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Application News

High Performance Liquid Chromatography

Analysis of Organic Acids in Beer by Nexera Organic Acid Analysis System

User Benefits

- Sensitive and precise analysis of organic acids in beer
- Simplified selection of analytial conditions depending on application goals
- Proven reliability of Organic Acid Analysis System

■ Introduction

During fermentation yeast generates numerous chemical compounds including ethanol, carbon dioxide, aldehydes, alcohols, fatty acids and organic acids (mainly acetic, citric, formic, lactic, malic, succinic, and pyruvic acid), which can influence not only flavor (sour, bitter or salty), but also the pH of beer. Presence of these acids can also inhibit growth of some bacteria, helping to improve shelf-life of beer. Therefore, the control of organic acid content in beer is important.

Ion-exchange chromatography (IC) with gradient elution or ion-exclusion chromatography (IEC) used in isocratic mode are established methods for analysis of organic acids. The acidic eluent usually applied for IEC improves separation of organic acids, but the sensitive conductivity detection is affected by low-ionization grade of the analytes at low pH.

The IEC method used in this application news involves continuous addition of pH-buffering reagent after separation, to increase the pH level to close to neutral. This not only reduces background noise, but also dissociates organic acids from the substance being analyzed, enabling electrical conductivity detection of organic acids with high sensitivity and selectivity. Fig. 1 shows a flow line diagram of the Nexera Organic Acid Analysis System.

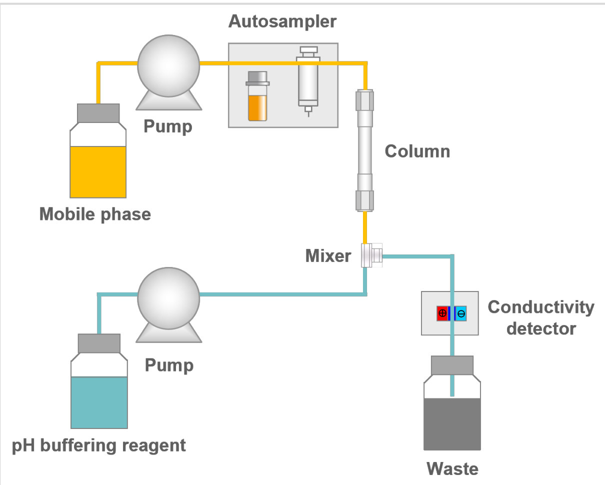

Figure 1: Flow line diagram of Nexera Organic Acid Analysis System

■ Experimental

Materials
The ultrapure water (ASTM Type 1) for preparation of the eluent, reagent and samples was prepared by arium® pro water purification system from Sartorius. All chemicals were of analytical grade.

Sample preparation
Organic acid standard stock solutions were prepared with final concentration of 0.1 mol/L. The beer bottles and cans containing samples were opened and the samples were degassed for 10 min in the ultrasonic bath. After filtration, the samples were diluted with ultrapure water (10-fold) and injected into the HPLC system.

Method
Shimadzu provides two different types of columns for the Nexera Organic Acid Analysis System. The High-Resolution* type was applied in this work for analysis of beer with the goal to separate a high number of organic acids. See analytical conditions in table 1.

Table 1: Analytical conditions

LC system	Nexera Organic Acid Analysis System
Column	2 x Shim-pack SCR-102H** (300 × 8 mm I.D., 7 µm); guard column SCR-102H** (50 × 6 mm I.D.)
Eluent	5 mM p-toluenesulfonic acid
Reagent	5 mM p-toluenesulfonic acid / 0.1 mM EDTA•4H / 20 mM BIS-TRIS
Eluent flow rate	0.8 mL/min
Reagent flow rate	0.8 mL/min
Column oven temperature	50 °C
Detection	Conductivity (53 °C cell temperature)
Autosampler temperature	Room temperature
Injection volume	20 µL
Analysis time	35 min

*Refer to Application News 01-00171-EN (High-Resolution type) and to Technical Report C190-E237A (High-Speed type)
**P/N 228-17893-91 (analytical); P/N 228-17924-91 (guard)

Results and Discussion

Resolution, Limits of Detection, Precision and Linearity
Good resolution (R), low limit of detection (LOD), high precision of retention time and peak area (n=6) were achieved for all organic acids relevant in beer analysis, as can be seen in table 2. The linearity (R^2) in the range of 0.05 mmol/L to 2 mmol/L was higher 0.99998.

