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Application News

Ultra High Performance Liquid Chromatograph

Trace Analysis Using the High-Sensitivity Cell of SPD-M40 X4 Photodiode Array Detector

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User Benefits

- The high-sensitivity cell of the photodiode array detector SPD-M40 X4 enables highly sensitive detection of trace components. In addition, the detector provides good linearity (up to 3 AU), enabling quantitative analysis over a wide concentration range.
- The low-dispersion injection mode of the autosampler minimizes peak dispersion caused by the system, contributing to enhanced sensitivity for the detection of trace components.

■ Introduction

Accurate quantification of trace components requires detection with a high signal-to-noise (S/N) ratio. Increasing the injection volume or sample concentration can be considered as approaches to improve S/N ratio. However, increasing the injection volume may adversely affect peak shape because the sample may not be properly retained at the column inlet due to the influence of the sample solvent. In addition, increasing the sample concentration may result in insufficient solubility of the sample. Therefore, when changes in injection volume or sample concentration are difficult, enhancing detector sensitivity is required for the detection of trace components. In this study, an example of highly sensitive detection of trace components using the photodiode array detector SPD-M40 X4 (high-sensitivity cell) installed in the Nexera™ X4 system (Fig. 1) is presented. In addition, the results of an evaluation of detector linearity are shown.



Fig. 1 Nexera™ X4

■ Analytical Conditions and Target Compounds

The target compound and analytical conditions are shown in Table 1. In this study, furosemide, a small-molecule drug, was used as a model sample. Using the high-sensitivity cell of SPD-M40 X4, the S/N ratio was compared with that of the conventional cell, and detector linearity was evaluated.

Table 1 Analytical Conditions and Target Compounds

System : Nexera X4	
Sample : Furosemide	
Mobile phase	
Pump A : 0.1% formic acid in water	
Pump B : Acetonitrile	
Column : Shim-pack Scepter C18-120 *1 (50 mm × 2.1 mm I.D., 1.9 µm)	
Analytical conditions	
B Conc.	: 40% (Isocratic)
Column Temp.	: 40 °C
Flow rate	: 0.5 mL/min
Mixer	: Micro mixer
Sample solvent	: Water / Acetonitrile = 1:1
Sample loop Vol.	: 15 µL
Injection Vol.	: 1 µL
Detection	: 273 nm (SPD-M40 X4, High-Sensitivity cell)
Sampling rate	: 10 msec
Time constant	: 25 msec

*1 P/N: 227-31012-03

■ Improvement of S/N Ratio Using High-Sensitivity Cell

Fig. 2 shows the chromatograms obtained by analyzing a 0.1 mg/L furosemide standard solution using SPD-M40 X4 (high-sensitivity cell) and SPD-M40 (UHPLC cell) as a comparison, with an injection volume of 1 µL. With the conventional SPD-M40, the peak was detected near the limit of detection. In contrast, the use of the high-sensitivity cell of SPD-M40 X4 provided an S/N ratio more than seven times higher, enabling reliable quantification.

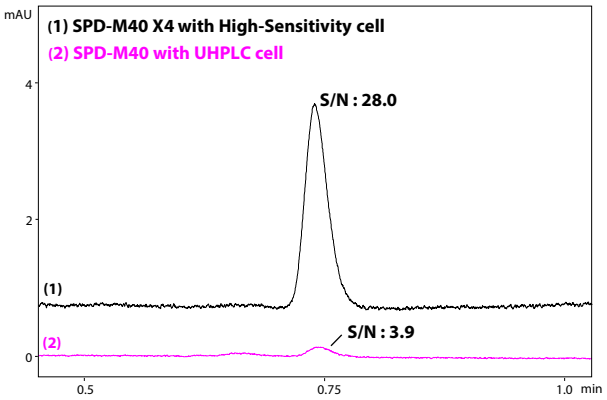


Fig. 2 Comparison of S/N Ratio between SPD-M40 X4 and SPD-M40

■ Linearity Evaluation of High-Sensitivity Cell

To evaluate the linearity of the high-sensitivity cell of SPD-M40 X4, a calibration curve was prepared using furosemide standard solutions at concentrations of 0.1, 0.5, 1, 5, 10, and 50 mg/L. Fig. 3 shows the calibration curve and coefficient of determination (r^2), and Table 2 shows the repeatability at each calibration point. Good linearity ($r^2 > 0.9999$) and repeatability were confirmed.

