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Application News

No. L578

High Performance Liquid Chromatography

Comparison of Separation and Selectivity of Nexera™ Organic Acid Analysis System and Reversed Phase-UV Method

Organic acids are organic substances that indicate acidity, and many are ionic compounds containing a carboxyl group. Organic acids are used in a wide range of fields, such as food, chemical, energy, and the environment. In any field, multi-component simultaneous analysis is required.

The simplest method for analysis of organic acids by HPLC is reversed phase separation-ultraviolet absorbance detection (reversed phase-UV method). Although analysis can be done using a combination of commonly used ODS column and UV detector, it is not always possible to obtain accurate quantitation results because organic acids generally have high polarity and thus are difficult to retain in ODS column. Furthermore, due to detection in a short wavelength range, the results are also strongly influenced by contaminants depending on the sample.

The Shimadzu Nexera organic acid analysis system employs the "post-column pH buffering electric conductivity detection method (post-column method)" in which organic acids are separated using ion exclusion chromatography and then mixed with a pH buffering reagent to enhance detection sensitivity. Moreover, results with excellent repeatability can be obtained easily by using a Shimadzu mobile phase and reagent kit for organic acid analysis, which includes the mobile phase and the pH buffering reagent.

In this article, comparative analyses of two different types of samples were conducted using the respective separation-detection methods. As introduced in the following, this experiment demonstrated that highly reliable results were able to be obtained in a short time with reversed phase-UV method depending on the sample, but the high selectivity of the post-column method was effective for analysis of diverse samples.

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Analysis by Reversed Phase-UV Method

This analysis was carried out using a Shim-pack™ GIST C18-AQ HP column for reversed phase separation. The detection wavelength was set to 210 nm. The Shim-pack GIST C18-AQ HP column is suitable for separation of organic acids and other highly polar compounds and can be used with aqueous mobile phases. The particle size is small (3 µm) and sharp peaks can be obtained, which is also an outstanding feature. Fig. 1 shows a chromatogram of the standard mixture, and Table 1 shows the analytical conditions.

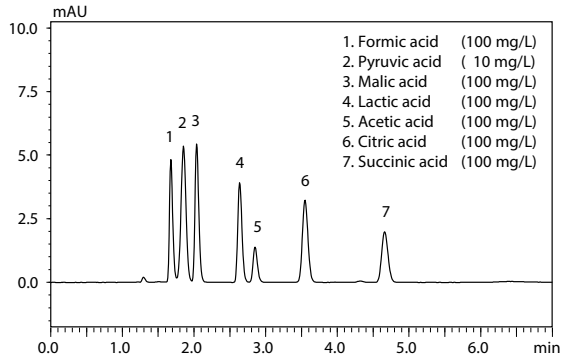


Fig. 1 Chromatogram of Standard Mixture (Reversed Phase-UV Method)

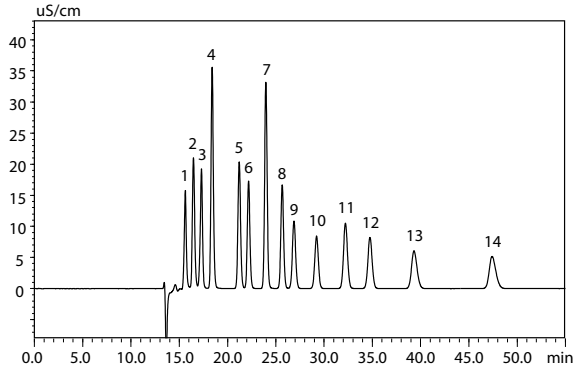
Table 1 Analytical Conditions (Reversed Phase-UV Method)

System	:	Nexera Series
Column	:	Shim-pack GIST C18-AQ HP (250 mm×3.0 mm I.D., 3 µm)*1
Mobile phase	:	10 mmol/L (Sodium) phosphate buffer (pH 2.6)
Flow rate	:	1.0 mL/min
Column temp.	:	40 °C
Injection vol.	:	4 µL
Vial	:	1.5 mL, IC, Polypropylene (manufactured by Shimadzu GLC Ltd.)*2
Detection	:	UV 210 nm

*1: P/N S227-30766-06, *2: P/N GLC-IVS-100

Analysis by Nexera Organic Acid Analysis System

This analysis was carried out by the post-column method using two Shim-pack SCR-102H columns in series for the ion exclusion chromatography. Fig. 2 shows a chromatogram of the standard mixture, and Table 2 shows the analytical conditions.



1. Phosphoric acid
2. Citric acid
3. Pyruvic acid
4. Malic acid
5. Succinic acid
6. Lactic acid
7. Formic acid
8. Acetic acid
9. Levulinic acid
10. Pyroglutamic acid
11. Isobutyric acid
12. Butyric acid
13. Isovaleric acid
14. Valeric acid

(1000 mg/L each)

Fig. 2 Chromatogram of Standard Mixture (Nexera Organic Acid Analysis System)

Table 2 Analytical Conditions (Nexera Organic Acid Analysis System)

System	:	Nexera organic acid analysis system
Column	:	Shim-pack SCR-102H (300 mm×8.0 mm I.D., 7 µm)*3×2
Guard column	:	Guard column SCR-102H (50 mm×6.0 mm I.D.)*4
Mobile phase	:	5 mmol/L *p*-Toluenesulfonic acid*5
Flow rate	:	1.0 mL/min
pH Buffering solution	:	5 mmol/L *p*-Toluenesulfonic acid, 20 mmol/L Bis-tris, 0.1 mmol/L EDTA*5
Mixer	:	Piping unit J*6
Column temp.	:	40 °C
Injection vol.	:	10 µL
Vial	:	1.5 mL, IC, Polypropylene (manufactured by Shimadzu GLC Ltd.)
Detection	:	Conductivity detector

*3: P/N S228-17893-91, *4: P/N S228-17924-91,
*5: P/N S228-61465-91, *6: P/N S228-21747-91

Sample 1: Analysis of Sports Drink

Sports drink was diluted 10 times with water, and an analysis was conducted by the two methods. Fig. 3 shows the chromatogram obtained by reversed phase-UV method, and Fig. 4 shows the chromatogram obtained by the Nexera organic acid analysis system.

