**
- **Sensitivity and peak capacity are improved by more than 40% and 20% respectively with smaller 1.8 and 2.7 µm particle columns as compared to larger 3.5 µm columns**
- **50 mm sub-2-µm and superficially porous 2-3 µm columns can achieve similar performance to a 100 mm 3.5 µm column in half the analysis time**
- **High efficiency columns with LC/MS are useful for separating analytes from matrix components, thereby preventing the possibility of ion suppression**
- **UHPLC columns paired with MS detection are a powerful combination for separating overlapping peaks from complex mixtures**
- **When separating closely eluting isobaric compounds with MS, optimizing the LC system for ultra-low dispersion (reducing extra-column volume by 50%) can improve resolution by >30% for better quantitation, sensitivity is also improved by 30%**
- **Fast MS scan rates can be used to optimize peak width and peak capacity, however faster rates will also generate more baseline noise, thus reducing sensitivity; scan rates should be optimized according to the specific analytical needs**
- **Mobile phase options should be considered with MS detection to optimize peak shape and selectivity; sensitivity can also be increased by >350%**

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