SCIENTIFIC INSTRUMENT NEWS
2015 Vol. 6 SEPTEMBER
Technical magazine of Electron Microscope and Analytical Instruments.
Technical Explanation

Introduction of ChromasterUltra Rs 6440 Fluorescence detector

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1. Introduction

The ChromasterUltra Rs is an ultra-high performance liquid chromatography (UHPLC) system delivering high-separation, high-sensitivity analysis. Two system features combined allow various analytical variations: a system pressure resistance of 140 MPa, which allows the use of mobile phases prone to increases in analysis pressure, and the LaChromUltra II column series using a microfine packing agent. Now, the new ChromasterUltra Rs 6440 Fluorescence detector joins the lineup as a detector for the ChromasterUltra Rs series.

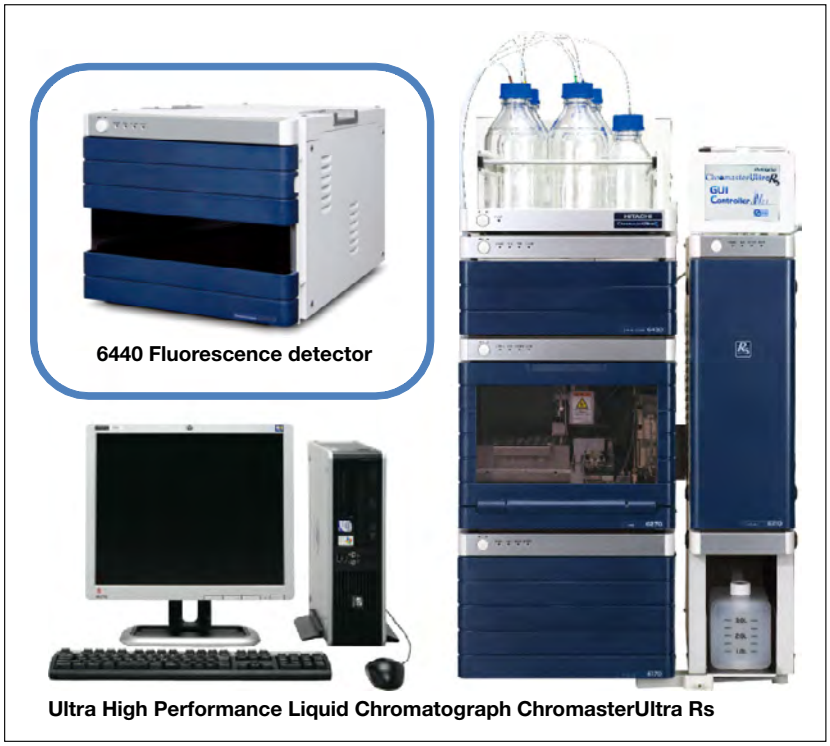

Fig. 1 External appearance

2. ChromasterUltra Rs 6440 Fluorescence Detector Features

2-1 Sharp peak shape

The ChromasterUltra Rs 6440 Fluorescence detector uses 0.1 mm inner-diameter tubing from the column to the detector and a 3 µL (irradiated volume) flow cell. Comprehensive reduction of the factors causing peak dispersion provides the greatest possible inhibition of component peak dispersion and delivers sharp chromatogram peak shapes.

Figure 2 is an example of 15-component analysis of a polycyclic aromatic hydrocarbon (PAH). This class of compounds has multiple benzene rings, and findings on its carcinogenicity have also been reported. The 15-component PAH was successfully separated in 14 minutes. To allow high-sensitivity detection of each component in conjunction with these results, the detection wavelength of the ChromasterUltra Rs 6440 Fluorescence detector is also switched by a timing program during measurement.

