Method for the Analysis of Inorganic and Organic Acid Anions in All Phases of Beer Production Using Gradient Ion Chromatography

Stephanie Boyles, *Analytical Laboratory, Coors Brewing Company, BC600, Golden, CO 80401*

ABSTRACT

An accurate analytical method has been developed to separate and quantify nine organic acids (acetate, lactate, formate, pyruvate, succinate, malate, maleate, oxalate, and citrate) and six inorganic anions (fluoride, chloride, bromide, nitrate, sulfate, and phosphate) in all phases of beer production, from wort to the final package. The organic acids and inorganic anions were separated in a 43-min chromatographic run. This method uses gradient ion chromatographic separation using the Dionex Omni-Pac PAX-500 column with suppressed conductivity detection. Possible interferences, such as coelution of analytes and matrix effects in the beer, were resolved and verified. The quantitative measurement of anions in beer products has not been previously available by isocratic ion chromatographic methodology. With gradient ion chromatography, the inorganic anions in beer can be accurately separated and quantified without the problem of interferences with organic acids. The linearity, precision, and percent recovery of the method throughout all phases of beer production also were determined. Sample treatment requires a dilution with Milli-Q water followed by a C_{18} Sep-Pak cleanup and filtration through a 0.45-μm nylon filter. This method provides an accurate, quantitative, and simultaneous measurement of inorganic anions and organic acids in beer products.

Keywords: Gradient ion chromatography, Inorganic anions, Organic acids

Inorganic anions from brewing water and organic acid anions from malt and yeast metabolism during fermentation have an important impact on beer flavor and physical appearance (2). The measurement of the concentrations of inorganic and organic acid anions, in all phases of beer production, can be used to help track metabolic products of fermentation and correlate beer flavor trends.

A variety of methods exists for the determination of inorganic anions and organic acid anions in beer and wort. There are several ion chromatographic methods available to separately determine inorganic anions (3,4) and organic acid anions. Ion-exclusion chromatography has been used for determining six organic acids in beer and wort (1). The simultaneous determination of organic and inorganic acid anions in beer products was not previously available by ion chromatographic methodology.

An accurate analytical method was therefore developed to separate and quantify the nine organic acid anions (acetate, lactate, formate, pyruvate, succinate, malate, maleate, oxalate, and citrate) and six inorganic acid anions (fluoride, chloride, bromide, nitrate, sulfate, and phosphate). All phases of beer products, wort through package and competitors' products, were investigated. The objective was to separate both inorganic and organic acid anions in one analysis run, using gradient ion chromatography with suppressed conductivity detection. The biggest obstacle was finding the optimum gradient conditions for the best resolution.

MATERIALS AND METHODS

Gradient Chromatography Instrumentation

The ion chromatographic system for this analysis included a gradient pump, an anion trap precolumn, an anion micromembrane suppressor, and the AutoRegen (Dionex, Sunnyvale, CA) accessory for the regenerant. The sample loop size was 10 μl, and the eluent flow rate was 1.0 ml/min. The separation of the inorganic and organic acid anions was accomplished in a 43-min analysis run, with a 15-min reequilibration step, using gradient ion chromatography with the Dionex Omni-Pac PAX-500 column (Dionex, Sunnyvale, CA) and suppressed conductivity detection. This column, consisting of 55% divinylbenzene cross-linked macroporous beads with ion exchange sites, allows for different separation mechanisms all on one column, i.e., ion exchange, ion suppression, or reverse phase. This gives the operator the flexibility to change the column selectivity by changing the eluent concentrations and/or composition for better resolution.

Eluent Preparation

All eluents and standards and the regenerant were prepared using Milli-Q deionized water (Millipore Corporation, Bedford, MA) or the equivalent (water with resistance of at least 17 MΩ and free of ionized and organic impurities). To prepare the first

eluent, 760 ml of deionized water was degassed for 10 min by sparging with helium. The eluent bottle was opened and 140 ml of 100% methanol and 100 ml of 100% ethanol were added. The bottle was closed and sparged to mix and degas for 5 min, but not longer, to avoid evaporating the solvent. The eluent bottle was opened again, and 13 μl of 50% NaOH solution (19.1N), carbonate-free, was added for a final NaOH concentration of 0.25 mM. The eluent bottle was closed and pressurized.

The second eluent was prepared by degassing 770 ml of deionized water for 10 min by sparging with helium. The eluent bottle was opened, and 230 ml of 100% ethanol was added. The bottle was closed and sparged to mix and degas for 5 min, but not longer, to avoid evaporating the solvent. The eluent bottle was opened again, and 4.1 ml of 50% NaOH (19.1N), carbonate-free, was added for a final eluent concentration of 79 mM NaOH. The eluent bottle then was closed and pressurized.

It is essential to keep hydroxide eluents under helium pressure at all times to prevent absorption of carbon dioxide into the eluents, which leads to the production of carbonate contaminants and causes high background conductivity and erratic results.

