Improvement of pharmaceutical impurity analysis by reducing the effects of stray light and room temperature fluctuations on PDA detector

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1. Introduction

A photodiode array detector (PDA) is widely used in HPLC analysis and is indispensable for the analysis of pharmaceuticals. The accurate determination of trace impurities has a great importance in both pharmaceutical drug development and drug manufacturing. Moreover, the impurity analysis should simultaneously quantify the high concentration of active pharmaceutical ingredients (API) and low concentration of impurities. Therefore, a detector with constant baseline output in combination with low peaks and a wide dynamic range is needed.

In principle, the stray light generated during the detection process significantly affects the dynamic range of the PDA. Also, the response of a PDA is affected by room temperature fluctuations that can cause baseline fluctuations.

We performed impurity analysis in pharmaceutical products using a newly developed PDA which reduced the effect of stray light and room temperature fluctuations.

2. Effect of stray light on absorbance

In general, absorbance is expressed in terms of the intensity of incident light on the flow cell and the intensity of transmitted light through the sample cell, according to Lambert-Bert's law.

$$A = -\log\frac{I}{I_0}$$

A : Absorbance
I : Real-time light intensity transmitted through the flow cell
I_0 : Incident light intensity

If the unexpected light is emitted by the spectroscope, the correct absorbance cannot be measured. Unexpected light during detection is commonly referred to as "Stray Light". The influence of stray light on the absorbance is expressed by the following equation.

$$A = -\log\frac{I+\Delta}{I_0+\Delta}$$

Δ : stray light intensity

If the absorbance in the flow cell is high, the transmitted light intensity from the cell will be small. In such cases, the effect of stray light intensity on the absorbance is more pronounced. Fig. 1 illustrates the influence on the absorbance of stray light when the ratio of stray light intensity to incident light was changed to 0 - 0.5%. In cases when the stray light intensity is larger, the calculated absorbance value is smaller than the ideal value, and the linearity will be affected especially in the region exceeding 2AU. To expand the dynamic range of a PDA ,stray light reduction is necessary, such as reflection and scattering of light by the optical element itself or at the spectrometer, and unexpected reflection and dispersion of light at the grating.

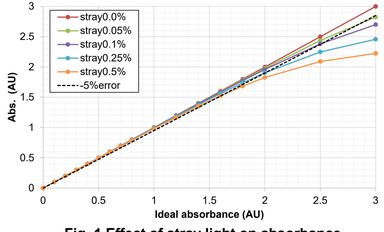

Fig. 1 Effect of stray light on absorbance

Designed to reduce the effect of these causes of stray light, newly developed PDA (SPD-M40, Shimadzu corporation) reduces overall stray light to a third of that of previous PDAs. It thereby achieves a linearity of 2.5 AU as a specification value. (The actual value is typically more than 2.5 AU.) This compares very favorably to that of a UV-VIS detector.

3. Effect of Ambient Temperature Fluctuation on the Baseline

To confirm the effect of baseline fluctuations due to ambient temperature fluctuations on quantitative accuracy, samples were successively injected and analyzed as the ambient temperature was varied over 5 °C. Analytical conditions and ambient temperature settings conditions are shown in Table 1.

Table 1 Analytical Conditions

HPLC system	: Nexera™ system (Shimadzu corporation)
Column	: Shim-pack HRC-ODS (3.0 mm I.D.× 250 mm L.)
Mobile Phase	: 70% Methanol
Flow Rate	: 0.2 mL/min
Column Temperature	: 40 °C
Injection Volume	: 1 µL (5 mg/L Caffeine)
Detection	: PDA at 273 nm
Cell Temperature	: 40 °C
Room Temperature	: 20 to 25 °C (profile indicated in Fig. 2)

The resulting chromatograms are shown in Fig. 2. Table 2 indicates the reproducibility of peak area values for the peaks detected in the chromatograms. Compared to a non-temperature-controlled detector, the baseline is stable, so good reproducibility was obtained even if the ambient temperature fluctuated. The SPD-M40 minimizes the effects of room temperature using a triple temperature control function (Advanced TC-Optics), which independently controls the temperature of the detector cell, light source lamp, and spectrometer.

