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Original Research Article

A new HPLC method for simultaneously measuring chloride, sugars, organic acids and alcohols in food samples

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ABSTRACT

This paper introduces an original, rapid, efficient and reliable HPLC method for the accurate and simultaneous quantification (g/L) of chloride in samples containing sugars, organic acids and alcohols. Separation was achieved using a HI-Plex H column at 35 °C, with H_2SO_4 (0.005 N) as the mobile phase at a flow rate of 0.4 mL/min. The column effluent was monitored by a Refractive Index (RI) detector. A linear response was achieved over NaCl concentrations of 0.25–2.5 g/L and 5–40 g/L. The analytical method inter- and intra-run accuracy and precision were better than ±10.0%. Investigating the mechanism of detection using different chloride and sodium s reviled that this method can be used for determining the total concentration of chloride salts when in suspension. This method was successfully applied to 15 samples of commercial food products and the salt content obtained from this method was compared with 3 other methods for salt determination. The (HI-Plex H) column was designed for determining the concentrations of sugars, organic acids and alcohols when in solution. Hence, application of our new methodology would allow the determination of sugars, alcohols and organic acids in samples derived from seawater-based fermentation media as well as samples from salty food and dairy products.
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1. Introduction

Sodium chloride (NaCl) is a common flavour and preservative component present in many food products such as cheeses, butter and pickles. Also media used in fermentations could contain high salt concentrations as seawater has been suggested as an alternative to the use of freshwater in some fermentations such as bioethanol production (Lin et al., 2011; Zaky et al., 2014). Hence, there is a need for an accurate and rapid method for NaCl determination during the manufacturing processes.

Classical titration methods, Mohr (Doughty, 1924) and Volhard (modified) (Schales and Schales, 1941), which are based on the use of silver nitrate ($AgNO_3$), are still widely used for the determination of NaCl (Leong et al., 2014; Rajković et al. 2010). However, those methods are associated with several limitations such as: a) time consuming, b) results are sensitive to the pH and the presence of heavy metals in the sample, c) they can have false end points, d) difficult to automate, and e) the safe disposal of silver compounds after testing (Wolfbeis and Hochmuth, 1984). Silver nitrate is considered as a very toxic and corrosive compound even at very low concentrations (Zhao and Wang, 2011). Hence, the chloride analyser has been suggested as a method, this is a rapid test but still requires $AgNO_3$ to operate (Johnson and Olson, 1985).

In general, use of HPLC is a convenient and accurate analytical method suitable for a variety of samples including the quantification of organic and inorganic compounds. However, obtaining an accurate quantification of NaCl using HPLC in samples containing sugars has proven difficult due to similar retention times for Cl^- and sugars especially glucose and sucrose (Sims, 1995). As those two sugars are most abundant in food products, the use of HPLC for measuring sugar content is limited in many food samples because they contain salt. In addition, the hydrolysis of cellulosic materials

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to monomeric sugars for second generation biofuel generates a considerable amount of salt during the neutralisation, so using HPLC for sugar quantification requires a method that separates sugars from salts in order to obtain an accurate analysis for both sugars and salts.

Chromatographic methods are the best analytical techniques for the quantification and identification of mono and oligosaccharides in food products (Duarte-Delgado et al., 2015). In fact, HPLC is the preferred method for sugar quantification according to the guidelines of the Association of Official Analytical Chemists (AOAC, 1993; Sims, 1995).

During HPLC analysis of a bioethanol fermentation sample, an unexpected peak appeared with a retention time (R_t) of 10.90 min (Fig. 1). The fermentation experiment was carried out using a medium that was prepared using natural seawater instead of freshwater. Further investigation revealed that this unexpected peak correlated with the concentration of chloride salts present in seawater. This study was thus carried out to validate this observation with an aim of introducing a new rapid and accurate HPLC method for the determination of chloride salts in the presence of sugars.

2. Materials and methods

2.1. Chemicals

Chemicals and solvents used in this study were HPLC or analytical grade purchased from authorized manufacturers and suppliers. Distilled water was used as a solvent for preparing the mobile phase and samples.

