

# Capillary Zone Electrophoretic Analysis of the Minor Anions Present in Orange Juice and Orange Pulpwash

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Capillary zone electrophoresis (CZE) was used to determine the chloride, sulfate, and nitrate concentrations of 35 orange juice, 10 orange pulpwash, and 3 water samples from juice-processing plants. Chloride and sulfate concentrations were determined by indirect photometry at 254 nm, and nitrate concentration was determined by direct UV detection at 214 nm. The concentrations of the anions in these samples were as follows: 7.7–97.4 ppm ( $\text{Cl}^-$ ), 19.1–107.6 ppm ( $\text{SO}_4^{2-}$ ), <0.2–3.52 ppm ( $\text{NO}_3^-$ ) for orange juice; 12.3–60.2 ppm ( $\text{Cl}^-$ ), 71.5–128.3 ppm ( $\text{SO}_4^{2-}$ ), <0.2–1.13 ppm ( $\text{NO}_3^-$ ) for pulpwash; 21.7–92.9 ppm ( $\text{Cl}^-$ ), 19.1–96.2 ppm ( $\text{SO}_4^{2-}$ ), <0.2–13.3 ppm ( $\text{NO}_3^-$ ) for processing plant water. The concentrations of these anions for two orange juice and two pulpwash samples were also determined by high-performance ion chromatography (HPIC), and these values were compared to those obtained by capillary zone electrophoresis. These results showed good correlation for chloride and sulfate; however, HPIC was unable to detect nitrate due to interference by other compounds in the orange juice/orange pulpwash and low concentration of nitrate in these samples. The effect of calcium and carbohydrate concentrations on the separation and quantitation of these three anions by CZE was also investigated. Calcium addition to the electrolyte caused retention time shifts which improved the separation of the aforementioned anions from citric acid. Carbohydrate addition had the effect of increasing retention times, peak broadening, and peak height reduction for each of the three anions. Results indicated that orange juice samples from Brazil had consistently lower levels of chloride than those from Florida, Israel, California, Arizona, Mexico, and Belize.

**Keywords:** *Capillary electrophoresis; anions; orange juice; pulpwash*

## INTRODUCTION

Capillary electrophoresis is an analytical tool which has not yet found ready acceptance by the food industry. It is a high-efficiency chromatographic method which under standard operating conditions provides  $10^5$ – $10^6$  theoretical plates (independent of column length). Capillary electrophoresis offers several advantages over the more conventional HPIC method for the analysis of anions in foods. These include shorter analysis times, short column regeneration times, high separation efficiency, minimal solvent/sample consumption, and low column cost.

Several methods have been employed to determine the chloride, sulfate, and nitrate content of orange juice (OJ) and orange pulpwash (OP).

Pulpwash is orange juice solids that are recovered during the processing of orange juice. The recovery process involves countercurrent extraction of finisher pulp with water. Methodology has been developed by Petrus and Attaway (1980) to detect the addition of pulpwash to orange juice by examining the differences in the visible and ultraviolet absorption and fluorescent excitation and emission characteristics of orange juice and orange pulpwash.

Nitrate levels in OJ have been determined colorimetrically by a number of researchers (Park et al., 1983; Wallrauch, 1971; Rebelein, 1967). According to Park et al. (1983) the concentration of nitrate in orange juice is typically less than 5 mg/L. Roya and Gimenez (1974) analyzed 50 Spanish orange juice samples for their nitrate content and found an average of 1.34 mg/L with a range of 0.45–2.80 mg/L. Wallrauch (1971) analyzed 91 pure orange juice samples and found that 25% of the

samples analyzed had nitrate levels >10 mg/L. This author attributed these high nitrate levels to the use of nondeionized water during reconstitution of the concentrate. A similar study by Benk et al. (1971) of 39 commercial orange juice samples showed a nitrate concentration range of 0.2–2.8 mg/L. Orange pulpwash analysis by Park et al. (1983) showed an average nitrate content of 26.5 mg/L with a range of 8.7–81.9 mg/L.

