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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 ≈ 0.05 µs cm^{-1} of complex formed with the Dionex IonPac AS11-HC-5 µm 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 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)) µg 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) µg 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 validate applied for analyzed of standard solution and also to study the interaction of Analysis at room temperature.

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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 (Shafqat ullah *et al*., 2012; Adou *et al*., 2012; Maher Al-Dabbas *et al*., 2012). 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 (Ehling and Cole, 2011). 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 (Hardy and Sanderson, 2010). 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 (Janati *et al*., 2012). 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 (Lacey *et al*., 2009; Martin *et al*., 2005), 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 (Krueger, 2012). 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 (Gundogdu and Yilmaz, 2012). IC system with conductivity Detector has advantages of 1µl /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 (Pohl, 2010).

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.

1-Experimental :- (www.dionex.com/en-s/webdocs/110622-AN273-IC-OrganicAcids-Anions-Wine-Jun2011-PN2727-01; Al-Sowdani and Al-Salman, 2012)

A dionex Ion Pac AS11-HC; 5µm, 4×250 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 5mM NaOH as eluent with DI. Water (18.2Ωm.cm resistivity).

2-Recommended Equipment: -(Al-Sowdani *et al*., 2015)

Ion Chromatography System (metrohm 690) having conductivity detector with the Dionex IonPac AS11-HC-5 µm Column and LKB Isocratic pump C2150.

Reagents and standards

- Deionized (DI) water, 18.2Ω-Cm resistivity.
- Sodium hydroxide (NaOH), IC grade, BDH chem.
- industrial Orange juice from locally market
- Standard solution1000µg/ml for each anion prepared.
- Stock standard solution 100 µg/ml for each anion prepared.

Table 1. Method parameters

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

Five different concentration 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 0.45µm) 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 CO_2 appeared as the carbonate peak in all samples, but did not interfere with the interest peaks (Xu *et al*., 2012; USEPA method 300.0, Rev. 2.1. Determination of inorganic anions by ion chromatography; 1993).

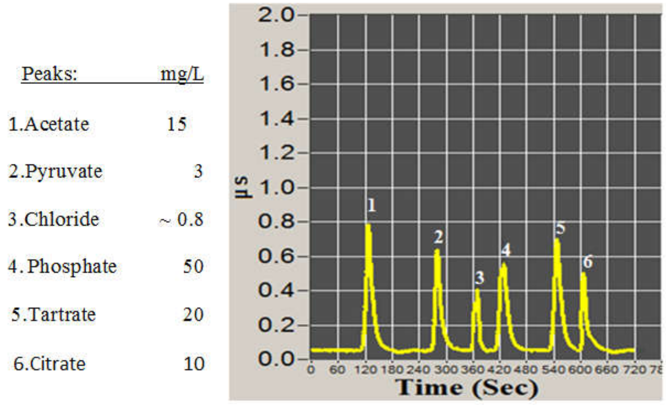

Figure 1. Peaks of 6 Anions in industrial beverage

Under the established conditions listed in Table 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))µg ml^{-1} consecutively, the typical calibration peaks are shown in Figures 2,3: A and B. The unknown concentrations of chloride

