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Technical Report

New Data Processing Method for Photodiode Array Detector

Principle and Summary of i-DReC (Intelligent Dynamic Range Extension Calculator)

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Abstract:

A new data processing method for a photo diode array (PDA) detector, Intelligent Dynamic Range Extension Calculator (i-DReC) enables the automatic calculation of peak area and height, utilizing spectrum similarity in the high concentration range where UV signal is saturated. When the integrated chromatographic peak area exceeds a user-defined threshold value, i-DReC automatically shifts the chromatographic profile to a wavelength with less UV absorption to prevent signal saturation. The absorption ratio between the original target wavelength and the wavelength used by the i-DReC function is applied as a correction factor to the peak area of the acquired chromatogram, thereby calculating the peak area and height at the original target wavelength. The i-DReC dramatically extends the linear dynamic range of calibration curves, enabling reliable quantitation of high concentration samples without need for sample dilution and reinjection, which would otherwise be required.

Keywords: PDA data processing, dynamic range extension, Nexera X2, UHPLC

1. Basic Principle of i-DReC

High concentration samples can produce saturated UV spectral absorbance, which significantly affects peak area calculation and causes a loss of linearity in the relationship of peak area to concentration. The i-DRec calculates an absorbance ratio between the original target wavelength and another wavelength that provides less absorbance in a spectrum on the down-slope of the chromatographic peak where neither wavelength's absorbance is saturated. The corrected peak area and height are then calculated by multiplying the measured peak area and height by the absorbance ratio.

■Calculation Algorithm

1. i-DRec is automatically applied when the intensity of a target peak exceeds the user-defined threshold value. If the threshold value is not exceeded, i-DReC is not applied.
2. The wavelength used for correction by i-DReC (λb) can be set either manually or automatically. When set manually, λb is a user-defined parameter. When set automatically, λb is determined as follows:
   - A UV spectrum is acquired at the retention time of the target peak.
   - The spectrum is analyzed to determine an appropriate wavelength for which the absorbance is not saturated, which is then set as λb.
3. The chromatogram at λb is extracted from the 3D data and integrated to determine peak area and height.
4. A UV spectrum is extracted from the chromatogram (at the original target wavelength (λa)) at a point on the down-slope of the peak (between the peak apex and peak end) where the absorbance of neither λa nor λb are saturated.
5. An absorption ratio (k) is calculated from the spectrum in (4). The intensity (Ia) of the spectrum at λa is divided by the intensity (Ib) of the spectrum at λb, as follows:

   $k = Ia/Ib$
6. Peak area and height of the measured peak in the chromatogram at λb are corrected by the absorption ratio to determine the effective area and height at λa, as follows:
   Peak area at λa = (peak area at λb) × k
   Peak height at λa = (peak height at λb) × k

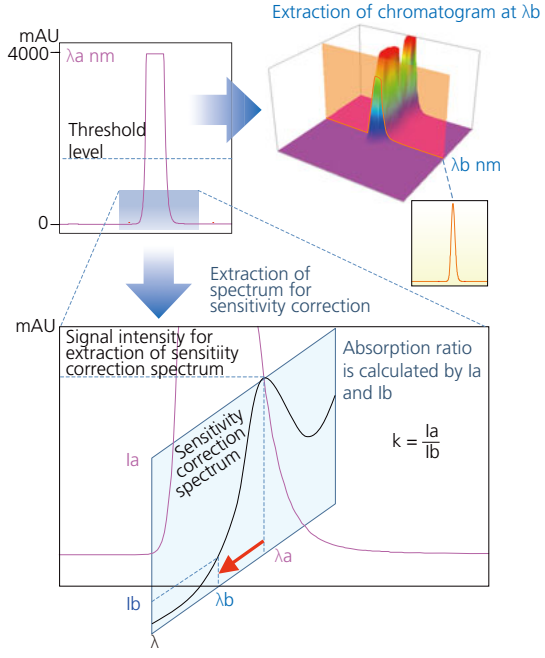


Fig. 1 Basic principle of i-DReC

1. Analytical & Measuring Instruments Division

2. Examples of i-DReC Applications

2-1. Extending the Linear Dynamic Range of Calibration Curves

This section demonstrates the extension of a calibration curve's linearity into a high concentration range, using standard solutions of Rhodamine with concentrations ranging from 0.01 g/L to 10 g/L. The following conditions were used for analysis.

