



## Estimation of Some Carboxylic Acids and Inorganic Anions in Industrial Fruit Juice Beverage by Ion Chromatography

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### ABSTRACT

Carboxylic acids and anions from Industrial fruit juice beverage are detected by measuring the suppressed conductivity detection at baseline  $\approx 0.05 \mu\text{S cm}^{-1}$  of complex formed with the Dionex IonPac AS11-HC-5  $\mu\text{m}$  Column. This method, high speed, efficient, cheap, very Recoveries and isocratic phase. Method has been developed and validated for the Estimation of Organic acid and anions using 5 mM sodium hydroxide as mobile phase having PH 9 Mobile phase was pumped at flow rate of  $1.0 \text{ mL min}^{-1}$  and isocratic elution using through column. The Conductivity detection method was performed at 12 minutes. Method was successfully following the IC Isocratic. Calibration curve was very linear in concentration range [(2-10) and (3-15)]  $\mu\text{g mL}^{-1}$  for Chloride and Phosphate Consecutively with correlation coefficient Range  $R^2(0.9890-1.0)$  for inorganic anion and Calibration curve (5-25)  $\mu\text{g mL}^{-1}$  for each carboxylic acid but non-linear ( $n=3$ ). The determine of lower limits of detection (LLOD) and quantitation (LLOQ) in standard Analyzed for inorganic anions, Also; method detection limit (MDL) for each anion in Organic acids. Recovery was found to be in the range 96.6-101.3% and precision less than 1%. Developed method was validating applied for analyzed of standard solution and also to study the interaction of Analysis at room temperature.

**Keywords:** Industrial beverages; Organic and inorganic anions; Saccharide compounds

### INTRODUCTION

Carboxylic acids of low and middle molecular weights such as acetic acid, pyruvic acids tartaric acid and citric acid play important role in quality of beverages made of industrial materials and then it has great importance in the industry of industrial juices so that color, taste and smile of juice is depended upon type of organic acid, found in Beverage; for example lemon industrial beverage, orange industrial beverage contain, high percentage of these acids, in addition; that it contains organic acids, phenol compounds and saccharides and high percentages of inorganic ions such as chloride, phosphate, organic acid profiles are monitored to certain determine the industry of industrial juices; so that the juices makers also monitor the concentration of various organic acids to ensure the quality of their juices[1-3]. In general speaking; these acids have bad absorption in case of applying technic of UV to be separate from its raw material and then its estimate will be hard due to presence of phenol compounds or its derivatives and saccharides causing interventions in the absorption determinate values and thus it is lessen the measurements sensitivity [4]. Ionization of carboxylic acids can be satisfactorily measurement for category charged by conductive detector by using separation columns accompanies with suppress columns, in case of using interventions causing this method validation for separation and assessment these acids and since that these acids of low molecular weights causing its separation successively according to the increasing PKa values and upon this foundation [5]. International Agency of Foods determined the validity of carbonized Industrial fruit juices through percentages of organic acids and inorganic ions, also; the percentages of its possibly contents of phenolic toxic compounds [6].

The IC method with suppress columns of conductivity can also have the ability to estimate trace amounts of undesirable impurities in sample preparations that can originate from the raw Industrial materials used in the manufacturing process [7,8]. The column that used for determination of carboxylic acids and anions, IonPac AS11-HC, is a high-capacity, hydroxide-selective anion-exchange column that provides low retention time and high resolution to separations of inorganic anions, low molecular weight organic anions, and polyvalent anionic species [9]. Therefore, the Ion Pac AS11-HC is best suited for the analysis of anions samples that are well-specifications. As result, short time analysis and very perfect analysis for most separation samples [10]. IC system with conductivity Detector has advantages of 1  $\mu\text{L}/\text{min}$  flow rate and low eluent concentration that consumption, allows continuously run and available IC system without additional consumable replacement aliments, very easily on equilibrating and recalibration typically need after each start – up. The Ion chromatography method according for estimate number of samples from carboxylic acids and anions by IC system with high sensitivity and reproducibility method [11].

