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Application Note 123

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Determination of Inorganic Anions and Organic Acids in Fermentation Broths

INTRODUCTION

Fermentation broths are used in the manufacture of biotherapeutics and many other biologically derived products using recombinant genetic technology. Broths are also used for the production of methanol and ethanol as alternative energy sources to fossil fuels. In addition, many food and beverage products such as alcoholic beverages, vinegars, fermented vegetables, sauces, and dairy products are all prepared by controlled fermentation processes. Fermentation monitoring is also important in detection of spoilage of fruit juices and food products. Recently, attention has been given to characterizing the ingredients of fermentation broths because carbon sources and metabolic by-products can impact the yield of the desired products. Carbohydrates (glucose, lactose, sucrose, maltose, etc.) are carbon sources essential for cell growth and product synthesis, while alcohols (ethanol, methanol, sugar alcohols, etc.), glycols (glycerol), and organic anions (acetate, lactate, formate, etc.) are metabolic by-products, many of which reduce desired yields. Fermentation broths are complex mixtures of nutrients, waste products, cells and cell debris, and desired products, such as antibiotics. Many of these ingredients are nonchromophoric and cannot be detected by absorbance.

Organic and inorganic anions are ionic and therefore can be determined by ion chromatography using suppressed conductivity detection. Suppressed conductivity is a powerful detection technique with a broad linear range and very low detection limits. Nonionic compounds are not detected. Suppression lowers the background conductivity caused by the eluent and effectively increases the conductivity of the analyte.[1, 2] Anion-exchange chromatography is a technique capable of separating complex mixtures of organic acids and inorganic anions. For complex samples like fermentation broths, the high resolving power of ion-exchange chromatography and the specificity of suppressed conductivity allow the determination of ionic fermentation broth ingredients, with little interference from other broth ingredients.[3-5] Although biosensor and flow-injection analysis methods are commonly used to evaluate fermentation broths,[6, 7] these techniques cannot simultaneously determine multiple compounds. Gel permeation chromatography with refractive index detection, and anion-exchange chromatography with UV-VIS detection, have been used for analysis of fermentation broths, but both are limited by poor selectivity and sensitivity.[8, 9] Anion-exchange chromatography with suppressed conductivity monitors, by direct injection, a large number of different compounds simultaneously, using a single instrument and chromatographic method.[10]

This application note describes the use of two different anion-exchange columns, with suppressed conductivity detection, to analyze common organic and inorganic anions in yeast and bacterial fermentation broths. The yeast Saccharomyces cerevisiae in yeast extract-peptone-dextrose (YPD) broth and the bacteria Escherichia coli in Luria-Bertani (LB) broth are common fermentation broth cultures and represent eukaryotic and prokaryotic systems. Both fermentation broth cultures are complex and contain undefined media ingredients, and thus are a great challenge for most separation and detection technologies. These formulations also contain carbohydrates, sugar alcohols, alcohols, and glycols that have been analyzed using the CarboPac™ PA1, PA10, and MA1 anion-exchange columns with pulsed amperometric detection.[11]

In the methods outlined in this application note, the selectivities of the IonPac® AS11 and IonPac AS11-HC anion-exchange columns are compared for the determination of anionic analytes in fermentation broths. The IonPac AS11 column packing consists of an alkanol quaternary ammonium latex bonded to a microporous crosslinked ethylvinylbenzene core. The AS11-HC (high capacity) latex is bonded to a macroporous crosslinked ethylvinylbenzene core. Due to the greater surface area of its core, the AS11-HC has six times more anion-exchange capacity than the AS11. Both columns are designed for separation of organic and inorganic anions using sodium hydroxide gradients. Organic solvents can be added to eluents to modify the selectivity of these columns.

Expected detection limits, linearity, selectivity, stability, and precision for organic and inorganic anions in fermentation broths are reported for the IonPac AS11 and AS11-HC columns using the Dionex DX-500 BioLC® system with suppressed conductivity detection.

EQUIPMENT

Dionex DX-500 BioLC system consisting of:
- GP40 Gradient Pump with degas option
- ED40 Electrochemical Detector
- LC30 or LC25 Chromatography Oven
- AS3500 Autosampler

PeakNet Chromatography Workstation

REAGENTS AND STANDARDS

Reagents

Sodium hydroxide, 50% (w/w) (Fisher Scientific and J. T. Baker)

Deionized water, 18 MΩ-cm resistance or higher was used for preparing all standards and eluents. Water that was used to prepare YPD broth was filter sterilized by passage through a 0.2-µm filter.

Standards

Lactic acid (Fisher Scientific)
Succinic acid (Aldrich Chemical Co.)
Pyruvic acid, sodium salt (Fisher Scientific)
DL-Isocitric acid, trisodium salt (Sigma Chemical Co.)
n-Butyric acid, sodium salt (Sigma Chemical Co.)
Sodium formate (Fisher Scientific)
Phenylacetic acid (Sigma Chemical Co.)
Propionic acid, sodium salt (Sigma Chemical Co.)
Maleic acid, disodium salt (Sigma Chemical Co.)
Oxalic acid, sodium salt (Fluka Chemika)
L-Malic acid (Eastman Chemical Co.)
Pyrophosphoric acid (Fluka Chemika)
Trichloroacetic acid (Fluka Chemika)
Chloroacetic acid (Aldrich Chemical Co.)
Glycolic acid (Sigma Chemical Co.)
L-Glutamic acid (Sigma Chemical Co.)
Fumaric acid (Fluka Chemika)
D-Gluconic acid, sodium salt (Sigma Chemical Co.)
Oxalacetic acid (Sigma Chemical Co., and Fluka Chemika)
Methylmalonic acid (Sigma Chemical Co.)
5-Keto-D-Gluconic acid, potassium salt (Sigma Chemical Co.)
2-Keto-D-Gluconic acid, hemicalcium salt (Sigma Chemical Co.)
Valeric acid (Aldrich Chemical Co.)
Isovaleric acid (Sigma Chemical Co.)
Isobutyric acid (Sigma Chemical Co.)
Sodium bromate (Fluka Chemika)
Sodium arsenate, dibasic, 7-hydrate (J.T. Baker Chemical Co.)
Sodium acetate, anhydrous (Fluka Chemika)
Sodium fluoride (Fisher Scientific)
Sodium nitrate (Fisher Scientific)
Sodium chloride (Fisher Scientific)
Potassium phosphate, dibasic, anhydrous (Fisher Scientific)
Citric acid, monohydrate (Fisher Scientific)
Sodium bromide (Aldrich Chemical Co.)
Sodium sulfate, anhydrous (EM Science)
Sodium carbonate, monohydrate (Fisher Scientific)

