

Technical Report

Using i-PeakFinder™, an Automatic Peak Integration Algorithm, to Provide Labor Savings and Improve the Efficiency of Analytical Operations —Example of its Application to Organic Acid Analysis—

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Abstract:

As the speed of analysis increases, the analysis time required per sample has shortened, which has improved productivity. On the other hand, there is concern that the increasing amount of data generated will require a much longer time for peak integration and data analysis processes. During data analysis, if contaminant peaks overlap with target component peaks, it may not be possible to process the samples adequately using conventional automatic peak integration methods. Manual peak integration 1) is troublesome and time consuming; and 2) can affect data consistency due to differences between results from different analysts. As a solution for such problems, the Technical Report entitled "A New Peak Integration Algorithm for LabSolutions (C191-E044)" described an Analytical Intelligence functionality called i-PeakFinder. The i-PeakFinder software simulate the operations of experts, and allows users with any level of knowledge or experience to acquire highly reliable data, without troublesome or time-consuming procedures. In the following example of the application of the algorithm to organic acid analysis, only simple settings are needed in order to accurately and simultaneously analyze the increasing amounts of data generated from faster sample analysis.

Keywords: Peak integration algorithm, i-PeakFinder, organic acid analysis, Shim-pack Fast-OA

1. Efficiency Bottleneck in Analytical Operations

Improved instrument and column performance has enabled faster analysis speeds and higher throughput. As analysis times per sample get shorter, the volume of data that must be processed continues to increase. On the other hand, peak integration remains a very time-consuming process. Consequently, there are concerns about the huge amount of time now required for data analysis.

When analyzing samples using low UV wavelength detection or under other HPLC conditions that do not provide high selectivity, and when analyzing foods or other samples with complicated compositions, it is not uncommon for peaks from contaminant components to overlap with the peaks from the target components being analyzed. In the case of organic acid analysis using electrical conductivity detection, negative peaks are detected for alcohol and other non-electrically conductive sample components.

Conventional automatic peak integration processes sometimes do not integrate target peaks appropriately if the chromatogram includes contaminant peaks that overlap with target component peaks or includes negative peaks (Fig. 2).

It is possible to perform a certain amount of batch peak integration using a time program (Fig. 3), but overlap by contaminant components must be treated by manual peak integration on an individual basis. Consequently, this does not avoid problems with 1) troublesome and time-consuming procedures and 2) data inconsistency due to differences between the results from different analysts. Therefore, given the increased quantity of data generated from faster sample analyses, there is a need for a peak integration method capable of accurate batch data analysis, and requiring only simple settings.

Shim-pack Fast-OA (Three 7.8mm I.D. × 100mm columns)

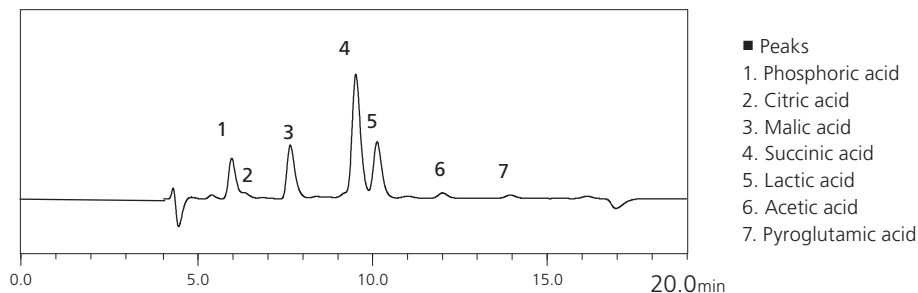


Fig. 1 Analysis of Organic Acids in Cooking Sake

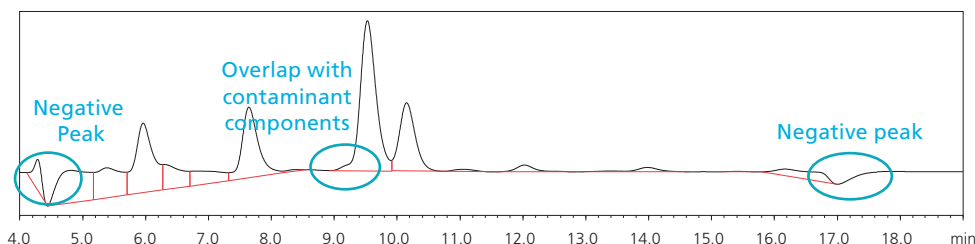


Fig. 2 Conventional Peak Integration Process

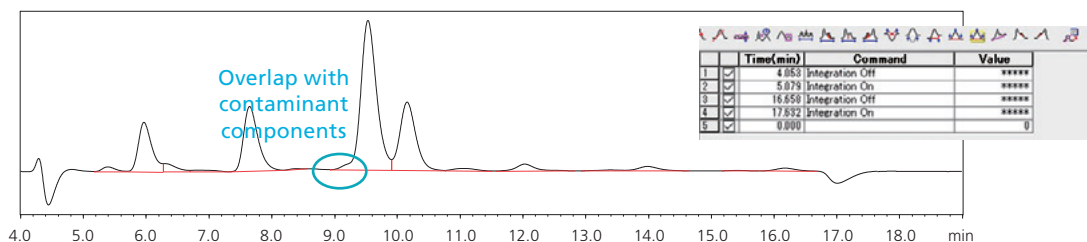


Fig. 3 Negative Peaks Eliminated by a Time Program for Peak Integration (Conventional Method)