Orange juice results reported by Wallrauch and Faethe (1988) showed a maximum sulfate concentration of 150 mg/L. Spanish orange juice samples were reported (Roya and Gimenez, 1974) to have an average sulfate concentration of 145.3 mg/L with a maximum of 211.3 mg/L. Navel orange juice (Park et al., 1983) was shown to have an average concentration of 72 mg/L with a maximum of 87 mg/L. Orange pulpwash (Park et al., 1983) was shown to have an average sulfate concentration of 104 mg/L with a maximum of 160 mg/L.

The chloride content (potentiometric measurement) of orange juice has been shown to be quite variable (Wallrauch and Faethe, 1988; Souci et al., 1989; Roya and Gimenez, 1974). Reported chloride concentration levels from these studies ranged from 21 to 87 mg/L.

In this work, CZE was used to analyze the nitrate, chloride, and sulfate concentration of 35 orange juice, 10 orange pulpwash, and 3 processing plant water samples. Selected OJ and OP samples were also analyzed for their minor anion content by high performance ion chromatography (HPIC). Because HPIC is considered by many to be the method of choice for anion analysis a comparison of the results obtained by these two techniques was used to determine the efficacy of CZE for minor anion analysis of OJ and OP.

The effect of calcium ion and carbohydrate concentration on the qualitative and quantitative analysis of chloride, sulfate, and nitrate in OJ was also examined.

It was hypothesized that the minor anion concentration differences in OJ and OP could be used to detect the undeclared addition of this material to commercial orange juice.

## MATERIALS AND METHODS

Orange juice and pulpwash samples were diluted to 10.0° Brix (Canlab refractometer, Model 302). These samples were centrifuged at 2000g for 10 minutes at 4 °C (Sorvall, Model SS-1) to remove pulp. The supernatant was passed through a 0.2  $\mu$ m syringe filter (Millipore).

Anions were analyzed on a Waters Quanta (Milford, MA) 4000 capillary electrophoresis system. Separation of the anions was achieved on a 60 cm  $\times$  75  $\mu$ m fused silica column (Polymicro Technologies Inc., Phoenix, AZ). The system was equipped with a negative power supply for anion separation. The applied voltage was 20 kV. Sample introduction into the capillary was by a 30-s hydrostatic injection from a height of 10 cm. The electrolyte solution for the separation of chloride and sulfate was 5 mM sodium chromate containing 0.40 mM OFM-BT which was adjusted to pH 8.0 with lactic acid. OFM-BT is an electroosmotic flow modifier (EOF-M) which is used to reverse the direction of the electroosmotic flow. The EOF-M was obtained as a 20 mM concentrate from Waters (NICE-Pak OFM Anion-BT). Indirect UV detection was employed for these two anions at 254 nm. The electrolyte solution for nitrate analysis was 10 mM sodium chloride containing 0.40 mM OFM-OH (NICE-Pak OFM Anion-OH) which was adjusted to pH 8.0 with sodium hydroxide. Direct UV detection was used for nitrate at 214 nm.

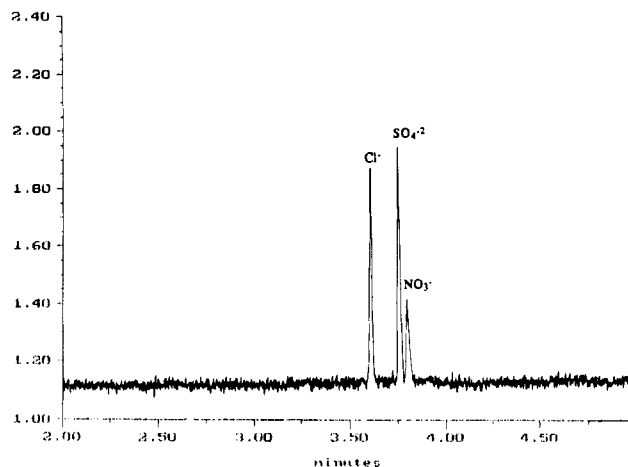
HPIC analysis of chloride, sulfate and nitrate was performed on a Dionex 4000i ion chromatography system equipped with a conductivity detector. An AS4A anion exchange column (4 mm  $\times$  25 cm) was used with an isocratic mobile phase of 2.0 mM sodium carbonate/0.75 mM sodium bicarbonate.

