PO-CON23006E

SHIMADZU
Excellence in Science

Analysis of Organic Acids in Beer by Ion-Exclusion Chromatography and Post-Column pH-Buffering Conductivity Detection

AOAC 2023 M037

Uwe Oppermann[1], Vadim Kraft[1], Robert Ludwig[1], Gesa J. Schad[1]
1 Shimadzu Europa GmbH, 47269 Duisburg, Germany

Introduction

Yeast generates during fermentation 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 [1].

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.

This poster presents an IEC method for analysis of organic acids in beer with pHbuffered conductivity detection. The method involves continuous addition of pHbuffering reagent after separation on the column, to adjust 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. Figure 1 shows a flow line diagram of the Nexera Organic Acid Analysis System.

Different combination of column length, mobile phase and column temperature can be selected, according to the analytical requirements. Shimadzu provides two different types of columns for the Nexera Organic Acid Analysis System [2, 3]. The high-resolution type [2], was applied in this work for analysis of beer with the goal to separate a high number of organic acids. Shimadzu studied both types in terms of varied capacity of stationary phase, as well as temperature as a factor influencing selectivity. A list of experimentally determined values of retention time obtained on two Shim-pack SCR-102H columns, connected in series, using different column oven temperature [2] was used to identify optimum separation conditions.

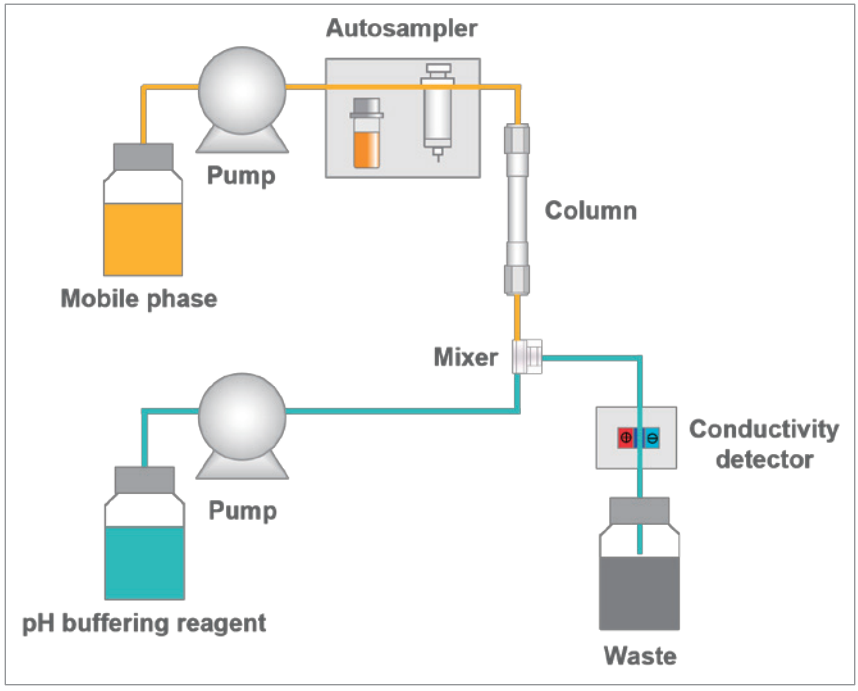


Figure 1: Flow line diagram of Nexera Organic Acid Analysis System

Method

Analytical Conditions

Table 1: Analytical conditions of IEC of organic acids

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 ° cell temperature)
Autosampler temperature	: Room temperature
Injection volume	: 20 µL
Analysis time	: 35 min

Preparation of Standard Solutions and Samples

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 in the HPLC system.

Results

Resolution, Limits of Detection, Precision and Linearity

Baseline resolution, low limit of detection, good precision of retention time and peak area (n=6) as well as proven linearity in the range of 0.05 mM to 2 mM were achieved for all organic acids relevant in beer analysis by IEC using the Nexera Organic Acid Analysis System, as can be seen in table 2.

Table 2: Results for resolution R, limits of detection LOD, precision and linearity in the IEC analysis of organic acid standard solutions

Component	Resolution	LOD [µM]	%RSD t_R	%RSD area	R^2
Citric acid	---	3.24	0.017	1.7	> 0.99999
Pyruvic acid	2.1	1.51	0.017	0.1	> 0.99999
Malic acid	2.7	1.91	0.015	1.2	> 0.99999
Succinic acid	6.1	1.91	0.016	0.5	> 0.99999
Lactic acid	2.9	4.98	0.015	0.5	> 0.99999
Formic acid	3.7	3.27	0.015	1.2	> 0.99999
Acetic acid	3.4	4.38	0.016	0.3	> 0.99999
Pyroglutamic acid	5.2	6.22	0.016	0.5	> 0.99998

Analysis of Beer Samples

Figure 2 shows an overlay of chromatograms presenting analysis of five beer samples and a 0.1 mM standard mixture of organic acids. The content of analyzed organic acids is presented in table 3.

