

In the development of drugs or chemical materials, the profiling of the active ingredient and impurities in raw materials and finished products is an important process. For the profiling of impurities, it is important to detect the active ingredient as well as all impurities with high sensitivity.

If the sample is prepared in a high concentration for the high sensitivity analysis of impurities, the impurity peaks may be buried under the main component peak and the impurities may not be detected. Thus, there is a limit to the injectable concentration and the detector which allows the detection with as high sensitivity as possible is desired. For Hitachi ultra high speed chromatograph ChromasterUltra Rs 6430 Diode Array Detector, a total reflection capillary flow cell (optical path length of 10 mm) was newly developed. With the installation of a low volume flow cell for little light loss, the high resolution performance and detection sensitivity required for an UHPLC detector were achieved. A high sensitivity flow cell (optical path length of 65 mm) is also available for the analysis requiring even higher sensitivity.

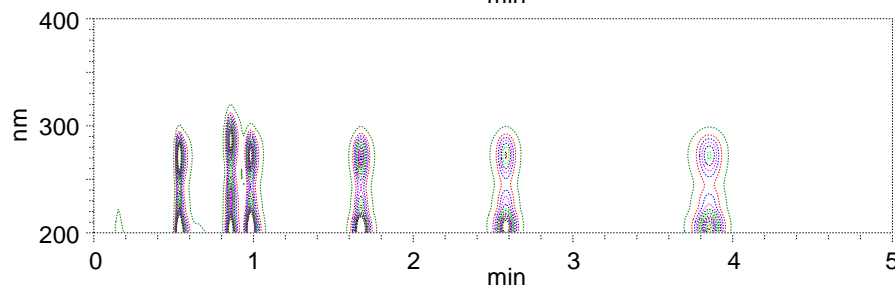
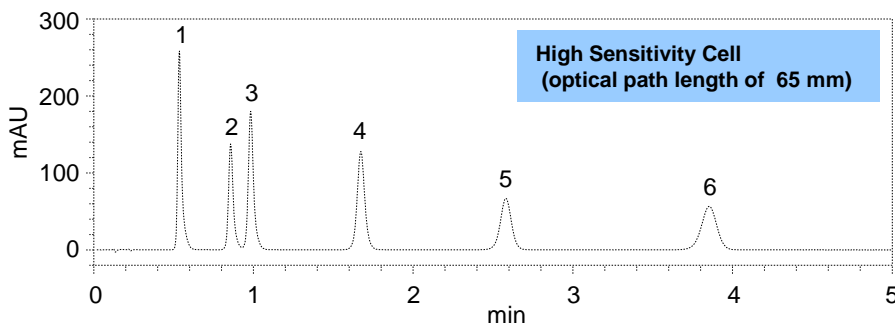
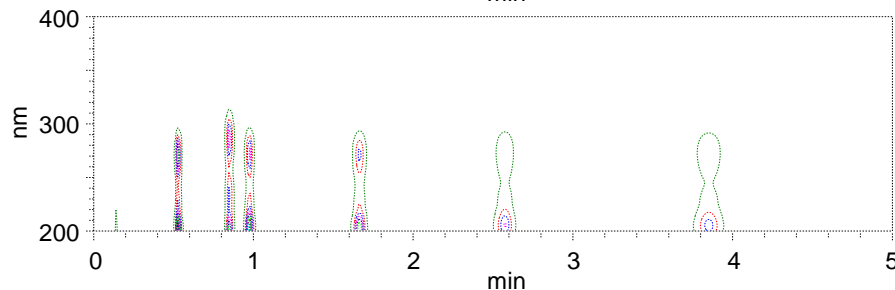
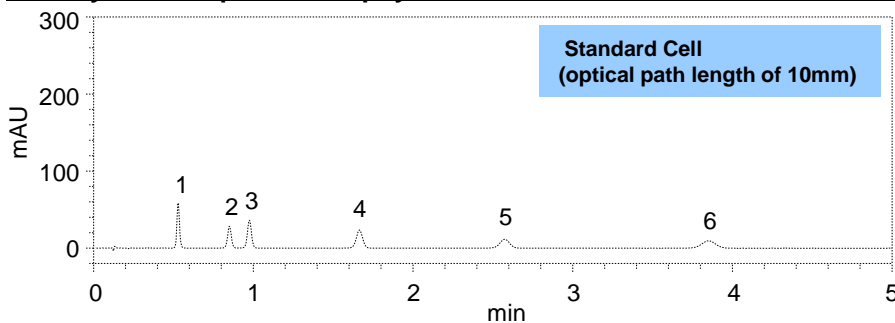
This time, this 6430 Diode Array Detector was used and the comparison of the detection sensitivities and confirmation by the spectra were performed by using a model sample prepared with theophylline as the main component and the impurities (related substances).



