# UHPLC Analysis by Chromaster Series

There is an industry trend towards reducing the operating cost of HPLC by shortening the analysis time or reducing the solvent consumption. Chromaster series HPLCs are available not only with the conventional 40 MPa pressure-resistant system but also with the product lineup consisting of the 5160 Pump and 5260/5280 Autosampler, which are compatible with the 60 MPa pressure-resistant system. This series is widely used as the entry-point models when switching a system from HPLC to UHPLC.

The system resistant to 60 MPa pressure, when combined with a UHPLC column packed with 2.0 µm or smaller particles, allows ultra high speed analysis. In this study, several considerations when converting from HPLC analysis to UHPLC analysis are presented.

High Performance Liquid Chromatograph Chromaster®

## Optimization of Analysis System

- ✓ In high speed analysis, a poor peak shape can result from dispersion outside the column when the system volume, contributed by parts such as tubing and detector flow cells, is large. In order to obtain the optimal resolution capability offered by a UHPLC column, a small internal diameter should be selected for the tubing connecting the sample introduction port to the detector cell inlet. The detector cell should also be changed to one with a smaller volume to ensure that diffusion is minimized. In addition, a gradient mixer with a small volume should be selected if gradient elution is employed.
- ✓ The effects of sample dispersion were confirmed by comparing an HPLC system with standard tubing connections to a UHPLC system with a low diffusion tubing kit and semi-micro flow cell compatible with high pressure.
  Table 1 shows the analysis conditions and changes made when switching to the UHPLC system from the HPLC system. The low dispersion tubing kit is used for the UHPLC system (4).
- ✓ Figure 1 compares the chromatograms and Table 2 shows the comparison results for the theoretical number of plates and symmetry factor. By reducing the system volume, the peak widening is reduced and the high-throughput, high-resolution analysis becomes possible.

Table 1 Changes When Switching from HPLC to UHPLC System

| | | HPLC system | UHPLC system |
|---|---|---|---|
| 1 | Column | LaChrom II C18 (5 µm) 2.0 mm I.D.x50 mm | |
| | Column temperature | 40°C | |
| 2 | Mobile phase | A) $H_2O$/ B) $CH_3CN$=40/60 | |
| | Flow rate | 0.4 mL/min | |
| 3 | Sample injection vol. | 1.0 µL | |
| | Samples | 1. Uracil (0.05 mg/mL), 2. Methyl benzoate (0.5 µL/mL)<br>3. Naphthalene (0.18 mg/mL),4. Butyl benzoate (1.5 µL/mL) | |
| 4 | Mixer volume | 700 µL (conventional) | 200 µL (semi-micro) |
| | Mixer – Autosampler tubing inside diameter | Φ 0.8 mm | Φ 0.25 mm |
| | Autosampler loop volume | 100 µL | 40 µL |
| | Autosampler – Column tubing inside diameter | Φ 0.25 mm | Φ 0.1 mm |
| | Column – Detector tubing inside diameter | Φ 0.25 mm | Φ 0.1 mm |
| 5 | Detector flow cell | Standard (volume 13 µL) | High pressure resistant semi-micro (volume 3 µL) |
| 6 | Detector conditions | Response=0.1 s, Sampling period = 400 ms | Response=0.05 s, Sampling period =10 ms |

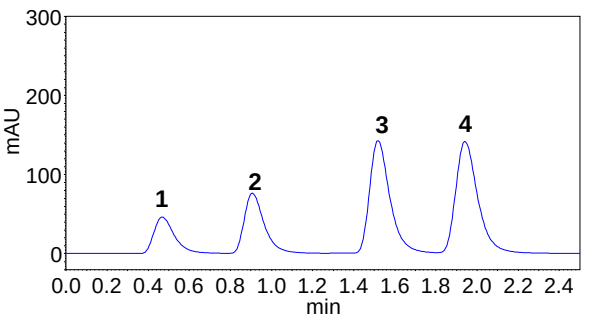


Figure 1-1 HPLC System

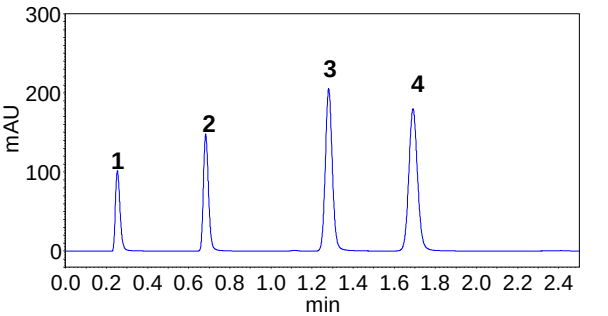


Figure 1-2 UHPLC System

Table 2 Number of Theoretical Plates and Symmetry Factor

| | | HPLC System | | UHPLC System | | Ratio of Number of theoretical plates (%) |
|---|---|---|---|---|---|---|
| | | Number of theoretical plates | Symmetry factor | Number of theoretical plates | Symmetry factor | HPLC/UHPLC |
| 1 | Uracil | 111 | 1.512 | 625 | 1.358 | 17.8 |
| 2 | Methyl benzoate | 446 | 1.504 | 3819 | 1.224 | 11.7 |
| 3 | Naphthalene | 1181 | 1.447 | 7045 | 1.115 | 16.8 |
| 4 | Butyl benzoate | 1630 | 1.433 | 7885 | 1.099 | 20.7 |

