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Improving Impurity Analysis in Photodiode Array Detection

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Introduction

When conducting impurity analysis of pharmaceuticals, researchers need to detect trace-level impurities along with the main ingredient, which is present at a high concentration. To accomplish this accurately requires a detector that provides linearity over a wide concentration range.
Furthermore, a high S/N (signal-to-noise) ratio is also required of the detector in order to provide high-accuracy quantitation of any impurities present at trace levels. Improving S/N might be achieved by increasing the sample concentration or injection volume, but increasing the sample concentration could decrease the solubility of the main ingredient, which is already present at a high concentration. Also, the common technique of increasing the solution temperature to facilitate dissolution of the main component may result in its decomposition. Finally, if the injection volume is increased, peak shape distortion may occur due to the effect of the sample solvent. Therefore, to detect trace impurities at a reasonable sample injection volume and concentration range, a high-sensitivity detector is essential.
Here, we introduce our evaluation of the sensitivity and linearity obtained in impurity analysis using the new SPD-M30A photodiode array detector incorporated with a high-sensitivity flow cell with an 85 mm path length.

Materials

Reagents and standards

Reagents: Ketoprofen and formic acid were purchased from Sigma-Aldrich. Water was made in-house using a Millipore Milli-Q Advantage A10 Ultrapure Water Purification System. Acetonitrile and methanol were purchased from Honeywell.

Standard solutions: 10 mg of ketoprofen were dissolved in 10 mL of water. It was diluted with water to 500, 200, 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01 mg/L. They were transferred to a 1.5 mL vial for analysis.

System

The standard solutions were injected to a Shimadzu Nexera X2 UHPLC system consisting of two LC-30AD pumps, DGU-20A5R degassing unit, SIL-30AC autosampler, CTO-30A column oven, SPD-M30A photodiode array detector equipped with a high-sensitivity flow cell, and a CBM-20A system controller. A SPD-M20A photodiode array detector equipped with a semi-micro cell was used for comparison.

Results

Sensitivity

Ketoprofen, a non-steroidal anti-inflammatory drug (NSAID), was used as the sample. Use of the high-sensitivity 85-mm path length flow cell, now available as an option for the new SPD-M30A photodiode array detector, makes it possible to conduct analysis of trace-level impurities, which up to now has been difficult to achieve. To compare its performance with that of the conventional SPD-M20A model (with 5-mm path length and 2.5-µL semi micro cell), 2 µL of 0.02 mg/L ketoprofen standard solution was injected using the same analytical conditions, and the respective S/N values were verified. Fig. 1 shows the analysis results, and Table 1 shows the analytical conditions. Comparison of the results indicates that with use of the high-sensitivity cell, the S/N for SPD-M30A is more than 10 times that of the peak for the SPD-M20A, which is just over the detection limit (defined as an S/N > 3), demonstrating excellent quantitative performance.

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Table 1: Analytical Conditions

System	: Nexera X2
Column	: Shim-pack XR-ODSIII (150 mm L. x 2.0 mm I.D., 2.2 µm)
Mobile Phase	: A: 0.1 % Formic acid in water
	B: Acetonitrile
Time Program	: B Conc. 30 % (0 min) - 70 % (10 min)
Flow Rate	: 0.5 mL/min
Column Temperature	: 40 °C
Injection Volume	: 2 µL
Detection	: 254 nm
Flow Cell	: High-sensitivity cell (SPD-M30A)
[Autosampler rinse settings]	
Rinse Solution	: R0: Water/Methanol=50/50 (v/v)
	R1: Acetonitrile
	R2: Acetonitrile
	R3: Acetonitrile
Rinse Mode	: Before and after aspiration
Rinse Method	: Rinse port → Rinse pump
External Rinse	: R0: 0 sec, R3: 2 sec
Internal Rinse	: R0 → R1, rinsing volume 300 µL each
Injection Port Rinse	: R0, R1

