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Application News

No. L538

High Performance Liquid Chromatography

Impurity Analysis in Pharmaceutical Products with the Advanced Photodiode Array Detector SPD-M40

Impurities in pharmaceuticals must be appropriately controlled to ensure product safety. The ICH guidelines on impurities in pharmaceutical substances [1] and impurities in drug products [2] have established specific thresholds for impurities. If the content of impurity in pharmaceuticals is more than 0.1 %, it is necessary to identify its structure, perform the safety evaluation and report to the regulatory authorities.

Therefore, the accurate determination of trace impurities is of great importance in both pharmaceutical drug development and drug manufacturing.

The following procedure is commonly used to measure the concentration of impurities in pharmaceutical products using area percentage normalization: prepare and analyze the standard solution at a concentration such that the height of the main peak will be within the linear range of the detector, then analyze multiple dilutions of the sample.

Since the new photodiode array detector SPD-M40 has completely eliminated the effects of stray light during detection, it not only provides a wide dynamic range (linearity up to 2.5 AU as a specification value, typical value is more than 2.5 AU) but also achieves low noise and high sensitivity.

Moreover, the SPD-M40 has little fluctuation in its baseline thanks to Advanced TC-Optics (triple temperature control of the cell, light source, and optical system), making it ideal for analyzing trace impurities in pharmaceuticals.

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Table 1 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
Flow Rate	: 1 mL/min
Column Temp.	: 40 °C
Injection Volume	: 2 µL
Detection	: SPD-M40 at 256 nm
Flow Cell	: Standard cell

Table 2 Gradient Time Program

Time (min)	A. Conc	B. Conc
0	70	30
4	40	60
6	10	90
8	10	90
8.01	70	30
10	STOP	

Linearity

This section presents examples of impurity analyses with SPD-M40 using ketoprofen, a nonsteroidal anti-inflammatory drug.

Analytical conditions and gradient time programs for mobile phases are shown in Table 1 and Table 2, respectively.

To confirm the linearity of ketoprofen with SPD-M40, 0.5-800 mg/L of standard solutions were prepared and analyzed. Fig. 1 shows the chromatograms of the ketoprofen standard solutions, and Fig. 2 shows the calibration curve of ketoprofen.

Excellent linearity ($R^2 \geq 0.999$) was obtained over a wide range of concentrations, from 0.5 to 800 mg/L.