### Aim of Study

To confirmation an efficient, comprehensive IC and quality control (QC) methods for the analysis at carboxylic acids and inorganic anion in industrial fruit beverages, this method must separate all anions, the application method to create an IC based method for determining anions especially Carboxylic acids from industrial beverages and shown to be fast, active, and sensitive method.

### Suitability System

A system that suitability to test samples was developed for successfully routine application of the calibrate method to determine the accuracy and precision of the system by making five injections of standard solution for each anion Table 3 shown all parameters that requirement for development method.

## EXPERIMENTAL SECTION

A dionex Ion Pac AS11-HC; 5  $\mu\text{m}$ , 4 $\times$ 250 mm (P/N 052960) was chosen for this separation because its structure are similar to the aromatic phenyl groups structures. The separation of all anions in sample can be completed within Run time 12 min using a 5 mM NaOH as eluent with DI. Water (18.2  $\Omega\text{m}$ .cm resistivity) [12,13].

### Recommended Equipment

Ion Chromatography System (metrohm 690) having conductivity detector with the Dionex IonPac AS11-HC-5  $\mu\text{m}$  Column and LKB Isocratic pump C2150 [14].

**Table 1: Method parameters**

Parameters	Conditions
Description Column	Deonex IonPac AS11-HC Analytical, 4 $\times$ 250 mm (P/N052960)
Iso Critic Mobil phase	5 mM NaOH
Detection System	Conductivity Detection
Back ground conductivity	0.05 $\mu\text{S cm}^{-1}$
Flow Rate	1.0 mL/min
Temperature	25 $^{\circ}\text{C}$
Pressure Background	200 Bar
Run Time	12 Min
Injection Volume	100 $\mu\text{L}$

### Reagents and standards

- Deionized (DI) water, 18.2  $\Omega\text{-Cm}$  resistivity.
- Sodium hydroxide (NaOH), IC grade, BDH chem.
- Industrial Orange juice from locally market
- Standard solution 1000  $\mu\text{g}/\text{ml}$  for each anion prepared.
- Stock standard solution 100  $\mu\text{g}/\text{ml}$  for each anion prepared.

Five different concentrations as working standard solution for each anion.

### Sample Preparation

Dilute Industrial fruit juice beverage samples 1:10 D.I water and filter through A filter paper (Millipore USA 0.45  $\mu\text{m}$ ) prior to analysis.

### Sample Analysis

A number of carboxylic acids and anions in samples were studied; the various Organic acids and inorganic anions were diagnosed by comparing their retention times with the standards anions solution. The concentrations of all the anions were determinate by using the 6-anion standard mixture shown in Figure 1, which were accurately quantified from their respective calibration curves. As noted in the chromatograms of the selected samples, dissolved  $\text{CO}_2$  appeared as the carbonate peak in all samples, but did not interfere with the interest peaks [15,16].

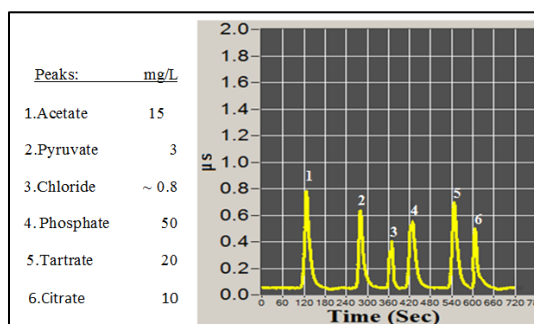


Figure 1: Peaks of 6 anions in industrial beverage

Under the established conditions listed in Tables 1-4, a linear calibration graph for chloride and phosphate ion was obtained in the range of [(2.0-10.0) and (3.0-15.0)]  $\mu\text{g ml}^{-1}$  consecutively, the typical calibration peaks are shown in Figures 2A and 2B as well as in Figure 3A and 3B. The unknown concentrations of chloride and phosphate ions were estimated by comparison with the standard calibration curve.

