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Application Data Sheet

No. AD-0056

Nexera X2- UHPLC

Quantification of Unresolved Peaks in HPLC Analysis Using Novel Intelligent Peak Deconvolution Analysis (*i*-PDeA) Method

Partially overlapping of chromatographic peaks is an often-encountered issue in HPLC analysis of complex samples due to co-elution. Although the conventional PDA detection method allows the use of different wavelengths to improve the resolution, overlapped peaks were not yet possible to be displayed separately and quantified accurately. Recently, Shimadzu Corporation developed a novel data analysis technique for PDA detection, the intelligent Peak Deconvolution Analysis (*i*-PDeA) [1]. By utilizing derivative spectrum chromatograms (DSC), the *i*-PDeA technique offers an unprecedented tool enabling deconvolution, visualization and quantification of overlapped peaks in HPLC analysis with a PDA detector. This Application Data Sheet shows an example of using *i*-PDeA method for deconvolution and quantification of unresolved Paracetamol and Caffeine peaks.

❑ Analysis Conditions

Mixed samples of Caffeine, Paracetamol and Methyl Paraben were analyzed on a UHPLC system with photodiode detector SPD-M30A (details see Table 1). The chromatograms of 260 nm are shown in Fig. 1. It can be seen that Paracetamol and Caffeine peaks were overlapped partially under this condition. Obviously, the unresolved peaks were not suitable for quantitative determination of the compounds by calibration curve method.

Table 1: Analytical conditions

System	: Nexera SR UHPLC
Column	: Shim-pack XR-ODS II (50mmL × 3.0mmID, 2.2 µm)
Mobile phase	: methanol /water (50:50 v/v)
Flow rate	: 0.8 mL/min
PDA model	: SPD-M30A (with SR-Cell)
Wavelength	: 210~400 nm
Injection volume	: 2 µL

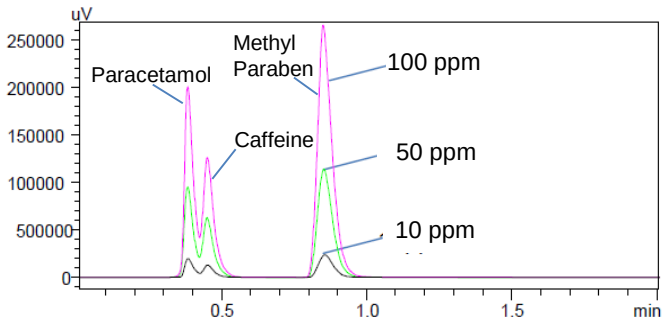

Fig 1: Chromatograms with Paracetamol and Caffeine peaks overlapped by PDA detector at 260 nm

❑ Deconvolution of Unresolved Peaks

The new *i*-PDeA data processing technique enables deconvoluting overlapped peaks completely if their UV spectra are different.