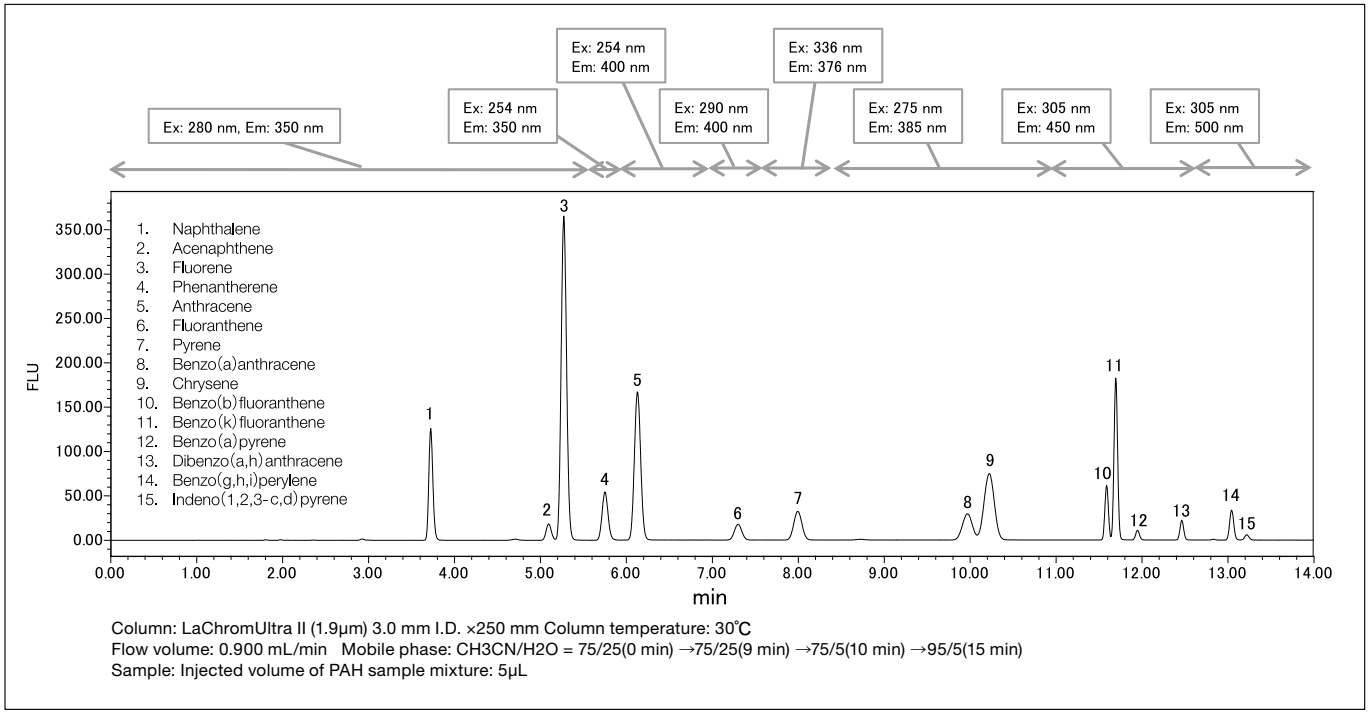

Fig. 2 Analysis of PAH using timing program

2-2 Increased dynamic range

A new “wide mode” has also been added to the existing dynamic range. As shown in Fig. 3, wide mode increases the calibration curve range approximately 5-fold compared to the existing standard mode.

As a result, sensitivity can also be raised by increasing injection volume, and concentration differences in components also allow simultaneous measurement of components not possible previously.

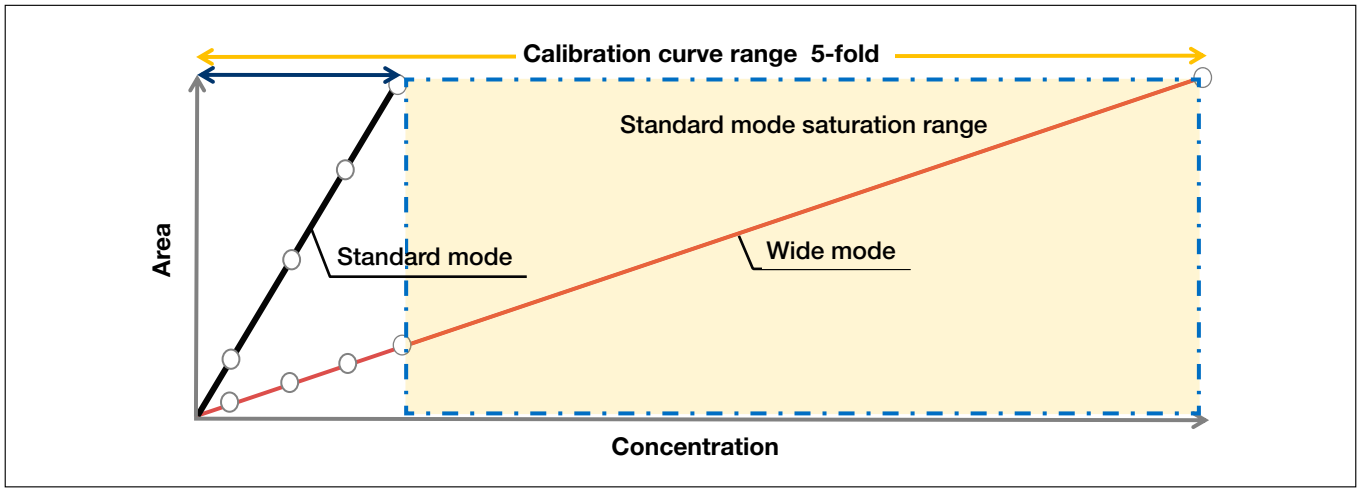

Fig. 3 Dynamic range comparison

As a comparison to chromatograms produced by dynamic range setting, Fig. 4 shows measurement results in standard mode and wide mode for the PAH shown in Fig. 2. The Fluorene peak detected at approximately 5.3 minutes is not quantifiable in standard mode due to peak saturation, but the availability of wide mode allows peak detection.