Analytical Conditions

Pump	: Shimadzu LC-30AD×2
Detector	: Shimadzu SPD-M30A
Column oven	: Shimadzu CTO-20AC
Controller	: Shimadzu CBM-20Alite
Autosampler	: Shimadzu SIL-30AC
Mobile phase	: Ammonium formate buffer 45% / ACN 55%
Column	: Shimadzu Shim-pack VP-ODS (4.6 mmL. × 150 mmI.D., 5.0 µm)
Flow rate	: 1 mL/min
Column temp.	: 40 °C
Sampling	: 80 msec
Slit width	: 1 nm
Time constant	: 80 msec
Wavelength range	: 190 nm-700 nm
Cell light path	: 10 mm
Injection volume	: 2 µL

Fig. 2 shows the UV absorbance spectrum of Rhodamine, A Calibration curve was created based on peak area in the extracted chromatogram at 554 nm, the wavelength of maximum absorbance, and is shown in Fig. 3a. At 1 g/L or greater concentration, the calibration curve exhibits the loss of linear relationship between peak area and concentration.

Fig. 3b shows the same calibration curve with i-DReC applied to extend the linearity into the high concentration range. In this example, 347 nm was selected manually as the wavelength for correction, and the spectrum used for sensitivity correction was extracted at an intensity of 700 mAU. The original peak area and the corrected peak area calculated by i-DReC is shown in Table 1. After correction by i-DReC, the calibration curve based on the corrected peak areas exhibited excellent linearity with an unweighted correlation factor of 0.9999078 and 0.9995750 weighted by 1/(concentration)^2 over the concentration range of 0.01 g/L to 10 g/L.

Fig. 3C shows the error in concentration values obtained by inverse estimation using the i-DReC corrected calibration curve with weighting of 1/(concentration)^2. Even though the i-DReC corrected calibration curve extended the linear range of the original calibration curve by an order of magnitude, over the full range of concentration, the error in calculated concentration value was within 5%.

Table 1 Calibration points of Rhodamine samples

#	Conc.(g/L)	Peak area avarage (uAUsec) (n=2)	
		Original	i-DReC
1	0.01	267,847	267,847
2	0.02	544,266	544,266
3	0.08	2,089,341	2,089,341
4	0.1	2,622,781	2,622,781
5	0.2	5,255,999	5,255,999
6	0.5	12,072,748	**12,282,271**
7	0.8	18,539,104	**19,887,814**
8	1	21,823,608	**24,644,792**
9	2	33,708,885	**49,250,552**
10	5	53,883,445	**126,813,723**
11	8	65,182,276	**198,990,013**
12	10	71,500,307	**245,336,353**