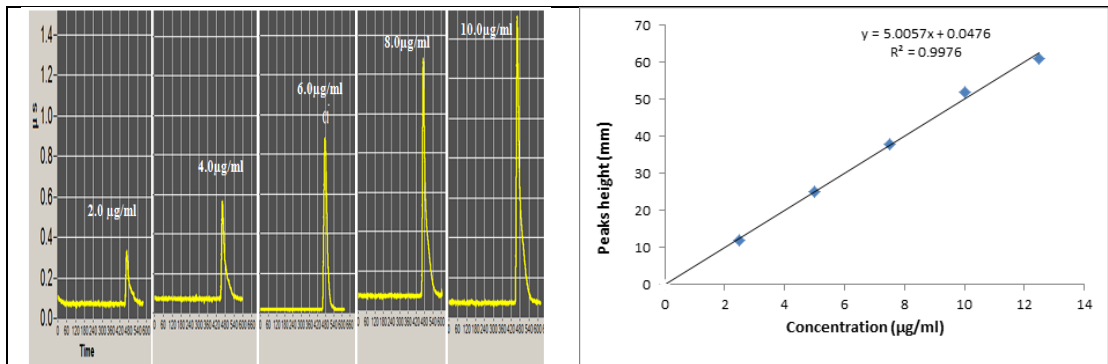


Figure 2: A) Peaks of chloride ion in industrial beverage; B) Standard calibration curve for chloride ion

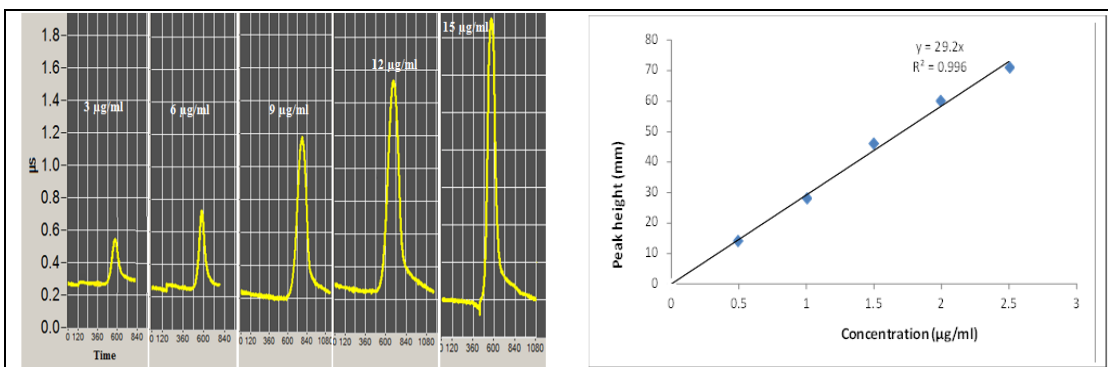


Figure 3: A) Peaks of phosphate ion in industrial beverage; B) Standard calibration curve for phosphate ion

Through the non-linear standard calibration curve for carboxylic acids, the concentration of the unknown of weak organic acids can be calculated by Kolarauch theoretical method [17,18] and dilution low for solutions in industrial juices, the concentration of the acids were calculated by Titration method versus different volumes and a constant concentration of sodium hydroxide that shown in Figure 4.

