Monitoring of Anionic Residuals Encountered in Bioprocessing by Capillary Electrophoresis

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Introduction

Characterization and quantification of the bioprocess additives, buffers, and ions in biological samples collected during the downstream processing of biopharmaceutical and biotherapeutics is a key component that can impact product quality.

Organic acids and inorganic ions are one such class of compounds. Regulatory agencies require that the active and inactive ingredients of pharmaceutical product be tested for identity, strength, quality and purity.

Quantification of these compounds can be challenging due to their complexity and presence of process matrix.

Capillary Electrophoresis is an ideal technique for separation and quantification of these compounds. This methodology offers many advantages over conventional analytical methods including automation, increased accuracy, and improved sensitivity.

Methodology

Anion residuals (Chloride, Sulfate, Bromide, Azide, Fluoride, Nitrate, Phosphate and Acetate) were detected and quantified using a 60cm (length) 75µM bare fused silica capillary with a UV detector set at 230nm with indirect detection; Capillary temperature was set at 25°C. The capillary was initially conditioned with 5 minute rinse of 0.1M NaOH followed by 1 minute rinse of CE grade water. The anion separation was performed at 30KV for 8 minutes with reverse polarity and 1minute voltage ramp.

Parameter	Specification
Electrophoresis System	Beckman Coulter ProteomeLab PA 800 Capillary Electrophoresis System
Capillary	Silica bare fused 75 µm × 50 cm, 2" (Beckman Coulter)
Anion Markers	Anion Standards (Beckman Coulter)
Capillary Temperature	25°C
Sample Temperature	20°C
UV Parameters	Wavelength – 230 nm; Data rate – 4 Hz; Filter – Normal Resolution; Peak width – 16-25 Absorbance - Indirect

Proteome Lab PA800 CE system

RESULTS

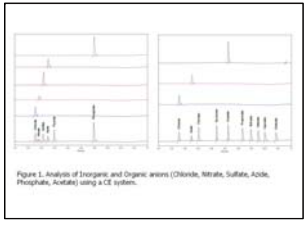

Figure 1. Analysis of Inorganic and Organic anions (Chloride, Nitrate, Sulfate, Azide, Phosphate, Acetate) using a CE system.

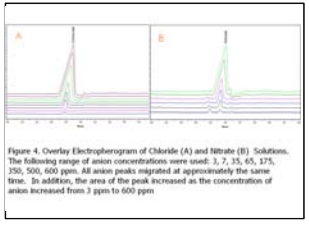

Figure 4. Overlay Electropherogram of Chloride (A) and Nitrate (B) Solutions. The following range of anion concentrations were used: 3, 7, 35, 65, 175, 350, 500, 600 ppm. All anion peaks migrated at approximately the same time. In addition, the area of the peak increased as the concentration of anion increased from 3 ppm to 600 ppm

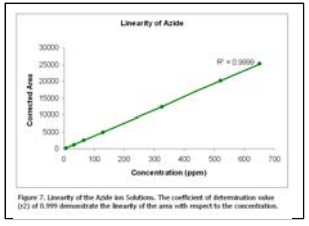

Figure 7. Linearity of the Azide ion Solutions. The coefficient of determination value (r2) of 0.999 demonstrate the linearity of the area with respect to the concentration.

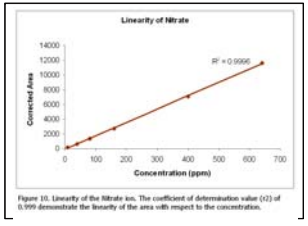

Figure 10. Linearity of the Nitrate ion. The coefficient of determination value (r2) of 0.999 demonstrate the linearity of the area with respect to the concentration.

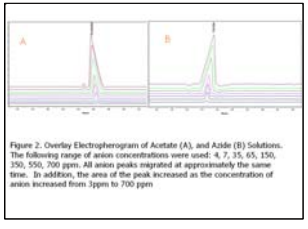

Figure 2. Overlay Electropherogram of Acetate (A), and Azide (B) Solutions. The following range of anion concentrations were used: 4, 7, 35, 65, 150, 350, 550, 700 ppm. All anion peaks migrated at approximately the same time. In addition, the area of the peak increased as the concentration of anion increased from 3ppm to 700 ppm

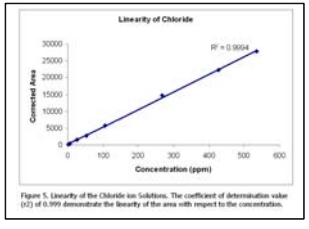

Figure 5. Linearity of the Chloride ion Solutions. The coefficient of determination value (r2) of 0.999 demonstrate the linearity of the area with respect to the concentration.

