Simultaneous analysis of inorganic anions, and organic acids by CZE

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Warren CR, Adams MA (2004) Capillary electrophoresis of the major anions and cations in leaf extracts of woody species. Phytochemical Analysis 15: 407-413

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 a modified version of the method for amino acids and sugars (Warren and Adams 2000). It has been optimized for inorganic anions and organic acids

1.1 Detection

Most organic acids and inorganic anions 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. 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 cetyltrimethylammonium bromide (CTAB).

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 2,6 PDC/CTAB background electrolyte

Background electrolyte (BGE) should be prepared fresh every couple of days. It continually absorbs atmospheric CO_2 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 2,6 PDC, 0.5 mM CTAB:

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;
   ? g 2,6 PDC
   ? g CTAB
   approx 90 mL of double DI water.
3. Place on a magnetic stirrer and dissolve benzoate and MTAB
4. Adjust pH to 5.6 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: 25°C
Polarity: negative to positive
Run voltage:25 kV
Run time: 30 min
Detector: 220 nm
Sample injection: 5 psi*sec
Purge cycles (3): 0.1 M HCl (120s), BGE (240s)

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

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Table 1 Linearity, sensitivity and reproducibility of the method for analysis of inorganic anions and organic acids. The linearity of detector response (peak area/MT) was assessed with standards over the range 0.1 to 5 mM. The detection limit was calculated at a signal-to-noise ratio of three. Reproducibility was determined by repeated (n=4) injection of an extract from needles of *P. pinaster*.

Cation	Linearity correlation (r^2)	Detection limit (mM)	Migration time (RSD %)	Peak area (RSD %)
Chloride	0.988	0.036	0.32	8.7
Nitrate	0.989	0.040	ND	ND
Sulphate	0.999	0.001	0.23	5.3
Oxalate	0.982*	0.005	ND	ND
Formate	0.999	0.001	ND	ND
Tartarate	0.992	0.002	ND	ND
Malate	0.999	0.002	0.08	2.6
Citrate	0.985*	0.002	0.14	5.3
Succinate	0.992	0.004	ND	ND
Pyruvate	0.984	0.007	ND	ND
Acetate	0.989	0.010	ND	ND
Lactate	0.994	0.003	ND	ND
Phosphate	0.996	0.001	0.07	2.4

* Correlation co-efficient for exponential regression

ND Not detected

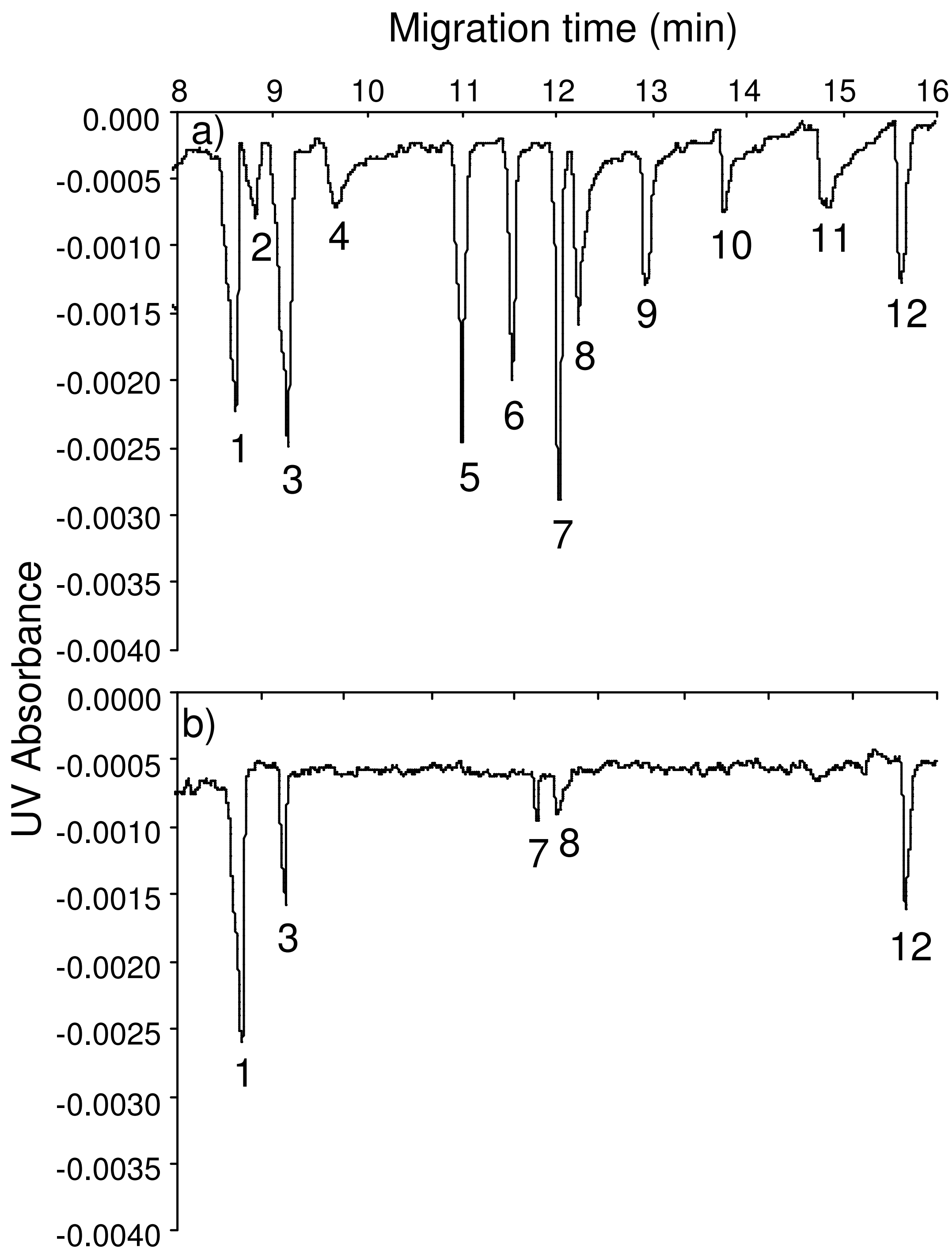

Figure 1 Inorganic anions and organic acids in (a) standard solution with each analyte at 1 mM, and (b) hot water extract of foliage from *P. pinaster*. Peaks are: 1, chloride; 2, nitrate; 3, sulphate; 4, oxalate; 5, formate; 6, tartarate; 7, malate; 8, citrate; 9, succinate; 10, pyruvate; 11, acetate; 12, phosphate. CE conditions as described in the text.