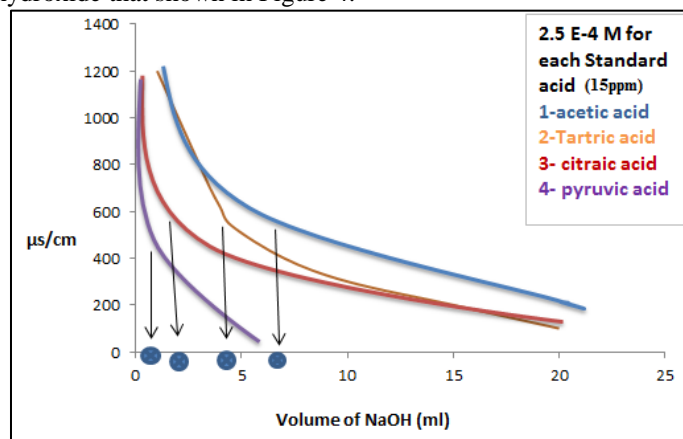


Figure 4: Non-linear standard calibration curve for carboxylic acids

## RESULTS AND DISCUSSION

### Organic Acid Analysis

Organic acids such as citrate or malate are often introduced into carbonated beverages in definite proportions to impart a particular flavor. For carbonated Industrial fruit beverage, some organic acids may be present naturally in the fruit. In addition, the presence of some organic acids can be used to reveal potential food adulteration [19,20]. One way to separate and detect organic acids with ion exchange chromatography method by using suppressed conductivity detection, the Ion Pac AS11-HC column is anion exchange column designed for efficient separation of low or medium molecular weight aliphatic and aromatic organic acids. Separation mechanism with weakly ionized acids are depend on differences in their PKa. Strong inorganic acid anions are not retained by the stationary phase and elute in the exchanged volume of the column, so the use of conductivity for detection may be regarded as non-specific in much the same way as refractive index is for compounds separated by IC and so the anion must be separated form one anther before they reach the detector. The sensitivity of the conductivity cell is depend on the dissociated constant (PKa) of the acid form of the anion, poorly dissociated constant for organic acid with PKa ~7 lead to weak detected by conductivity cell [21]. So the organic acid are separated depend on increasing PKa value, so; this process need to the Dionex Ion Pac AS11-HC high sensitivity column with the standard eluent 5 mM NaOH Table 1.

### Inorganic Anions

Chloride and phosphate such as inorganic anions present in industrial carbonated beverages are usually derived from the water and some preservatives that used in production. Some anions, however, such as phosphate may be added deliberately to impart a particular flavor or acidity test. The water can be monitored by ion chromatography to ensure purity and consistency, while the final product is monitored to keep its quality and testing. Inorganic anions are separated by anion-exchange chromatography, and monitored by suppressed conductivity detection; Table 1 lists the optimum conditions for experimental. When performing gradient elution on the AS11 column, a very dilution hydroxide eluent system is used instead of a carbonate eluent, because of its high elute and lower background conductivity when suppressed conductivity column is used, an Anion Suppressor Column (ASC) should be installed between the gradient pump and the injection valve to minimize baseline shifts resulting from the elution of anionic contaminants in the eluent. The separations of inorganic and organic anions in a variety of industrial carbonated beverages using the AS11 column. The samples were degassed and diluted 1:10 prior to injection. The sodium hydroxide concentration is a good enough eluent for anion and organic acid [22,23].

### Effect of Column and Eluent on the Separation [24]

Separation column is suitable used in this method, the Dionex IonPac AS11-HC (5 µm), column has high efficiency to separate different anions, the linear gradient ranged between 10 minutes for all anions while the total time of separation is 12 minutes. Six consecutive peaks appearance in chart, the distinct peaks cause of validation method

and sensitivity to separation and determination of organic acids; medium height peaks refers to limited concentration for inorganic and organic anions. Organic acids and inorganic anions can be detected under baseline  $\approx 0.05 \mu\text{S cm}^{-1}$  by suppressed conductivity with 5 mM NaOH as eluent, this eluent can be prepared directly after experimental perfect.

### Effect of Column Temperature on the Separation

The effect of temperature on the separation column AS11-HC 5 $\mu\text{m}$  that is used to separate and estimate all anions were studied. The separation of standard on the column, with temperature 25°C. As expected, increment the column temperature decreased retention time, and baseline separation of the standards were achieved in the temperature range, however, preferably used temperature 25°C in all measurements [25]. Column temperature could cause some other compound to interfere with detection of the analysis, but in this column; a good separation for the sample at room temperature.

### Method Performance (Reproducibility, Linearity and Detection Limits)

The method reproducibility was determined by making consecutive injection of all samples. Excellent RSD for retention time and peaks height were acquired as shown in Table 2. Lower limit of detection (LLOD), quantitation (LLOQ) and method detection limit (MDL) are the concentrations that give signal to noise ratio of 3:1 or 10:1 for LLOD and LLOQ respectively which can be detected and verified by the relation of standard deviation of response (SD) to the slope or T-Test:

$$\text{LLOD} = 3.3 \text{ SD/S}$$

$$\text{LLOQ} = 10 \text{ SD/S}$$

$$\text{MDL} = \text{SD} \times t_{95\%}$$

LLOD, LLOQ and MDL were calculated using the single-sided student's test method (at the 95% confidence limit). Using five consecutive injection at all anion sample and standard, the testing determined standard deviation values for calculating LLOD, LLOQ and MDL [26,27]. It is important to note that carboxylic acids are a low dissociation acids and have different Pka values. therefore, linear and correlation coefficient values cannot be measured in the calibration curve of these acids, so; LLOD and LLOQ values cannot be measured because these values based on the correlation coefficient (R2) and slope (S), but the MDL based on the test factor (t) and Standard deviation (SD). Table 2 lists all these values:

**Table 2: Regression statistics of the proposed method with LLOD, LLOQ, Intercept, Slope and MDL**

Ions	Retention Time(min)	R2	Std Er	Std Er Est	Intercept	Slope	LOD $\mu\text{g mL}^{-1}$	LOQ $\mu\text{g mL}^{-1}$	MDL $\mu\text{g mL}^{-1}$
Chloride	6	0.989	0.507	0.5	0.523	3.52	0.172	1.152	-
Phosphate	7	1	0	0	0	23	0.552	3.698	-
Acetate	2	-	0.443	0.434	-	-	-	-	2
Pyruvate	5	-	0.42	0.4	-	-	-	-	2.2
Tartrate	9	-	0.455	0.4	-	-	-	-	2.4
Citrate	10	-	0.601	0.581	-	-	-	-	1.9

### Accuracy/Recovery Studies

The accuracy of an analytical method is determined by how close the test results obtained by that method equal to the true value. It can be determined by recovery studies, where a known amount of standard is accurate in the sample to be analyzed. The results of accuracy studies are shown in Table 3 and it is evident that the method could be used for accurate determination within the desired range [28].

### Precision

Precision of method, reported as %RSD, was estimated by measuring repeatability (intra-day assay) on five replicate injection for all concentration anions shown in Table 4, and intermediate precision (inter-day variation) was studied for two days using intermediate concentration solution for each anion (n=3) [29,30]. All the results given in Table 4

**Table 3: Method accuracy from recovery assays for the studied compounds analyses**

Ions	Conc. ( $\mu\text{g mL}^{-1}$ )	Recovered conc. ( $\mu\text{g mL}^{-1}$ )	Recovery %	RSD %
Chloride	6	5.8	96.6	0.0982
Phosphate	9	8.8	97.7	0.1338
Acetate	15	15	100	0.101
Pyruvate	15	15.2	101.3	0.2
Tartrate	15	14.8	98.6	0.1521
Citrate	15	14.9	99.3	0.1111

**Table 4: Intra and inter-day precision and accuracy of standard analysis (n=3)**

Inter-day			Intra-day			
RSD %	Recovery %	Found ( $\mu\text{g mL}^{-1}$ )	Recovery %	Found ( $\mu\text{g mL}^{-1}$ )	Conc. ( $\mu\text{g mL}^{-1}$ )	Ions
0.1141	96.6	5.8	96.6	5.8	6	Chloride
0.091	98.8	8.9	97.7	8.8	9	Phosphate
0.2001	100	15	100	15	15	Acetate
0.1322	100	15	101.3	15.2	15	Pyruvate
0.2189	100	15	98.6	14.8	15	Tartrate
0.0987	100	15	99.3	14.9	15	Citrate

## CONCLUSION

Ion chromatography method with conductivity detector has been applied successfully to the analysis of carbonated industrial beverages for a variety of inorganic and Organic components. Total analysis time is approximately 12 minutes for IC analysis to separation of 6 anions on the Dionex IonPac AS11-HC (5  $\mu\text{m}$ ) due to the need for column re-equilibration. The method which described in this application it's a rapid and gave excellent separation for ions in industrial carbonated beverages. IC with suppressed conductivity detection is a well-established technique for the determination of a wide range of organic acid and anions. Therefore, IC has become an invaluable analytical tool to analyzed the carbonated beverages industry. The suppressed conductivity detection offers high sensitivity for all anions, including various Organic acids and anions at low concentrations. The specificity and sensitivity of this method allow sample treatments without complex procedures such as extraction and/or derivative also the processes ensure good peaks height, retention time and precisions. In addition, the recovery study shows good accuracy of the method.

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