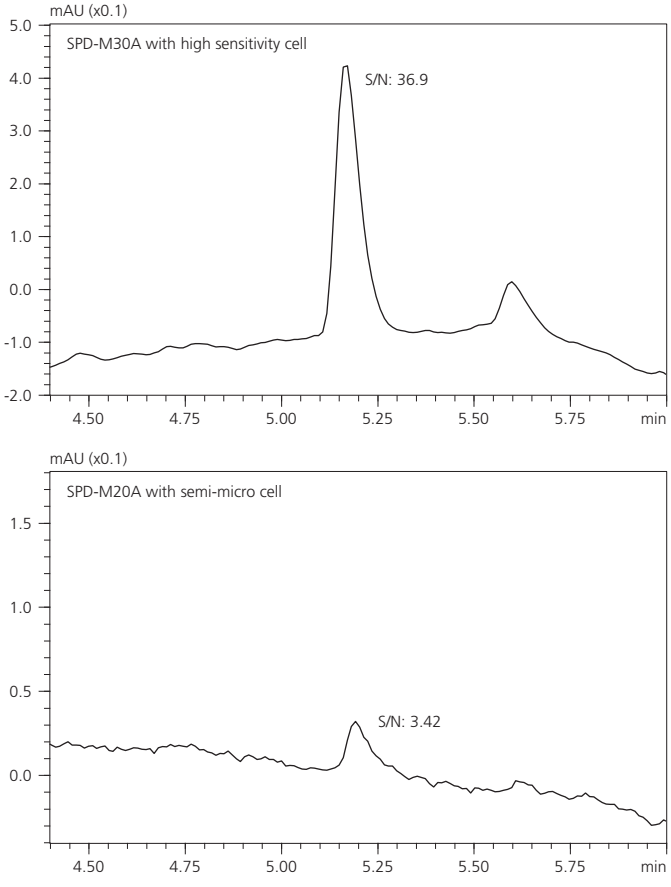


Fig. 1: Chromatogram Obtained with 0.02 mg/L Ketoprofen Standard Solution

Linearity

To check the linearity of ketoprofen detection using the SPD-M30A with a high-sensitivity flow cell, we conducted measurements using a sample concentration range of 0.01 – 500 mg/L. Fig. 2 shows the calibration curve generated using a concentration range of 0.01 – 50 mg/L. Excellent linearity was obtained, with a correlation coefficient (R2) greater than 0.999. Fig. 3 shows the calibration curve generated using a concentration range from 0.01 – 500 mg/L. Since the linearity was not maintained when the concentration exceeded 50 mg/L, we used the Intelligent Dynamic Range Extension Calculator (*i*-DReC) in LabSolutions software to extend the range of the calibration curve. With that, the correlation coefficient (R2) improved to better than 0.999, and the error associated with each calibration point improved to less than 5 %.

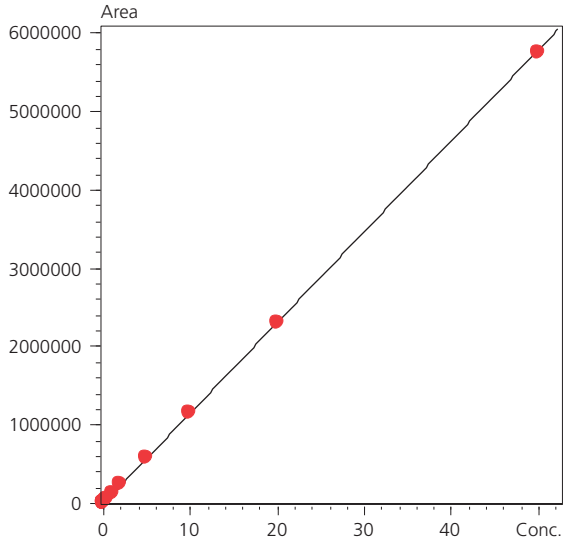
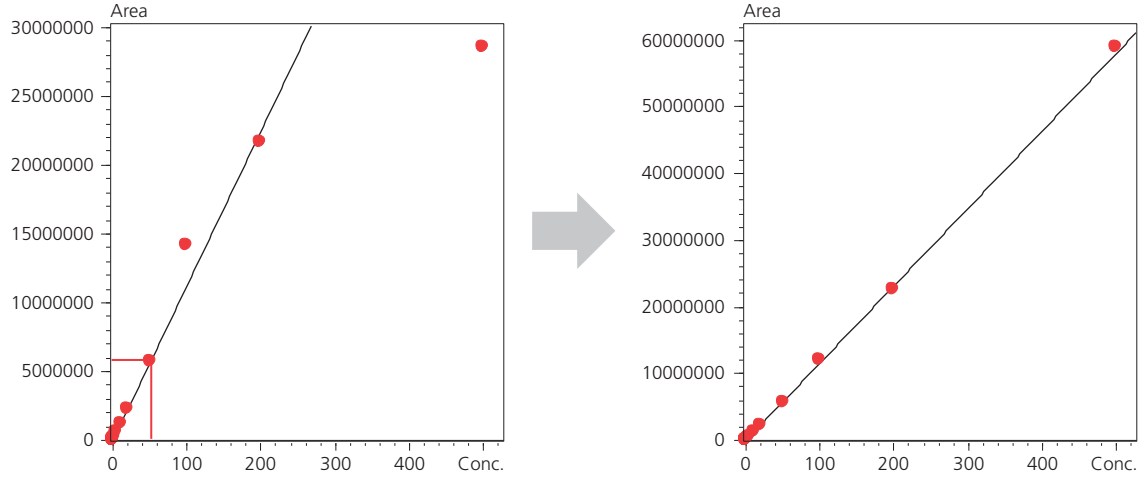


Fig. 2: Linearity of Ketoprofen (0.01 - 50 mg/L)



Linear range without *i*-DReC: 0.01-50 mg/L

Linear range with *i*-DReC: 0.01-500 mg/L

Fig. 3: Linearity Correction by *i*-DReC (0.01 - 500 mg/L)

Impurity Analysis

Fig. 4 shows the chromatogram obtained using the 500 mg/L ketoprofen solution. When the ketoprofen peak intensity exceeds 4 AU, the signal saturates, but if the *i*-DReC feature is applied, a corrected area value is automatically calculated to maintain linearity. Thus, it becomes possible to directly calculate the impurity content of the sample using the area percentage method. Those values are displayed at the peak tops in Fig. 4. Impurities at extremely low concentration levels on the order of 0.001 % could also be detected.