2.2. Chromatography

The HPLC system consisted of a JASCO AS-2055 Intelligent auto sampler (JASCO, Tokyo, Japan) and a JASCO PU-1580 Intelligent HPLC pump (JASCO). Chromatographic separation of sodium chloride (NaCl) as well as all other components under investigation in this study (organic and inorganic salts, sugars, organic acids and alcohols) was achieved at 35 °C using a Hi-Plex H column (7.7 × 300 mm, 8 μm) (Agilent Technologies, Inc., UK) and a Jasco RI-2031 Intelligent refractive index detector (Jasco). The mobile phase was 0.005 N H_2SO_4 at a flow rate of 0.4 mL/min. The mobile phase solution was also used for flushing the syringe of the auto sampler. The injected volume was 10 μL and the analysis was completed in 12 min for determination of Cl^- salts only, 16 min for determining Cl^- salts and sugars and 32 min to include the determination of organic acids and ethanol. A blank sample of distilled water was used to verify the purity of the water being used as solvent. The goodness-of-fit of various calibration models were evaluated by visual inspection and the correlation coefficient as well as intra and inter-run accuracy and precision values.

2.3. Preparing a stock solution of NaCl for peak identification

Stock solutions at the concentration of 40.00 g/L from 3 different NaCl grades (analytical grade from Fisher 99.85%, rock salt Lab grade from Fisher and salt food grade from SAXA) were prepares at 4 levels (40.00, 20.00, 10.00, 5.00 g/L) to identify the peak under investigation.

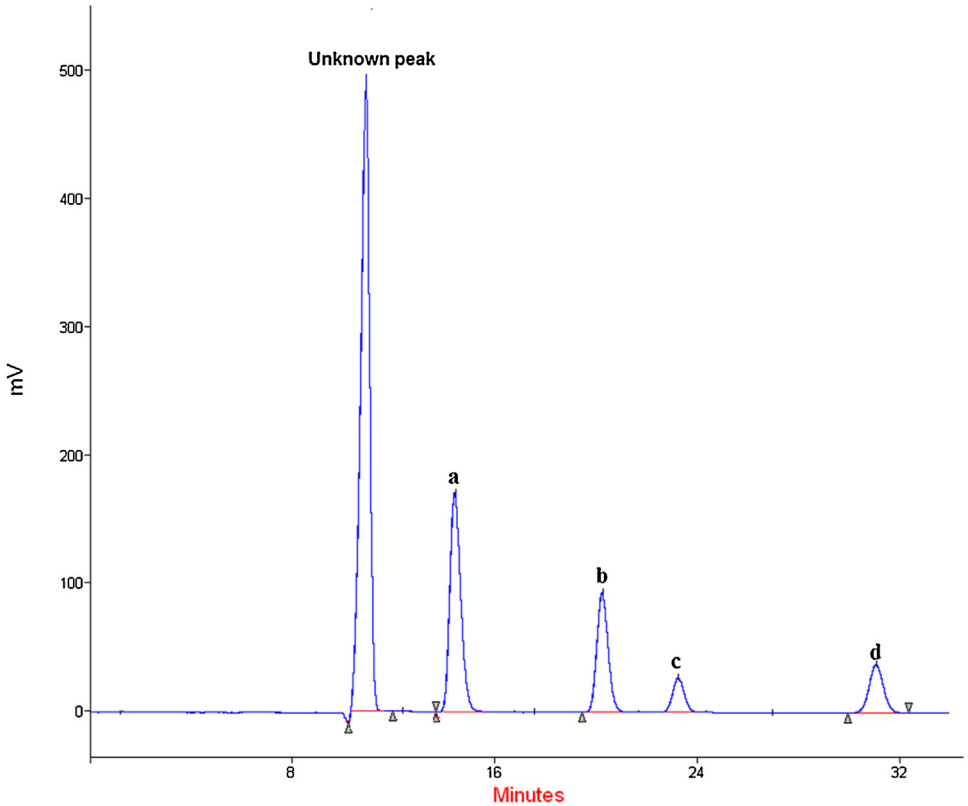

Fig. 1. Peaks present on a HPLC chromatogram from fermentations media using seawater based media.
Peaks analogous to standards for glucose (a), glycerol (b), acetic acid (c) and ethanol (d) are labelled along with an unknown peak which was eluted after 10.90 min.

Table 1
Detection and retention time (R_t) of different chemical compounds using HI-Plex H column.

