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Application News

No.L451

High Performance Liquid Chromatography

i-DReC (Intelligent Dynamic Range Extension Calculator), a New Photodiode Array Detector Function; Impurity Analysis Application

Using the Intelligent Dynamic Range Extension Calculator (*i*-DReC), a new data analysis function for the photodiode array detector, peak area and peak height can be calculated using spectrum similarity, even in a high-concentration region where the detector signal is saturated. Here, we explain the principle of *i*-DReC, and introduce an example of analysis that applies this principle in determining the area percentage of an impurity in a pharmaceutical substance.

Fundamental Principle of *i*-DReC Function

When analyzing a high-concentration sample, a peak may exceed the dynamic range of the detector and prevent a correct area value from being obtained. Using *i*-DReC, the target peak area (height) can be determined by multiplying a sensitivity coefficient calculated from a spectrum chosen at the base of the peak by the peak area (height) obtained from a chromatogram chosen at a wavelength showing low absorption. The process flow is as follows.

1. Acquire spectra in the elution region of the target peak that exceeded the dynamic range.
2. A search of the spectra for a correction wavelength λb where the intensity value is within the dynamic range is automatically conducted in the shorter or longer wavelength regions of designated wavelength λa.
3. The chromatogram at the correction wavelength λb is chosen, and peak integration is conducted. Then, in this chromatogram, the peak corresponding to the peak to be corrected is identified. The "peak area or peak height" of this peak will be used as the data for conducting the correction.
4. On the downslope of the saturated peak, the spectrum associated with the time at which the intensity corresponds to the "sensitivity correction spectrum extraction intensity" is automatically extracted, and sensitivity coefficient k is determined as the ratio of intensity Ia at wavelength λa to intensity Ib at wavelength λb.
   k = Ia/Ib
5. The target peak area (height) is automatically calculated by multiplying the sensitivity coefficient (k) by the peak area (height) of the chromatogram obtained in Step 3.
   Peak area = (correction wavelength peak area) × k
   Peak height = (correction wavelength peak height) × k

Using this function, it is possible to correct the linearity of the high-concentration components as shown in Fig. 2.

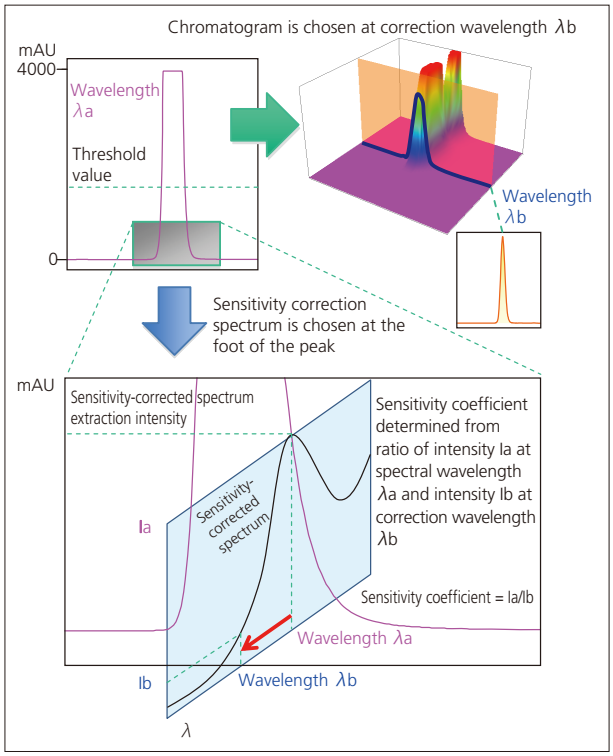

Fig. 1 Fundamental Principle of *i*-DReC

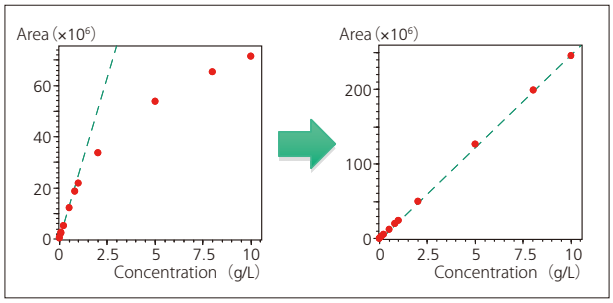

Fig. 2 Linearity Correction by *i*-DReC

Application in Impurity Analysis

For analysis of impurities in pharmaceuticals, to ensure reliability, trace impurities at less than about 0.1 % with respect to the principle component are calculated based on two or more analyses using the following procedure.

1. A sample solution is prepared at a concentration such that the height of the primary peak is sufficiently within a range that ensures the linearity of the detector (typically less than 1 AU), and is then analyzed.
2. A high-concentration sample solution that will permit stable detection of the trace level impurity is prepared, and then analyzed. Often, the primary peak will exceed the dynamic range of the detector.
3. The area of the impurity peak observed in Step 1 is calculated by dividing the impurity peak area of Step 2 by the concentration ratio (dilution rate) between the samples prepared in Steps 1 and 2.
4. The impurity content percentage is determined using the area percent calculated from the sum of the impurity area obtained from the above calculation and the primary peak area of Step 1.