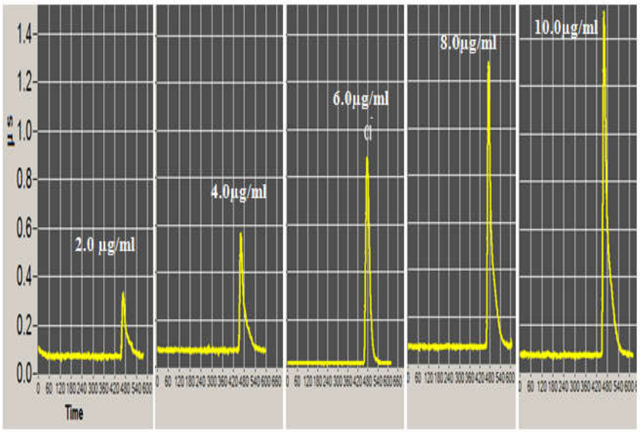

Figure 2: A:Peaks of Chloride ion in industrial beverage

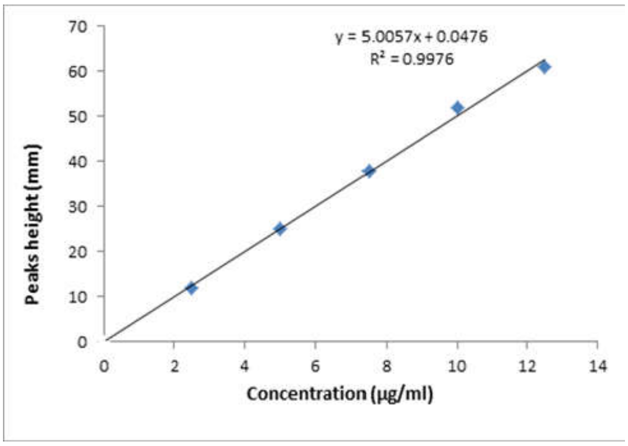

Figure 2:B:Standard Calibration Curve for Chloride ion

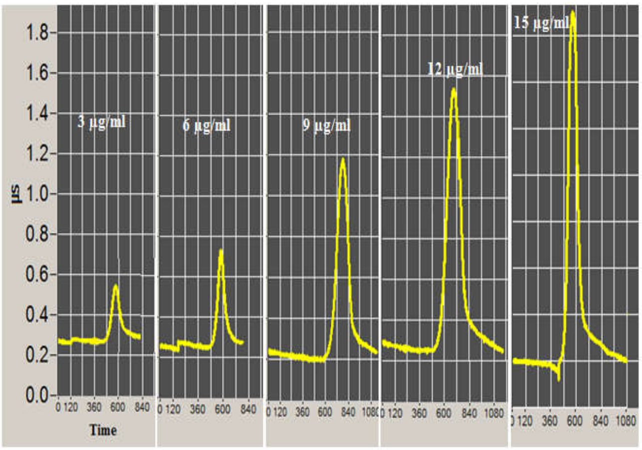

Figure 3. A:Peaks of phosphate ion in industrial beverage

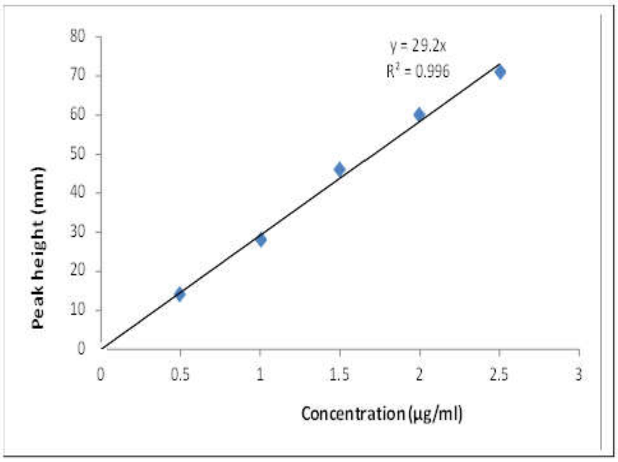

Figure 3. B:Standard Calibration Curve for phosphate ion

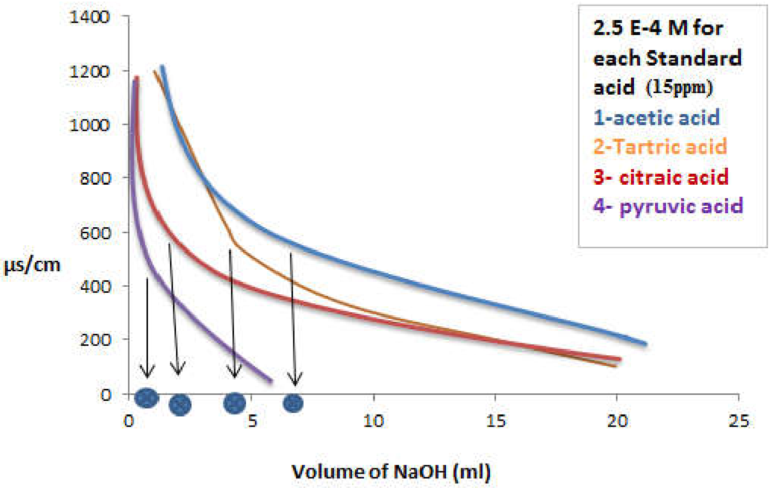

Figure 4. Non-linear standard calibration curve for carboxylic acids

and phosphate ions were estimated by comparison with the standard calibration curve. 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 (Lobo *et al*., 2015; Ribeiro and Ana, 2015) 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.

