

Analysis of Organic Acids in Aqueous Samples

Application

Food and Beverage Analysis

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Abstract

The ZORBAX SB Aq column was successfully used to separate organic acids by Ion Suppression Chromatography at low pH using Reversed Phase Liquid Chromatography columns and Diode Array Detection. Two standard mixtures of organic acids were separated and a number of softdrinks and wines were analyzed.

Introduction

Organic acids are an important class of compounds that must be analyzed in different samples, for example, fruit drinks, wines, and many other aqueous samples. Under most reversed-phase liquid chromatography (RPLC) conditions, organic acids are difficult to retain and hence to analyze.

For this type of analysis, a number of major issues must be considered: Under most usually applied reversed-phase separation conditions these acids are ionized and show little or no retention. The application of ion suppression chromatography (ISC) is a convenient and straightforward approach to increase retention and selectivity. In ISC the pH of the eluent must be about 2 pH units lower than the pK_a -values of the analyte acids. Under such conditions, acids are in their molecular form and can be retained and separated.

In addition, under ISC-conditions the eluent is usually nearly completely aqueous, which may result in collapsing many standard bonded stationary phases. Specific RPLC-phases, for example, the ZORBAX SB Aq column can easily be applied under completely aqueous conditions. Furthermore, under acidic eluent conditions, many RPLC-columns are not very stable over an acceptable period of time.



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It was found, however, that the use of bulky side chains significantly improves column/bonded phase longevity under acidic conditions [1]. This concept of stable bond technology has resulted in a number of StableBond (SB) reversed phase columns. This group of columns has already proved their substantial column longevity under acidic eluent conditions [2]. The ZORBAX SB Aq column was specifically designed to operate under completely aqueous conditions without phase collapsing. At the same time the column longevity under acidic conditions is excellent.

Experimental

The organic acids selected for study are listed in Table 1.

Table 1. Organic Acids Selected for Study

Acid	MW	Formula	Std #	RT (min)
Tartaric	150.09	C ₄ H ₆ O ₆	1, 2	1.86
Malic	134.09	C ₄ H ₆ O ₅	1, 2	2.15
Lactic	90.08	C ₃ H ₆ O ₃	1	2.23
Ascorbic	176.12	C ₆ H ₈ O ₆	2	2.28
Acetic	60.05	C ₂ H ₄ O ₂	1, 2	2.42
Citric	192.12	C ₆ H ₈ O ₇	1, 2	2.94
Succinic	118.09	C ₆ H ₄ O ₆	1	3.19
Maleic	116.07	C ₄ H ₄ O ₄	2	3.47
Fumaric	116.07	C ₄ H ₄ O ₄	1, 2	4.50

Samples and Sample Preparation

Two standard solutions were prepared due to co-elution of lactic acid and ascorbic acids:

- Standard solution 1: tartaric acid, malic acid, lactic acid, acetic acid, citric acid, succinic acid, and fumaric acid.
- Standard solution 2: tartaric acid, malic acid, ascorbic acid, acetic acid, citric acid, maleic acid, and fumaric acid.
- Typical concentrations of the standard solutions were between 0.1 mg/mL for maleic acid and 2.5 mg/mL for citric acid.

Orange, apple, and grape juices, lemonades, and white wines were successfully analyzed. Sample preparation consisted of filtration (0.45 µm) and occasional dilution of the samples.

Chromatographic Conditions

The different organic acids were analyzed in the ISC mode. The eluent pH was adjusted at least 2 pH units below the pK_a-value of the acids. Detection was performed using Diode Array Detection (DAD).

