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Peak Deconvolution Analysis with Photo Diode Array Detector

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Toshinobu Yanagisawa, Yasuhiro Mito,
Minori Nakashima, Yusuke Osaka, Junichi Masuda,
Okiyuki Kunihiro, Masami Tomita,
Shimadzu Corporation, Kyoto, Japan

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Summary

A photo diode array detector is one of the most commonly used detectors in a high-performance liquid chromatography system. The detector's resolution, sensitivity, linearity and stability have been improved by incorporating sophisticated technology, such as a newly developed capillary cell, a temperature electric control of optical system, and an improved digital signal processing technique.
In addition, from a software point of view, we have developed a peak deconvolution analysis function in order to process a derivative spectrum chromatogram extracting with derivative spectrum values at the specified wavelength. The effect of a target component can be canceled by differentiating at the wavelength of the maximum or minimum intensity of the spectrum.
This function utilizes the selectivity of a derivative spectrum chromatogram to separate unresolved peaks, to detect impurities and to quantitate target peaks on leading or tailing peaks, eliminating the effect from background data. This feature has been applied to several test samples. The evaluation results reported in this study illustrate a new solution for peak separation and impurity analysis.

What is *i*-PDeA, new method for peak separation?

The *i*-PDeA (Intelligent Peak Deconvolution Analysis) is a new calculation method for peak separation utilizing spectrum information. This function can provide alternative peak separation which cannot be performed on the HPLC chromatogram. The *i*-PDeA is based on the spectrum data acquired by a photodiode array detector such as the SPD-M30A, SPD-M20A. Although there is insufficient peak separation, it can extract a single peak as shown in Figure 1.

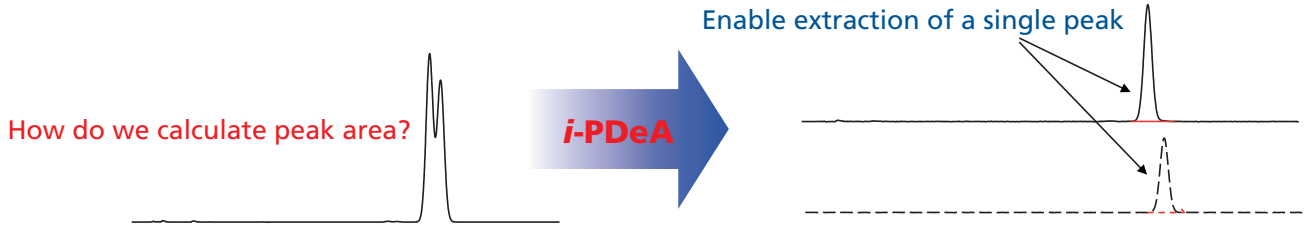

Figure 1: Outline image of the *i*-PDeA function

Principle of the *i*-PDeA

Figure 2 shows an example of two peaks that are not completely separated. Figure 3 (a) at the left side shows spectrums of the two peaks shown in the Figure 4. Figure 3 (b) shows a derivative spectrum of the two peaks shown in Figure 4. The slope of the spectrums in Figure 3 (a) is zero at the λmax or λmin wavelength. In the derivative spectrum in Figure 3 (b), the derivative values become zero at the λmax and λmin wavelengths. When the derivative value of the target single peak is zero, the other peak has a certain derivative value if λmax (or λmin) is different from each other. The *i*-PDeA function can extract derivative spectrum chromatograms as just one chromatographic peak by selecting the λmax or λmin wavelength of the other peak as show in figure 4. By using *i*-PDeA, derivative spectrum chromatograms of each component in the non-separated peaks can be displayed, eliminating the mutual effect. The *i*-PDeA can't separate two peaks if the λ max and λmin of the peaks are perfectly the same because the *i*-PDeA utilizes the spectrum difference of two peaks.

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[Features of the *i*-PDeA function]

- Extraction of a single peak from co-eluted peaks using derivative spectrum chromatograms
- Improvement of quantitation accuracy of non-separated peaks using derivative spectrum chromatograms
- Optimized processing of peak integration methods such as tailing peak treatment, vertical or baseline separation for non-separated peaks is not required