(Optional, includes the parts prepared by a customer)

ChromasterUltra_{RS}

Analysis Example of Theophylline and Related Substance Standard Samples



<Analytical Conditions>

Column : LaChromUltra II C18 (1.9 μm)
2.0 mm I.D. × 50 mm
Eluent : 20 mM KH₂PO₄ / CH₃CN
= 95 / 5 (v/v)
Flow rate : 0.7 mL/min
Column temp. : 30°C
Detection wavelength : DAD 200-400 nm
(275 nm)
Injection vol. : 3 μL

<Theophylline and Related Substances>

1. 3-Methylxanthine (3-MX)
2. 1,3-Dimethyluric acid (1,3-DMU)
3. Theobromine
4. Theophylline
5. β-Hydroxyethyltheophylline (β-HET)
6. Caffeine

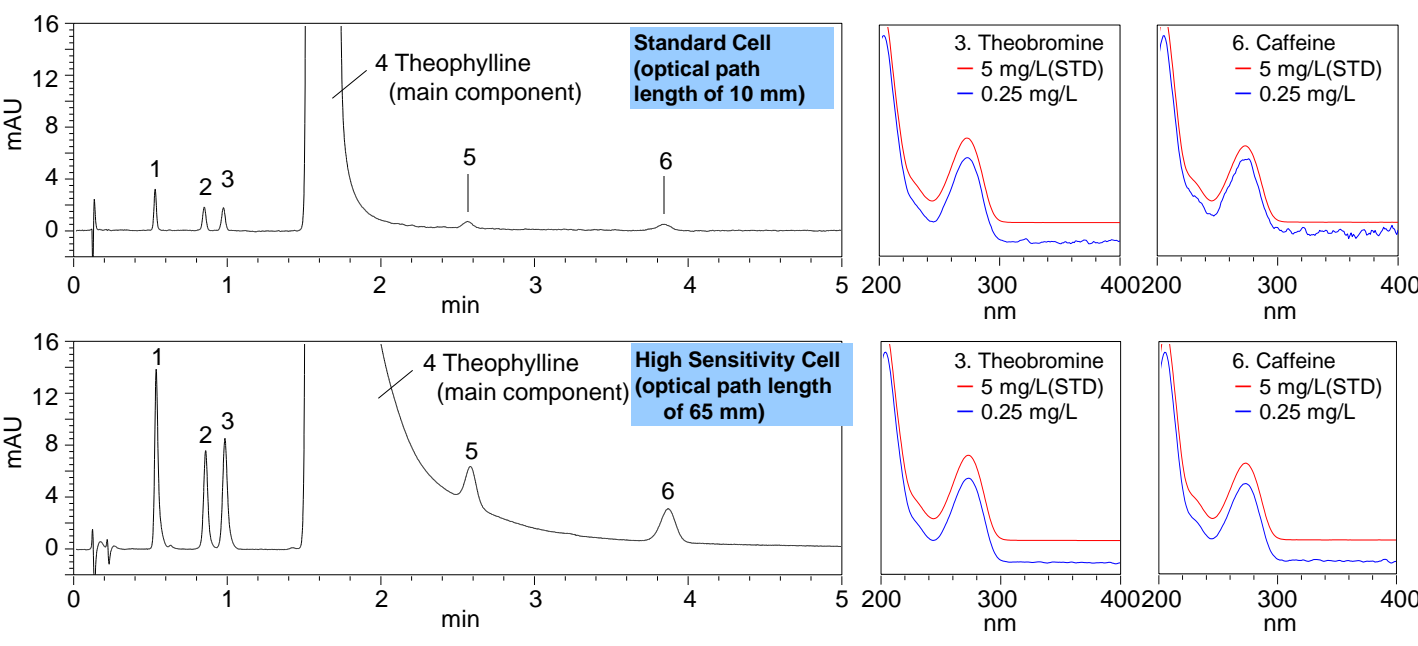
<Preparation Method>

The stock solution was prepared by dissolving with the eluent.
The stock solutions were mixed and serially diluted with the eluent to prepare the mixed standard solution of 6 components.