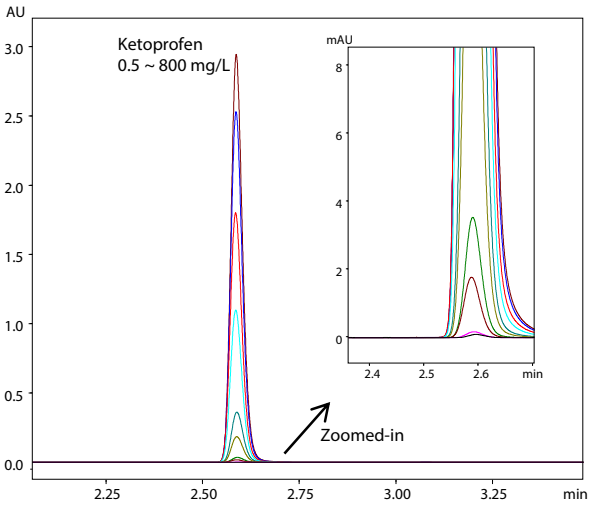

Fig. 1 Chromatograms of the Ketoprofen Standard Samples

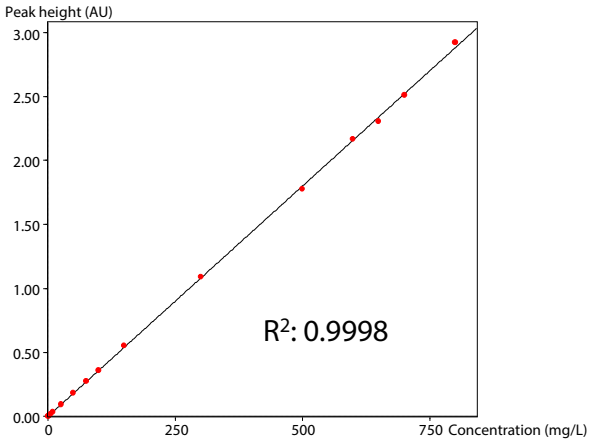

Fig. 2 Calibration Curve for Ketoprofen

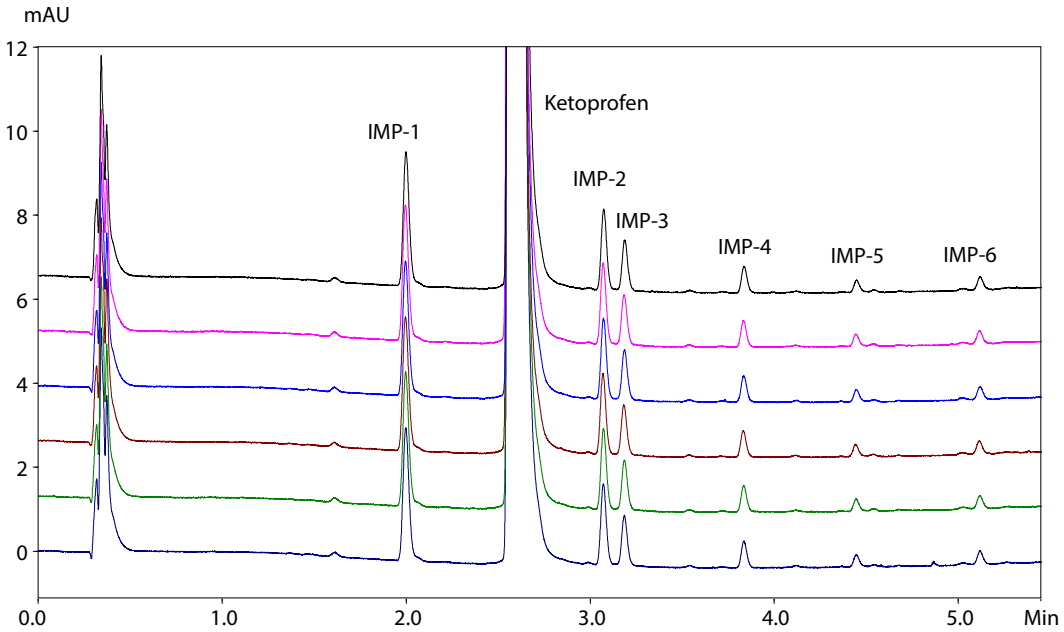

Fig. 3 Overlapped Chromatograms of Ketoprofen 700 mg/L Standard Solution (n = 6)

Impurity Analysis

Fig. 3 shows the chromatograms of six repeated analyses of 700 mg/L ketoprofen standard solution with peak heights of approximately 2.5 AU. Table 3 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.

Table 3 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

Peak Purity and Spectral Confirmation

One of the main advantages of PDA detection for impurity analysis is the possibility of performing a peak purity assay and impurity spectral confirmation. Fig. 4 shows the purity curve of the ketoprofen peak. No impurities were detected within the peak elution interval. Fig. 5 shows the UV spectrum of Impurity 3 with an area percentage concentration of 0.046 %. This result shows that informative spectra can be obtained even at very low concentrations.

Results for pharmaceutical analyses obtained using SPD-M40 are highly reliable due to its high performance over a wide range of concentrations.

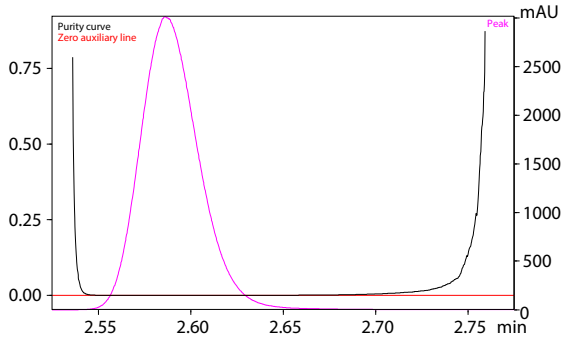

Fig. 4 Purity Curve of the Ketoprofen Peak

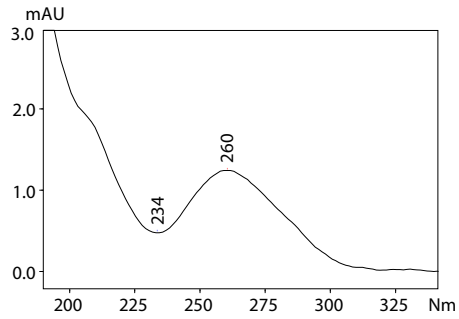

Fig. 5 UV Spectrum of Impurity 3

References

1) Revision of Guidelines for Impurities in New Drug Substances (Evaluation and Licensing Division, PMSB, dated December 16, 2002, Notification No. 1216001).
2) Revision of Guidelines for Impurities in New Drug Substances (Evaluation and Licensing Division, PMSB dated June 24, 2003, Notification No. 0624001).

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