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Application News

No. L539B

High Performance Liquid Chromatography

Improvement of Quantitative Performance for Ibuprofen Using UV Cut-Off Filter on SPD-M40

Ibuprofen is an example of a nonsteroidal anti-inflammatory drug (NSAID) and is widely used as an antipyretic or analgesic agent. It has been reported that during tests to confirm stability during storage, decomposition products were generated due to temperature, acidity, light irradiation, etc. of the surroundings. In particular, the area percentage of 4-Isobutylacetophenone, one of the decomposition products, increased by about 40 % after the 72-hour light irradiation test.

The Nexera™ Series photodiode array detector SPD-M40 is equipped with a UV cut-off filter that excludes light in the ultraviolet range, in order to ensure more stable detection of compounds that are prone to photodegradation. Here we introduce examples of improving the quantitation of ibuprofen using the UV cut-off filter function of the SPD-M40.(1)

＊This function was developed with the help of the comments by Lion Corporation.

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UV Cut-Off Filter

A photodiode array detector (PDA) irradiates the sample cell with white light (including ultraviolet light, which is relatively high-energy), spectrally separates the transmitted light, and measures the absorbance of a sample at a specific wavelength. Fig. 1 shows a diagram of a flow cell without the UV cut-off filter in use. Photodegradation can occur in the flow cell for analytes that are easily degraded by UV light.

As a consequence, accurate quantitation may be affected both by simultaneous determination of decomposition products with different light absorption characteristics, and by underestimation of the quantity of the target analyte.

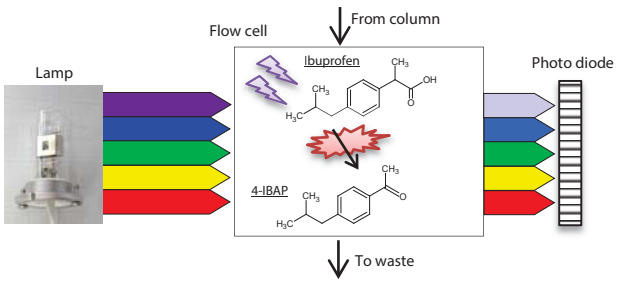

Fig. 1 **UV Cut-Off Filter Flow Cell (UV Cut-Off Filter Disabled)**

Fig. 2 shows a diagram of the flow cell with the UV cut-off filter in use. The short-wavelength, high-energy UV light can no longer enter the flow cell. Decomposition of the target analyte is suppressed due to the filter, and quantitation results are more reliable, no longer being affected by decomposition products.

Fig. 4 shows the chromatograms of the standard samples and the spectrum of the ibuprofen peak analyzed without using the UV cut-off filter. The calibration curve is shown in Fig. 5, and the error on each calibration point is shown in Table 2. In the low

The SPD-M40 detector uses a filter with a cut-off wavelength of around 240 nm. This completely cuts out incident light in the ultraviolet region <220 nm, and provides a 90 % reduction for wavelengths up to 240 nm.

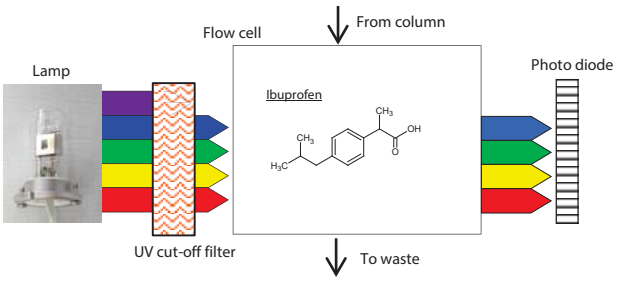

Fig. 2 **UV Cut-Off Filter Flow Cell (UV Cut-Off Filter Active)**

The user can choose whether to use the UV cut-off filter by modifying the method file (Fig. 3). It is easy to apply different conditions to each method even within the same analytical batch.

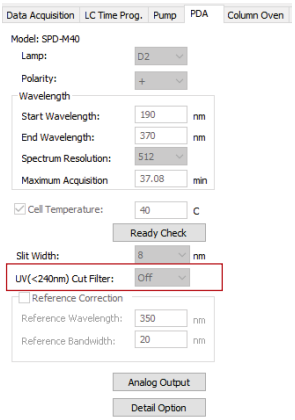

Fig. 3 **UV Cut-Off Filter Function Setting Screen for the SPD-M40**

Effects of UV Cut-Off Filter Use on the Analysis of Ibuprofen

Ibuprofen standard solutions (5 to 75 mg/L) were analyzed with and without the use of a UV cut-off filter. The analytical conditions are shown in Table 1.

Table 1 **Analytical Conditions**

Column	:	Shim-pack Velox™ C18 (3 mm × 100 mm, 2.7 µm)
Mobile phase	:	0.1 % Formic acid aq./Acetonitrile =2/3 (v/v)
Flow rate	:	0.4 mL/min
Column temp.	:	40 °C
Injection vol.	:	10 µL
Detection	:	SPD-M40 at 262 nm (190 - 400 nm)

concentration range, the intercept of the calibration curve is relatively high due to the influence of decomposition products with large absorption coefficients. As a result, there is significant error on the calibration curve in the low concentration region.