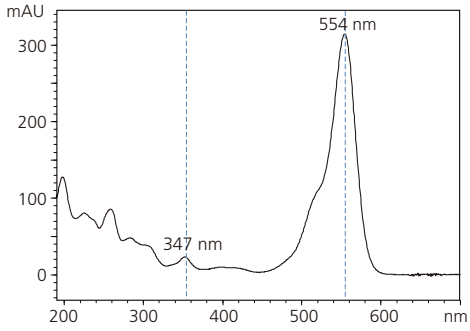
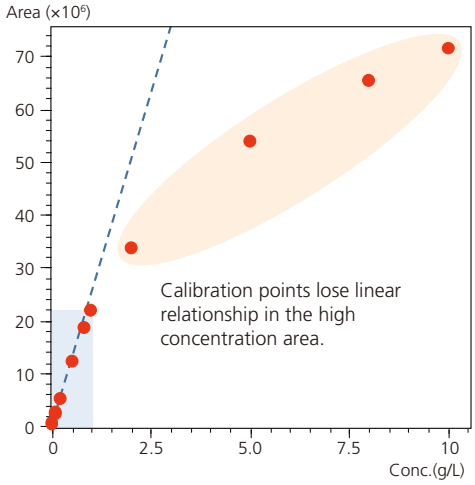
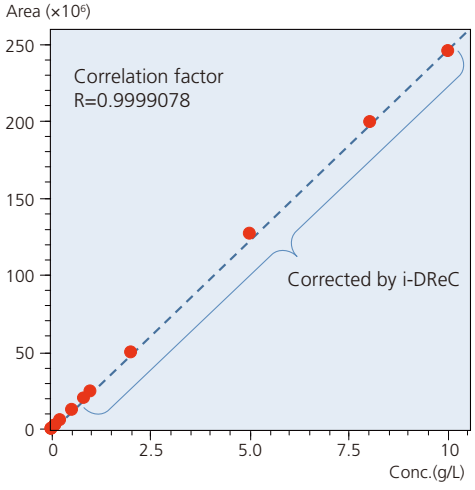


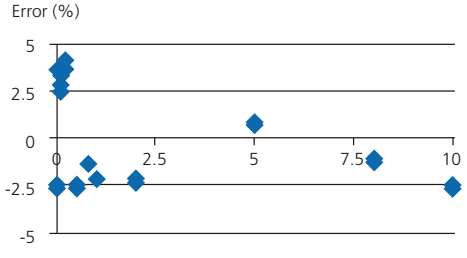
Fig. 2 Spectrum of Rhodamine



(a) Calibration curve at 554 nm



(b) Linearity range extended by i-DReC



(c) Error of corrected calibraition points

Fig. 3 Calibration curve of Rhodamine

2-2. Simultaneous Quantitation of Main Component and Minor Impurities

This section demonstrates the use of i-DReC to simultaneously quantify a relatively high concentration major component in a pharmaceutical sample and the relatively low concentration impurities. Samples in which the concentration of the main component ranged from 0.01 g/L to 1 g/L where analyzed using the SPD-M30A photodiode array detector equipped with the high sensitivity cell. Fig. 4 shows the calibration curve for the main component based on peak area in the extracted chromatogram at 250 nm.

Analytical Conditions

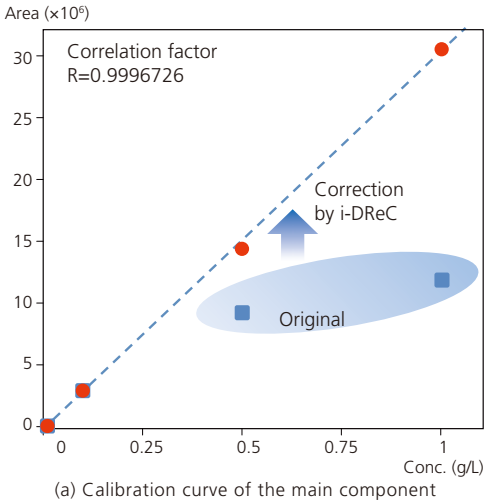
Pump	: Shimadzu LC-30AD×2
Detection	: Shimadzu SPD-M30A
Column oven	: Shimadzu CTO-20AC
Controller	: Shimadzu CBM-20Alite
Autosampler	: Shimadzu SIL-30AC
Column	: Shimadzu Shim-pack XR-ODS (150 mmL. × 3.0 mmI.D., 2.2 µm)
Mobile phase A	: 5% MeCN + 0.05% TFA
Mobile phase B	: 95% MeCN + 0.05% TFA
Time program	: 2% (0–1.2 min) →2–98% (1.2–8.9 min) → 98% (8.9–10.8 min) → 98–2% (10.8–11.1 min) →STOP (14 min)
Flow rate	: 1 mL/min
Column temp.	: 40 °C
Sampling	: 160 msec
Slit width	: 8 nm
Time constant	: 160 msec
Wavelength range	: 190 nm-700 nm
Cell light path	: 85 mm
Injection volume	: 1 µL