2. Peak Integration Algorithm

Shimadzu's peak integration algorithm, shown in Table 1, is based on Analytical Intelligence functionality. The system and software simulate the operations of experts, and allow users with any level of knowledge or experience to acquire highly reliable data, without troublesome or time-consuming procedures.

i-PeakFinder requires only simple parameter settings and can automatically integrate peaks, including shoulder peak detection, even

in complex chromatograms with waveforms that previously required manual peak integration by an analyst. In contrast, Peakintelligence, which is intended for specific method packages, uses AI technology to eliminate the need to adjust parameter settings prior to analysis. For this report, i-PeakFinder, which can be used for all LC and LCMS data, was used to analyze the data from an organic acid analysis.

Table 1 Applicability and Features of the Peak Integration Algorithm

	i-PeakFinder	Peakintelligence™
Applicability	New peak integration algorithm for LabSolutions. Can be used for LC, LCMS, and GC.	Optional LabSolutions Insight™ software. For primary metabolite and cell culture profiling LC-MS/MS method packages
Features	Analytical Intelligence functionality Fully automatic integration function detects peaks accurately, without the need to configure special parameter settings.	Analytical Intelligence functionality Enables expert-level data analysis, using expert peak integration skills learned by AI. No parameter setting adjustments are required prior to analysis.



ANALYTICAL INTELLIGENCE

Automated support functions utilizing digital technology, such as M2M, IoT, and Artificial Intelligence (AI), that enable higher productivity and maximum reliability.

3. Automatic Peak Integration Using i-PeakFinder

i-PeakFinder, which requires simpler parameter settings than conventional methods, can be expected to provide the following benefits.

- Enables accurate shoulder peak detection.
- Baseline processing can be changed easily.
- Reliable peak tracking improves reproducibility.
- Enables correct peak integration, even with fluctuations from baseline drift.

It is easy to switch between the conventional method and i-PeakFinder. The peak integration method can be selected depending on the given situation. For example, the conventional method can be selected to maintain consistency with past data.

There are three basic parameter settings for peak detection: baseline processing, peak integration range, and peak detection threshold. The baseline processing setting can be selected based on the sample or the purpose of the test (Fig. 4).

If there are peaks that do not need to be detected in the first or second half of the chromatogram, specify [Integration range] settings that avoid those peaks, thereby enabling appropriate peak integration.

The [Detection Threshold] setting is used to determine the S/N ratio, based on estimated noise values calculated using Shimadzu's unique algorithm, and then detect peaks higher than a given threshold value. Increasing the [Detection Threshold] value results in the detection of multiple separate small peaks collectively, as a single peak. For comparison purposes, the chromatograms in Fig. 6 show the results of changing the [Detection Threshold] setting from 5 (default value) to 40. (The chromatograms are enlarged from the chromatogram in Fig. 5 between 4 minutes and 11.5 minutes.) Detection methods can be adjusted intuitively.

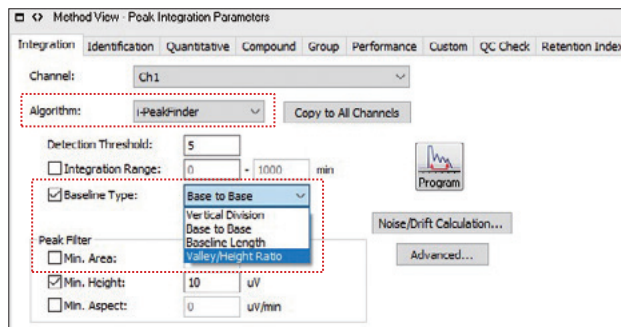


Fig. 4 Tab Page for i-PeakFinder Settings (Method View [Integration Parameter] Tab Page)

