A Fast and Accurate Method for the Analysis of Organic Acids and Anions in Fermentation Broth

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Introduction

Fermentation is commonly used in many industrial applications including the manufacture of recombinant biotherapeutics, and food and beverage production. During fermentation numerous by-products are produced that can have a considerable effect on the fermentation yield and also the taste, aroma, and other characteristic properties of the food and beverages. Organic acids and inorganic ions are one class of compounds that impact fermentation product quality. Capillary electrophoresis is an ideal technique for the separation and quantification of these compounds as they are both charged and polar. In this poster we describe a simple and rapid capillary electrophoresis method for the determination of anions and organic acids in fermentation broth. This method utilizes a dynamic capillary coating and indirect UV detection to provide resolution of 4 anions and 11 organic acids that contribute to overall product quality in beer. Both final product and in-process beer samples were evaluated for anions and organic acids.

Methods and Materials

Sample and Standard Preparation

All samples were diluted 2 and 4 fold with 0.1N NaOH
Samples tested included a fermentation, wort, and final beer product.
All standards were purchased from Sigma Aldrich. A stock standard solution was prepared and diluted with 0.1N NaOH to the concentrations listed in the table below.

Component	Stock Standard mg/L	Level 1 mg/L	Level 2 mg/L	Level 3 mg/L	Level 4 mg/L
Chloride	400	10	20	40	100
Nitrate	150	3.75	7.5	15	37.5
Sulfate	400	10	20	40	100
Oxalate	200	5	10	20	50
Formate	200	5	10	20	50
Malate	200	5	10	20	50
Citrate	400	10	20	40	100
Succinate	400	10	20	40	100
Pyruvate	200	5	10	20	50
Acetate	400	10	20	40	100
Lactate	400	10	20	40	100
Phosphate	1200	30	60	120	300
Benzoate	400	10	20	40	100
Sorbate	400	10	20	40	100

Instrumentation

All separations were performed on a P/ACE™ MDQ capillary electrophoresis instrument fitted with a UV detector with indirect detection at 230 nm.

Separation Chemistry

The Anion Analysis kit (Beckman Coulter, Inc). This kit utilizes a ~~dynam~~ic coating and reverse polarity which reverses the electro-osmotic flow.

Capillary and Conditioning

A 75 µm ID bare fused-silica capillary 60 cm total length, 50 cm effective length, was used for all separations. Capillary temperature was set at 25°C.
A new capillary was conditioned with a 5 minute rinse of 0.1M NaOH followed by a 1 minute rinse with distilled deionized water.
Between runs the capillary was reconditioned with a 0.5 minute rinse with 0.1M NaOH and a 0.5 minute rinse with distilled deionized water.

Separation Conditions

- Rinse with Anion Coating solution at 20 psi for 0.5 minutes
- Rinse with Anion Separation Buffer at 20 psi for 0.5 minutes
- Pressure injection of sample for 8 seconds at 0.5 psi
- Water plug injection
- Separation at 30 kV for 8 minutes with reverse polarity and a 1 minute voltage ramp

Indirect Detection

Many small ions and organic acids are UV transparent and require indirect UV detection. Indirect detection involves adding a chromophore to the background electrolyte. When the analyte passes the detector a decrease in absorbance will be observed. 32Karat Software has a setting for indirect detection which will reverse the signal displaying the negative peaks as positive peaks (Figure 1). 32Karat with the Caesar Algorithm has the ability to integrate both positive and negative peaks so both UV transparent and absorbent molecules can be detected.

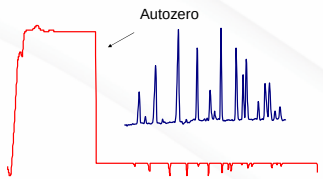

Figure 1. Indirect Detection
The red trace is the direct detection and the blue trace is the inverse signal.

Anion and Organic Acid Separation

4 Anions and 10 organic acids are separated within 6 minutes. Both UV transparent and absorbent compounds can be detected and quantitated. To determine if Butyrate can also be detected by this method, the standard solution was spiked with Butyrate and the peak is well resolved from both Benzoate and Sorbate as shown in Figure 2.

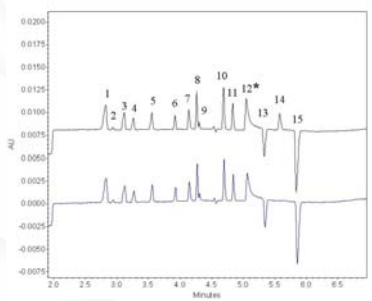

Figure 2. Standard Solution and Standard solution spiked with Butyrate.
* The phosphate peak shaped can be improved and the tailing eliminated by using a HCl rinse instead of the water rinse in the method.