Nitrate analysis of selected orange juice and pulpwash samples was performed on a Cobas Fara II automated centrifugal analyzer (Hoffman-La Roche).

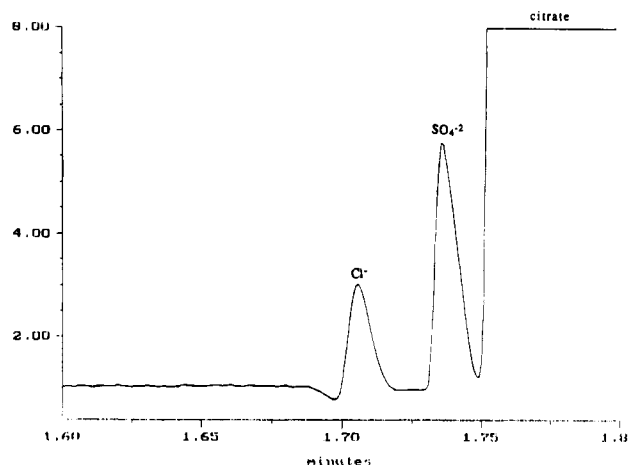
Two separate experiments were conducted to determine the effect of calcium ions and carbohydrate concentration on the separation and quantitation of chloride, sulfate, and nitrate by capillary zone electrophoresis (CZE). A solution containing the three anions each at a concentration of 10 ppm was analyzed by CZE employing the sodium chromate electrolyte previously described. To this solution was added 10% calcium hydroxide at increasing concentrations (from 0 to 200  $\mu$ L/50 mL of electrolyte; pH was maintained at 8.0). The second experiment consisted of a solution of chloride, sulfate, and nitrate at concentrations of 2.5, 15.0, and 1.1 ppm, respectively. This solution was analyzed by CZE employing sodium chromate as the electrolyte (conditions previously described) with increasing concentrations of glucose added to the electrolyte (from 0 to 20% w/w).

## RESULTS AND DISCUSSION

Capillary zone electrophoresis (CZE) employing indirect photometry (254 nm) afforded a rapid (<4 min) and baseline resolved separation of a chloride, sulfate and nitrate standard (Figure 1). Application of this technique to orange juice (OJ) or orange juice pulpwash (OP) samples resulted in faster elution times for chloride and sulfate with nitrate coeluting with sample constituents (Figure 2). The more rapid elution times for these anions can be explained by the high organic acid content of OJ and OP. Citric acid is the major organic acid present in these samples and has an average concentration of 0.70% (AFNOR, 1988) with a retention time of approximately 1.7 min by CZE (under our elution conditions). The high level of citric acid in orange juice



**Figure 1.** Electropherogram of a standard aqueous solution of chloride (1 ppm), sulfate (1 ppm), and nitrate (0.5 ppm) employing indirect photometry at 254 nm; sodium chromate electrolyte (5 mM) containing OFM-BT (0.40 mM).



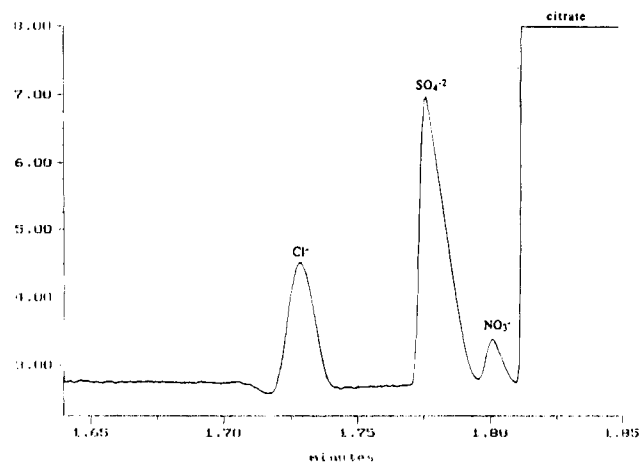
**Figure 2.** Electropherogram of a representative orange juice sample employing indirect photometry at 254 nm; sodium chromate electrolyte (5 mM) containing OFM-BT (0.40 mM).

and pulpwash samples resulted in a higher ionic strength within the capillary which resulted in more rapid elution of chloride, sulfate, and nitrate.

