Improvement of quantitative performance for photodegradable compounds using UV cut-off filter on photodiode array detector

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1. Introduction

Analysis using a photodiode array detector (PDA) makes it possible to monitor various absorption spectra using light beam of a wide range of wavelengths, but light beam also includes high energy wavelengths such as UV. Therefore, there are compounds which are easily decomposed by light beam and difficult to analyze accurately. Ibuprofen is a type of non-steroidal anti-inflammatory drug (NSAID) and widely used as an antipyretic and analgesic agent. It has been reported that during tests to confirm stability during storage, decomposition products were generated due to temperature, light irradiation, etc. of the surroundings. In particular, the area percentage of 4-Isobutylacetophenone, one of the decomposition products, was increased by about 40 % after the 72-hour light irradiation test. Therefore, we performed quantitative analysis of ibuprofen using an ultra-high performance liquid chromatograph (UHPLC; Nexera™ PDA System) with a UV cut-off filter excluding light beam in the short wavelength ultraviolet region, and examined its usefulness for the analysis of photodegradable compounds.

2. Experimental

2-1. Analytical system

Shim-pack Velox™ C18 (3 mm × 100 mm, 2.7 µm) (Shimadzu Corporation) was used as an analysis column.

The ibuprofen standard stock solution was diluted and subjected to LC analysis at a concentration of 5-75 mg/L.

As an analysis system, a PDA detector SPD-M40 (Shimadzu Corporation) equipped with a UV cut-off filter was used. The other units used the Nexera system (Shimadzu Corporation).

Figure 1: Nexera system

2-2. 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. Figure 2 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 beam. Figure 3 shows the image in the flow cell when using the UV cut-off filter. Since the short wavelength UV light with high energy is not irradiated to the flow cell, the decomposition of the target compound at the time of detection can be suppressed, and quantification can be performed without being affected by the decomposition products.

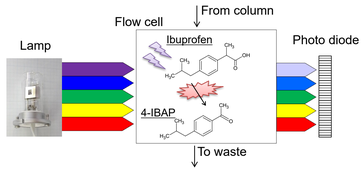

Figure 2: UV cut-off filter flow cell (UV cut-off filter disabled)

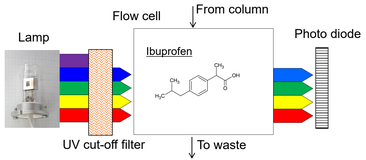

Figure 3: UV cut-off filter flow cell (UV cut-off filter active)

2-3. Analytical conditions

Ibuprofen standard solutions (5-75 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 condition

Mobile phase	:	A: 0.1 % Formic acid aq. B:Acetonitrile A/B=2/3 (v/v)
Flow rate	:	0.4 mL/min
Column	:	Shim-pack Velox C18 (3 mm × 100 mm, 2.7 µm)
Column temp.	:	40° C
Wave length	:	264 nm (at PDA) (190 – 400 nm)
Injection vol.	:	10 µL

3. Results

3-1. Comparing the spectrum of ibuprofen

The UV spectrum of the ibuprofen standard sample is shown in Figure 4. It was confirmed that short wavelength UV light of 240 nm or less was significantly reduced by using a UV cut-off filter.

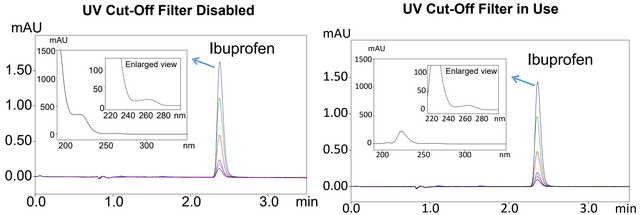

Figure 4: Comparison of chromatograms and spectrum of ibuprofen samples

3-2. Linearity comparison

The calibration curve obtained by standard sample analysis without the UV cut-off filter is shown in Figure 5, and the calibration point error rate is shown in Table 2. Figure 6 shows the calibration curve obtained by standard sample analysis with the UV cut-off filter, and Table 3 shows the calibration point error rate. From the comparison of the two results, It is considered that the intercept of the calibration curve is high due to the influence of the large decomposition products of the light absorption coefficient in the low concentration region. As a result, it is suggested that the calibration curve error rate in the low concentration region is large.

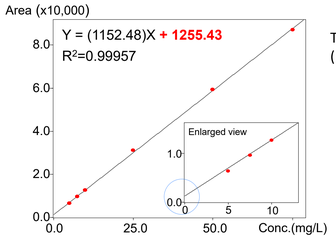

Figure 5: Calibration curve of Ibuprofen (UV cut-off filter disabled)

Table 2: Error on calibration points (UV cut-off filter disabled)

Conc. (mg/L)	Area	Error(%)
5	6,418	-10.4
7.5	9,615	-3.28
10	12,692	-0.76
25	31,164	3.8
50	59,361	0.84
75	87,085	-0.7

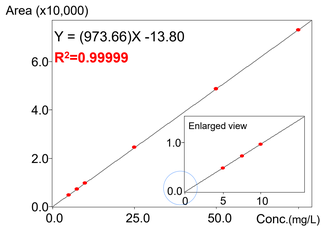

Figure 6: Calibration curve of Ibuprofen (UV cut-off filter in use)

Table 3: Error on calibration points (UV cut-off filter in use)

Conc. (mg/L)	Area	Error(%)
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

3-3. Comparison of reproducibility in low concentration area

Table 4 shows the results of quantitative analysis for the 5 mg/L standard sample, which is the minimum concentration point on the calibration curve (LLOQ). Using the UV cut-off filter, the difference from the expected value was much smaller, compared to without the UV cut-off filter. There was also improved reproducibility (peak area).

Table 4: Quantitative results for 5 mg/L sample

UV cut-off filter disabled

	R.T.	Area	Conc.(mg/L)
1	2.366	6,462	4.517
2	2.38	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**

UV cut-off filter in use

	R.T.	Area	Conc.(mg/L)
1	2.354	4,882	5.028
2	2.352	4,843	4.988
3	2.35	4,844	4.99
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**

4. Summary

It was shown that good linearity can be obtained with a calibration curve in a wide concentration range using the UV cut-off filter for the quantitative analysis of the photolytic compound ibuprofen. Consequently, it is considered that an analysis system using the UV cut-off filter is useful for quantitative analysis of components that are easily decomposed by short wavelength UV light beam.