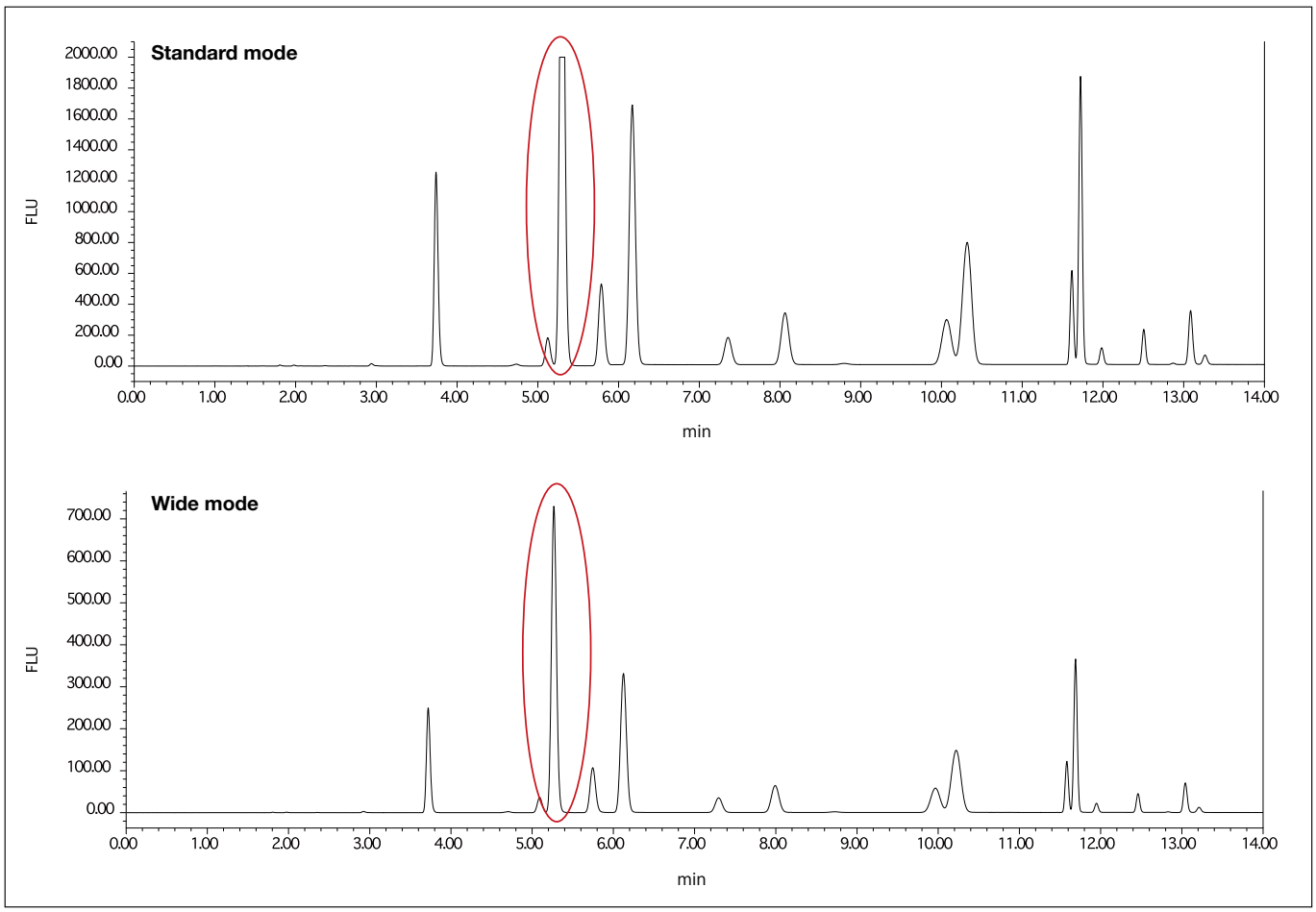

Fig. 4 Comparison to chromatogram produced by dynamic range setting

3. Introduction to applications

Amino acid analysis is one UHPLC chromatogram application where interest exists. Though Hitachi High-Tech Science has existing applications for its dedicated amino acid analyzer (Model L-8900) employing post-column derivatization capable of precise quantification, and likewise, for its general-purpose HPLC systems (Chromaster series), here we introduce an additional application for ultra-high performance amino acid analysis using the ChromasterUltra Rs 6440 Fluorescence detector.