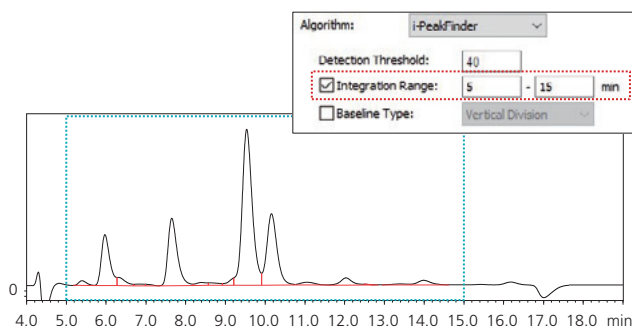


Fig. 5 Specifying the [Integration range] Settings

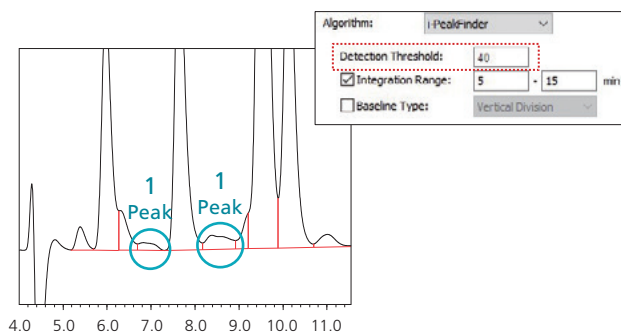
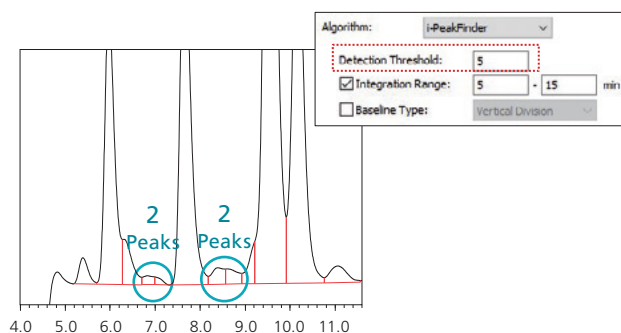


Fig. 6 Specifying the [Detection Threshold] Settings

4. Example of the Use of i-PeakFinder for Organic Acid Analysis

Organic acids in cooking sake were analyzed using the post-column pH-buffered electrical conductivity detection method, which enables selective and high-sensitivity detection of organic acids, in combination with Shim-pack Fast-OA high-speed organic acid analytical columns. The analytical conditions (with overlapping injection) are indicated in Table 2. i-PeakFinder was specified as the peak integration algorithm, and peak integration was performed automatically. The corresponding peak integration results are shown in Fig. 7, and the area reproducibility results are shown in Table 3.

- [Integration range]: 5 to 15 min
- [Detection Threshold]: 40

The results show that baseline processing was the same for the entire chromatogram. Also, excellent area %RSD values of 1.5 % or less were obtained for most of the organic acids. i-PeakFinder integrates the peaks automatically and provides highly accurate results as soon as all the analysis steps are finished. This provides labor savings by improving the efficiency of analytical operations, regardless of the expertise level of the analysts (Fig. 8).

Table 2 Analytical Conditions

Column	: Shim-pack Fast-OA with three columns (100 mm L. × 7.8 mm I.D., 5 μm)
Guard column	: Shim-pack Fast-OA (G) (10 mL × 4.0 mm I.D.)
Mobile phase	: 5 mmol/L p-toluenesulfonic acid (Mobile phase, included in the mobile phase reagent set for organic acid analysis)
Flow rate	: 0.8 mL/min
pH buffering solution	: 5 mmol/L p-toluenesulfonic acid 20 mmol/L Bis-Tris 0.1 mmol/L EDTA (pH-buffering reagent, included in the mobile phase reagent set for organic acid analysis)
Flow rate	: 0.8 mL/min
Detection	: Conductivity detector (CDD-10Avp)
Oven Temperature	: 50 °C
Injection volume	: 10 μL

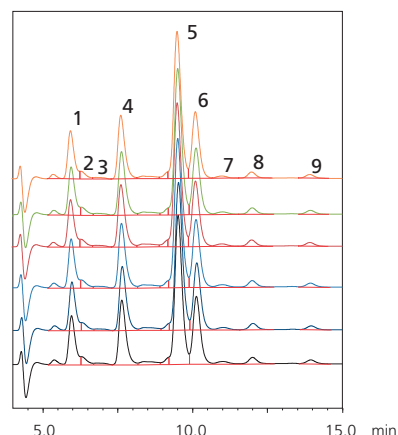


Fig. 7 Chromatogram of Cooking Sake ([Detection Threshold] = 40, n = 6)

Table 3 Area and Area Reproducibility Using i-PeakFinder for Organic Acids in Cooking Sake (n = 6)

	Organic Acid	Area (Mean Value)	Area Reproducibility (%RSD)
1	Phosphoric acid	145038	1.24
2	Citric acid	22133	0.96
3	Pyruvic acid	7734	1.34
4	Malic acid	209206	1.17
5	Succinic acid	515023	1.13
6	Lactic acid	236157	1.15
7	Fumaric acid	12480	3.14
8	Acetic acid	24466	0.83
9	Pyroglutamic acid	15195	1.76

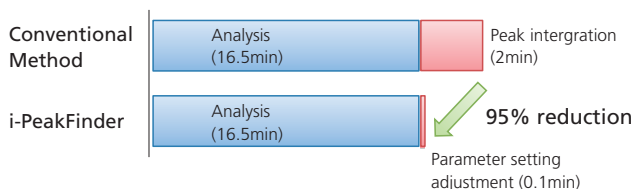


Fig. 8 Sample Analysis and Data Analysis Time per Sample (for Continuous Analysis of 50 Cooking Sake Samples)

Note: For details regarding Shim-pack Fast-OA columns for high-speed organic acid analysis, refer to the Technical Report entitled "High-Speed Analysis of Organic Acids Using Shim-pack Fast-OA and pH-Buffered Electrical Conductivity Detection (C190-E237A)."

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