Culture and Media

Bacto YPD Broth (DIFCO Laboratories, Cat# 0428-17-5)
Bacto Yeast Extract (DIFCO Laboratories, Cat# 0127-15-1)
Bacto Peptone (DIFCO Laboratories, Cat# 0118-15-2)
LB Broth (DIFCO Laboratories, Cat# 0446-17-3)
Yeast, S. cerevisiae; Bakers Yeast type II (Sigma Chemical Co., Cat# 45C-2)
Bacteria, E. coli (donated by SRI International)

Table 1. Chromatographic Conditions

	Conditions	
	System 1	**System 2**
Column:	IonPac AS11 Analytical (P/N 44076) IonPac AG11 Guard (P/N 44078) ATC-1 Anion Trap Column (P/N 37151)	IonPac AS11-HC Analytical (P/N 52960) IonPac AG11-HC Guard (P/N 52962) ATC-1 Anion Trap Column (P/N 37151)
Flow Rate:	2.0 mL/min	1.5 mL/min
Injection Volume:	10 µL	10 µL
Oven Temperature:	Ambient	30 °C
Detection (ED40):	Suppressed conductivity, ASRS®, AutoSuppression® recycle mode, 300 mA	Suppressed conductivity, ASRS, AutoSuppression recycle mode, 300 mA
Eluents:	A: Water B: 5 mM sodium hydroxide C: 100 mM sodium hydroxide	A: Water B: 5 mM sodium hydroxide C: 100 mM sodium hydroxide
Gradient:	0.5–38 mM sodium hydroxide: 0.5 mM sodium hydroxide, hold for 2.5 min; 0.5–5 mM sodium hydroxide in 3.5 min; 5–38 mM sodium hydroxide in 12 min.	1–60 mM sodium hydroxide: 1 mM sodium hydroxide, hold for 8 min; 1–15 mM sodium hydroxide in 10 min; 15–30 mM sodium hydroxide in 10 min. 30–60 mM sodium hydroxide in 10 min; 60 mM sodium hydroxide, hold for 2 min.
Method:	Time (min) / A (%) / B (%) / C (%) 0.0 / 90 / 10 / 0 2.5 / 90 / 10 / 0 6.0 / 0 / 100 / 0 18.0 / 0 / 62 / 38 18.1 / 90 / 10 / 0 25.0 / 90 / 10 / 0	Time (min) / A (%) / B (%) / C (%) 0.0 / 80 / 20 / 0 8.0 / 80 / 20 / 0 18.0 / 85 / 0 / 15 28.0 / 70 / 0 / 30 38.0 / 40 / 0 / 60 40.0 / 40 / 0 / 60 40.1 / 80 / 20 / 0 50.0 / 80 / 20 / 0
Typical Background Conductivity:	0.5 mM sodium hydroxide: 0.5–1 µS 38 mM sodium hydroxide: 2–3 µS	1 mM sodium hydroxide: 0.5–1 µS 60 mM sodium hydroxide: 2–3 µS
Typical System Operating Backpressure:	12.4 Mpa (1800 psi)	15.2 Mpa (2200 psi)

CONDITIONS

See "Conditions" (Table 1).

PREPARATION OF SOLUTIONS AND REAGENTS

Sodium Hydroxide Eluents

5 mM Sodium Hydroxide

It is essential to use deionized water of high resistance (18 MΩ-cm) that is as free of dissolved carbon dioxide as possible. Carbonate is formed in alkaline eluents from carbon dioxide. Carbonate, a divalent anion at high pH, binds strongly to the columns and causes a loss of chromatographic resolution and efficiency. Carbonate can be removed by placing an anion trap column (ATC-1, P/N 37151) between the pump and the injection valve. Commercially available sodium hydroxide pellets are covered with a thin layer of sodium carbonate and should not be used. A 50% (w/w) sodium hydroxide solution is much lower in carbonate and is the preferred source for sodium hydroxide.

Dilute 0.524 mL of 50% (w/w) sodium hydroxide solution into 2000 mL of thoroughly degassed water to yield 5 mM sodium hydroxide. Keep the eluents blanketed under 5–8 psi (34–55 kPa) of helium at all times.

100 mM Sodium Hydroxide

Follow the same precautions described above for the 5 mM sodium hydroxide eluent. Dilute 10.4 mL of 50% (w/w) sodium hydroxide solution into 1990 mL of thoroughly degassed water to yield 100 mM sodium hydroxide. Keep the eluents blanketed under 5–8 psi (34–55 kPa) of helium at all times.