Conditions

Column:	4.6 mm × 150 mm × 5 µm ZORBAX SB-Aq
Mobile phase:	20 mM aqueous phosphate buffer pH 2.0/acetonitrile=99/1(v/v)
Flow rate:	1.0 mL/min
Injection volume:	1 µL
Column temperature:	25 °C
Detection:	UV-DAD detection wave- length/window 210/8 nm, reference wavelength 360/80 nm
Sample preparation:	Filtration over 0.45-µm filter

Equipment

- Agilent 1100 series HPLC
- Vacuum degasser
- Quaternary pump
- Autosampler
- Thermostatted column compartment
- DAD
- Agilent ChemStation + software

Statistics

In order to check the quality of this analysis protocol, the reproducibility of standard organic acid mixtures, and linearity and minimum detectable amount for maleic acid were determined.

Results

Reproducibility

The organic acid standard (solution 2) was analyzed six times, see Tables 2 and 3. It is assumed that the reproducibility for the analysis of standard solution 1 would be very similar to that for standard solution 2.

Table 2. Retention Times (min)

Compound	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Average	% RSD
Tartaric acid	1.888	1.888	1.890	1.889	1.888	1.887	1.888	0.05
Malic acid	2.184	2.186	2.190	2.186	2.186	2.186	2.186	0.09
Ascorbic acid	2.312	2.315	2.324	2.314	2.315	2.317	2.316	0.18
Acetic acid	2.443	2.440	2.445	2.442	2.441	2.441	2.442	0.07
Citric acid	3.031	3.032	3.038	3.027	3.022	3.028	3.030	0.18
Maleic acid	3.635	3.636	3.639	3.635	3.636	3.653	3.639	0.19
Fumaric acid	4.746	4.742	4.739	4.741	4.739	4.733	4.740	0.09

The reproducibility of retention times (RTs) was <0.2% relative standard deviation (RSD).

Table 3. Observed Peak Areas, (mAU*s), for Seven Replicate Runs

Compound	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Average	% RSD
Tartaric acid	100.40	100.50	100.70	100.30	100.60	100.50	100.50	0.14
Malic acid	107.00	107.20	106.90	107.50	107.00	107.10	107.12	0.20
Ascorbic acid	220.80	221.40	220.20	220.10	219.30	217.60	219.90	0.60
Acetic acid	118.80	118.90	118.90	119.20	118.90	118.40	118.85	0.22
Citric acid	168.80	168.50	168.60	169.80	169.90	168.30	168.98	0.41
Maleic acid	500.00	500.10	501.10	501.50	501.30	499.30	500.55	0.17
Fumaric acid	657.40	658.00	657.90	658.20	657.50	655.90	657.48	0.13

The reproducibility of the peak area was <0.6 % RSD.

Linearity

Seven solutions with different concentrations of maleic acid were each analyzed twice. A calibration curve was calculated using ChemStation software, and shown in Figure 1. The correlation factor was 0.99994.

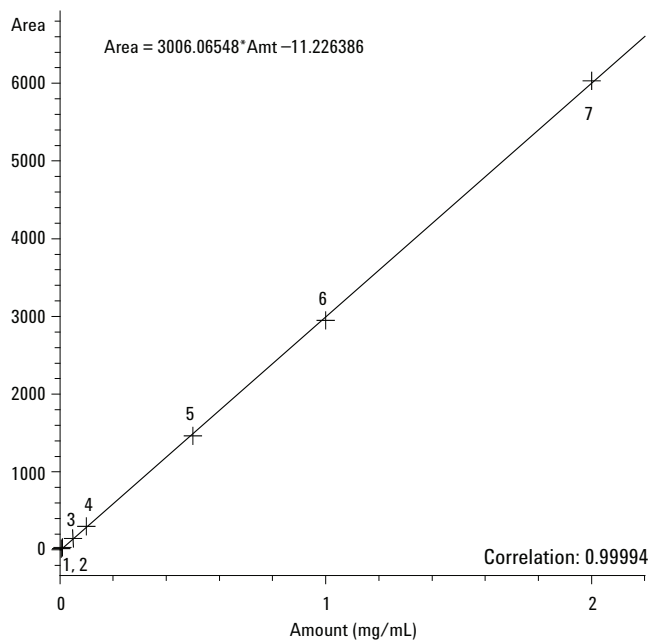


Figure 1. Calibration curve for maleic acid.

