UV Cut-Off Filter of a Photodiode Array Detector Improves the Quantitativity of L-Ascorbic Acid Through Its Photoprotection

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Abstract
A photodiode array detector (PDA) is frequently utilized as a detector in high-performance liquid chromatography (HPLC) system for a detection of various compounds. A PDA emits a light of a wide range wavelength including ultraviolet light (UV) which has a possibility to induce photodegradation of analyzed compounds. If so, target compounds might be degraded when analyzed in this HPLC system. Therefore, photoprotection during HPLC analysis is required for the accurate analysis. In this study, the protective effect of a UV cut-off filter, which can cut off UV especially at shorter wavelength (< 240 nm), on the quantitativity of a photodegradable compound L-ascorbic acid (AA) was examined. A UV cut-off filter prevents AA from photodegradation, followed by the improvement of several parameters of a calibration curve. Furthermore, this protective potency was significant in the case that photodegradability of AA was enhanced with the conditions such as a small injection volume and a low flow rate. This study strongly suggests that the UV cut-off filter is a useful equipment when analyzing photodegradable compounds.

Keywords: UV cut-off filter; L-Ascorbic acid; Photoprotective effect; HPLC; PDA

1. Introduction
A photodiode array detector (PDA) is frequently used as a detector in a high-performance liquid chromatography (HPLC) system. A PDA utilizes a wide range wavelength of light emission for a detection of various compounds having ultraviolet light (UV) and visible light absorption. It is possible to detect the almost all target compounds after the column separation. On the other hand, light emission contains UV at shorter wavelength, which might induce photodegradation more than that at longer wavelength. There are various pharmaceuticals which are affected by photolysis, such as β-blockers, calcium channel blockers, antibiotics, non-steroidal anti-inflammatory drugs, hyperlipidemia agents and anti-epileptic drugs [1-7]. These pharmaceuticals might be degraded immediately after photolysis. For example, cyanocobalamin, which is utilized as a vitamin agent, is known as a photodegradable compound, and degraded completely by room-light irradiation within 1 hr [8]. Photodegradation has a crucial effect on both the quality and quantity of compounds and might make it difficult to analyze photodegradable compounds accurately. Its protection is needed to obtain the accurate analysis of photosensitive compounds.

To suppress photodegradation of analyzed compounds with a PDA detection, a UV cut-off filter is equipped with a SPD-M40 PDA detector (Shimadzu Corporation, Kyoto, Japan). This filter could cut off UV especially at shorter wavelength (< 240 nm). The main component of this filter is glass. Transmittance of UV at 240 nm is 25%, and that of at 200 nm is 0%. It is expected that the UV cut off filter would protect photodegradable compounds from photodegradation. Previous studies indicate the photoprotective performance of a UV cut-off filter on photodegradable pharmaceuticals such as ibuprofen and naproxen (data not shown). A UV cut-off
filter protected them from photodegradation followed the improvement of the accuracy. However, UV absorption maxima of ibuprofen and naproxen are around 230 nm, and they were not immediately degraded as described in the previous reports [9,10]. In our previous study, naproxen and L-ascorbic acid (AA) in a solution were irradiated by a black light lamp for 1 hr. The residual amount of naproxen was 95.82% while that of AA was 34.99% [11]. These reports suggest that AA is more photosensitive than naproxen, and its analysis through a PDA detector might be interrupted due to light emission especially at shorter wavelength. AA is oxidized to dehydroascorbic acid (Fig. S1) and this reaction proceeded immediately by the photo-irradiation [12]. This photochemical reaction might be induced by utilized photo-irradiation emitted from a PDA. If so, a UV cut-off filter exerts protective potency for AA degradation and might improve the quantitativity.

In this study, the protective effect of a UV cut-off filter in the PDA detector on AA photodegradation was evaluated. To determine the improvement of the quantitativity of AA, its calibration curves were prepared with and without a UV cut-off filter. AA determination was performed by the following HPLC system. The purpose of this research is to evaluate the utility of a UV cut-off filter for the analysis of photodegradable compounds. The concept of this study is summarized in Fig. 1.