Stock Standards

Solid standards were dissolved in water to 10 g/L anionic concentrations. These were combined and further diluted with water to yield the desired stock mixture concentrations.

The solutions were kept frozen at -20 °C until needed. For determinations of linear range, combine 10-g/L solutions of chloride, bromide, and citrate to make a 1-mg/L standard mix solution. Dilute with water to concentrations of 800, 600, 400, 200, 100, 80, 60, 40, 20, 10, 4, and 1 µg/L. Standard solutions of acetate, bromide, nitrate, sulfate, phosphate, and citrate were also prepared for estimating lower detection limits and linearity at concentrations of 10, 8, 6, 4, 2, 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.08, 0.06, 0.04, 0.02, and 0.01 mg/L. Chloride was prepared at concentrations of 2.4, 1.9, 1.5, 1.0, 0.49, 0.24, 0.19, 0.15, 0.098, 0.048, 0.024, 0.019, 0.014, 0.0097, 0.0048, and 0.0024 mg/L.

SAMPLE PREPARATION

Yeast Fermentation Broth Culture—Standard Media

In a sterile 500-mL Erlenmeyer flask, dissolve 10 g of Bacto YPD Broth (DIFCO Laboratories, Cat# 0428-17-5) in 200 mL filter-sterilized water. Bacto YPD Broth contains 2 g Bacto Yeast Extract, 4 g Bacto Peptone, and 4 g dextrose (glucose) per 10 g. Dissolve 1.0 g yeast (S. cerevisiae; Bakers Yeast type II; Sigma Chemical Co., Cat# 45C-2) in the YPD broth. Cap the flask with a vented rubber stopper. Incubate the culture in a 37 °C shaking water bath (500–600 rpm) for 24 h, removing aliquots at designated time points and placing them on ice. For this study, samples were taken after the addition of yeast at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 24-h intervals. The incubation starts when yeast is added to the medium. Aliquots are centrifuged at 14,000 × g for 10 min and diluted 10- and 100-fold in purified water. Diluted supernatant (10 µL) was analyzed directly.

Heat-inactivated yeast fermentation broth supernatant was spiked with anions for the recovery and stability study. To inactivate the culture, broth supernatant was diluted 10-fold, and heated in boiling water for 10 min. An aliquot of heat-inactivated supernatant was then diluted another 10-fold using 100 µg/mL lactate, acetate, formate, pyruvate, sulfate, oxalate, phosphate, and citrate. The final concentration of each anion was 10 µg/mL. Another aliquot of heat-inactivated yeast culture supernatant was diluted 100-fold in water, serving as an unspiked "blank".

E. Coli Fermentation Broth Culture—Standard Media

LB Broth is dissolved to a concentration of 25 g/L with water, heated to a boil, and autoclaved for 15 minutes at 121 psi. A liter of LB broth contains 10 g of tryptone, 5 g yeast extract, and 10 g of sodium chloride per 25 g. The culture was incubated and sampled as described for the yeast standard media.

RESULTS AND DISCUSSION

Selectivity

IonPac AS11

Figure 1A shows the separation of the common fermentation broth anions using an IonPac AS11 column set with a 0.5–38 mM NaOH gradient (Table 1, System 1) flowing at 2.0 mL/min. The organic and inorganic anions were well-resolved. The analytes were eluted from the column in less than 20 min. The retention times of the anions in Figure 1A are listed in Table 2. In general, monovalent anions eluted first, followed by di- and trivalent anions.

IonPac AS11-HC

Figure 1B shows the analysis of common fermentation broth anions using the IonPac AS11-HC column. Analytes were eluted using a 1–60 mM sodium hydroxide gradient (Table 1, System 2) flowing at 1.5 mL/min. A stronger eluent was needed to elute anions from this column due to its higher capacity. The higher capacity improves resolution of early eluting peaks. For example, lactate and acetate are better resolved on the AS11-HC than the AS11. The elution order of the AS11-HC is similar to the AS11. Table 2 also summarizes the retention times of different anions on the AS11-HC column. These results demonstrate that the AS11-HC column has