No.	Substance	Rt (min)
1	Sodium bicarbonate ($NaHCO_3$)	NA
2	Sodium carbonate (Na_2CO_3)	NA
3	Sodium fluoride (NaF)	NA
4	Sodium sulphate (Na_2So_4)	10.48
5	Sodium bromide (NaBr)	10.5
6	Potassium chloride (KCl)	10.55
7	Magnesium chloride ($MgCl_2$)	10.55
8	Sodium chloride (NaCl)	10.55
9	Maltose ($C_{12}H_{22}O_{11}$)	12.5
10	Sucrose ($C_{12}H_{22}O_{11}$)	12.52
11	Tri-sodium phosphate (Na3PO4)	12.65
12	Lactose ($C_{12}H_{22}O_{11}$)	12.7
13	Tri-sodium citrate (Na3C6H5O7)	13.38
14	Citric acid ($C_6H_8O_7$)	13.42
15	Glucose ($C_6H_{12}O_6$)	14.45
16	Galactose ($C_6H_{12}O_6$)	15.35
17	Xylose ($C_5H_{10}O_5$)	15.35
18	Fructose ($C_6H_{12}O_6$)	15.73
19	Mannitol ($C_6H_{14}O_6$)	16.08
20	Sorbitol ($C_6H_{14}O_6$)	16.65
21	Arabinose ($C_5H_{10}O_5$)	16.7
22	Succinic acid ($C_4H_6O_4$)	19.25
23	Sodium succinate ($C_4H_4Na_2O_4$)	19.27
24	Lactic acid ($C_3H_6O_3$)	20.08
25	Glycerol ($C_3H_8O_3$)	20.33
26	Formic acid (CH_2O_2)	21.28
27	Sodium acetate ($C_2H_3NaO_2$)	23.28
28	Acetic acid (CH_3COOH)	23.28
29	Ethanol (C_2H_6O)	30.63

2.4. Preparation of different stock solutions from various components for peak detection

Stock solutions of 29 different salts, sugars, organic acids and alcohols (Table 1) were prepared at a concentration of 20 g/L. Each component was injected separately into the system at a concentration of 10 g/L in order to test the ability of the method for detecting and determining the retention time (R_t) of each component. The stock solutions were used to prepare 5 mixed solutions (A–E) from components that had different R_t to test the separation efficiency of the method. Fig. 2 shows the components that were used in each mixed solution.

2.5. Preparation of stock solutions of NaCl for calibration standards, and quality control samples

Calibration solutions of NaCl (Fisher, UK) at concentrations of 40.00, 20.00, 10.00, 5.00, 2.50, 1.00, 0.50 and 0.25 g/L were prepared in distilled water. These solutions were freshly prepared in triplicate on the day of analysis and the experiment was repeated on four different days. The results were used to generate a standard curve as well as to investigate intra- and inter-run variation.

Quality control samples of NaCl in distilled water at concentrations of 30.00, 15.00, 7.50, 3.00, 2.00 and 1.00 g/L were prepared in quadruplicate. The quality control samples were used to validate the accuracy and reproducibility of the standard curves.