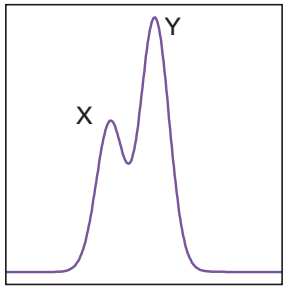

Figure 2: Insufficiently separated chromatogram

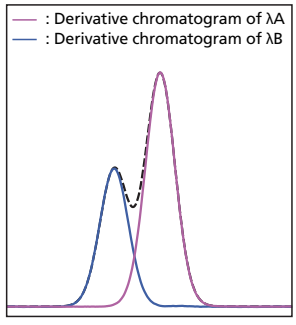

Figure 4: Fully separated chromatograms

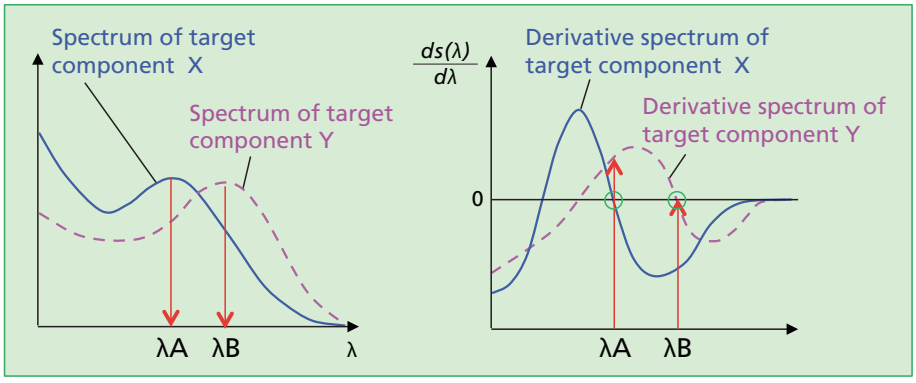

Figure 3: Spectrum of the peaks for two target samples
(a) Normal spectrum acquired with PDA detector (Left)
(b) Derivative spectrum of two peaks (Right)

Application of the *i*-PDeA

Separation for regioisomeric compounds of benzene derivatives

Separation of regioisomers frequently has problems since the chemical structure is very similar to each other although it is important for synthetic chemistry. One typical example, the separation of *o*-aminophenol and *p*-acetamide phenol, is shown in Figure 5. In this example, since the wavelength for which the derivative chromatogram of *p*-acetamide phenol (the blue colored spectra) is zero at 244.8 nm, the derivative chromatogram at 244.76 nm only indicates *o*-aminophenol (the pink colored). Conversely, the derivative chromatogram at 269 nm indicates p-acetamide phenol only. Even when there are co-eluted chromatogram peaks, the *i*-PDeA function provides the solution for complete separation.

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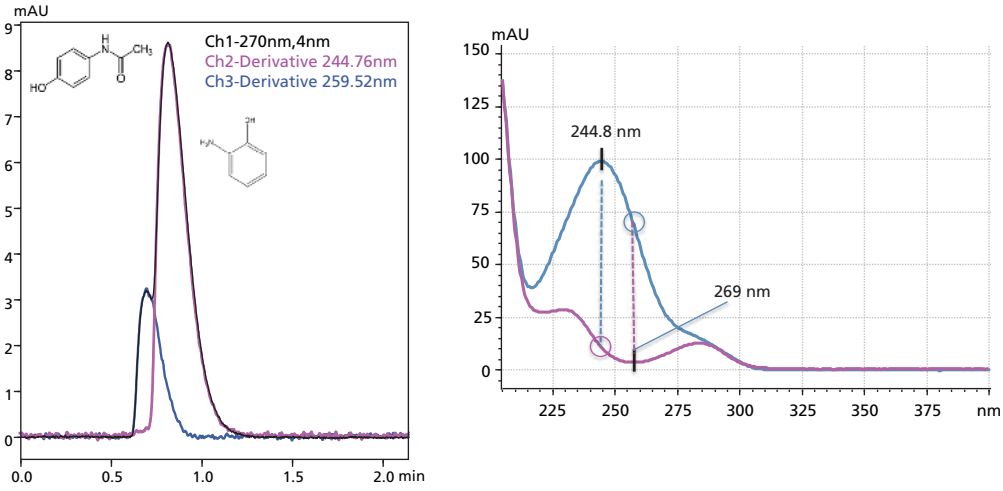

Figure 5: Separation of *o*-aminophenol and *p*-acetamide phenol
Left: Separated chromatogram of each compound (overlapped)
Right: Each spectra of compound

Quantification based on the derivative chromatogram

This shows the *i*-PDeA method applied to another quantitative application. Chromatograms of *o*- and *p*-Chrolophenols are shown in Figure 6. This separation can be performed by the *i*-PDeA function with a derivative spectrum chromatogram at 281 nm for *o*-Chrolophenol and at 274.9 nm for *p*-Chrolophenol, respectively.