2. Experimental

2.1. Chemicals

All regents and organic solvents used were of special grade or HPLC grade. AA and acetic acid were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). Milli-Q (18.2 mΩ/cm) water was prepared by using a Milli-Q water purification system (Merck, Darmstadt, Germany).

2.2. Sample preparation

To make a calibration curve, test solutions of AA were prepared as follows; AA was initially dissolved in Milli-Q water (10 g/L), and this solution was diluted further by the addition of Milli-Q water to make a concentration of 100 µg/L-1 g/L. 1 mL of diluted sample solutions in glass vials were used for the HPLC analysis.

2.3. HPLC analysis

HPLC analysis for evaluating of AA quantification was performed on a Prominence HPLC system except for a PDA detector, which is composed of an LC-20AB pump, an SIL-20AC auto-sampler, a CBM-20A system controller, a DGU-20A3 degasser, and a CTO-20A column oven (Shimadzu Corporation, Kyoto, Japan). An SPD-M40A (Shimadzu Corporation), which was a component of a Nexera HPLC system and has a UV cut-off filter, was used as a PDA detector, and the HPLC system was operated using LabSolutions software (Shimadzu Corporation). The analytical column was a Develosil XG-C30M-3 column (4.6 x 150 mm, particle size 3 µm, Nomura Chemical CO., Ltd., Aichi, Japan). The column was kept at 40 ºC during the analysis. Isocratic separation was achieved using a mobile phase consisting of 0.1% acetic acid (v/v). Basic analytical condition was as follows; a flow rate was maintained at 1.0 mL/min, and an injection volume was 20 µL. Detection wavelength was set at 270 nm. A flow rate was changed to 0.1, 0.25 or 0.5 mL/min when evaluating the flow rate dependency of AA quantification. Injection volume was changed to 5, 10 or 40 µL when evaluating the injection volume dependency. Retention time of AA in the each flow rate were as follows; 1.86 min (1.0 mL/min, k’=0.208), 3.66 min (0.5 mL/min), 7.31 min (0.25 mL/min) and 18.34 min (0.1 mL/min).

2.4. Evaluation of the protective effect of UV cut-off filter on AA quantification

HPLC analysis of AA was performed by the conditions mentioned above and obtained results were utilized to make calibration curves. Slope, intercept and correlation coefficient of calibration curves were calculated using Microsoft Excel. Values obtained in the presence or absence of a UV cut-off filter, which was switched using LabSolutions, were compared to evaluate the protective effect of it.

3. Results and discussion

3.1. Protective effects of a UV cut-off filter on AA

In this study, the effect of a UV cut off filter on AA
quantitativity, especially its calibration curves, was evaluated. The HPLC chromatogram of an AA solution and the UV spectrum of AA were shown in Fig. 2. Retention time of AA was 1.86 min, and the result of UV absorption spectral analysis by means of the PDA detector indicate that AA has characteristic absorption between 200 and 300 nm, showing absorption-maximum wavelength value at 243 nm.

The results of AA quantitative analysis in the presence or absence of a UV cut-off filter were shown in Table 1. The mean values of peak areas of AA using a PDA detector with a UV cut-off filter at each concentration were higher compared to those of without a UV cut-off filter. Also, the values of RSD (%) without a UV cut-off filter in five times analysis were higher compared to those with a UV cut-off filter, showing that the results of AA analysis without a UV cut-off filter had less accuracy than those with the filter. These differences were significant at the lower concentration of AA. These data indicate that AA seems to be degraded by photo-irradiation in the detector, followed by the variation of peak area of AA. Both UV and visible light (200-800 nm) are utilized in a SPD-M40A. It is already reported that their emissions induce the photodegradation of AA in an aqueous media [12]. AA is oxidized to corresponding dehydroascorbic acid by photo-irradiation [12], which has no UV absorption at 270 nm. In this experiment, injected AA was flowed into the flow cell and irradiated in the PDA detector, and some of AA might be degraded. This photodegradation was remarkable in the case of that concentrations of AA were low. Furthermore, it is possible that a UV cut-off filter might act as a protective agent for AA degradation. With a UV cut-off filter, peak areas were higher and RSD values were lower at the lower concentration. These results showed that a UV cut-off filter make it possible to suppress photodegradation of compounds and perform more accurate analysis.