In samples like sports drinks that contain comparatively few contaminants, satisfactory separation and selectivity, were able to be obtained also by reversed phase-UV method as the result obtained by Nexera organic acid analysis system. High speed analysis can be done in such case using reversed phase-UV method that allows shorter analysis time compared to that of Nexera organic acid analysis system.

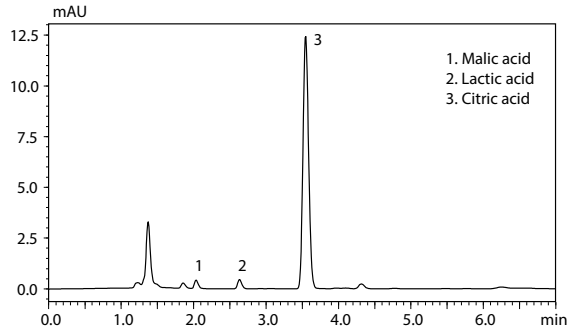


Fig. 3 Chromatogram of Sports Drink (Reversed Phase-UV Method)

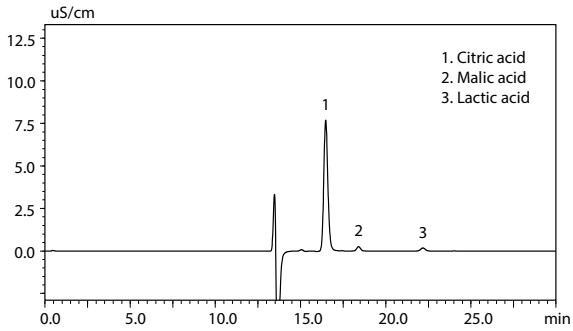


Fig. 4 Chromatogram of Sports Drink (Nexera Organic Acid Analysis System)

Sample 2: Analysis of Red Wine

Next, a commercial red wine was diluted 10 times with water, and the sample was analyzed by the two methods. Fig. 5 and Fig. 6 show the chromatograms obtained by reversed phase-UV method and the Nexera organic acid analysis system, respectively.

As shown in Fig. 5, when red wine was analyzed by reversed phase-UV method, a large number of peaks originating from contaminants were detected. Since these peaks cause poor separation and misidentification, reliability of the analysis results is decreased. Moreover, strongly retained hydrophobic compounds may make analysis time longer because the mobile phase does not contain an organic solvent. Therefore, those compounds may affect the separation of next analysis.

In contrast, the Nexera organic acid analysis system was not affected by contaminants, and results with high selectivity were obtained, as shown in Fig. 6. Thus, when analyzing samples containing many contaminants such as red wine, highly reliable results can be obtained in the shortest possible time by using the Nexera organic acid analysis system.

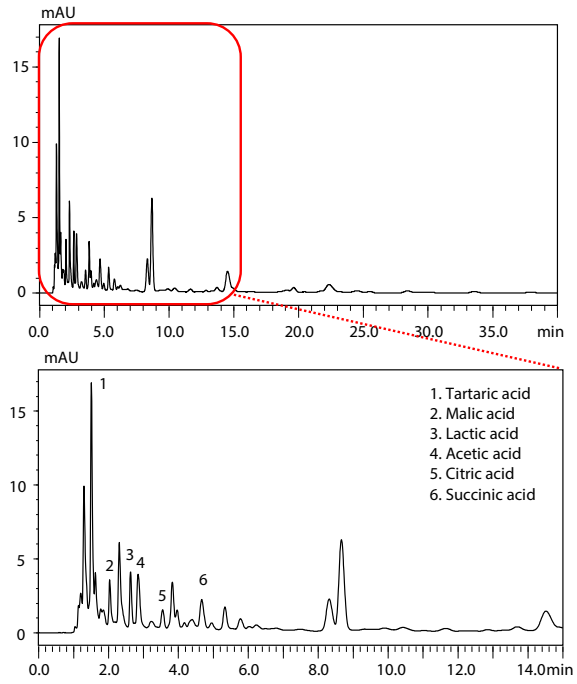


Fig. 5 Chromatogram of Red Wine (Reversed Phase-UV Method; Top: Total Chromatogram, Bottom: Enlargement)

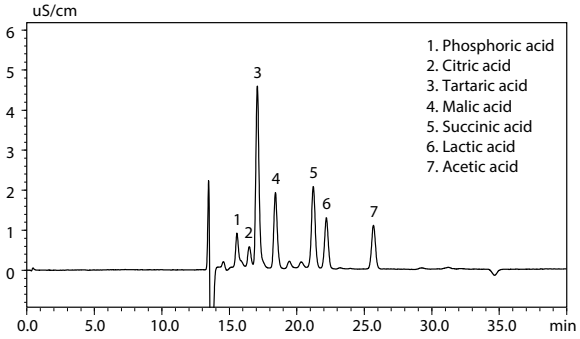


Fig. 6 Chromatogram of Red Wine (Nexera Organic Acid Analysis System)

Conclusion

A comparative evaluation of two methods of reversed phase-UV method and post-column method by the Nexera organic acid analysis system was conducted using same sample analyses. As a result, the appropriate selection from these two methods depending on the samples affords reliable results efficiently because some samples can be analyzed by reversed phase-UV method whereas others require post-column method.

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