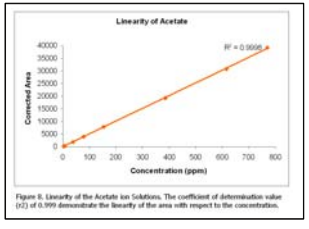

Figure 8. Linearity of the Acetate ion Solutions. The coefficient of determination value (r2) of 0.999 demonstrate the linearity of the area with respect to the concentration.

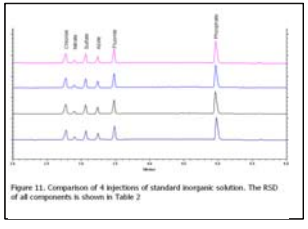

Figure 11. Comparison of 4 injections of standard inorganic solution. The RSD of all components is shown in Table 2

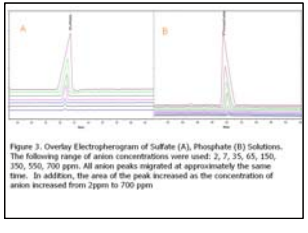

Figure 3. Overlay Electropherogram of Sulfate (A), Phosphate (B) Solutions. The following range of anion concentrations were used: 2, 7, 35, 65, 150, 350, 550, 700 ppm. All anion peaks migrated at approximately the same time. In addition, the area of the peak increased as the concentration of anion increased from 2ppm to 700 ppm

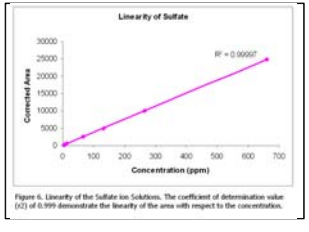

Figure 6. Linearity of the Sulfate ion Solutions. The coefficient of determination value (r2) of 0.999 demonstrate the linearity of the area with respect to the concentration.

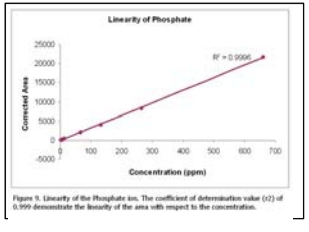

Figure 9. Linearity of the Phosphate ion. The coefficient of determination value (r2) of 0.999 demonstrate the linearity of the area with respect to the concentration.

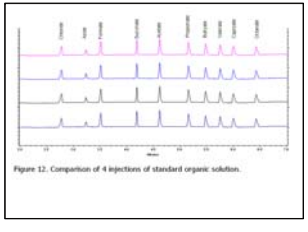

Figure 12. Comparison of 4 injections of standard organic solution.

Anion	PPM	Analysis 1	2	3	4	5	6	RSD
Chloride	107	5414	6002	556	5934	6138	6140	5%
Sulfate	132	5747	5519	5558	5676	5607	5602	2%
Acetate	154	7597	7782	7689	7922	7831	7935	2%
Phosphate	132	4303	4303	4367	4350	4439	4406	1%
Azide	130	4803	4838	4786	5005	4945	4919	2%
Nitrate	80	1402	1436	1561	1513	1497	1493	4%

Anion	PPM	Analysis 1	2	3	4	5	6	RSD
Chloride	107	2.83	2.83	2.83	2.83	2.83	2.83	0%
Sulfate	132	3.13	3.12	3.12	3.12	3.12	3.12	<1%
Acetate	154	4.60	4.60	4.60	4.61	4.60	4.61	<1%
Phosphate	132	4.96	4.97	4.96	4.97	4.96	4.96	<1%
Azide	130	3.30	3.31	3.31	3.31	3.31	3.31	<1%
Nitrate	80	2.94	2.95	2.95	2.95	2.95	2.95	<1%

Table 1. Reproducibility of Corrected Area and Migration time with Anions (Chloride, Sulfate, Acetate, Phosphate, Azide, Nitrate) in a typical BDS matrix.

Anion	PPM	Analysis 1	2	3	4	RSD
Chloride	20	2.78	2.77	2.77	2.77	<1%
Nitrate	20	2.90	2.90	2.90	2.90	<1%
Sulfate	20	3.07	3.07	3.07	2.07	<1%
Azide	10	3.25	3.24	3.24	3.24	<1%
Fluoride	10	3.49	3.48	3.48	3.48	<1%
Phosphate	50	4.97	4.96	4.96	4.96	<1%

Anion	PPM	Analysis 1	2	3	4	RSD
Chloride	20	1525	1482	1500	1511	1%
Nitrate	20	314	304	300	311	2%
Sulfate	20	1135	1111	1116	1106	1%
Azide	10	666	677	672	675	1%
Fluoride	10	1422	1390	1446	1499	3%
Phosphate	50	2020	2017	2037	2021	<1%

Table 2. Migration time and Corrected Area Reproducibility

CONCLUSIONS

A CE based method for detection and quantification of 8 anion residuals (Chloride, Sulfate, Bromide, Azide, Fluoride, Nitrate, Phosphate and Acetate) was successfully developed for maximum reproducibility.

The speed and reproducibility of the assay makes it an excellent alternative to other ion analysis techniques