Reproducibility and Linearity

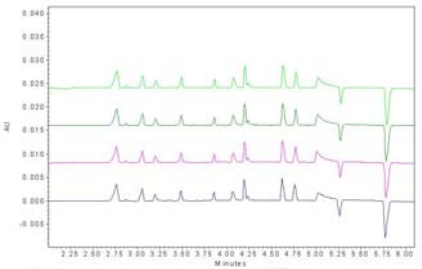

Figure 3. Comparison of 4 injections of standard solution RSD for all components < 0.2% as shown in Table 1 below.

Table 1. Migration Time Reproducibility

Component	Run 1	Run 2	Run 3	Run 4	% RSD
Chloride	2.77	2.77	2.77	2.77	0.07
Nitrate	2.87	2.87	2.87	2.88	0.11
Sulfate	3.05	3.05	3.06	3.06	0.08
Oxalate	3.20	3.20	3.20	3.20	0.07
Formate	3.49	3.49	3.49	3.49	0.08
Malate	3.85	3.85	3.85	3.85	0.11
Citrate	4.07	4.07	4.07	4.07	0.08
Succinate	4.20	4.20	4.20	4.20	0.08
Pyruvate	4.23	4.24	4.24	4.24	0.10
Acetate	4.62	4.63	4.63	4.63	0.07
Lactate	4.76	4.77	4.77	4.77	0.13
Phosphate	5.03	5.01	5.02	5.02	0.10
Benzoate	5.26	5.27	5.27	5.28	0.14
Sorbate	5.77	5.78	5.77	5.78	0.12

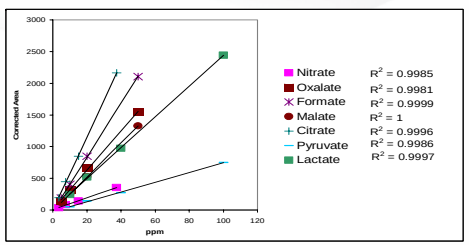

Figure 4. Standard curves for 7 of the components of the standard solution.

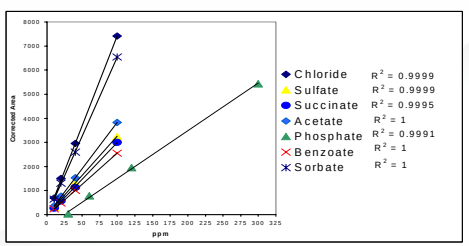

Figure 5. Standard Curves for remaining 7 components of the standard solution.

Analysis of Fermentation and Final Product Analysis

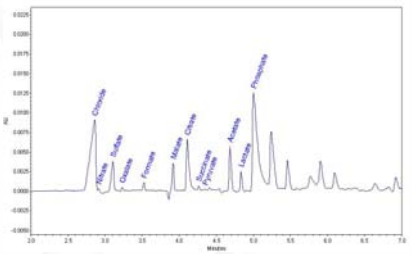

Figure 6. Wort Sample

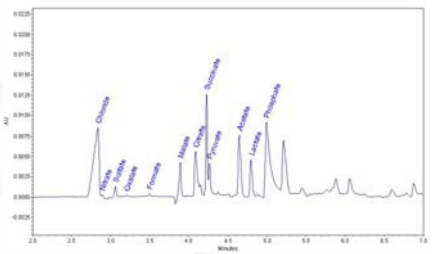

Figure 7. Fermentation Sample

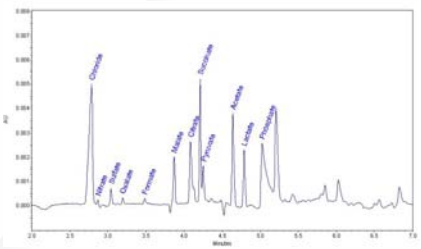

Figure 8. Final Beer Product

Summary

- The Beckman Coulter Anion Analysis kit provides a simple and fast method for the identification and quantitation of anions and organic acids.
- 11 organic acids and 4 anions in beer were ~~in~~ separated in a total run time of 8 minutes.
- Samples require no preparation other than a dilution with 0.1M NaOH.
- The method is linear and reproducible and can detect both UV-transparent and UV absorbent organic acids.
- The speed and reproducibility of this method make it an excellent alternative to other ion analysis techniques.