The parameters of calibration curves of AA with and without a UV cut-off filter were as follows; slope (x 10,000): 1898.7, intercept: -13699.5 and correlation coefficient: 0.99988 in the case of without a UV cut-off filter, and slope (x 10,000): 1904.1, intercept: -2802.4 and correlation coefficient: 0.99997 in the case of with a UV cut-off filter. Prepared calibration curves in both conditions were shown in supporting information (Fig. S2). The values of slope of both calibration curves were similar, but those of intercept were different. The value of intercept without a UV cut-off filter (-13699.5) was lower than that of with a UV cut-off filter (-2802.4). AA photodegradation at the lower concentration contributes to the decrease of the intercept value of the calibration curve. Also, intercept and correlation coefficients of both conditions were comparable. This is because peak areas of AA at higher concentration were the same level among two groups. In the case that peak areas of AA at higher concentration (25 mg/L >) were removed, due to the significance of the decrease of peak areas of AA at lower concentrations, the value of slope without a UV cut-off filter (15265.1) was lower than that of with a UV cut-off filter (17918.0). These results showed that a presence of UV cut-off filter could improve the quantitativity of AA.

3.2. Evaluation of protective effects of a UV cut-off filter on AA degradation in the photodegradable conditions.

In generally, photodegradation reaction is promoted when the irradiation time is prolonged or the concentration of the substrate is low. It is possible that AA might be more degradable in the case that an injection volume into an HPLC system and a flow rate are low. The effects of an injection volume and a flow rate on the parameters of calibration curves of AA with or without a UV cut-off filter were shown.

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**Table 1.** Peak area of AA detected by a PDA detector in the presence or absence of a UV cut-off filter.

| Concentration (mg/L) | UV cut-off filter (-) | UV cut-off filter (+) |
|----------------------|----------------------|----------------------|
|                      | Mean | RSD (%) | Mean | RSD (%) |
| 0                    | 0    | -       | 0    | -       |
| 0.1                  | 1491 | 25.6    | 1663 | 6.9     |
| 0.25                 | 3746 | 17.7    | 4255 | 8.1     |
| 0.5                  | 6751 | 32.1    | 8333 | 6.9     |
| 1                    | 14538| 13.3    | 17880| 3.8     |
| 2.5                  | 35455| 28.4    | 42937| 8.1     |
| 5                    | 71226| 28.3    | 85281| 4.2     |
| 10                   | 154026| 18.2 | 180398| 1.1     |
| 25                   | 445795| 5.7  | 467879| 2.8     |
| 50                   | 905837| 6.4  | 945767| 3.2     |
| 100                  | 1896827| 2.9  | 1921905| 1.7     |
| 250                  | 4736805| 1.6  | 4750722| 1.2     |

Data were based on five replicate analyses.
Chromatography

Table 2. The effect of an injection volume on slope, intercept and correlation coefficient of AA calibration curves in the presence or absence of a UV cut-off filter.

|        | 5 µL   | 10 µL  | 20 µL  | 40 µL  |
|--------|--------|--------|--------|--------|
|        | (-) (+) (+) (+) | (-) (+) (+) (+) | (-) (+) (+) (+) | (-) (+) (+) (+) |
| Slope (×10,000) | 536.8 | 747.0  | 924.2  | 1845.7 | 1815.1 | 3523.6 | 3533.1 |
| Intercept | -21003.3 | -3009.9 | -17018.5 | -2262.4 | -17871.0 | -1810.4 | -6180.9 | 15847.1 |
| Correlation coefficient | 0.99277 | 0.99990 | 0.99896 | 0.99966 | 0.99969 | 0.99979 | 0.99963 |

These parameters were calculated using Microsoft Excel. (+) and (-) mean the presence and absence of a UV cut-off filter. Calibration curves were prepared in the range of 0-250 mg/L except for 5 µL (-) and 0.1-250 mg/L (5 µL (-)).