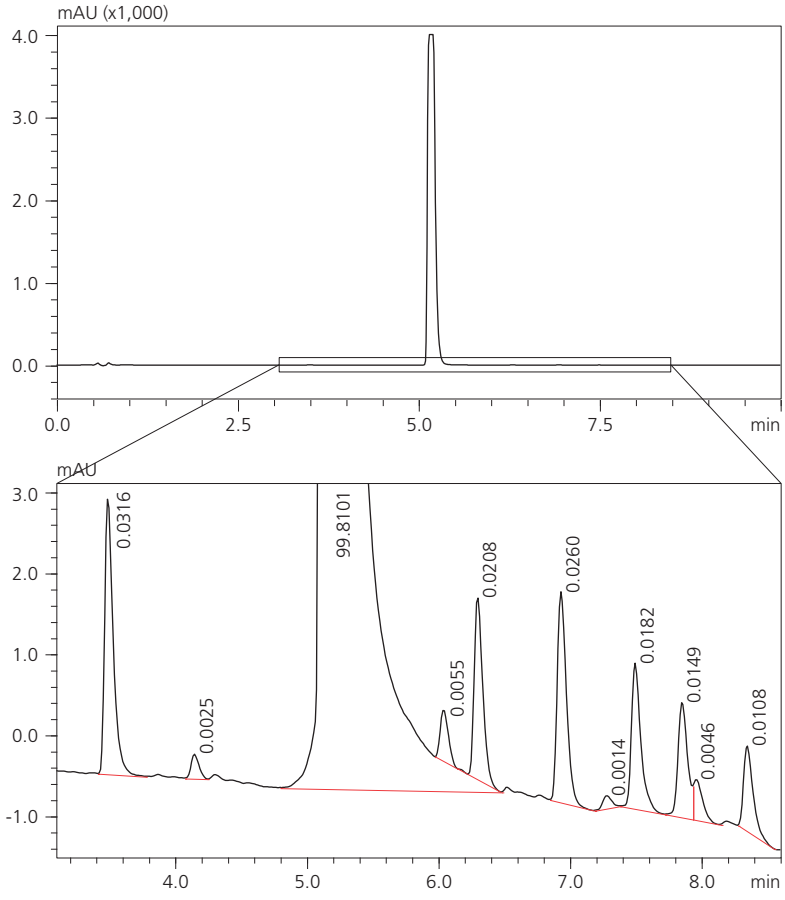


Fig. 4: Chromatogram of Ketoprofen Standard Solution (500 mg/L)

Carryover Verification

It is important to control carryover to maintain linearity over a wide concentration range. If carryover becomes excessive, quantitation accuracy will deteriorate in the region of low concentrations. Fig. 5 shows the results of ketoprofen carryover verification. A sequence with a low-concentration standard solution (0.01 mg/L), a high-concentration standard solution (500 mg/L), and a blank were injected in that order. By utilizing the internal and external needle rinse operations, which are a feature of the Nexera X2 series autosampler, carryover was completely suppressed.

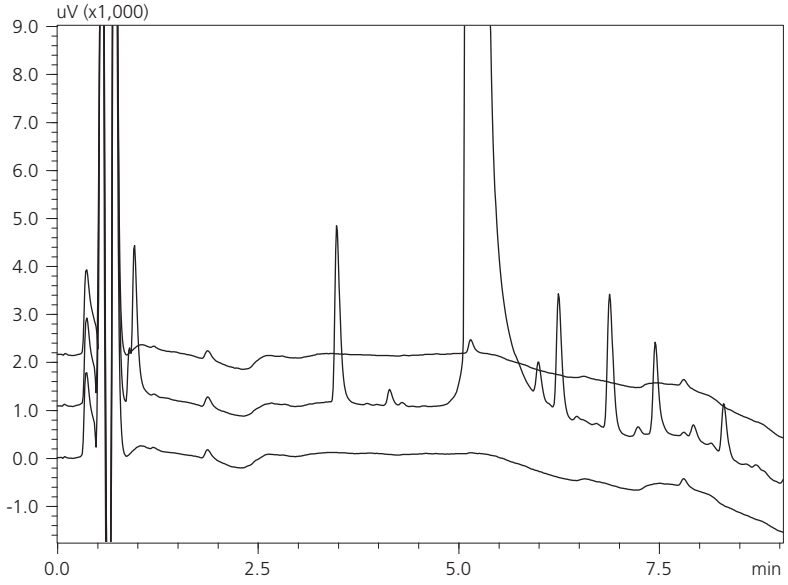


Fig. 5: Ketoprofen Carryover Test (Upper: 0.01 mg/L, Middle: 500 mg/L, Lower: Blank)

Conclusions

1. The SPD-M30A equipped with a high-sensitivity cell gave more than 10 times the sensitivity compared to a traditional semi-micro cell.
2. The *i*-DReC function successfully extended the linear range of the sample concentration by a factor of ten.

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