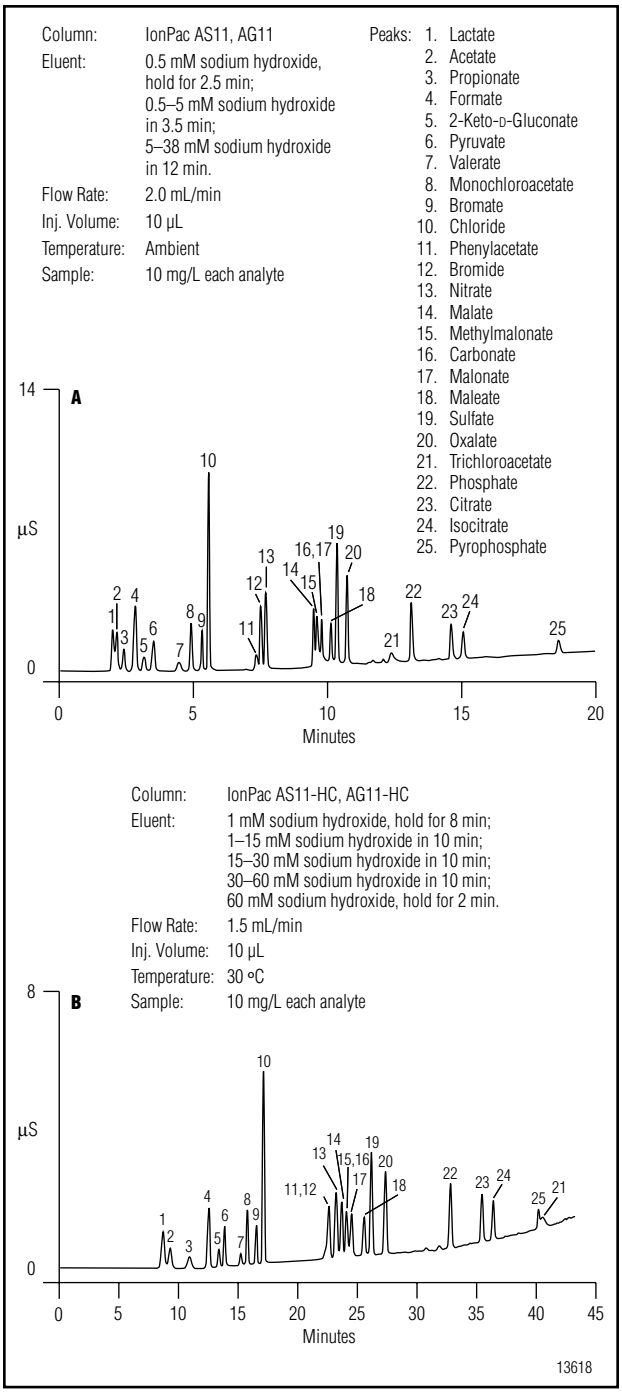

Figure 1. Common organic and inorganic anions found in fermentation broths analyzed on the IonPac AS11 and AS11-HC columns with suppressed conductivity.

slightly different selectivity than the AS11. For example, the AS11 column elutes phenylacetate, bromide, and nitrate several minutes before malate; the AS11-HC elutes these compounds much closer to malate. Also, trichloroacetate elutes before phosphate on the AS11, but elutes after pyrophosphate on the AS11-HC. The high capacity of the AS11-HC permits larger sample loads.

Table 2. Retention Times for Common Organic and Inorganic Anions

	Retention Times (Minutes)	
Analyte	**IonPac AS11/AG11**	**IonPac AS11-HC/AG11-HC**
Fluoride	2.3	8.7
Gluconate	2.3	8.2
Lactate	2.5	8.8
Acetate	2.6	9.5
Glycolate	2.6	9.4
Propionate	2.9	11.0
Isobutyrate	3.2	12.3
Formate	3.4	12.4
Butyrate	3.6	12.8
2-Keto-D-Gluconate	4.0	13.1
Pyruvate	4.3	13.5
Isovalerate	4.3	13.7
Valerate	5.1	14.8
Monochloroacetate	5.5	15.3
Bromate	5.8	16.1
Chloride	6.1	16.7
Phenylacetate	8.0	21.7
Bromide	8.2	21.9
5-Keto-D-Gluconate	8.3	20.1
Nitrate	8.4	22.4
Glutarate	N/A	22.5
Succinate	10.1	22.9
Malate	10.1	23.0
Carbonate	N/A	23.5
Methylmalonate	10.2	23.4
Malonate	10.4	23.8
Maleate	10.7	24.9
Sulfate	11.0	25.4
Oxalate	11.4	26.6
Fumarate	11.4	26.8
Oxalacetate	12.2	29.2
Trichloroacetate	13.5	39.0
Phosphate	13.9	31.8
Arsenate	15.1	33.9
Citrate	15.5	34.4
Isocitrate	16.0	35.3
Pyrophosphate	19.7	39.1

N/A - Not available

Table 3. Estimated Lower Detection Limits (10-µL Injection)		
	System 1 AS11	
	ng	µg/L
Acetate	2	200
Chloride	0.5	50
Bromide	4	400
Nitrate	3	300
Sulfate	1	100
Phosphate	4	400
Citrate	4	400

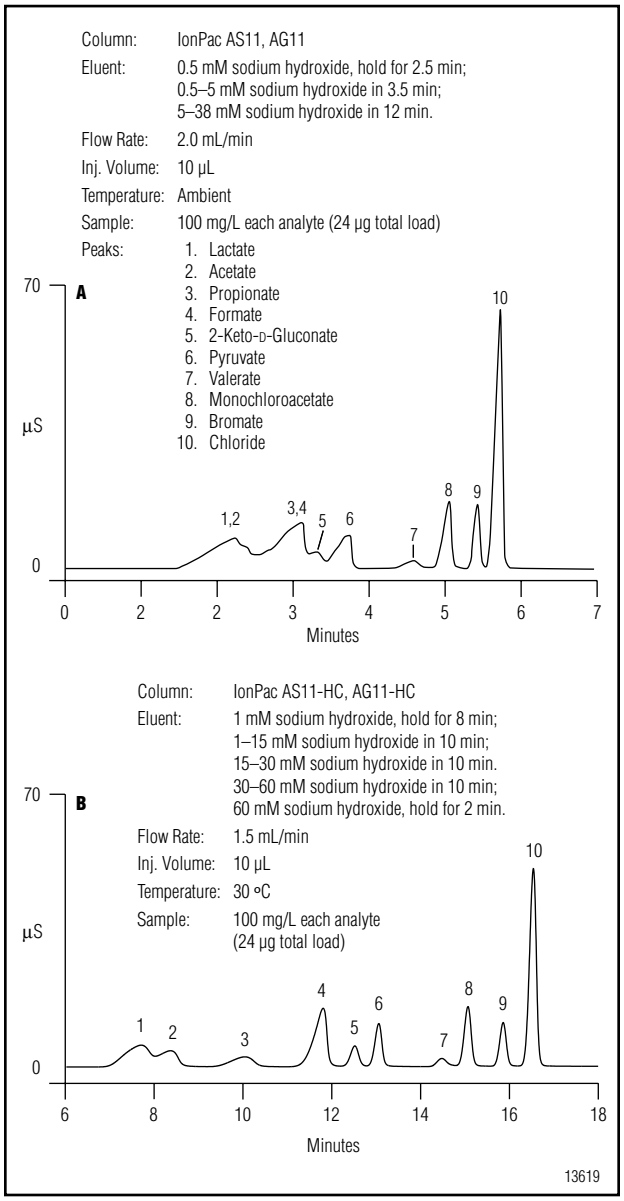

Figure 2. *Separation of early eluting organic and inorganic anions at high levels (24 µg total load) using the IonPac AS11 and AS11-HC.*

