PO-CON1357E

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Novel Methods for Detection and Quantitation of Impurities Using a New High Sensitivity Photodiode Array Detector

HPLC 2013 FUND-37

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1. Introduction

The presence of impurities in commercial products may seriously affect their quality, resulting in more stringent requirements for impurity monitoring. For this reason, researchers require a more sensitive UHPLC detector.

We have developed a new high sensitivity photodiode array detector with novel data processing methods to detect and quantitate very trace amounts of impurities present in complex samples.

2. High Sensitivity Analysis of Impurities

The SPD-M30A photodiode array detector adopts the high-sensitivity and low-dispersion capillary cell, SR-Cell, which achieves a 0.4 × 10^{-5} AU noise level. The SPD-M30A offers the ultimate in UHPLC analysis, realizing high-sensitivity and high-resolution. The excellent sensitivity and wider dynamic detection range enables simultaneous analysis of a major ingredient and impurity with the ratio of concentrations as low as 0.005% of the main peak (Fig. 1).

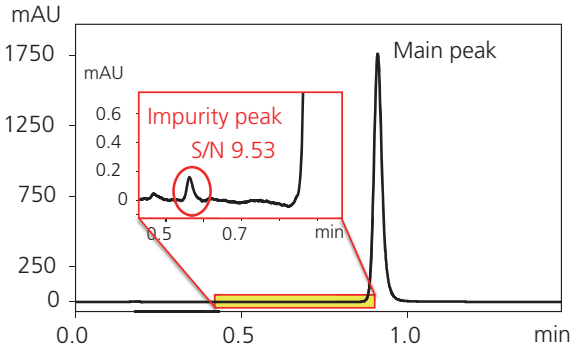

Fig. 1 Chromatogram of ethyl 4-hydroxybenzoate of 0.005% content

3. New Data Processing Method *i*-PDeA

A new data processing method, *i*-PDeA (Intelligent Peak Deconvolution Analysis), allows complete separation of co-eluted peaks and can provide a solution of the problem for determination of the peak purity.

PRINCIPLE

Fig. 2 is an example of two peaks that are not completely separated. Fig. 3 shows UV spectrum of each peak. Derivative spectra will be as shown in Fig. 4. At λmax and λ min of UV Spectrum, the derivative values become zero regardless of the signal intensity. Thus, for example, the derivative chromatogram at the λmax of Compound Y (Fig. 5, pink line) has no effect from compound Y, and then the peak of Compound X can be observed clearly.

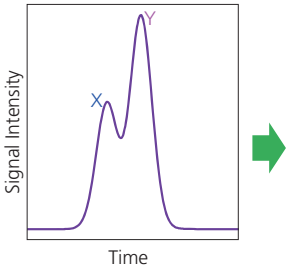

Fig.2 non-separated peaks

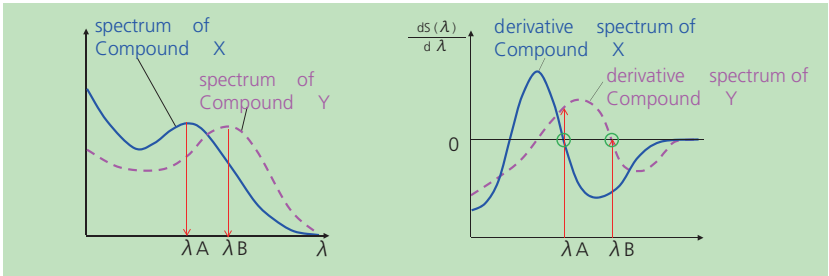

Fig. 3 UV Spectra of each component

Fig. 4 Derivative spectra of each component

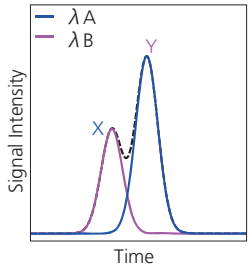

Fig. 5 Derivative chromatograms

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4. Quantitation of a Mixture of Two Components with *i*-PDeA

Valerophenone (VP) standards (1, 10, 50, 100, 200 mg/mL) were analyzed by the UHPLC system equipped with the SPD-M30A photodiode array detector. The data was processed with *i*-PDeA at λmax of difluorobenzophenone (DFBP) to create the calibration curve (Fig. 7). The mixture of VP and DFBP in 5 different ratios (DFBP/VP = 100/1, 100/10, 100/50, 100/100, 100/200) were analyzed and the concentration of VP was quantitated with i-PDeA at same wavelength (Fig. 8).

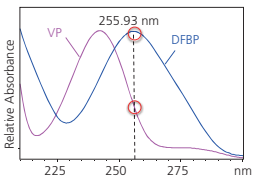

Fig. 6 UV Spectra of Pure VP and DFBP

Sample Conc. [mg/mL]	Area	Observed Conc. [mg/mL]	Error [%]
1	2,984	0.964	-3.62
10	30,368	9.876	-1.24
50	151,922	49.439	-1.12
100	310,801	101.149	1.15
200	613,207	199.572	-0.21

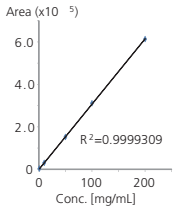

Fig. 7 Calibration data of VP standard sample by derivative chromatogram

Ratio (DFBP/VP)	Area	VP Conc. [mg/mL]	Error [%]
100/1	3,167	1.023	2.30
100/10	30,372	9.878	-1.22
100/50	153,206	49.856	-0.29
100/100	309,596	100.757	0.76
100/200	620,556	201.964	0.98