In ultra-high performance amino acid analysis, amino acids brought to reaction with a previously derivatized reagent (pre-column derivatization) were separated in an ODS column. The derivatized reagent used was NBD-F (4-fluoro-7-nitro-2,1,3-benzoxadiazole). Though this technique also allows detection with a UV detector, its combination with a fluorescence detector makes ultra-high sensitivity analysis possible (i.e., fmol order amino acid detection). Using this technique, 19 amino acid components were separated in 9 minutes.

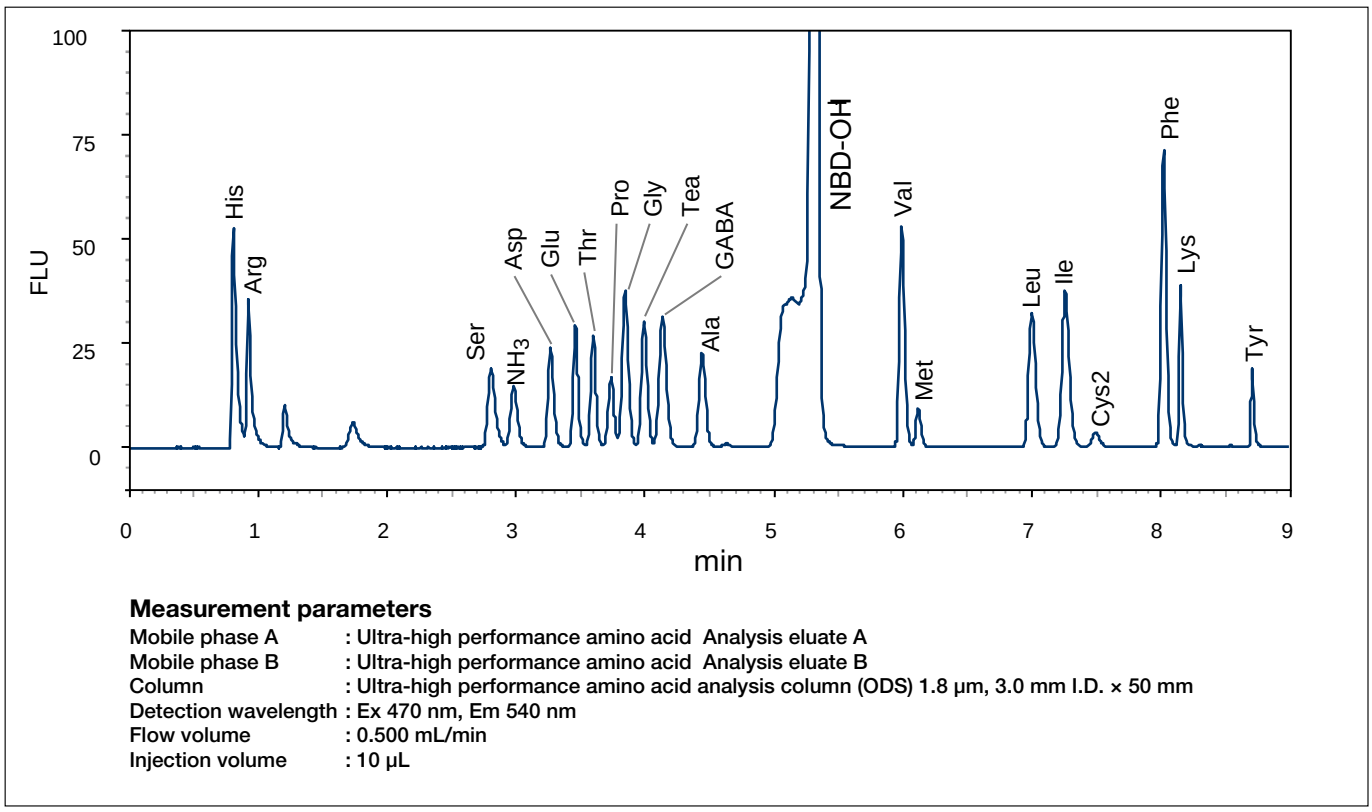

Fig. 5 Measurement of NBD-F amino acid standard sample with ChromasterUltra Rs Fluorescence detector system

4. Closing remarks

Fluorescence detectors use two wavelength parameters in measurement, an excitation wavelength and a fluorescence wavelength. This technique allows measurement with generally higher selectivity for the target substance and higher sensitivity than a UV detector and is well-suited for analysis of trace components. Future efforts will focus on seeking new applications using the ChromasterUltra Rs 6440 Fluorescence detector.

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