Minimum Detectable Amount

The noise of the baseline was 0.03 mAU. The minimal detectable peak height was 0.09 mAU. This peak height is equivalent to a concentration of 0.24 mg/L maleic acid.

Chromatographic Examples

Examples of the separation of organic acids in standards, orange juice, apple juice, and white wine using the ISC mode on the ZORBAX SB-Aq column, are shown in Figures 2–6.

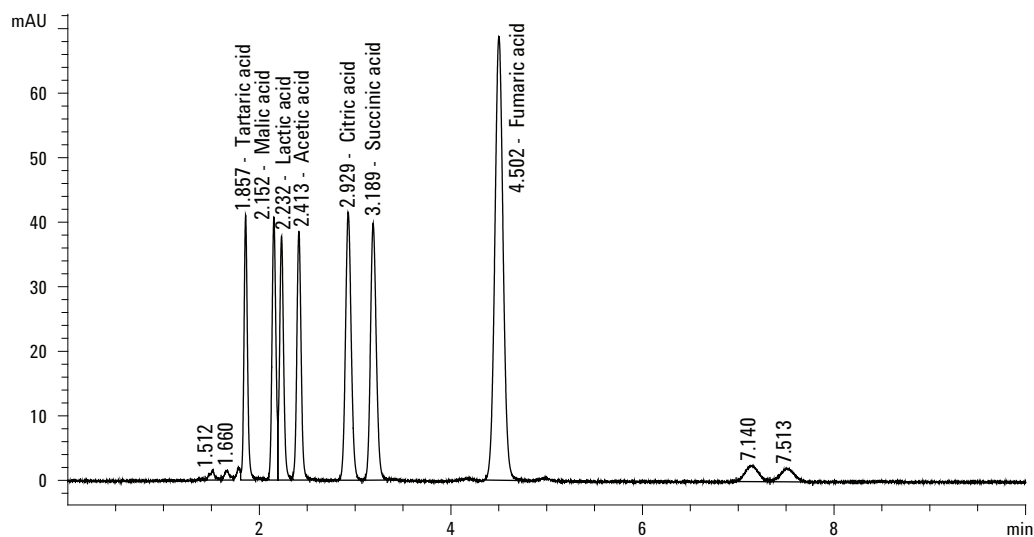


Figure 2. Standard mixture 1.

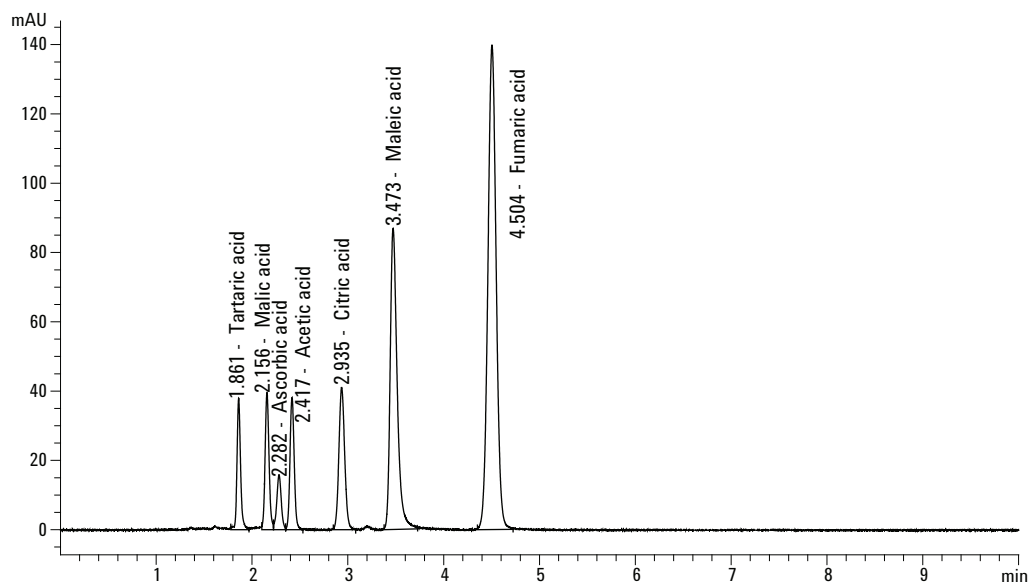


Figure 3. Standard mixture 2.