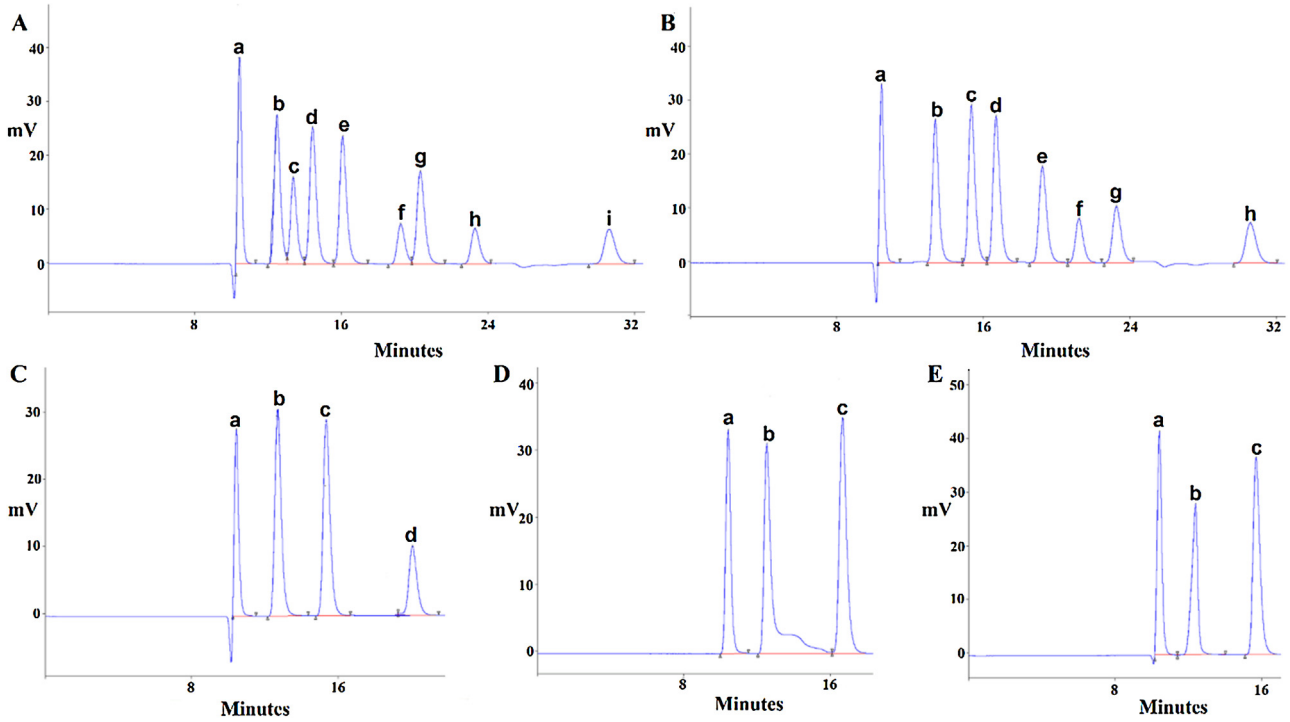

Fig. 2. HPLC chromatograms from mixed solutions of different components.
The chromatograms show the separation of different components from 4 solutions of mixed components. Peaks analogous to standards as following;
A: the mixture solution was prepared from 9 components at a concentration of 2.22 g/L for each component. Sodium chloride (a), maltose (b), sodium citrate (c), glucose (d), mannitol (e), sodium succinate (f), glycerol (g), sodium acetate (h), ethanol (i)
B: the mixture solution was prepared from 8 components at a concentration of 2.5 g/L for each component. Potassium chloride (a), citric acid (b), galactose (c), arabinose (d), succinic acid (e), formic acid (f), acetic acid (g), ethanol (h)
C: the mixture solution was prepared from 4 components at a concentration of 2.5 g/L for each component. Magnesium chloride (a), lactose (b), xylose (c), lactic Acid (d)
D: the mixture solution was prepared from 3 components at a concentration of 3.33 g/L for each component. Sodium sulphate (a), sucrose (b), sorbitol (c)
E: the mixture solution was prepared from 3 components at a concentration of 3.33 g/L for each component. Sodium bromide (a), sodium phosphate (b), fructose (c)

2.6. Validation of the procedures

The selectivity of the methodology (the validation of the peak and retention time) was evaluated by using different NaCl solutions of different grades (analytical grade, rock lab grade and food grade). Different NaCl grades were used to make sure the peak at 10.90 min correlate to the Cl^- in the seawater and not any other organic substances from the sea.

The validation was carried out following formerly reported procedures (Marin et al., 2007; Shah et al., 2000). Validation of the chromatographic method was carried out for two concentration ranges; high concentration range (40.00 to 5.00 g/L) and low concentration range (2.50 to 0.25 g/L) and was determined by applying 4 sets of calibrations in triplicate at 4 levels for each concentration range. Quality control samples at 6 concentrations and from different stock solutions were also applied.