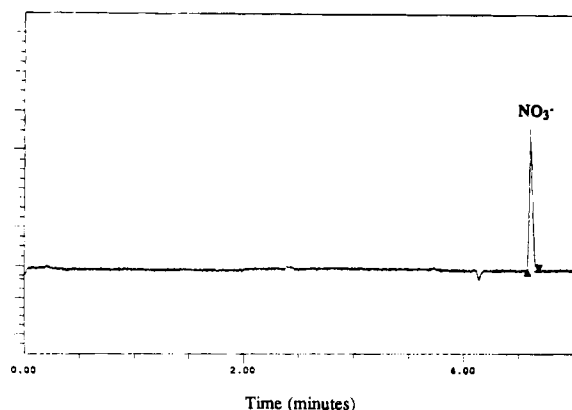
Due to the coelution of nitrate and citric acid, the effect of calcium ions on the relative mobility of nitrate was studied by the addition of 5–200  $\mu$ L of a 10% calcium hydroxide solution to 50 mL of electrolyte. This addition resulted in nitrate separation from citric acid (by indirect photometric detection) with a concomitant increase in the retention time of sulfate (Figure 3). These changes in anion mobility appear to be due to their complexation with calcium.

Because chloride, sulfate, and citrate are virtually transparent at 214 nm, direct detection of nitrate at 214 nm was investigated. This detection mode in conjunction with sodium chloride as the electrolyte resulted in a significantly improved (with respect to the lack/reduced detection of interfering compounds) analysis of nitrate in OJ and OP samples (Figure 4).

Results from CZE analysis of the 35 orange juice, 11 orange pulpwash, and 3 processing plant samples for chloride and sulfate concentration (indirect photometry at 254 nm) and nitrate (direct photometry at 214 nm) are shown in Table 1. Detection limits for each of these anions (based on a sample signal 3 $\times$  baseline noise) were 0.2 ppm. Linear results (correlation coefficients of 0.997–1.000) were obtained over a wide concentration



**Figure 3.** Electropherogram of a representative orange juice sample employing indirect photometry at 254 nm; sodium chromate electrolyte (5 mM) containing calcium hydroxide (50  $\mu$ L of a 10% solution) and OFM-BT (0.40 mM).



**Figure 4.** Electropherogram of a representative orange juice sample employing direct photometry at 214 nm; sodium chloride electrolyte (10 mM) containing OFM-OH (0.40 mM).

range for chloride (0.2–110 ppm), sulfate (0.2–125 ppm), and nitrate (0.2–75 ppm).

Examination of these results indicated that orange juice samples from Brazil had consistently lower levels of chloride than those from Florida, Israel, California, Arizona, Mexico, and Belize. In addition, all eight Floridian samples had nitrate levels less than the CZE limit of detection (0.20 ppm) used in this study. Because other samples had nitrate levels which were also <0.20 ppm, geographical distinction could not be based on nitrate. Due to the wide range observed for sulfate this anion would not be useful for sample geographical determination. Although the number of samples used in this study was small, chloride content may be a useful parameter in conjunction with metal data (McHard et al., 1979) for the authentication of Brazilian orange juice.

Range and mean results for orange juice, orange pulpwash, and processing plant water samples are shown in Table 2. The results from this study show that the relationship among chloride, sulfate, and nitrate concentrations could not be used to detect the addition of orange pulpwash to orange juice. The inability to detect this form of adulteration by anion analysis was due to the wide natural range of these anions in both oranges and the processing plant water used to produce pulpwash.

Table 3 shows the results obtained from the analysis of two orange juice and two orange pulpwash samples

