

Speed vs. Sensitivity?

The Influence of Peakwidth and Flow Rate on Sensitivity in UHPLC-MS for Bioanalytical Quantitation.



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Overview

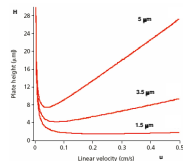
With the development of UHPLC chromatographical peak widths have been reduced to 2 sec and below (see examples). UHPLC allows faster analysis and at the same time higher chromatographical resolution and better sensitivity.

The particle size has a major influence on the C coefficient in the van Deemter equation (s. below). Small particles result in a reduced plate height (i.e. increased efficiency) of columns. Equally important is the fact that with increased flow rates there is no loss in column efficiency.

Empirical van Deemter eq. (Knox)

$$H = A + \frac{B}{U} + CU$$

H Plate height
A coefficient Eddy diffusion
B coefficient longitudinal diffusion
C coefficient resistance mass transfer
u linear solvent velocity



UHPLC leads to reduced peak widths and to increased peak heights (while retaining area under the peak).

Thus UHPLC provides a significant gain in separation efficiency, since it is linked to several chromatographic quantities of peak width:

The number of theoretical plates:

$$N = 5.545 / (t_p / W_{0.5})^2$$

The resolution between 2 compounds a & b:

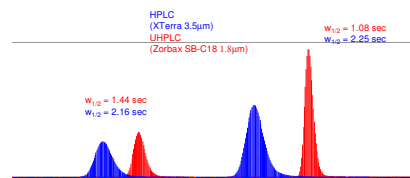
$$R_{ab} = 2(t_{Rb} - t_{Ra}) / (W_a + W_b)$$

Introduction

Resolution, sensitivity, reproducibility and speed of analysis are of major importance in bioanalytical laboratories. However the sharp peaks in UHPLC could potentially create problems for quantitation by mass spectrometry where at least 10-15 data points across a peak are required.

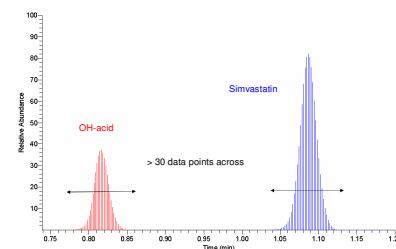
The poster presents a systematic study of the correlations between peak width, flow rate and sensitivity for a number of different substances in plasma samples. Data from a triple quadrupole are presented and analysed in terms of cycle time and sensitivity and reproducibility. The influence of a heated ESI source (H-ESI) as well as split flow into the MS source have been studied.

Peak width / Signal height



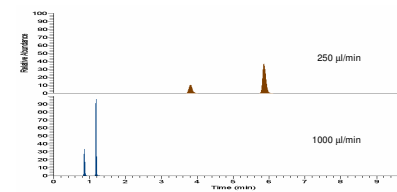
Shown is a comparison of the peak widths at half height ($W_{0.5}$) obtained with HPLC (Waters XTerra 3.5 µm particles, blue) and UHPLC (Agilent Zorbax SB-C18 1.8 µm particles, red) at a flow of 400 µL/min.

Peak width / Data points



Across a typical UHPLC peak the Quantum Discovery can acquire >30 data points in SRM mode. Required for quantitation are 10-15 data points.

Flow rate / Speed



Shown is the influence of flow rate on cycle time for Simvastatin/OH-Simvastatin separation. The cycle time in this example can be reduced by a factor of 4 going to high flow rate. In combination with the significantly improved resolution in many cases a reduction of the cycle time by a factor of 10 can be achieved.

Conclusions

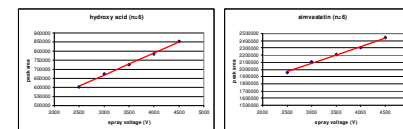
- Flow rate/sensitivity for 2mm ID columns : Maximum sensitivity was achieved at around 600 µL/min. Sensitivity at this flow was roughly 2 times higher than at 200 µL/min, the normal flow rate for such columns. At 1000 µL/min the sensitivity is roughly equal to the values achieved at 200 µL/min. Thus high flow rates translate into short cycle times with no loss in sensitivity, but allowing a significant throughput improvement.
- Sensitivity: UHPLC increased sensitivity (as area under a peak) generally by a factor of 2 compared to high end HPLC columns at equal flow rates. This is mainly a result of the narrow peakwidths.
- In SRM/ MRM mode on triple quads enough scans/peak are generated across UHPLC peaks (> 30 scans for a 1 sec peak with the Quantum Discovery).
- Sharp peaks result in excellent resolution, a crucial advantage for the analysis of complex mixtures (e.g. identification of metabolites, proteomics)
- The stability of the hardware (Flux Rheos Allegro UHPLC pump, CTC autosampler) even with difficult samples (plasma, protein digests) has been demonstrated. Values for retention times and area are reproducible over hundreds of injections.
- A postcolumn split is not a suitable solution.

H-ESI parameters

Four different parameters for the heated electrospray source (H-ESI) have been explored (see below) in order to maximise sensitivity for the 2 compounds described above:

- Spray voltage (top graphic)
- Sheath gas (middle graphic)
- Auxiliary gas (middle graphic)
- Vaporizer temperature (i.e. temperature of the auxiliary gas, bottom graphic)

For Simvastatin (right panel below) and its hydroxy acid (left panel) the highest signal intensities have been obtained with the highest spray voltages and gas flows. A dramatic reduction of the peak area at high temperature (T>300°C, data not shown) suggests a thermic degradation of the analytes, which is compound dependent (less pronounced for the hydroxy acid).



Acknowledgement:

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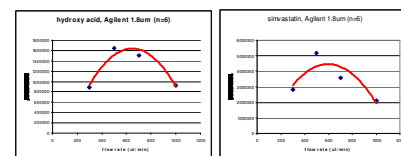
Sample & Setup



Sample: Simvastatin & its hydroxy acid @ 50 ng/mL
Spiked plasma samples: Protein precipitation with EtOH/MeCN 50/50 + centrifugation (14000 RPM at 4°C)
Pump: Flux Rheos Allegro UHPLC pump
Injector: CTC HTS-PAL with Rheodyne UHP injection valve option
MS: Thermo Quantum Discovery™
System control: Completely controlled by Thermo Xcalibur™

The precursor → product ion transitions monitored were previously optimized:
Simvastatin hydroxy acid: m/z= 419.3 → m/z= 199.2, in negative mode
Simvastatin: m/z= 435.2 → m/z= 319.1, in positive mode

Flow rate / Sensitivity



Six injections per data point were acquired.

Under optimised H-ESI conditions (see below) the best sensitivity is observed at around 600 µL/min for a number of compounds. Increasing the flow rate further (1000 µL/min) leads to a sensitivity achieved around 250 µL/min.

A post column split led to a reduction of the signal intensity by up to 50%. The reasons for this unexpected behaviour are under investigation