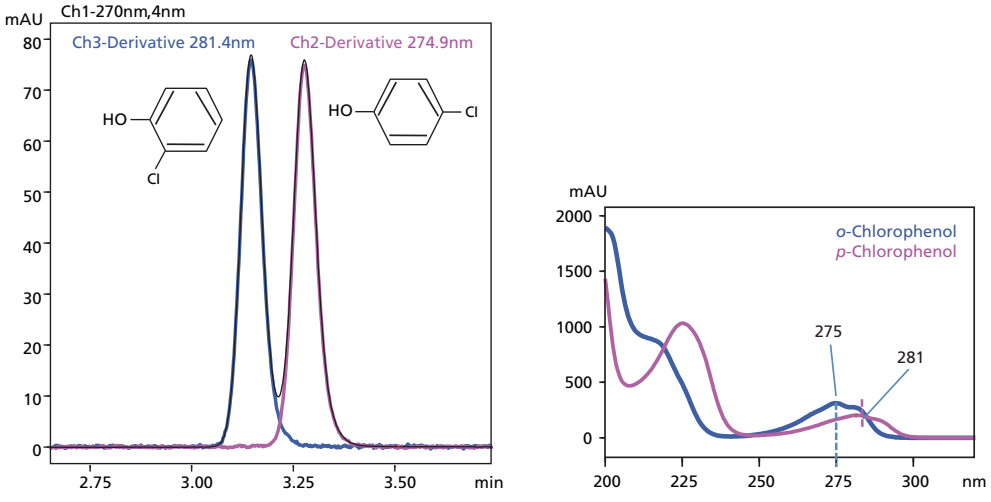

Figure 6: Chromatograms of *o*- and *p*- Chrolophenol

In terms of quantification, the calibration curve can be made based on each derivative spectrum chromatogram directly as with a normal calculation. The calibration curves associated with *o*-Chrolophenor (at left side) and *p*-Chrolophenol (at right side) are shown in Figure 7. Both calibration curves were calculated with each peak area on the derivate spectrum chromatogram, not the normal peak area. The obtained calibration curves indicate good results as each contribution ratio is 0.9999 or more for excellent linearity.

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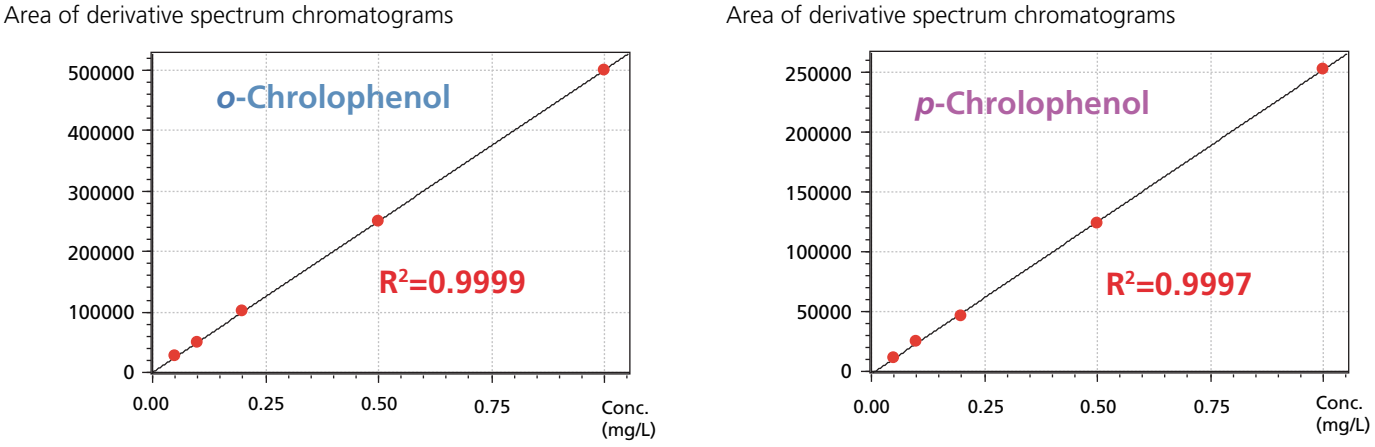

Figure 7: Calibration curve of *o*- and *p*-Chrolophenol

As long as using a derivative chromatogram for both standard and target samples, the quantification procedure is exactly the same for an insufficient separated chromatogram (co-eluted target peak).

Conclusion

- *i*-PDeA, a novel data processing concept, is a useful tool for the separation for co-eluted peaks.
- *i*-PDeA is performed based on the spectrum information acquired by a photodiode array detector.
- A derivative spectrum chromatogram, which is a chromatogram trace at the wavelength with zero point of derivative plot, can provide a single peak of the target even with a co-eluted peak.
- A derivative spectrum chromatogram can also be used for quantitative analysis as a single peak. LabSolutions software has a function for this calibration

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