# Volume of Detector Flow Cell

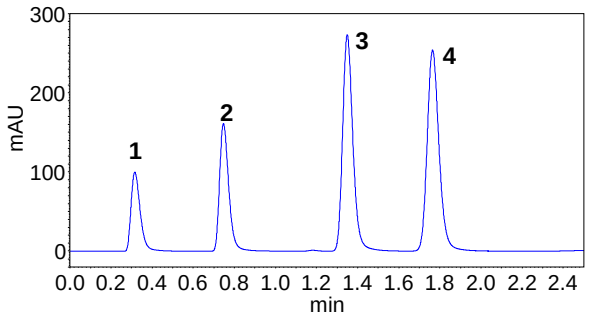


Figure 3-1 A-1) Standard Cell (Volume 13 µL)

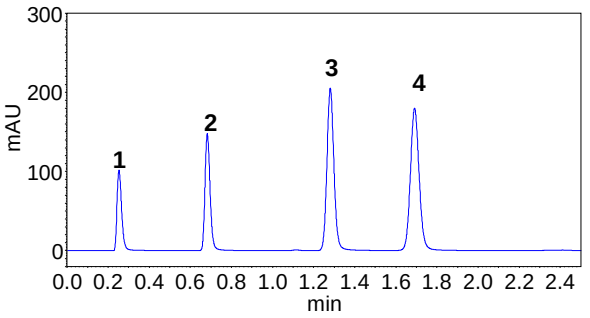


Figure 3-2 A-2) High Pressure Resistant Semi-micro Cell (Volume 3 µL)

✓When a UHPLC column is used, the detector cell volume greatly affects the resolution. The smaller the cell volume, the better the resolution becomes. However, the sensitivity is reduced as the optical path becomes shorter.

Table 3 Numbers of Theoretical Plates for Different Cell Volumes in UHPLC System

| | A-1 | A-2 |
|---|---|---|
| System | UHPLC System | |
| Detector flow cell | Standard cell | High pressure resistant semi-micro |
| Response (s) | 0.05 | |
| Collection interval (ms) | 10 | |
| 1. Uracil | 254 | 625 |
| 2. Methyl benzoate | 1461 | 3819 |
| 3. Naphthalene | 3673 | 7045 |
| 4. Butyl benzoate | 4684 | 7885 |

# Optimization of Detector Setting Values (Response Value and Collection Interval)

Table 4 Comparison of Numbers of Theoretical Plates for Different Response Values and Sampling Period

| | B-1 | B-2 |
|---|---|---|
| System | HPLC System | |
| Detector flow cell | Standard cell | |
| Response (s) | 0.1 | 0.05 |
| Sampling Period | 400 | 10 |
| 1. Uracil | 111 | 145 |
| 2. Methyl benzoate | 446 | 625 |
| 3. Naphthalene | 1181 | 1655 |
| 4. Butyl benzoate | 1630 | 2208 |

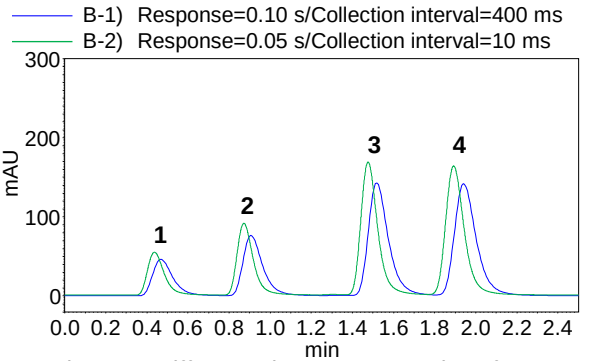


Figure 4 Difference in Response Values for HPLC System

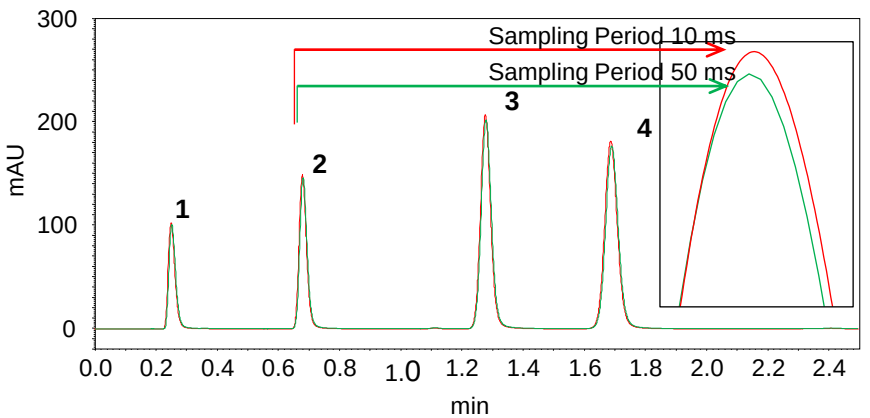


Figure 5 Comparison of Sampling Period for UHPLC System

✓The response value specifies the detector reaction speed. A smaller response value gives faster response, resulting in a sharper peak with an increased peak height. However, the noise is also increased and S/N will be reduced.
When setting the response value, the narrowest analyte peak obtained under the actual analytical conditions should be selected, then choose the response value which gives the required resolution and S/N.

✓The sampling period is the time in seconds used for the data processor to collect the data from the detector. A minimum of 20 data points are required for each peak. Care should be taken as the peak shape becomes poor when fewer data points are used.

<Main System Configuration>
Chromaster 5160 Pump, 5260 Autosampler, 5310 Column Oven, 5420 UV-VIS Detector

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.


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