This process, however, requires that at least two analyses should be conducted using at least two different solutions having different concentrations. It is the *i*-DReC feature that permits this calculation to work using just one analysis. In analysis using this feature, valid impurity content can be calculated just by analyzing the high-concentration sample adjusted for the probable trace concentration of the impurity in the drug product. Below is an example of impurity content determination in the new quinolone infectious disease treatment drug, ofloxacin, using the i-DReC feature.

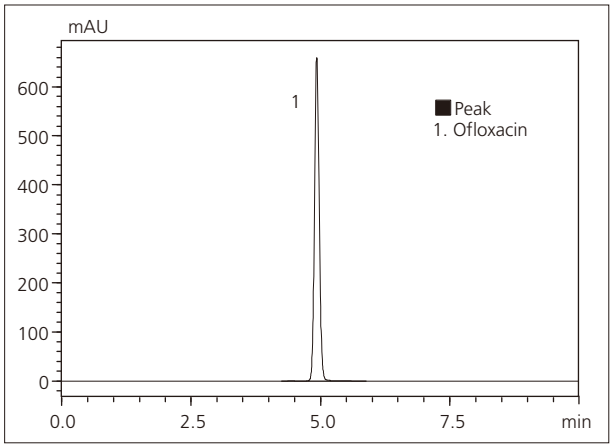

Fig. 3 Chromatogram of Ofloxacin at Appropriate Concentration

Table 1 Analytical Conditions

HPLC System	: Nexera X2
Column	: Shim-pack XR-ODS (75 mm L. × 3.0 mm I.D., 2.2 µm)
Mobile Phase	: As described in Japanese Pharmacopoeia
Flowrate	: 0.5 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 2 µL
Detection	: SPD-M30A at 294 nm, response 0.24 sec, sampling rate 240 msec.
Flow Cell	: Standard

First, 2 µL of a 200 mg/L ofloxacin solution is analyzed using the Nexera X2 system. It is assumed that the approximately 650 mA absorbance at the peak top is well within the linearity range of the detector.
Next, the chromatogram of a 10 g/L high-concentration solution of ofloxacin is shown, in addition to the expanded chromatogram showing the relevant portion. To reliably obtain the area values and abundance ratios of trace impurities, the concentration of ofloxacin becomes excessive to the point that its peak exceeds the dynamic range of the detector.

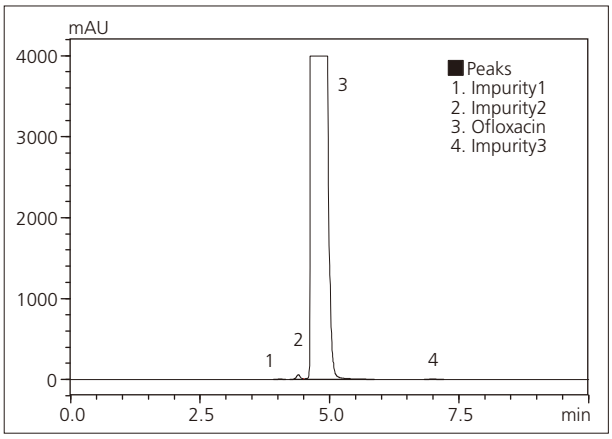

Fig. 4 Chromatogram of Ofloxacin at High Concentration

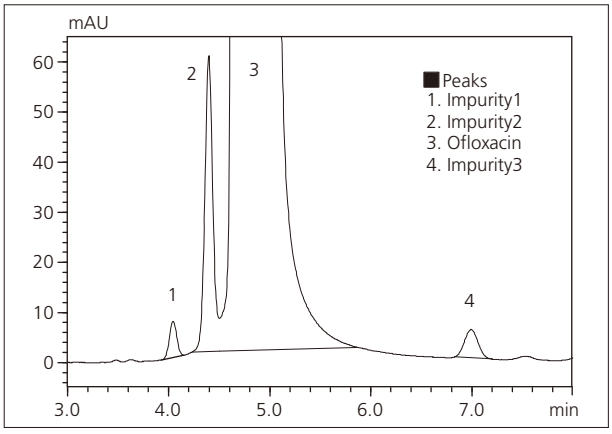

Fig. 5 Magnified Chromatogram of Ofloxacin at High Concentration

The following table compares the content values of impurities obtained using the traditional method with those calculated from the area values obtained by applying the *i*-DReC function to the saturated chromatogram to expand the dynamic range of the primary peak. It can be seen that using the *i*-DReC function allows results equivalent to those obtained by the conventional method to be achieved by conducting half the number of analyses.

Table 2 Comparison of Impurity Content with/without the *i*-DReC Function

(Area %)	Impurity1	Impurity2	Ofloxacin	Impurity3
Without *i*-DReC	0.04	0.358	99.545	0.057
With *i*-DReC	0.016	0.147	99.813	0.024
Conventional Method	0.017	0.153	99.731	0.025

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