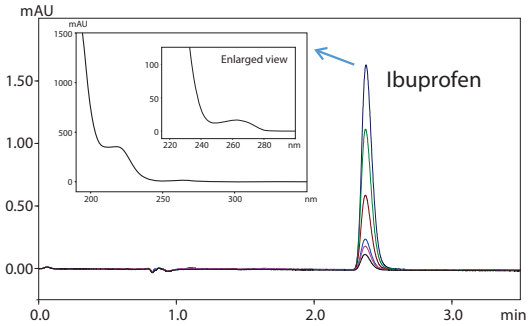

Fig. 4 **Chromatograms of Ibuprofen Standard Samples (5 to 75 mg/L) and Spectrum (75 mg/L) with UV Cut-Off Filter Disabled.**

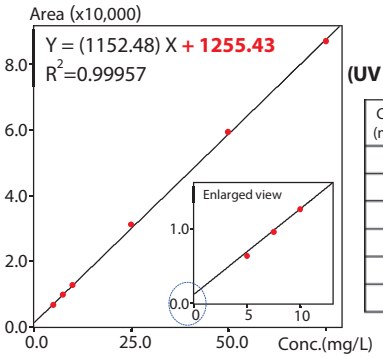

Table 2 Error on Calibration Points (UV Cut-Off Filter Disabled)

Conc. (mg/L)	Area	Error Rate (%)
5	6,418	**-10.40**
7.5	9,615	-3.28
10	12,692	-0.76
25	31,164	3.80
50	59,361	0.84
75	87,085	-0.70

Fig. 5 **Calibration Curve of Ibuprofen (UV Cut-Off Filter Disabled)**

Table 3 shows the results of quantitative analysis without using the UV cut-off filter for the 5 mg/L standard sample, which is the minimum concentration point on the calibration curve (LLOQ). After six consecutive repeats, the average result for the concentration of the analyte was >10 % lower than the expected value.

Table 3 Quantitative Results for 5 mg/L Sample (UV Cut-Off Filter Disabled)

	Retention Time (min)	Area	Conc. (mg/L)
1	2.366	6,462	4.517
2	2.380	6,319	4.394
3	2.376	6,508	4.558
4	2.378	6,339	4.411
5	2.377	6,371	4.439
6	2.378	6,468	4.523
Average	2.376	6411	**4.474**
RSD(%)	0.21	1.22	**1.51**

Fig. 6 shows the chromatograms of the standard samples and the spectrum of the ibuprofen peak analyzed with the use of the UV cut-off filter. The calibration curve is shown in Fig. 7, and the error on each calibration point is shown in Table 4. Since the short-wavelength UV light is eliminated by the UV cut-off filter, the influence of the decomposition products becomes negligible. Good linearity is achieved over the entire calibration range with reduced error and increased quantitative accuracy.

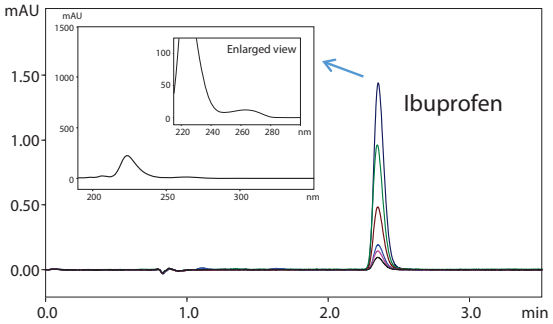

Fig. 6 **Chromatograms of Ibuprofen Standard Samples (5 to 75 mg/L) and Spectrum (75 mg/L) with UV Cut-Off Filter in Use.**

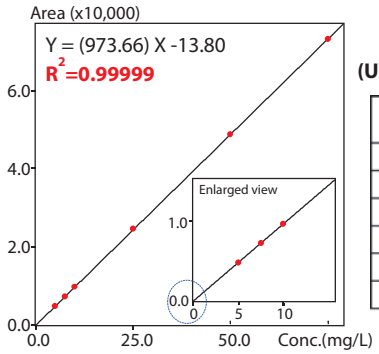

Table 4 Error on Calibration Points (UV Cut-Off Filter in Use)

Conc. (mg/L)	Area	Error Rate (%)
5	4,833	**-0.44**
7.5	7,290	0.02
10	9,657	-0.67
25	24,461	0.55
50	48,629	-0.08
75	73,003	-0.01

Fig. 7 **Calibration Curve of Ibuprofen (UV Cut-Off Filter in Use)**

Table 5 shows the results of the quantitative analysis using the UV cut-off filter for the 5 mg/L standard sample (LLOQ). The difference from the expected value is now much smaller, <0.1 %, compared to the previous case (UV cut-off filter off). There was also improved reproducibility (peak area).

The UV cut-off filter resulted remove this word to be useful for quantitative determination of components that are easily degraded by wavelengths in the UV range, particularly improving quantitation accuracy in the low concentration range.

Table 5 Quantitative Results for 5 mg/L Sample (UV Cut-Off Filter in Use)

	Retention Time (min)	Area	Conc. (mg/L)
1	2.354	4,882	5.028
2	2.352	4,843	4.988
3	2.350	4,844	4.990
4	2.353	4,859	5.005
5	2.353	4,829	4.974
6	2.351	4,840	4.985
Average	2.351	4,849	**4.995**
RSD(%)	0.06	0.38	**0.38**

Reference

(1) S. Farmer et al., "Forced Degradation of Ibuprofen in Bulk Drug and Tablets and Determination of Specificity, Selectivity, and the Stability Indicating Nature of the USP Ibuprofen Assay Method" Pharmaceutical Technology North America, **26** (5), 28-42 (May 2002)

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