[Analysis Example of Theophylline and Related Substance Standard Samples (5 mg/L each)]

By using the high sensitivity cell (optional) of 6430 Diode Array Detector, it was possible to obtain the peak height about five times higher than that by the standard cell.

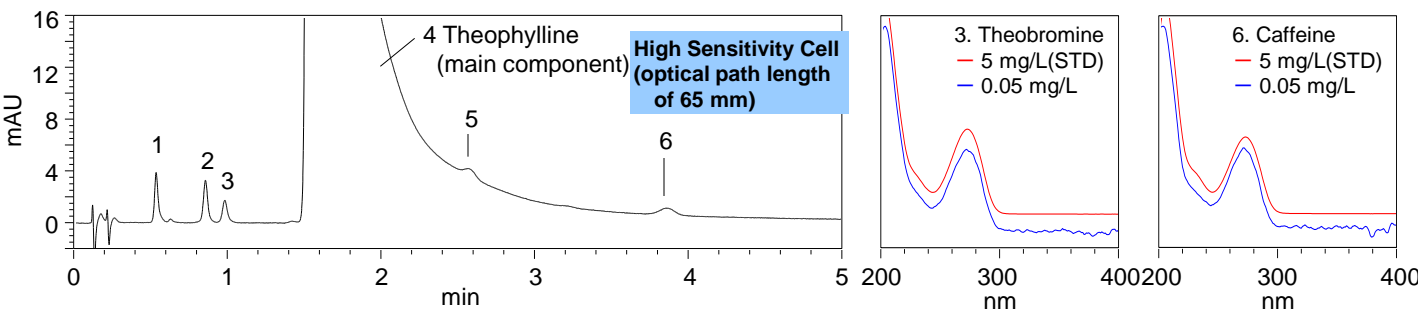
Analysis Example of Related Substances Added to Theophylline Standard Sample (0.005% each)



[Analysis Example of Related Substance Standard Samples (0.25 mg/L each) Added to Theophylline (5000 mg/L)]

[Comparison of Spectra]

Analysis Example of Related Substances Added to Theophylline Standard Sample (0.001% each)



The theophylline analyzed this time contains trace amounts of 1 (3-MX) and 2(1,3-DMU)

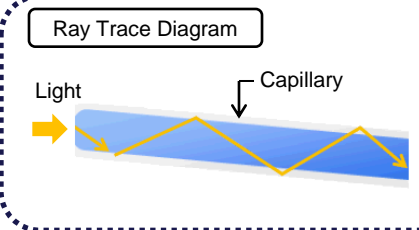
[Analysis Example of Related Substance Standard Samples (0.05 mg/L each) Added to Theophylline (5000 mg/L)]

[Comparison of Spectra]

<Preparation Method for Model Sample> Weigh to have the theophylline concentration of 0.5% and dissolve with the eluent. Add the mixed standard solution of the related substances (5 components) to have the concentration of 0.005% or 0.001%, relative to theophylline, and make up the volume with the eluent.

By using 6430 DAD, it was possible to reliably detect and quantitatively analyze each of the related substances contained at 0.005%, relative to theophylline, the main component. In addition, by using the high sensitivity flow cell, each of the related substances contained at 0.001% could be detected and quantitatively analyzed. The spectrum of each related substance was also compared with that of the standard sample so as to perform the qualitative analysis.

Total Reflection Capillary Flow Cell of 6430 Diode Array Detector



A quartz glass capillary is used in the flow cell path and a total reflection on the capillary interface is used. As a result, the loss of the light transmitted through the flow cell was successfully kept low. Therefore, it was possible to have the baseline performance at the level equivalent to that by the conventional instrument even when the flow cell optical path was extended, allowing the high sensitivity analysis. The standard installation includes the flow cell with the optical path length of 10 mm. The high sensitivity flow cell with the optical path length of 65 mm (optional) can also be selected if even higher sensitivity is required.

Main system configuration: ChromasterUltra Rs DAD system (6170 Binary Pump, 6270Autosampler, 6310 Column Oven, 6430 Diode Array Detector, Organizer), High sensitivity flow cell for 6430

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