**Table 1. Chloride, Sulfate, and Nitrate Concentration (Parts per Million) of Orange Juice, Orange Pulpwash, and Processing Plant Water Samples**

sample	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	NO <sub>3</sub> <sup>-</sup>
Valencia (Mexico)	29.9 ± 1.2	43.6 ± 2.4	0.58 ± 0.04
Valencia (Mexico)	23.5 ± 1.1	50.0 ± 2.2	0.26 ± 0.02
Valencia (Mexico)	31.5 ± 1.1	76.7 ± 2.8	<0.20
Valencia (Mexico)	19.5 ± 1.1	77.3 ± 3.1	<0.20
Valencia (Mexico)	30.2 ± 1.2	72.0 ± 2.6	0.36 ± 0.03
Victoria (Mexico)	16.0 ± 0.8	42.6 ± 1.3	<0.20
blend (Brazil)	17.9 ± 0.6	47.9 ± 2.4	0.52 ± 0.03
blend (Brazil)	11.0 ± 0.4	54.6 ± 1.4	0.53 ± 0.02
Hamlin (Brazil)	10.2 ± 0.3	26.4 ± 1.0	<0.20
Hamlin (Brazil)	9.4 ± 0.1	44.7 ± 2.0	0.43 ± 0.03
Pera (Brazil)	11.0 ± 0.3	77.6 ± 2.5	0.23 ± 0.01
Pera (Brazil)	11.7 ± 0.3	72.3 ± 2.1	0.32 ± 0.02
Valencia (Brazil)	7.7 ± 0.1	69.3 ± 2.2	0.32 ± 0.01
Valencia (Brazil)	10.2 ± 0.3	72.7 ± 2.0	0.36 ± 0.02
Valencia (Brazil)	9.5 ± 0.1	77.3 ± 2.6	<0.20
Hamlin (Florida)	31.6 ± 1.2	19.1 ± 0.6	<0.20
Hamlin (Florida)	22.4 ± 1.3	42.6 ± 1.4	<0.20
Hamlin (Florida)	25.3 ± 0.4	74.5 ± 2.2	<0.20
Pineapple (Florida)	39.2 ± 1.1	50.9 ± 2.3	<0.20
Pineapple (Florida)	26.9 ± 1.0	60.2 ± 2.8	<0.20
Valencia (Florida)	32.2 ± 1.4	84.6 ± 2.2	<0.20
Valencia (Florida)	25.3 ± 0.7	72.5 ± 2.3	<0.20
Valencia (Florida)	37.8 ± 1.1	40.1 ± 1.2	<0.20
Valencia (Israel)	38.4 ± 1.5	60.2 ± 2.4	1.51 ± 0.08
Shamouti (Israel)	32.2 ± 1.0	44.5 ± 2.0	0.34 ± 0.02
Shamouti (Israel)	28.8 ± 1.1	56.2 ± 2.3	0.35 ± 0.01
blood (Italy)	77.1 ± 2.1	83.2 ± 2.3	0.53 ± 0.01
blood (Italy)	26.5 ± 1.1	60.3 ± 2.1	0.39 ± 0.02
navel (California)	26.7 ± 1.1	87.6 ± 1.4	<0.20
navel (California)	44.2 ± 1.2	70.3 ± 2.0	1.09 ± 0.01
navel (California)	75.8 ± 1.1	55.3 ± 1.4	0.38 ± 0.02
Valencia (California)	77.1 ± 2.3	70.1 ± 1.2	0.32 ± 0.01
Valencia (California)	48.8 ± 0.6	107.6 ± 3.4	3.52 ± 0.12
Valencia (Arizona)	97.4 ± 2.7	69.8 ± 1.3	<0.20
Valencia (Belize)	25.3 ± 0.9	74.5 ± 1.7	<0.20
pulpwash (Brazil)	13.6 ± 0.6	79.3 ± 2.2	0.86 ± 0.03
pulpwash (Brazil)	13.3 ± 0.3	76.0 ± 1.5	0.93 ± 0.05
pulpwash (Brazil)	12.3 ± 0.4	72.7 ± 3.0	0.90 ± 0.01
pulpwash (Brazil)	28.2 ± 1.1	71.5 ± 1.3	1.13 ± 0.03
pulpwash (Florida)	60.2 ± 1.3	107.3 ± 3.3	0.30 ± 0.01
pulpwash (Florida)	40.7 ± 1.1	98.3 ± 2.3	0.24 ± 0.01
pulpwash (Florida)	51.7 ± 0.9	126.5 ± 4.1	<0.20
pulpwash (Florida)	55.7 ± 1.2	95.5 ± 2.0	<0.20
pulpwash (Florida)	53.0 ± 0.9	128.3 ± 5.4	<0.20
pulpwash (Florida)	50.0 ± 2.3	109.4 ± 2.8	<0.20
processing plant water (Florida)	21.7 ± 0.4	19.1 ± 0.2	<0.20
processing plant water (Florida)	92.9 ± 3.4	96.2 ± 3.0	13.30 ± 0.72
processing plant water (California)	43.5 ± 1.9	19.9 ± 0.6	5.70 ± 0.33