Calibration graphics in the range of 5.00–40.00 g/L and the range of 2.50–0.25 g/L NaCl were plotted based on the peak areas of NaCl on axis y against the respective nominal concentrations on axis x. All calibration curves were required to have a correlation value of at least 0.998. The intra-run and inter-run accuracy and precision of the assay were assessed by the average relative percentage deviation (DEV%) from the nominal concentrations and the coefficient of variance (CV) values, respectively, based on reported guidelines (Marin et al., 2007; Shah et al., 2000). Precision (CV) and accuracy (DEV%) were calculated by the following Eqs. (1) and (2):

$$\text{CV}\ (\%) = (\text{SD}/\text{Average calculated concentration}) \times 100 \quad (1)$$

$$\text{DEV}\ (\%) = (1 - \text{Average calculated concentration}/\text{Nominal concentration}) \times 100 \quad (2)$$

Intra-run (n = 3) and Inter-run (n = 12) precision and accuracy of the analytical method were determined from the results of 2 groups of calibration curves run on 4 different days. Quality control samples containing the 6 concentrations of NaCl were evaluated from the obtained calibration curves. The intra-run precision and accuracy measurements were also performed on the quality control samples (n = 4).

2.7. Limit of quantification (LOQ) and Limit of detection (LOD)

LOQ was determined by considering the lowest concentration with a precision expressed by CV of lower than 20% and accuracy expressed by DEV% also lower than 20%, and a Signal-to-noise ratio (S/N) greater than 10.0. LOD was determined at the lowest detectable concentration with S/N greater than 3.0 (Marin et al., 2007; Shah et al., 2000).

2.8. Application of the method

Salt content in 15 food samples, purchased from a retail market, was determined as a NaCl using our new method. The results were compared with three other methods as listed below in Sections 2.8.3 to 2.8.4.

2.8.1. Sample preparation

Liquid samples (1–10) were filtered using a 0.45 μm syringe filter (Millipore, UK). Four grams of each solid sample (11–15) were placed into a 50 mL falcon tube. Then, 40 mL of a hot deionised water (85 °C) were added to each falcon tube. The samples were dissolved by vortexing the falcon tube for 5 min. The tubes were then incubated in a water bath at 85 °C for 10 min, then vortexed again for 1 min. The suspensions were filtered using a glass microfiber filter (pore size, 1.2 μm; Whatman®) then they were filtered again using a 0.45 μm syringe filters (Millipore, UK). Cheese samples (11 &12) were mashed in a porcelain mortar before salt extraction.

2.8.2. Using the HPLC method developed in this study for simultaneously measuring NaCl, sugars, organic acids and alcohols in food samples

Prepared food samples were injected (directly without dilution) into the HPLC system using auto sampler at an injection volume of 10.00 μL as described in Section 2.3. The total running time was 32 min. All measurements were carried out in triplicates. Means with standard deviations of triplicate determinations were reported.

2.8.3. Using ATAGO salt meter to measure salt content in food samples

ATAGO pocket salt meter (PAL-ES2, Japan) is a typical equipment used for testing the level of salinity in a solution, which is based on the conductivity of the sample. Prepared food samples were used directly (without dilution) as the equipment states that the measurement range from 0.1 to 50 g/L. All measurement were carried out in triplicates and the measuring cell was rinsed with distilled water after each test. Means with standard deviations of triplicate determinations were reported.

2.8.4. Using ion chromatography (IC) to measure NaCl content in food samples

Prepared food samples were diluted to an expected Cl^- range between 10 and 200 ppm. The Dionex ICS-1100 ion chromatography System (Thermo Scientific) was used to measure the Cl^- in the samples. Separation of Cl ions was achieved by using IonPac AG14A Carbonate Eluent Anion-Exchange Column while IonPac AS14A Carbonate Eluent Anion-Exchange Column was used as a guard column. The flow rate of the mobile phase was 1.4 mL/min containing; a) 3.5 mM Na_2CO_3 and 0.1 mM $NaHCO_3$. The separation was achieved at 30 °C and the total running time was 12 min.

The concentration of sodium chloride in the liquid samples was calculated using Eq. (1). The concentration of sodium chloride in the solid samples was calculated using Eq. (2).

$$C_{NaCl} = C_{Cl^-} \times \frac{molecular\ weight\ of\ NaCl}{molecular\ weight\ of\ Cl^-} \times D \quad (1)$$

$$C_{NaCl} = C_{Cl^-} \times \frac{molecular\ weight\ of\ NaCl}{molecular\ weight\ of\ Cl^-} \times D \times \frac{0.040L}{4.0g} \times 1000 \quad (2)$$

C_{NaCl} is the concentration of NaCl in the sample (g/L for liquid sample, g/kg for solid sample)

C_{Cl^-} is the concentration Chloride ion obtained from the IC method (g/L)

