

# Simultaneous analysis of inorganic anions, organic acids, amino acids and sugars by CZE

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## DISCLAIMER

Before using this or any other analytical method it is imperative that you check that it works with your samples. The bare minimum is to test accuracy and precision.

- Test accuracy by creating a standard curve by serial dilution of a sample and/or via spike and recovery tests. Both tests will show if the analysis is affected by the sample matrix.
- Test precision by repeated analysis of the same sample. It's best to do separate precision tests for the analytical method (replicate analyses of the same extract) and for the entire extraction and analysis procedure (extract the same sample several times and carry each extract through the analysis procedure). These tests will show you where poor precision is creeping into your analysis.

Remember that your results are qualitative if you rely on a standard curve with a purified analyte.

## 1 Introduction

The method described is for the simultaneous analysis of organic acids, amino acids and sugars by CE. It does not require derivatization or extensive pre-treatment of samples. Thus it is potentially much more rapid than chromatographic techniques. However, this method has several serious drawbacks:

1. **Reproducibility of migration time** is appalling; migration time consistently increases over the course of a run due to pH changes in the electrolyte as atmospheric CO<sub>2</sub> is absorbed. Thus the electrolyte must be changed frequently (every 5 samples) and peak identification is problematic. Peak ID cannot be automated but ends up being "intuitive".
2. **Electropherograms are typically complex** since many common solutes give an indirect UV response, e.g. inorganic anions, organic acids, carboxylic acids, amino acids, sugars, polyols. These problems can be partially overcome by fractionating samples and analysing zwitterions (amino acids) separately from inorganic and organic anions.
3. **Separation of some analytes is poor.** Separation of inorganic anions and organic acids is much worse than with techniques optimised for them (e.g. PDC/CTAB at pH 5.6, see dedicated method for analysis of inorganic anions and organic acids). Certain amino acids also present a problem for separation, e.g. glutamine and proline are only partially resolved.
4. **Limits of detection are much worse than with chromatographic techniques.** This particularly applies to arginine and polyalcohols which are only partially dissociated and give poor indirect UV response.

### 1.1 Detection

Most organic acids, amino acids and sugars lack chromophores and do not give a UV-Vis response. One option is to derivatize analytes with a UV-Vis-absorbing species. A potentially more elegant approach is to use indirect UV detection with a background electrolyte such as benzoate with a high UV absorbance. The background electrolyte provides a high background UV signal, when analytes pass the detector they reduce UV absorbance, thus analytes are detected as "negative peaks" against a high background. Those analytes that do absorb UV (e.g. nitrate, tryptophan, ascorbate) may produce a positive peak.

## 1.2 Separation

In CZE (capillary zone electrophoresis) analytes migrate according to their mass/charge ratio. Hence a prerequisite of CZE is that analytes are charged. This is achieved only under highly alkaline conditions (pH 12.0-12.1) where all analytes of interest are negatively charged and migrate towards the anode. The electro-osmotic flow (EOF) normally is towards the cathode, however better separation is achieved if the EOF is reversed to the direction of the anode (i.e. in the same direction as the analytes are migrating). This is achieved with the cationic surfactant myrisityltrimethylammonium bromide (MTAB).

## 2 Preparation of samples

Samples generally require little pre-treatment before analysis. Samples such as phloem and xylem sap only require dilution prior to analysis. Analytes can be extracted from leaves with hot water, methanol/chloroform/water, or aqueous ethanol. If samples contain arginine and mannitol or sorbitol they will have to be fractionated prior to analysis. This can best be achieved with strong cation exchange resin. Proteins also present a problem for analysis since they bond to the inside of the capillary and consistently increase migration time. Protein-rich samples can be either precipitated with TCA or ultrafiltered prior to analysis.

## 3 CE analysis

### 3.1 Benzoate/MTAB background electrolyte

Background electrolyte (BGE) should be prepared fresh every couple of days. It continually absorbs atmospheric CO<sub>2</sub> and the pH decreases with time. Adding more NaOH to old BGE will increase the pH, but it also increases sodium concentration, leading to an increase in separation current and non pH-related shifts in migration time.

To make 100 mL of 20 mM benzoate, 0.5 mM MTAB:

1. Clean electrode and calibrate pH meter using **fresh** pH standards (ca. pH 7 and 9 or 10).
2. To a 100 mL erlenmeyer flask add:
  - a magnetic stirrer bar;
  - 0.2882 g of sodium benzoate;
  - 0.01683 g of myrisityltrimethylammonium bromide;
  - approx 90 mL of double DI water.
3. Place on a magnetic stirrer and dissolve benzoate and MTAB
4. Adjust pH to 12.1 with 1 N NaOH
5. Make up to 100 mL with double DI water
6. Store in a tightly capped container

### 3.2 Operational conditions for analysis

Capillary: uncoated fused-silica (50 µm i.d. x 100 cm total length)

Cartridge and carousel temp: 22°C

Polarity: negative to positive

Run voltage: 25 kV

Run time: 30 min

Detector: 220 nm

Sample injection: 5 psi\*sec

Purge cycles (3): 1 M NaOH (90 s), 0.1 M NaOH (240 s), BGE (300 s).

### 3.3 Quantification of analytes

In CE the speed at which different analytes pass the detector varies systematically, as opposed to HPLC where all solutes migrate at the same speed. Peak area is a function of migration time: slower moving peaks spend longer in the detection window and have larger areas than similar, but faster moving peaks. To allow for this variation, peak areas are divided by migration time. An internal standard (Fucose) is typically used to correct for minor differences in the volume of sample loaded onto the capillary and all peak areas are calculated relative to the internal standard and are referred to as normalised peak areas:  
(analyte peak area / migration time)/( internal standard peak area / migration time)

1. Calculate normalised peak areas for analytes
2. Construct a standard curve with standards
3. From the normalised peak areas and the standard curve, calculate the analyte concentration in the extract.
4. Calculate the concentration in the plant.

### **4 References**

- Chen Z, Warren CR, Adams MA (2000) Separation of amino acids in plant tissue by capillary zone electrophoresis with indirect UV detection using aromatic carboxylates as background electrolytes. *Chromatographia* 51: 180-186.
- Soga T, Ross GA (1999) Simultaneous determination of inorganic anions, organic acids, amino acids and carbohydrates by capillary electrophoresis. *J Chromatogr* 837: 231-239
- Warren CR, Adams MA (2000) Capillary electrophoresis for the determination of the major amino acids and sugars in foliage: application to the nitrogen nutrition of sclerophyllous species. *J Exp Bot.* 51: 1147-1157