RESULTS AND DISCUSSION

1- 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

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

Ions	Retention Time(min)	R^2	Std Er	Std Er Est	Intercept	Slope	LOD µg mL^{-1}	LOQ µg mL^{-1}	MDL µg mL^{-1}
Chloride	6	0.9890	0.507	0.500	0.523	3.52	**0.172**	**1.152**	-
Phosphate	7	**1.0**	0.0	0.0	**0.0**	**23.0**	**0.552**	**3.698**	-
Acetate	2	-	**0.443**	**0.434**	-	-	-	-	2.0
Pyruvate	**5**	-	**0.420**	**0.400**	-	-	-	-	2.2
Tartrate	9	-	**0.455**	**0.400**	-	-	-	-	2.4
Citrate	10	-	**0.601**	**0.581**	-	-	-	-	1.9

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

Ions	conc. (µg mL^{-1})	Recovered conc. (µg mL^{-1})	% Recovery	%RSD
Chloride	**6**	**5.8**	**96.6**	0.09823
Phosphate	**9**	**8.8**	**97.7**	0.13381
Acetate	**15**	**15**	**100**	0.10099
Pyruvate	**15**	**15.2**	**101.3**	0.20001
Tartrate	**15**	**14.8**	**98.6**	0.15210
Citrate	**15**	**14.9**	**99.3**	0.11105

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

Intra-day				Inter-day		
Ions	conc. (µg mL^{-1})	Found (µg mL^{-1})	% Recovery	Found (µg mL^{-1})	% Recovery	%RSD
Chloride	**6**	**5.8**	**96.6**	**5.8**	**96.6**	0.11409
Phosphate	**9**	**8.8**	**97.7**	**8.9**	**98.8**	0.09101
Acetate	**15**	**15**	**100**	**15**	**100**	0.20010
Pyruvate	**15**	**15.2**	**101.3**	**15**	**100**	0.13224
Tartrate	**15**	**14.8**	**98.6**	**15**	**100**	0.21885
Citrate	**15**	**14.9**	**99.3**	**15**	**100**	0.09865

reveal potential food adulteration (Abrahamse and Bartowsky, 2012; Lopez-Rituerto *et al.*, 2009). 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 (Lateef, 2011), 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 Table1.

2- 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 Suppresser 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 elutent for anion and Organic acid (Ravi *et al.*, 2010; Serpen, 2012).

3- Effected of Column and elunt on the separation:-(Al-Salman *et al.*, 2017)

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 detect under baseline ≈ 0.05 µs cm^{-1} by suppressed conductivity with 5 mM NaOH as eluent, this eluent can be prepared directly after experimental perfect.

4- Effected Column Temperature on the separation:-(Tasnim *et al.*, 2010)

The effect of temperature on the separation column AS11-HC 5µm that its used to separate and estimate all anions were studied. The separation of standard on the column, with temperature 25^{o}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^{o}C in all measurements. Column temperature could cause some other compound to interfere with detection of the analysis, but in the this column; a good separation for the sample at room temperature.

5- Method performance (Reproducibility, linearity and Detection Limits):-(Al-Salman *et al*., 2017; Weikle, 2012)

The method reproducibility was determined by making consecutive injection of all samples. Excellent RSD for retention time and peaks height were acquire 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:

LLOD=3.3 SD/S

LLOQ=10 SD/S

MDL = SD × $\mathbf{t_{95\%}}$

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. 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 (R^2) and Slope (S),but the MDL based on the test factor (t) and Standard deviation (SD). Table 2 list all this values :

Accuracy/recovery studies (Yamakawa *et al*., 2012)

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 cold be used for accurate determination within the desired range.

Precision (Khosrokhavar *et al*., 2010; Cornelia and Elena, 2009)

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). All the results given in Table 4

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 µm) 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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