The 0.025N sulfuric acid regenerant was prepared by diluting 100 ml of the stock solution of sulfuric acid (7 ml of concentrated sulfuric acid per liter of deionized water) to 1 L using deionized water. The regenerant flow rate was 12–15 ml/min. The regenerant was made up fresh each time before starting the method. Approximately 250 ml of regenerant was pumped to waste through the suppressor and the AutoRegen cartridge before the regenerant was recycled and the method was started.

Sample Preparation

All samples, such as wort and fermenter samples, were first clarified by centrifugation and kept cold, not frozen, until the day of analysis. The only sample treatment necessary for all phases of the clarified beer products was a dilution with deionized water (1:1 for package beer and 1:4 for fermenter, storage, and wort). The samples then were passed through a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA) and filtered with a 0.45-μm nylon filter. The prepared samples then were placed into each autosample vial and capped for analysis.

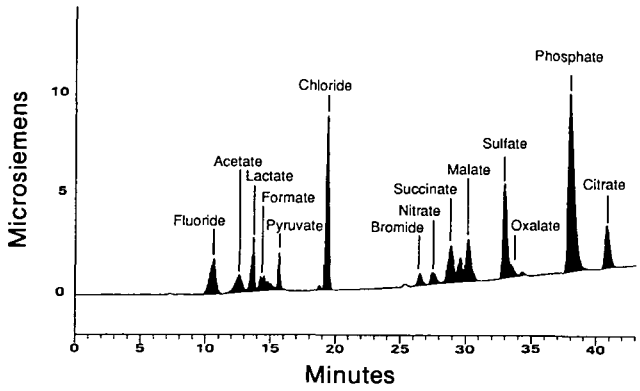

Fig. 1. Fermenter beer sample chromatogram.

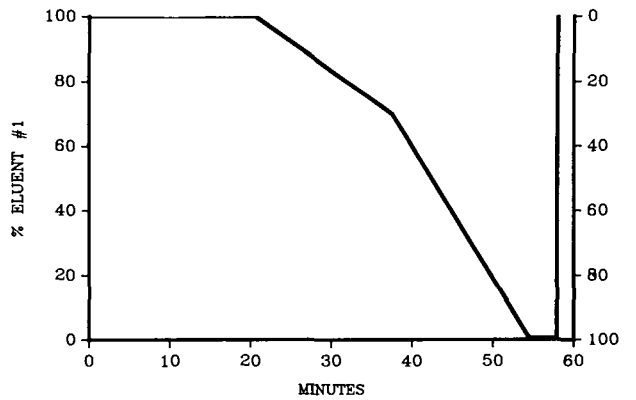

Fig. 2. Optimum gradient conditions. Eluent 1 = 0.25 mM NaOH in methanol + ethanol; eluent 2 = 79 mM NaOH in ethanol.

Standard Solution of Inorganic Anions and Organic Acids

A standard stock solution of inorganic and organic acid anions was prepared at 100, 200, or 400 mg/L by dissolving either the acid or the sodium or potassium salt (99+%) of each acid in 5% (v/v) ethanol to deionized water. The standard anion stock solution was stabilized to inhibit bacterial degradation for one month by adding 15 drops of chloroform. A serial dilution with deionized water of the standard stock solution was used for subsequent calibration.

The stock solution contained the following components in milligrams per liter in ionic form: lactate, 200; acetate, 200; citrate, 200; pyruvate, 200; succinate, 200; malate, 200; maleate, 200; formate, 200; bromide, 200; nitrate, 200; oxalate, 200; fluoride, 100; sulfate, 400; chloride, 400; and phosphate, 400.

Standardization and Quantification

A linear regression analysis of the peak areas versus the concentrations of each component in all of the working standard dilutions was used to calculate the slope, intercept, and correlation coefficient of each component. The equation of each component's calibration curve then was used to calculate the concentration from the peak areas of inorganic and organic acid anions present in each sample of that series of analyses.

RESULTS AND DISCUSSION

The biggest obstacle to overcome was finding the optimum gradient conditions for the best resolution. A chromatogram of a fermenter beer sample is shown in Figure 1, which demonstrates the resolution of these inorganic and organic acid anions. The objective of separating both inorganic and organic acid anions in one analysis run, using gradient ion chromatography with suppressed conductivity detection, was achieved with this method. The optimum operating gradient conditions are given in Figure 2. The background conductivity for 100% of eluent 1 and 2 was 1–3 and 3–7 μS, respectively.

To determine the precision, six replicate samples of packaged beer, prepared individually in an identical manner, were analyzed using the gradient inorganic and organic acid anions method.