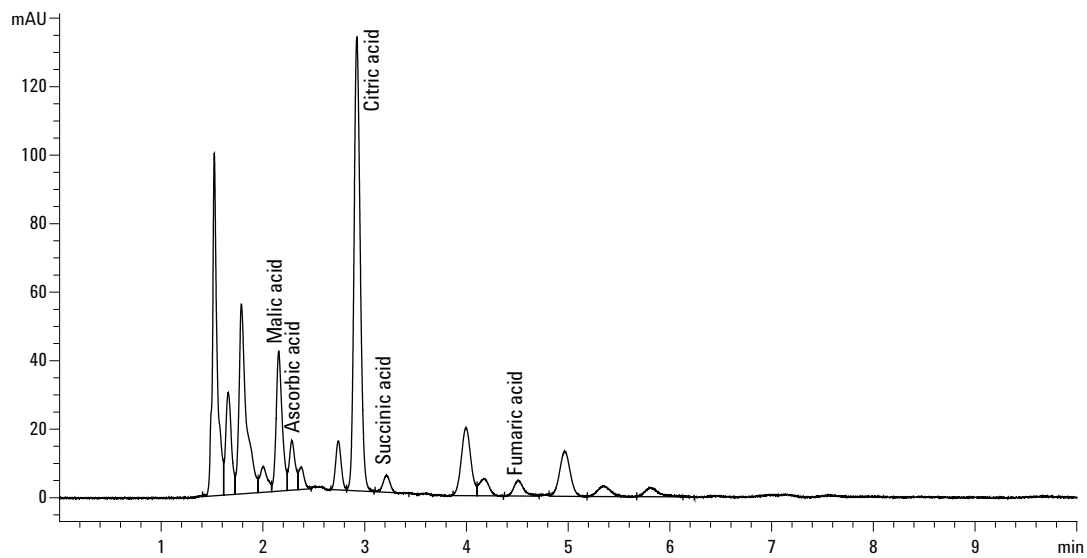


Figure 4. Orange juice.