Figure 2A shows early-eluting peaks from the analysis of 24-µg sample of fermentation broth anions analyzed on the AS11, and Figure 2B shows the same analysis on the AS11-HC. At this sample load, the AS11 is overloaded.

Detection Limits

The detection limits for a 10-µL injection of representative fermentation broth anions, in the absence of broth matrix, using the AS11 column, are shown in Table 3. The detection limit is defined as the minimum concentration required to produce a peak height signal-to-noise ratio of 3. The detection limit can be further decreased by increasing the injection volume above the 10-µL injection volume used for this application note. If increasing injection volume also increases sample load beyond the AS11 column capacity, the higher capacity AS11-HC can overcome this limitation. The detection limit can be further decreased by using smoothing algorithms available in PeakNet software and by using external water mode.

Table 4. Peak Area Precision (RSD, %)				
Analyte	Last 8 Hours	First 48 Hours	Second 48 Hours	96 Hour Period
Lactate	0.2	0.4	0.3	0.5
Acetate	0.2	0.7	0.4	0.6
Formate	0.1	0.3	0.2	0.4
Pyruvate	0.5	0.5	0.7	0.8
Chloride	0.6	0.5	0.4	0.5
Sulfate	0.3	1.2	1.3	1.3
Oxalate	0.4	0.8	0.5	1.2
Phosphate	0.7	1.6	0.5	1.9
Citrate	0.3	1.8	0.6	2.1
Retention Time Precision (RSD, %)				
Analyte	Last 8 Hours	First 48 Hours	Second 48 Hours	96 Hour Period
Lactate	0.1	0.2	0.2	0.3
Acetate	0.1	0.2	0.1	0.3
Formate	0.1	0.2	0.1	0.3
Pyruvate	0.0	0.2	0.1	0.3
Chloride	0.0	0.2	0.1	0.4
Sulfate	0.0	0.3	0.1	0.7
Oxalate	0.0	0.3	0.1	0.7
Phosphate	0.0	0.3	0.1	0.7
Citrate	0.0	0.3	0.0	0.7

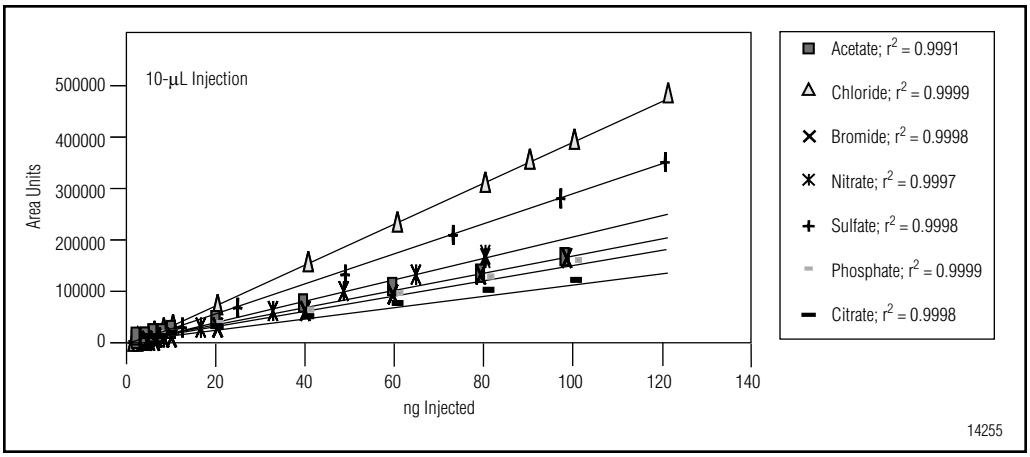

Figure 3. *Method linearity for IonPac AS11 with suppressed conductivity detection.*