TABLE I
Precision of the Method Used to Determine Inorganic and Organic Acid Anions

Component	Mean[a] (mg/L)	SD	Coefficient of Variation (%)
Fluoride	17.9	0.40	2.2
Acetate	49.9	1.25	2.5
Lactate	63.8	2.50	3.9
Formate	<LQL[b]	...	...
Pyruvate	33.5	0.75	2.2
Chloride	66.6	0.65	1.0
Bromide	15.4	0.62	4.0
Nitrate	9.4	0.36	3.8
Succinate	85.8	1.45	1.7
Malate	85.8	2.01	2.3
Sulfate	88.3	0.84	1.0
Oxalate	8.3	0.17	2.0
Phosphate	390.1	3.03	0.8
Citrate	101.1	1.78	1.8

[a] $n = 6$.
[b] Sample value less than lower quantification limit.

TABLE II
Limits of Linearity of the Method Used to Determine Inorganic and Organic Acid Anions

Component	Lower Limit (mg/L)	Upper Limit (mg/L)	Detection Limits (mg/L)
Fluoride	0.5	25.0[a]	0.2
Acetate	1.0	30.0[a]	0.5
Lactate	1.0	30.0[a]	0.5
Formate	1.0	100.0	0.5
Pyruvate	1.0	100.0	0.5
Chloride	2.0	200.0	1.0
Bromide	1.0	20.0[a]	0.5
Nitrate	1.0	20.0[a]	0.5
Succinate	1.0	100.0	0.5
Malate	1.0	100.0	0.5
Maleate	1.0	100.0	0.5
Sulfate	2.0	180.0	1.0
Oxalate	5.0	20.0[a]	0.5
Phosphate	5.0	300.0	1.0
Citrate	5.0	100.0	1.0

[a] Narrower range of linearity for fluoride, acetate, lactate, bromide, nitrate, and oxalate because of the limited loading capacity of the column, creating resolution difficulties.

TABLE III
Percent Recoveries from Spiked Samples

Component	Amount Added (mg/L)	Percent Recovery		
		Package Beer[a]	Fermenter[b]	Wort[b]
Fluoride	5.0	95.0	89.7	91.3
Acetate	10.0	83.2	87.8	76.2
Lactate	10.0	95.1	85.4	92.2
Formate	10.0	103.7	95.6	83.1
Pyruvate	10.0	88.1	85.8	97.6
Chloride	20.0	97.0	93.6	94.8
Bromide	10.0	87.0	101.3	90.5
Nitrate	10.0	99.3	101.7	113.3
Succinate	10.0	88.0	101.0	81.6
Malate	10.0	103.2	88.3	90.9
Maleate	10.0	94.2	106.1	105.4
Sulfate	20.0	104.3	97.4	104.4
Oxalate	10.0	<LQL[c]	106.5	117.8
Phosphate	20.0	94.2	77.8	97.3
Citrate	10.0	98.5	101.1	109.6
Range		83.2–104.3	77.8–106.5	76.2–117.8

[a] $n = 6$.
[b] $n = 4$.
[c] Sample value less than lower quantification limit.

All of the analytes had coefficients of variation of 4.0% or less. The results are summarized in Table I.

The linear range for most of the analytes in this method was from 0.5 to 300 mg/L. The narrower range of linearity for fluoride, acetate, lactate, bromide, nitrate, and oxalate was attributable to the limited loading capacity of the column. This was shown by decreased resolution with higher concentrations of each of these analytes. The adjustment of the sample preparation by a simple dilution of the samples resulted in an extended dynamic linear range. The upper and lower limits of linearity were represented by the method's linear response versus both high and low standard concentrations, or the full range for which the method produces a linear response. The lower quantification limit is determined by the lowest concentration in the linear range. The limits of detection were determined by serial dilution of the standard concentrations to the point of no detection by the method, i.e., no statistical difference from a blank. The limits of detection ranged from 0.2 to 1.0 mg/L for each analyte. The limits of quantification and detection for each analyte are given in Table II.

The percent spiked recoveries were determined by comparison of the initial levels of inorganic and organic acid anions in packaged beer, fermenter, and wort to each of the respective type of sample that was spiked with various amounts of inorganic and organic anions. The various amounts, shown in Table III, for the inorganic and organic acid anions were added as dilutions of the stock standard mixture to the diluted beer samples. The percent recoveries, as shown in Table III, ranged from 83.2 to 104.3% for packaged beer, from 77.8 to 106.5% for fermenter samples, and from 76.2 to 117.8% for wort. The slightly wider percent recovery ranges for wort and fermenter samples were attributable to the decrease in resolution for those analytes as a result of the limited loading capacity of the column.

CONCLUSIONS

This method provides an accurate and simultaneous determination of the inorganic and organic acid anions in all phases of beer production with a single sample injection and analysis. The inorganic anions in beer now can be accurately resolved and quantified without the problems caused by interferences from organic acids in the sample matrix. The optimum gradient conditions for the best resolution of both inorganic and organic anions in beer were determined for this method. The spiked recovery results indicated that the sample matrix interferences, using this method, were minimal for each phase of beer. This method for the quantitative measurement of inorganic and organic acid anions can be used to help track fermentations and to help predict and correlate beer flavor trends.

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