As shown in Fig. 4a, calibration points for concentrations above 0.5 g/L deviate from linear relationship. In this example, 280 nm was selected manually as the wavelength for correction, and the spectrum used for sensitivity correction was extracted at an intensity of 200 mAU. The absorption ratio was calculated and used to correct the peak areas and extend the linear dynamic range of the calibration curve, resulting in a correlation factor R of 0.9996726 weighted by 1/(concentration)^2 over the concentration range of 0.01 g/L to 1 g/L.

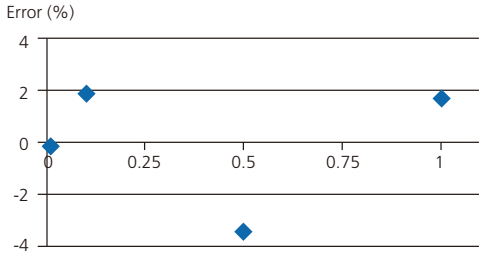
Fig. 4b shows the error in concentration values obtained by inverse estimation using the i-DReC corrected calibration curve with weighting of 1/(concentration)^2 is within 4%. The reproducibility of the peak area (n=6) of the main component and impurities, as well as the peak area ratio between the main component and impurities, is shown in Table 2. An example chromatogram for the sample is shown in Fig. 5. In this example, i-DReC was only applied to correct the peak area of the main component, which provided a saturated signal at 250 nm.

Table 2 Peak area reproducibility of the pharmaceutical sample

Peak	Retention Time(min)	Mean Area (µAUsec)	Area %RSD	Area Ratio(%)
①Main	4.634	31,123,746	0.06	--
②	5.448	925,522	0.12	2.974
③	3.900	64,161	0.08	0.206
④	4.910	32,810	0.15	0.105
⑤	5.091	15,103	0.16	0.049
⑥	4.487	9,487	0.26	0.030
⑦	4.226	7,981	0.28	0.026
⑧	4.975	7,981	0.44	0.026
Imp1	4.056	2,001	0.27	0.006
Imp2	4.331	2,440	0.85	0.008
Imp3	4.376	1,663	0.65	0.005