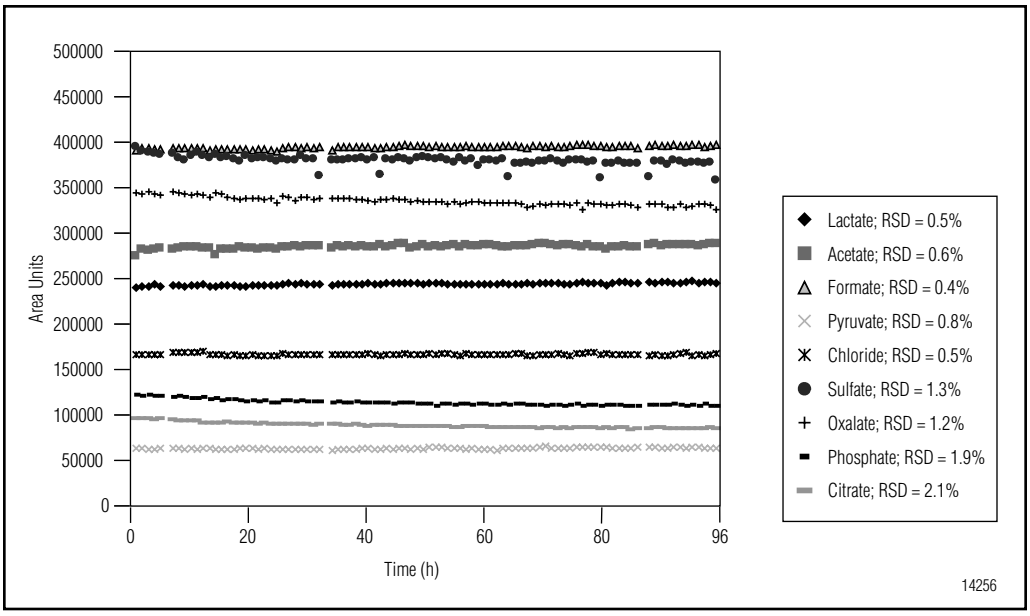

Figure 4. *Peak Areas during 4 day repetitive analysis of heat-inactivated yeast fermentation broth.*

Table 5. Recovery of Anions in the Yeast Fermentation Broth

Analyte	Percent Recovery
Lactate	100
Acetate	88
Formate	101
Pyruvate	99
Sulfate	101
Phosphate	100
Citrate	84

Linearity

Chloride, bromide, and citrate standards ranging from 1–1000 mg/L (10–10,000 ng) were injected (in triplicate) on the AS11 column. For these analytes, the peak area response was found to be linear over this range ($r^2 \geq 0.999$). Acetate, nitrate, sulfate, and phosphate were investigated over the concentration range of 0.1–12 mg/L (1–120 ng) and showed high linearity ($r^2 \geq 0.999$). Broad linear ranges help reduce the need to repeat sample analyses when components vary greatly in concentration. Representative calibration curves for the AS11 column is presented in Figure 3.

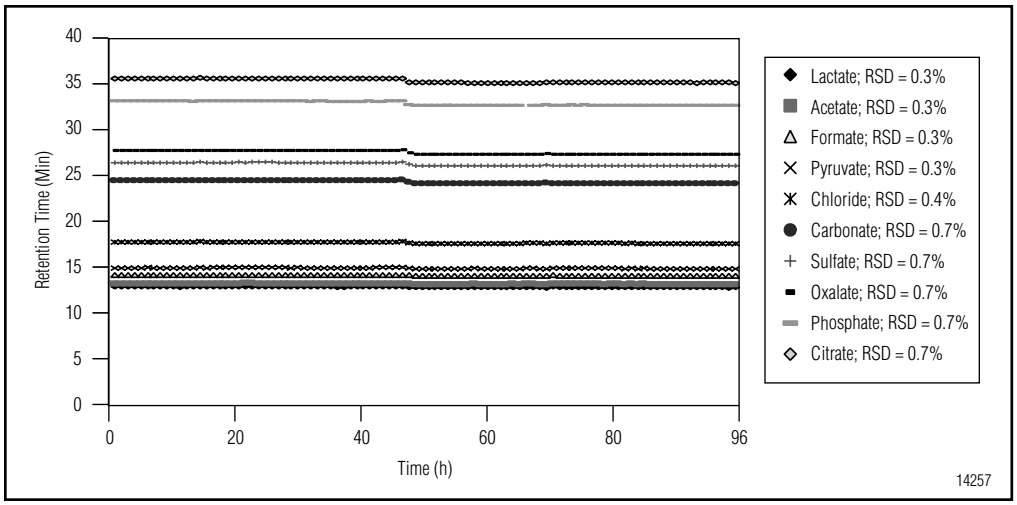

Figure 5. *Retention times during 4 day repetitive analysis of heat-inactivated yeast fermentation broth.*

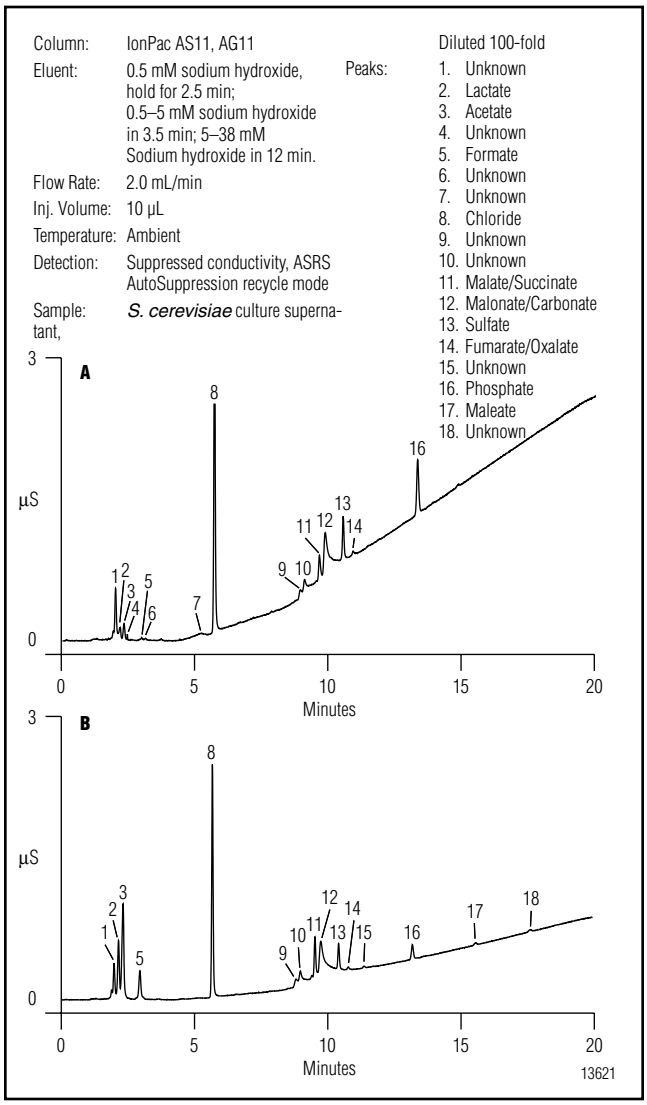

Figure 6. *S. cerevisiae fermentation broth culture (100-fold dilution) using the IonPac AS11 column at 0 h (A) and 24 h (B) of incubation.*