**Table 2. Range of Anion Concentration (Parts per Million) for Orange Juice, Orange Pulpwash, and Processing Plant Water Samples**

	mean	low	high
orange juice (35 samples)			
Cl <sup>-</sup>	31.1	7.7	97.4
SO <sub>4</sub> <sup>2-</sup>	62.5	19.1	107.6
NO <sub>3</sub> <sup>- a</sup>	0.44	<0.20	3.52
orange pulpwash (10 samples)			
Cl <sup>-</sup>	37.9	12.3	60.2
SO <sub>4</sub> <sup>2-</sup>	96.5	71.5	128.3
NO <sub>3</sub> <sup>- a</sup>	0.52	<0.20	1.13
processing plant water (3 samples)			
Cl <sup>-</sup>	52.7	21.7	92.9
SO <sub>4</sub> <sup>2-</sup>	45.1	19.1	96.2
NO <sub>3</sub> <sup>- a</sup>	6.4	<0.20	13.30

<sup>a</sup> Assuming <0.20 ppm = 0.20 ppm.

by both CZE and HPIC. There was good correlation between these two analytical techniques with respect to chloride and sulfate concentration. Due to the presence of interfering compounds and low concentra-

**Table 3. CZE and HPIC Concentration (Parts per Million) Results for Chloride, Sulfate, and Nitrate for Two Orange Juice and Two Orange Pulpwash Samples**

	CZE	HPIC
orange juice (Brazil)		
Cl <sup>-</sup>	17.2	17.9
SO <sub>4</sub> <sup>2-</sup>	47.3	47.9
NO <sub>3</sub> <sup>-</sup>	0.51	nd <sup>a</sup>
orange juice (Florida)		
Cl <sup>-</sup>	31.0	30.2
SO <sub>4</sub> <sup>2-</sup>	80.1	76.7
NO <sub>3</sub> <sup>-</sup>	<0.20	nd
pulpwash (Brazil)		
Cl <sup>-</sup>	27.4	28.2
SO <sub>4</sub> <sup>2-</sup>	68.6	71.5
NO <sub>3</sub> <sup>-</sup>	1.11	nd
pulpwash (Florida)		
Cl <sup>-</sup>	52.3	51.7
SO <sub>4</sub> <sup>2-</sup>	130.0	126.2
NO <sub>3</sub> <sup>-</sup>	<0.20	nd

<sup>a</sup> Not detected.

tion of nitrate in these samples, HPIC was unable to detect this anion. These results indicate that CZE would be the method of choice for nitrate analysis of orange juice (and pulpwash).

Due to the high carbohydrate content of OJ and OP, their effect on anion elution was studied by the addition of glucose (0–20%) to the electrolyte. Results from these experiments showed that as the concentration of glucose increased the retention times and peak areas increased/peak height decreased (when compared to standards) for each anion. This effect was most likely due to the viscosity change of the electrolyte solution which resulted in a slowing down of the mobility of the anions (Jandik and Jones, 1991). Therefore, accurate results for CZE analysis of these three anions can only be obtained when standard solutions contain the same relative carbohydrate concentration as the samples being analyzed.

## CONCLUSIONS

Capillary electrophoresis is a rapid, easy, and inexpensive analytical technique for the analysis of chloride, sulfate, and nitrate in orange juice/orange juice pulpwash. The technique does not appear to be useful for the detection of undeclared addition of pulpwash to orange juice samples. However, sample cleanup was facile, involving only sample dilution and filtration. Detection limits for these three anions were low (0.20 ppm) and were linear over a fairly wide concentration range. In addition to the low relative cost of the capillary column (~\$7), more than 400 injections can be made on a single column (with intermittent cleaning with sodium hydroxide). These factors in addition to the diversity of chemical components (cations, protein,

carbohydrates, etc.) which can be measured by CZE make it an ideal analytical tool for the food industry.

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