Table 3. The effect of a flow rate on slope, intercept and correlation coefficient of AA calibration curves in the presence or absence of a UV cut-off filter.

|        | 0.1 mL/min | 0.25 mL/min | 0.5 mL/min | 1 mL/min |
|--------|------------|-------------|------------|----------|
|        | (-) (+) (+) (+) | (-) (+) (+) (+) | (-) (+) (+) (+) | (-) (+) (+) (+) |
| Slope (×10,000) | 17950.6 | 17533.0 | 7082.4 | 7387.9 | 3797.1 | 3765.5 | 1644.5 | 1887.2 |
| Intercept | -604170.0 | -119830.3 | -82276.9 | -17359.9 | -20448.1 | -2653.2 | -7928.8 | -1051.4 |
| Correlation coefficient | 0.99757 | 0.99889 | 0.99958 | 0.99997 | 0.99993 | 0.99999 | 0.99991 | 0.99987 |

These parameters were calculated using Microsoft Excel. (+) and (-) mean the presence and absence of a UV cut-off filter. Calibration curves were prepared in the range of 0-250 mg/L except for 0.1 mL/min (-) and 0.5-250 mg/L (0.1 mL/min (-)).

In Table 2 and Table 3. Without a UV cut-off filter, AA showed a decrease of peak areas at lower concentration by reducing the injection volume, resulting in the lower intercept values. When an injection volume is 5 µL, the peak of AA at 0.1 mg/L was not detected in the absence of a UV cut-off filter (Table S1), and the intercept value was -21003.3 (Table 2). Furthermore, the smaller flow rate induced photodegradation of AA and a significant decrease of the intercept values. AA was not detected at the lower concentrations (0.1-1 mg/L) without a UV cut-off filter (Table S1). In the case of 0.1 mL/min, the intercept value was -604170.0 (Table 3). On the other hand, a UV cut-off filter weakened AA degradation in these analytical conditions. With a UV cut-off filter, AA showed a decrease of peak areas at the lower concentrations, but it was milder than those of without a UV cut-off filter. The intercept value was -3009.9 when an injection volume was 5 µL (Table 2), and the peak of AA at 0.1 mg/L was detected (Table A.1). Also, the intercept value was lower by being smaller flow rate, but it was rescued by a UV cut-off filter (Table 3). A part of AA might be degraded although a UV cut-off filter is activated. A UV cut-off filter shuts off the UV of which wavelength is less than 240 nm. This function has a contribution to the partial protection of AA due to UV at above 240 nm and visible light might induce photodegradation. As shown in above results, a UV cut-off filter could improve the detection sensitivity of AA especially when its photodegradation was enhanced by the HPLC conditions.

Conclusion

In this study, the utility of a UV cut-off filter was investigated. As described previous section, it is indicated that a UV cut-off filter is an effective tool for analyzing of photodegradative compounds. Without a UV cut-off filter, AA especially at the lower concentrations is degraded by photo-irradiation emitted by a detector. In contrast, a UV cut-off filter prevents AA from photodegradation, followed by the improvement of several parameters of calibration curve. Furthermore, this protective potency was significant in the case that photodegradability of analyzed compounds was enhanced such as a small injection volume and a low flow rate. This study strongly suggests that a UV cut-off filter is a useful equipment when analyzing photodegradable compounds.

Conflict of interests

There is no conflict of interest.

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Supporting information
Structure of AA and its photodegradation to dehydroascorbic acid (Fig. S1), calibration curves of AA with and without a UV cut-off filter (Fig. S2), and results of HPLC analysis of AA in the case that injection volume and flow rate were changing with and without a UV cut-off filter (Table S1 and Table S2) are available via the WEB at http://chromsoc.jp/Journal/SI.html.

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