Precision and Stability

The peak area and retention time RSDs were determined for replicate injections of common anions spiked into yeast fermentation broth. Anion standards were added to heat-inactivated S. cerevisiae fermentation broth culture supernatant to yield 10 mg/L spike concentrations and then analyzed repeatedly for 96 h (10-µL injections) on the AS11-HC column. Statistics for this experiment are presented in Table 4. Figures 4 and 5 show peak areas and retention times for every injection of this experiment. Peak area RSDs were 0.4–2.1% over 96 h. Retention time RSDs ranged from 0.3–0.7%. Retention times shifted slightly at 45 h (Figure 5) when the 100 mM sodium hydroxide eluent was replenished during the study. These results demonstrate that changing eluents can affect retention time precision.

Recovery from Sample Matrix

After correction for endogenous amounts, the measured levels of selected anions spiked into a heat-inactivated yeast fermentation broth culture were compared to their expected levels. These results are presented in Table 5, and show good recovery of anions from the yeast fermentation broth.

Yeast *(S. cerevisiae)* Culture

Yeast were grown in Bacto YPD broth at 37 °C for up to 24 h. Figure 6 shows the separation of fermentation broth ingredients in a yeast culture at the beginning (Figure 6A) and after 24 h (Figure 6B) of incubation. Lactate, acetate/glycolate, formate, valerate, methylmalonate, and citrate increased during the 24-h

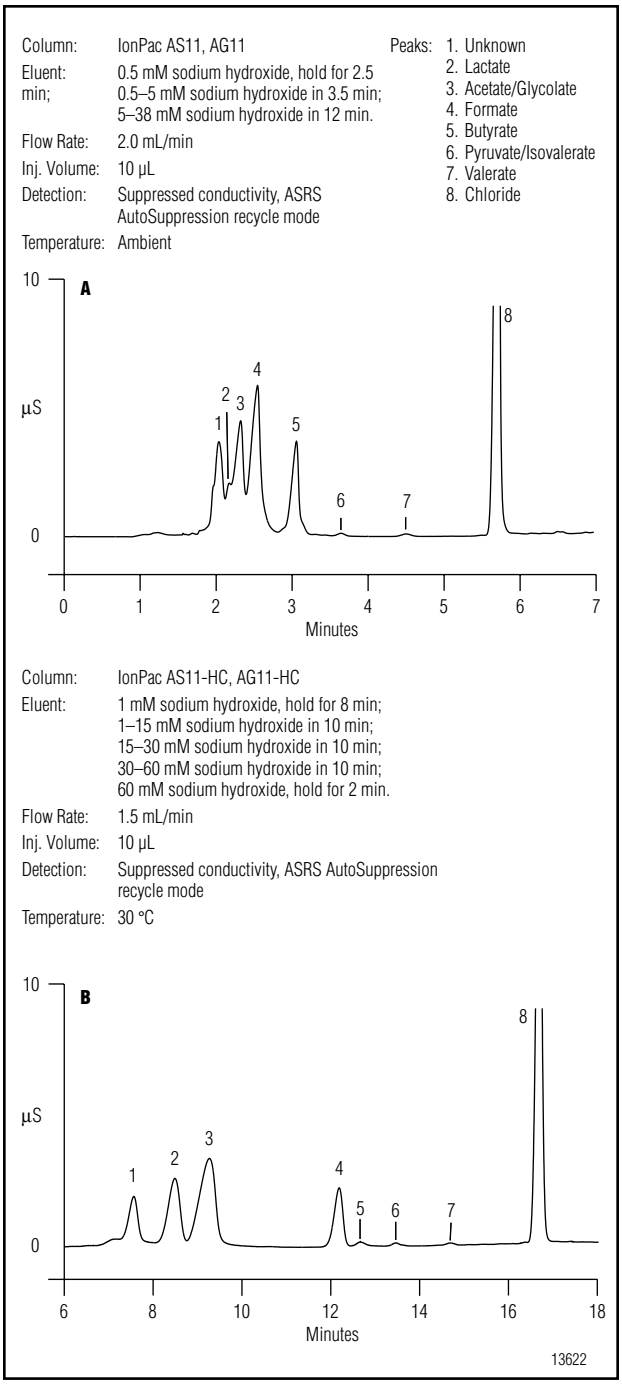

Figure 7. *S. cerevisiae fermentation broth culture (10-fold dilution) using the IonPac AS11 and AS11-HC column at 24 h of incubation.*