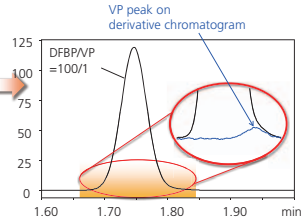

Fig. 8 Quantitation result of VP in DFBP/VP mixed samples

5. New Method of Dynamic Range Extension: *i*-DReC

Another new method, *i*-DReC (Intelligent Dynamic Range Calculation), is a technique that enables an extension in apparent dynamic range by shifting the detection wavelength when the linearity of the calibration curve cannot be maintained due to intensity saturation of the detector. This function avoids the unnecessary sample dilution or re-analysis, and, as a result, improves work efficiency.

PRINCIPLE

Fig. 9A shows an example of a saturated peak at the maximum wavelength (***λa***). First, the spectrum of the peak top is confirmed and the wavelength that the peak is not saturated (***λb***) is determined. The peak area on the chromatogram at ***λb*** is defined as ***Ab*** (Fig. 9B). Second, obtain the unsaturated spectrum and the signal intensities at λa and λb are determined as ***Ia*** and ***Ib***, respectively (Fig. 9C). The sensitivity correction factor ***k*** is calculated as follows:

$$k = Ia / Ib$$

Third, The peak area can be calculated as follows (Fig. 9D):

$$Aa = k \times Ab = Ia / Ib \times Ab$$

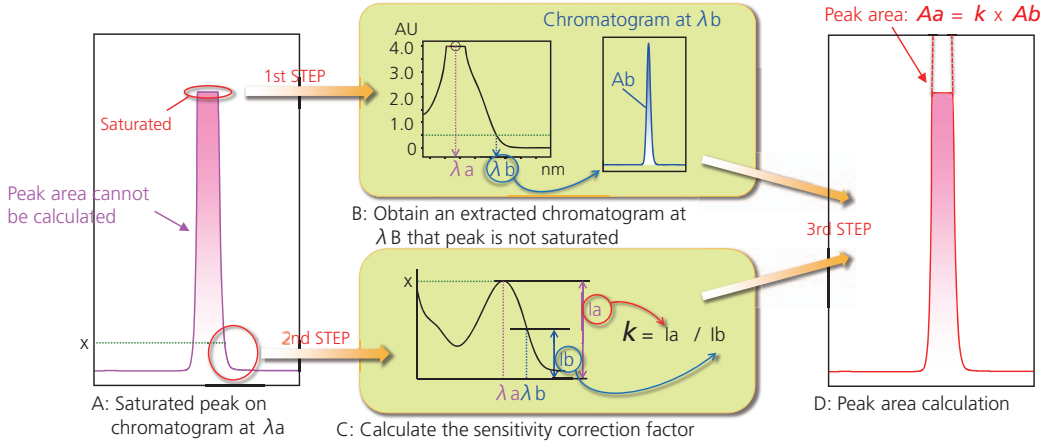

Fig. 9 Principle of i-DReC

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6. Quantitation of Pharmaceutical Samples with *i*-DReC

Pharmaceutical samples from 0.01 g/L to 1 g/L were analyzed by the UHPLC equipped with SPD-M30A photodiode array detector. Fig. 10 shows the calibration curve of the active ingredient with or without *i*-DReC. Calibration points showed the linearity even at 0.5 g/L or higher concentration, while they deviated downward without *i*-DReC.

Reproducibility of peak area (n=6) and area ratios of impurities to the active ingredient are shown in the Table 1. The chromatogram is shown in Fig. 11. *i*-DReC provided 0.06% RSD reproducibility of peak area for the main peak (active ingredient) and less than 1% RSD for its impurity peak of which area ratio was only 0.005% against the main peak.

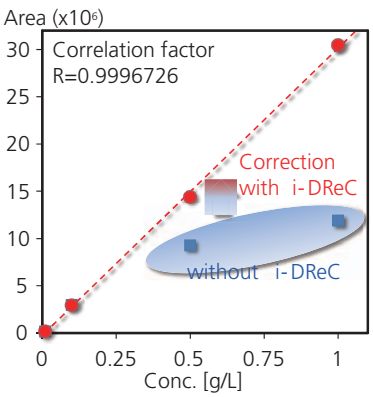

Fig. 10 Linearity evaluation

Table 1 Quantitation results of the pharmaceutical sample

Peak	Retention Time [min]	Mean Area [μAUsec]	Area %RSD	Area Ratio [%]
1, Main	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

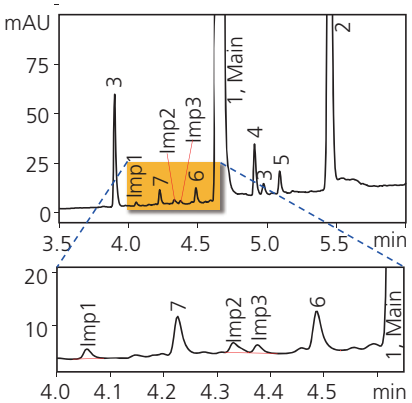

Fig. 11 Chromatogram of the pharmaceutical sample

First Edition: June, 2013

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