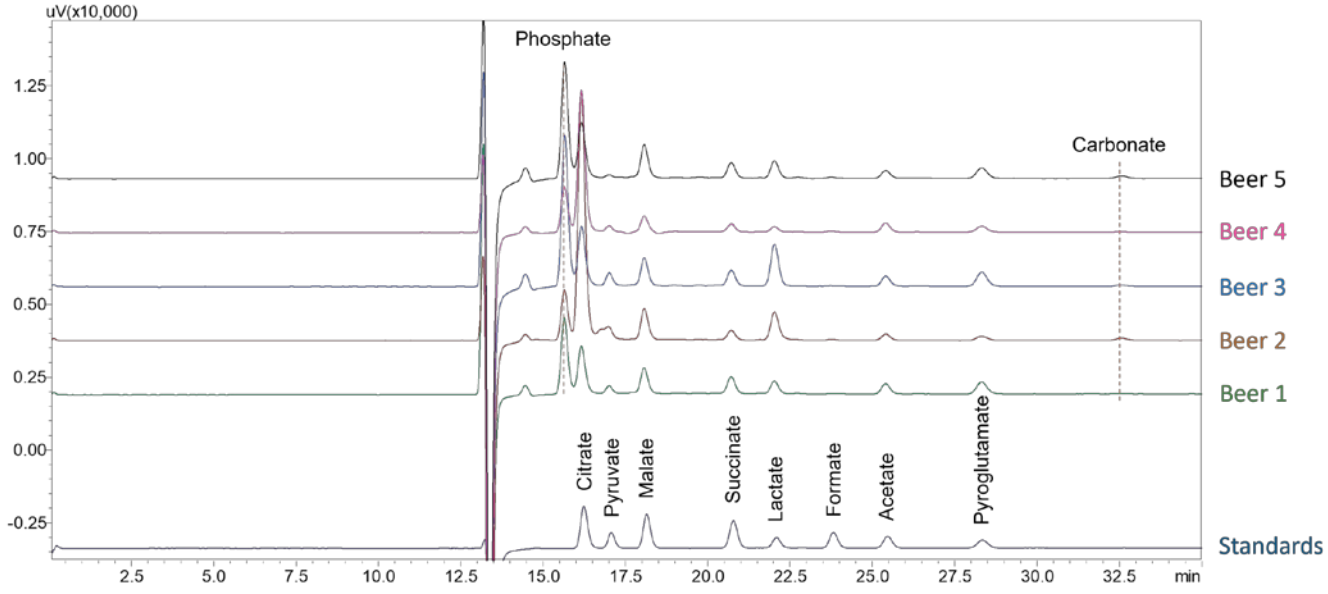


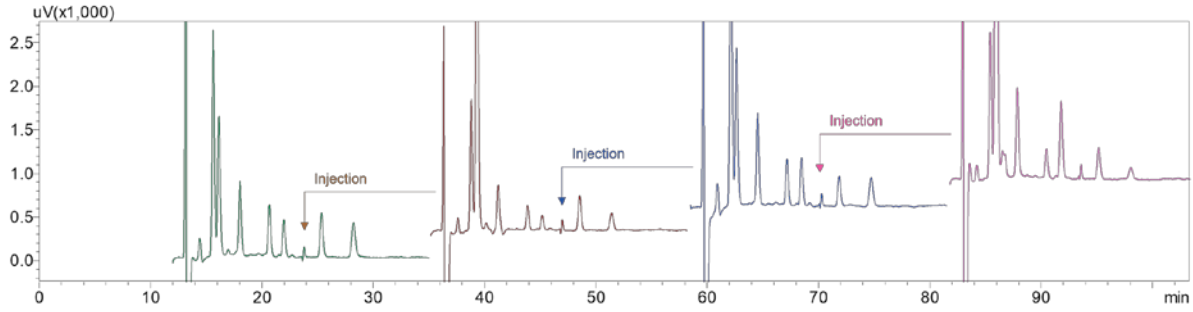
Figure 2: Overlay of chromatograms from analysis of beer samples and 0.1 mM standard solution

Table 3: Content of organic acids [mM] in analyzed beer sample

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

Overlap Function

The overlap injection function contributes to reduction in analysis time. Figure 3 demonstrates the function on an example of a batch analysis of beer samples, leading to a total time saving of 13 min per run starting with the 2nd injection.



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 [2]. 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.

References

[1] Anderson, H.E., Santos, I.C., Hildenbrand, Z.L., Schug, K.A. (2019). Analytica Chimica Acta, 1085, 1-20.
[2] Shimadzu Application News No. 01-00171-EN – Nexera Organic Acid Analysis System and its Application.
[3] Shimadzu Technical Report C190-E237A – High-Speed Analysis of Organic Acids Using Shim-pack Fast-OA and pH-Buffered Electrical Conductivity Detection.

First Edition: December, 2023

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/