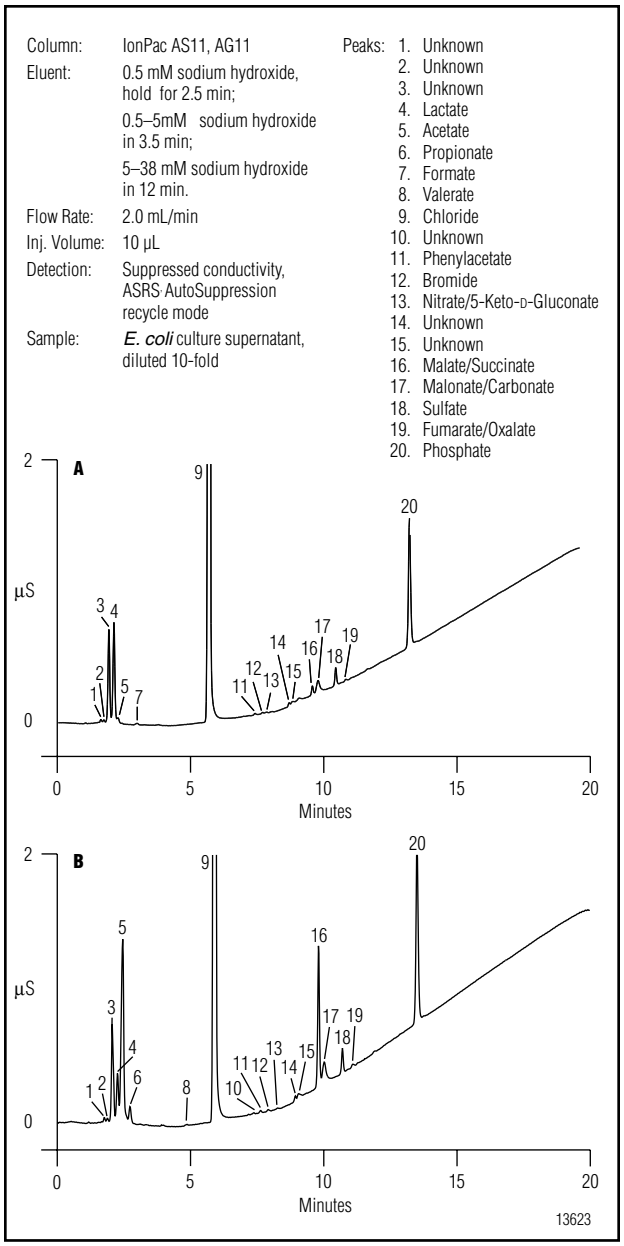

Figure 8. *E. coli fermentation broth culture using the IonPac AS11 column at 0 h (A) of incubation and the AS11 column at 24 h (B) of incubation.*

incubation. Table 6 lists the measured concentrations of these and other analytes during the 24-h incubation. Between 7 and 24-h, no additional time points were taken; however, substantial increases in the levels of lactate, acetate/glycolate, formate, and valerate occur. Some anions remained constant throughout the 24-h incubation, including chloride, malonate, sulfate, and oxalate/fumarate. Phosphate concentration decreased, presumably due to incorporation into the biomass (e.g., DNA, RNA, membrane phospholipids, etc.). At least 10 unidentified peaks were observed. The area units for eight of these peaks changed over the course of the incubation period. Changes in lactate, acetate, and formate concentrations are expected as a result of normal metabolic processes. Trending can be used to track culture status.

Table 6. Anions in Yeast Fermentation Broth During in 24 h Incubation											
		Broth Concentration (µg/mL)									
	Incubation Time (h)	0	0.5	1	2	3	4	5	6	7	24
Anions	Lactate	59	67	66	70	88	85	84	89	90	338
	Acetate	72	122	153	187	199	222	227	247	235	704
	Propionate	11	10	4	9	4	6	4	7	4	11
	Formate	7	10	11	13	11	14	6	7	6	21
	2-Keto-D-Gluconate	4	9	2	10	0	0	0	11	4	2
	Pyruvate	10	14	19	24	14	16	17	17	17	6
	Valerate	0	0	0	0	0	5	5	4	11	24
	Chloride	348	345	353	320	355	356	357	354	371	347
	Malate	0	7	13	9	8	15	15	12	15	11
	Methylmalonate	100	125	169	180	224	248	247	237	253	229
	Malonate	428	452	569	476	474	547	563	531	577	563
	Sulfate	68	68	79	63	67	65	64	63	61	57
	Oxalate	12	14	14	10	12	13	12	16	15	11
	Phosphate	165	124	92	62	55	57	58	58	59	62
	Citrate	0	0	0	13	15	12	13	13	10	0

Bacteria (*E. coli*) Culture

Bacteria (E. coli) was grown on LB broth for 24 h at 37 ºC. Figure 8A shows the anions present in this broth at the beginning of the culture, and Figure 8B shows anions after 24 h.

To examine anions at lower concentrations, injections of a more concentrated culture are needed and the AS11-HC column is the best choice. Yeast fermentation broth (diluted only 10-fold) was analyzed by both the AS11 and AS11-HC columns, and is presented in Figures 7A and 7B, respectively. The AS11 column did not resolve the first unknown peak from lactate, while the AS11-HC did. Lactate and acetate were better resolved on the AS11-HC. Butyrate was resolved from formate on the AS11-HC column. Furthermore, many of the trace components that could not be measured using a 100-fold dilution could be measured with a 10-fold diluted sample using the AS11-HC column.

Concentrations of anions were determined at different time points during incubation. After 24 h, lactate decreased, while acetate increased in concentration. Malate/succinate increased over this period. Chloride remained unchanged. Peaks having retention times equal to propionate and valerate were present after 24 h of incubation.

CONCLUSION

These results show that both yeast and bacterial culture fermentation broths can be analyzed for anion composition using ion chromatography and suppressed conductivity. Two columns (IonPac AS11 and AS11-HC) are available for fermentation broth analysis of organic acids and inorganic anions. The AS11-HC permits higher sample loading due to higher capacity. The high capacity of the column is able to resolve lactate, acetate, and formate. Complex mixtures of organic and inorganic anions can be monitored simultaneously during fermentation, providing the analyst with some of the information needed to optimize the fermentation.

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