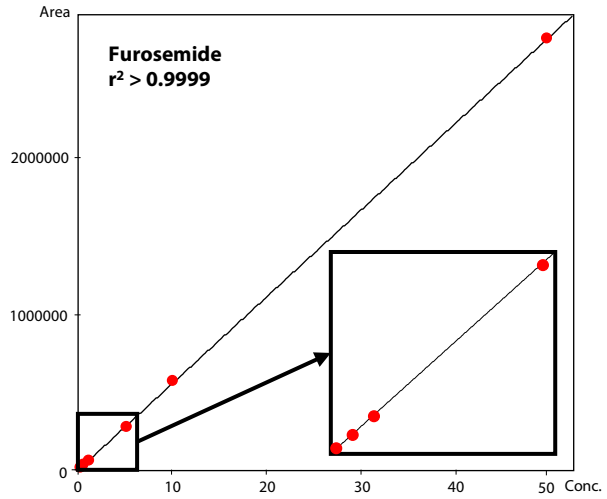


Fig. 3 Evaluation of Linearity of High-Sensitivity Cell

Table 2 Repeatability at Each Calibration Point (%RSD: n = 6)

	Calibration point concentration (mg/L)	Retention time	Peak area
1	0.1	0.11	1.34
2	0.5	0.05	0.40
3	1	0.04	0.67
4	5	0.04	0.46
5	10	0.03	0.51
6	50	0.04	0.53

■ Improvement of S/N Ratio Using Low-Dispersion Injection Mode

In high-performance liquid chromatography, minimizing sample band broadening both within and outside the column is important for achieving sharp peaks and a high S/N ratio. The autosampler of Nexera X4 minimizes system-induced peak dispersion through the low-dispersion injection mode, enabling highly sensitive analysis. Fig. 4 shows the effect of the low-dispersion injection mode on peak shape and S/N ratio for a furosemide standard solution (0.5 mg/L). Application of the low-dispersion injection mode improved peak height and increased the S/N ratio.

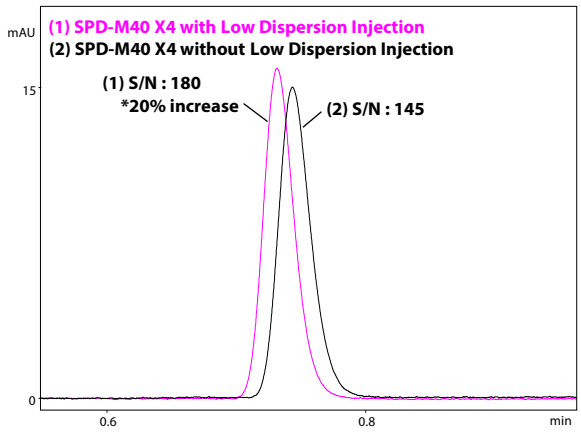


Fig. 4 Improvement of S/N Ratio Using Low-Dispersion Injection Mode

■ Conclusion

In this study, furosemide, a small-molecule drug, was used as a model sample, and the S/N ratio and linearity were evaluated using the high-sensitivity cell, an optional component of SPD-M40 X4. Analysis of a 0.1 mg/L furosemide standard solution showed that the peak detected near the limit of detection with conventional SPD-M40 achieved an S/N ratio more than seven times higher when using the high-sensitivity cell of SPD-M40 X4. In addition, a calibration curve prepared over a concentration range of 0.1–50 mg/L showed good linearity ($r^2 > 0.9999$). Furthermore, the use of the low-dispersion injection mode of the autosampler was shown to further improve the S/N ratio.

Related Application

1. Simultaneous Analysis of API and Related Impurities Using SPD-M40 X4 Photodiode Array Detector
   Application News No. 01-01126-EN

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