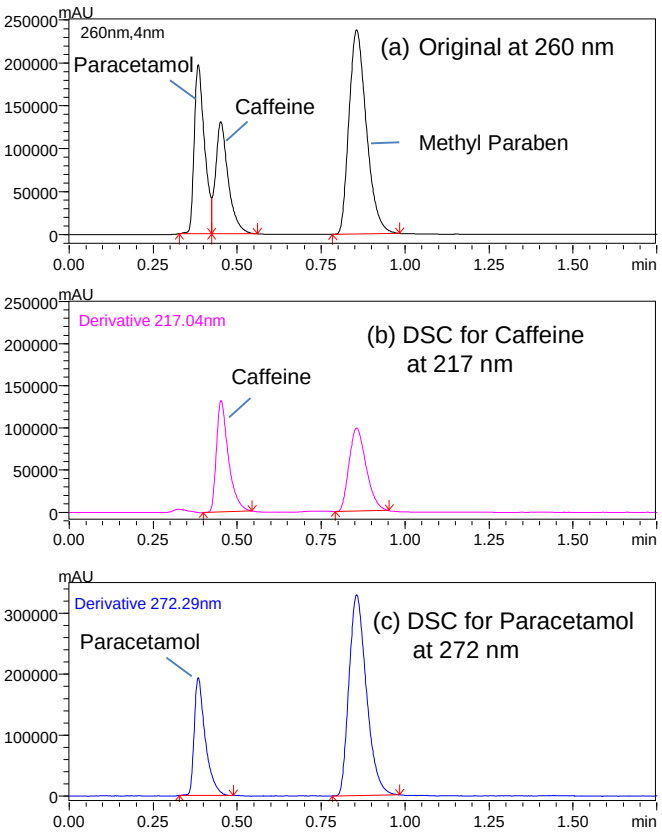

Fig 2: Original chromatogram (a) and derivative spectrum chromatograms (DSC) for Caffeine at 217 nm (b) and Paracetamol at 272 nm (c)

The result of deconvolution of the original chromatogram of the 100 ppm sample is shown in Figure 2(a). Selecting proper Derivative Zero Wavelengths [1] for Caffeine (λ = 217.04 nm) and Paracetamol (λ = 272.29 nm), the two overlapped peaks were deconvoluted completely and displayed separately in derivative spectrum chromatograms (DSC) as shown in Figures 2(b) and 2(c).

❑ Calibration Curves Based on DSC Peaks

As shown in Figure 3, linear calibration curves were established for Caffeine and Paracetamol for the testing range (10~100 ppm) based on their derivative spectrum chromatograms (DSC). Good linearity (R^2 > 0.999) was obtained for both compounds.

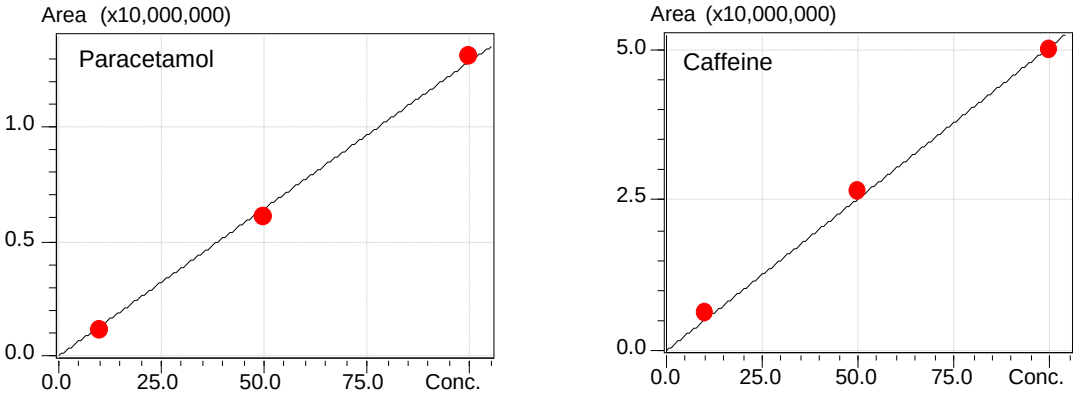

Fig 3: Linear calibration curves of Paracetamol and Caffeine for concentrations of 10, 50 and 100 ppm based on derivative spectrum chromatograms (DSC).

❑ Accuracy of Quantitative Method Based on DSC

A mixed sample of Paracetamol and Caffeine of 20.0 ppm was each prepared separately from stock solution. The concentrations of the sample was determined using the above DSC calibration method. The results were 19.71 ppm and 19.97 ppm for Paracetamol and Caffeine, respectively, corresponding to accuracy of 98.6% and 99.9%.

❑ Summary

Overlapped peaks of Paracetamol and Caffeine were deconvoluted successfully using the *i*-PDeA data processing method. The resolved peaks could be displayed in derivative spectrum chromatogram (DSC). A quantitative method based on the DSC was established and applied to a mixed sample. The accuracy of the results was 98.6% for paracetamol and 99.9% for caffeine.

❑ References

1. Toshinobu Yanagisawa, Technical Report, C190-E166, Shimadzu Corporation (2013)

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