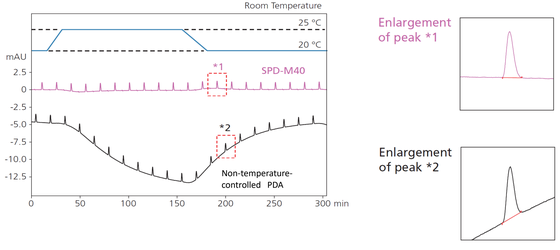

Fig. 2 Effect of Ambient Temperature Changes on Peak Integration

Table 2 Peak Area Reproducibility with Ambient Temperature Fluctuations

	SPD-M40	Non-temperature-controlled PDA
Peak Area Reproducibility (%RSD, n = 20)	0.62	1.87

4. Impurity analysis in pharmaceutical

The impurity analysis of ketoprofen, a non-steroidal anti-inflammatory drug using SPD-M40 that reduces stray light and suppresses baseline fluctuations due to ambient temperature fluctuations.

4-1. Analytical Condition

Analytical conditions are shown in Table 3.

Table 3 Analytical Conditions

Column	: Shim-pack Velox™ C18 (100 mm L. × 3.0 mm I.D., 2.7 µm)
Mode	: Low pressure gradient
Mobile Phase	: A)10 mM Sodium phosphate buffer (pH=2.6) B) Acetonitrile
Time program	: 30-60%B (0-4 min), 60-90%B (4-6 min), 90%B (6-8 min), 30%B (8-10 min)
Flow Rate	: 1.0 mL/min
Column Temperature	: 40 °C
Injection Volume	: 2 µL
Detection	: PDA at 256 nm
Sample	: Ketoprofen 0.5~800 mg/L

4-2. Result of Linearity

Fig. 3 shows the chromatograms of the ketoprofen standard solutions, and Fig. 4 shows the calibration curve of ketoprofen. Excellent linearity (R2 ≥ 0.999) was obtained over a wide range of concentrations, from 0.5 to 800 mg/L.

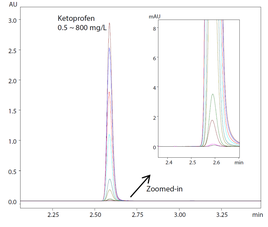

Fig. 3 Chromatograms of the Ketoprofen Standard Samples

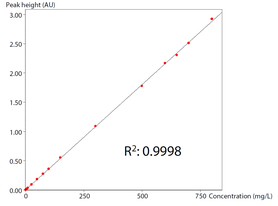

Fig. 4 Calibration Curve for Ketoprofen

4-3. Result of Impurity Analysis

Fig.5 shows the chromatograms of six repeated analyses of 700 mg/L ketoprofen standard solution with peak heights of approximately 2.5 AU. Table 4 shows the area percentage and area coefficient of variation (%RSD) of ketoprofen and each impurity component. The peak area RSD% of Impurity 1 (the only impurity with content >0.1 %) was less than 1 %, showing good reproducibility.

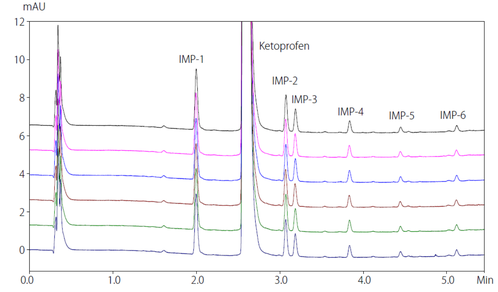

Fig. 5 Overlapped Chromatograms of Ketoprofen 700 mg/L Standard Solution

Table 4 Analytical Results for Each Component

No.	Compounds	Retention Time (min)	Area (%)	Area (%RSD)
1	Ketoprofen	2.583	99.704	0.001
2	Impurity 1	1.998	0.126	0.598
3	Impurity 2	3.072	0.075	0.543
4	Impurity 3	3.186	0.046	1.115
5	Impurity 4	3.834	0.025	1.644
6	Impurity 5	4.446	0.011	4.556
7	Impurity 6	5.118	0.012	3.355

5. Conclusions

- Stray light generated in the detection process of a PDA greatly affects absorbance linearity. SPD-M40 minimizes the influence of stray light and achieves a wide dynamic range.
- When the ambient temperature fluctuates, the baseline of a non-temperature-controlled detector fluctuates and the quantitative accuracy is affected. The triple temperature control function of the SPD-M40 minimizes baseline fluctuations.
- The SPD-M40 with a wide dynamic range and baseline stability enables the highly precise analysis of pharmaceutical impurities that require simultaneous detection of high-concentration major components and low-concentration impurities.