(a) Calibration curve of the main component



(b) Error of corrected calibration points

Fig. 4 Linearity evaluation

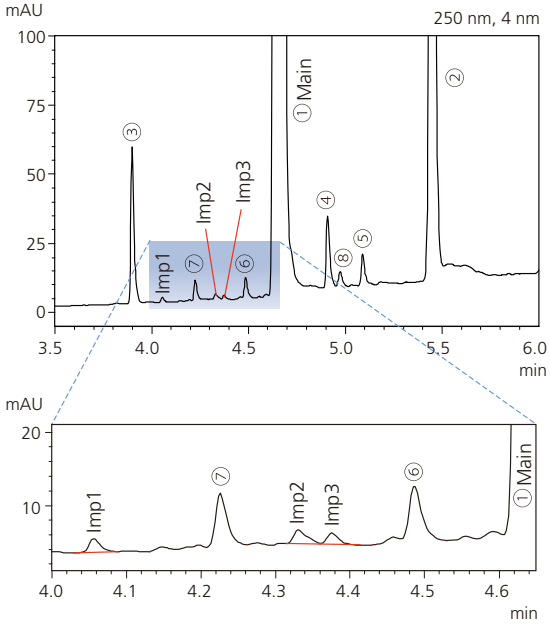


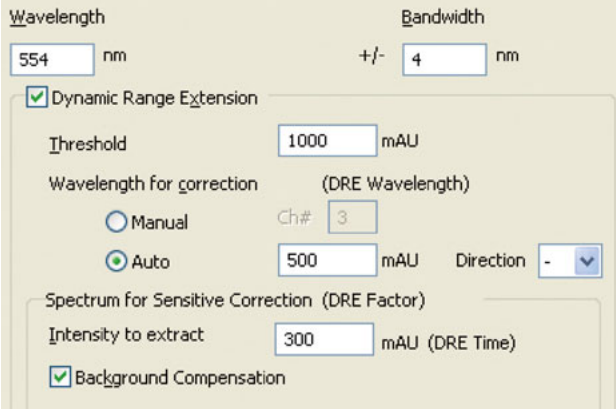
Fig. 5 Chromatogram of the pharmaceutical sample

As shown in Table 2, i-DReC correction provided peak area reproducibility of 0.06% RSD for peak 1, the main component. Peak area reproducibility for impurity peak 3 (Imp3), whose peak area was 0.005% of the main component, was less than 1%.

3. Summary of i-DReC Settings

i-DReC parameters are set as part of the data processing parameters for the photodiode array detector and can be applied in data processing methods for routine analysis without requiring post-run operations. The following is a summary of parameters and their descriptions.

1. i-DReC parameters are set in the multi chromatogram table.



Parameter	Description
Dynamic range extension	Select whether i-DReC is applied or not.
Threshold	When intensity at peak top is over the threshold, i-DReC is applied to the peak for correction.
Wavelength for correction (manual/auto)	Select whether wavelength for correction will be set manually or automatically.
Ch#	When "Manual" is selected, set the channel number of the chromatogram that will be used.
Intensity for correction wavelength	When "Auto" is selected, set the target intensity for determining an appropriate correction wavelength.
Direction (+/-)	When "Auto" is selected, set the direction to search for an appropriate correction wavelength. (+ = longer wavelength, – = shorter wavelength)
Intensity to extract	Set intensity for extraction of sensitivity correction spectrum.
Background compensation	Select whether background compensation is used or not for sensitivity correction spectrum.

The concentration range over which i-DReC effectively extends the linear dynamic range is dependent upon the shape of the spectrum of target peaks. The lower the slope of the spectrum around the correction wavelength, the more reliable the correction.

2. A mark indicating whether i-DReC has been applied, the wavelength used for correction, the retention time of the spectrum used for sensitivity correction, and the calculated sensitivity correction factor can be shown in the peak table and compound table.

Item	Description
Mark	C = peaks to which i-DReC was applied E1-E4 = error in i-DReC calculation
DRE wavelength	For peaks to which i-DReC was applied, this is the wavelength used for correction.
DRE factor	For peaks to which i-DReC was applied, this is the absorption ratio used for sensitivity correction.
DRE time	For peaks to which i-DReC was applied, this is the retention time of the spectrum used for sensitivity correction.

i-DReC requires that spectrum similarity is maintained across the peak. When peak separation is insufficient, i-DReC may not be able to be applied.

3. The peak area and height corrected by i-DReC can be used in normal quantitation processes. The simple implementation allows the seamless use of i-DReC for routine analysis.

4. Conclusion

i-DReC's ability to calculate corrected peak area and height for high concentration samples is made possible by the improved performance of the SPD-M30A photodiode array detector and the excellent reproducibility of the Nexera X2 system. The key features of i-DReC can be summarized as follows.

- Extension of the linear dynamic range using spectral similarity.
- Simultaneous quantitation of both low and high concentration compounds in a single injection.
- Requires the use of only one PDA detector.
- Standard samples are not necessary for correction.
- Simple method settings allow the use of i-DReC in routine anaysis.

i-DReC can be applied to samples containing a wide range of compound concentrations. The use of i-DReC improves the efficiency of sample pretreatment processes and laboratory productivity.

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