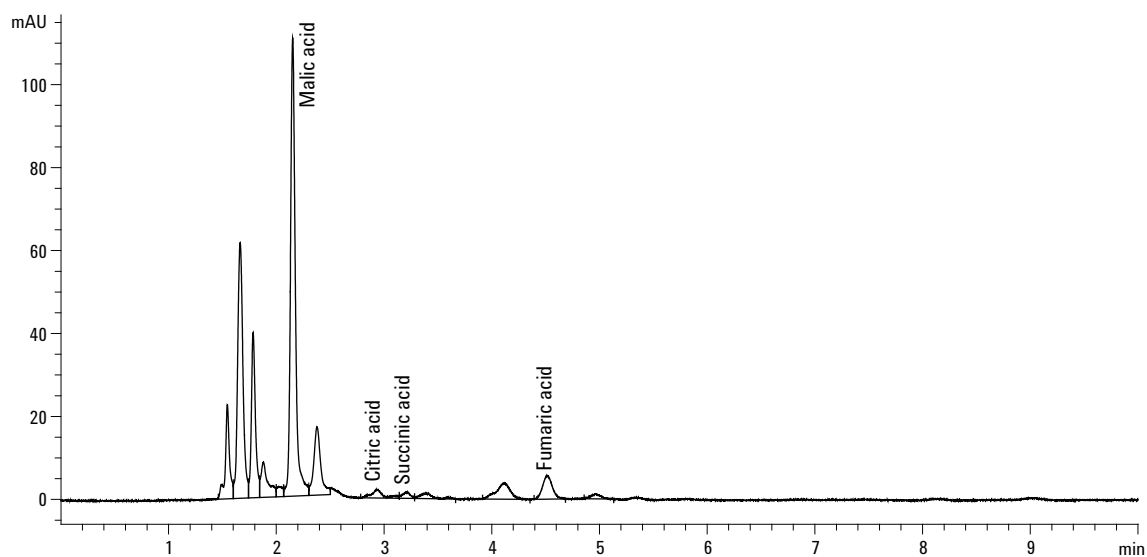


Figure 5. Apple juice.

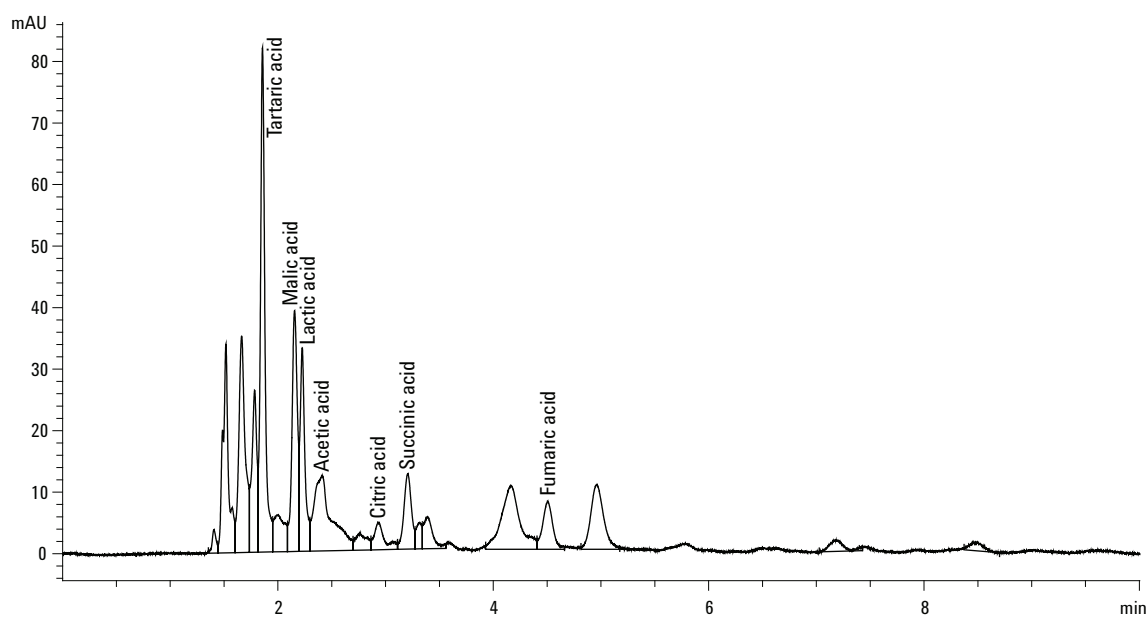


Figure 6. White wine.

Conclusions

- Ion suppression can be used to retain and analyze for organic acids.
- The ZORBAX SB-Aq column separates organic acids with good peak shape and retention at low pH (ion suppression) and under high aqueous conditions.
- This column has an alkyl bonded phase structure and is also designed to prevent phase collapse. At the same time, very hydrophilic compounds are retained under completely aqueous conditions.
- The ZORBAX SB-Aq column has a unique selectivity and resolution for very polar compounds.

References

1. J.J. Kirkland, J.H. Glajch, and R.D. Farlee, (1989) *Anal. Chem.*, **61**, (1): 2-11.
2. H.A. Claessens, (2001) *Trends in Analytical Chemistry*, **20**, no. 10.

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