Table 2: Results for resolution, limits of detection, and precision of organic acid standard solutions

Component	R	LOD [µmol/L]	%RSD t_R	%RSD area
Citric acid	---	3.24	0.017	1.7
Pyruvic acid	2.1	1.51	0.017	0.1
Malic acid	2.7	1.91	0.015	1.2
Succinic acid	6.1	1.91	0.016	0.5
Lactic acid	2.9	4.98	0.015	0.5
Formic acid	3.7	3.27	0.015	1.2
Acetic acid	3.4	4.38	0.016	0.3
Pyroglutamic acid	5.2	6.22	0.016	0.5

Analysis of Beer
Fig. 2 shows an overlay of chromatograms presenting analysis of five beer samples and a 0.1 mmol/L standard mixture of organic acids. The content of analyzed organic acids is presented in table 3.

Table 3: Content of organic acids [mmol/L] in analyzed beer samples

Component	Beer 1	Beer 2	Beer 3	Beer 4	Beer 5
Citric acid	0.988	5.626	1.152	3.203	1.092
Pyruvic acid	0.425	0.292	0.776	0.447	0.153
Malic acid	0.76	0.924	0.854	0.626	1.019
Succinic acid	0.512	0.408	0.505	0.391	0.536
Lactic acid	1.484	3.502	5.069	0.575	2.034
Formic acid	--	--	--	--	--
Acetic acid	0.648	0.283	0.627	0.547	0.451
Pyroglutamic acid	1.417	0.488	1.66	0.725	1.215

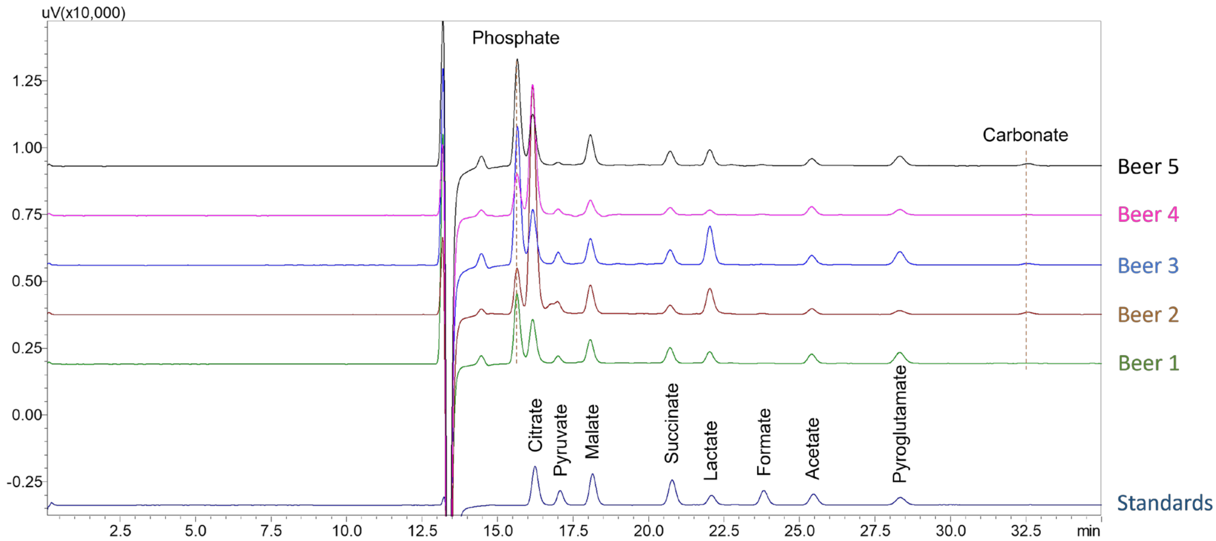

Figure 2: Overlay of chromatograms from analysis of beer samples and a 0.1 mmol/L standard solution

Conclusion

Selectivity and resolution of the separation of organic acids varies with column oven temperature and column capacity. Two 30 cm columns connected in series provided baseline separation for almost all measured organic acids in five beer samples. The ideal column temperature of 50 °C was selected based on tabulated retention indices (refer to Application News 01-00171-EN).

Furthermore, the method shows high repeatability for peak area and retention time. With an LOD in the range 1-6 µmol/L the method is also sensitive for the target compounds. The disadvantage of long analysis time can be compensated by the Shimadzu Overlap function for a series of analytical runs (refer to Application News No.L565 and Technical Report C190-E235).

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