D is the dilution factor for IC analysis

2.8.5. Using Flame Photometer (FP) to measure NaCl content in food samples

Prepared food samples were diluted to several concentrations to achieve the suitable concentration range of sodium (1–20 ppm). Diluted samples were then injected manually in a flame photometer (Sherwood 410, Halstead, UK) to determine the sodium content. The method of using this flame photometer was previously described by Helrich (1990). Butane and air were supplied as the source of flame in this experiment. The flow rate of fuel was adjusted to obtain the maximum sensitivity. Standard curve of sodium in the concentration range from 5 to 20 ppm was prepared and the signal of the 20 ppm standard was checked several times during the analysis.

The concentration of sodium chloride in the liquid samples was calculated using Eq. (3). The concentration of sodium chloride in the solid samples was calculated using Eq. (4). Means with standard deviations of triplicate determinations were reported.

$$C_{NaCl} = C_{Na} \times \frac{molecular\ weight\ of\ NaCl}{molecular\ weight\ of\ Na} \times D \quad (3)$$

$$C_{NaCl} = C_{Na} \times \frac{molecular\ weight\ of\ NaCl}{molecular\ weight\ of\ Na} \times D \times \frac{0.040L}{4.0g} \times 1000 \quad (4)$$

C_{NaCl} is the concentration of NaCl in the sample (g/L for liquid sample, g/kg for solid sample)

C_{Na} is the concentration sodium ion obtained from the FP method (g/L)

D is the dilution factor for FP analysis.

3. Results and discussion

3.1. Chromatography and peak identification

HPLC analysis of a fermentation medium, prepared in natural seawater, resulted in an unidentified peak with a R_t of 10.90 min in addition to the expected fermentation products (Fig. 1). Normally, seawater from open seas and oceans contains around 34 g/L of different salts (Yen et al., 2016; Zaky et al., 2016). NaCl represents about 85% (about 28 g/L) of the seawater's total salts and therefore NaCl was a potential candidate for the unknown peak. Using analytical grade NaCl at 4 concentrations revealed a peak at R_t 10.90 min whose peak area correlated with the peak areas observed for the NaCl standards. This was confirmed when peak areas of different grades of NaCl (rock salt Lab grade and table salt food grade) were compared retention time and peak areas of the unknown peak (Fig. 3). These findings along with the sharpness and symmetrical resolution of the peak indicated the potential of this method for the quantitative measurement of NaCl.

Substantially, the elution order of the analytes appeared in Fig. 1 which was NaCl, glucose, glycerol, acetic acid and ethanol can be explained. These analytes have a mixed polarizability and they elute as a function of their size, and their electrical charge; apart from NaCl, the first to elute are the large neutral molecules (glucose then glycerol) and subsequently the charged molecules (acetic acid) and smaller neutral molecules such as ethanol.

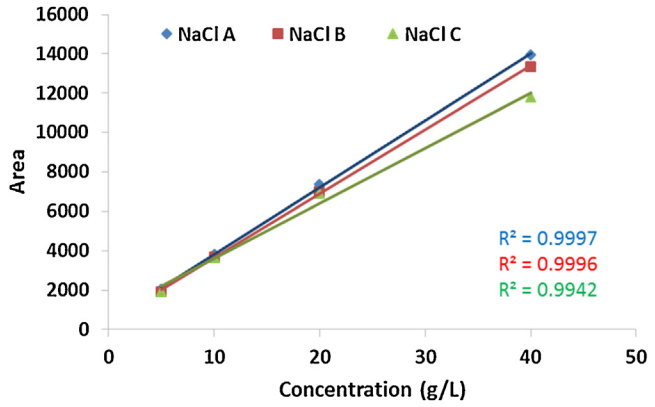

Fig. 3. Quantification of standard solutions of different NaCl grades.
This figure shows the linearity of a slandered solutions of 3 NaCl grades at 4 concentrations:
NaCl A, analytical grade NaCl from Fisher 99.85%; NaCl B, rock salt Lab grade from Fisher;
NaCl C, table salt food grade from SAXA.

3.2. The method's ability to detect different salts, sugars, organic acids and alcohols

Table 1 shows a list of chemical compounds that were tested by the HPLC method investigated in this study. The results revealed that sodium carbonate, sodium bicarbonate and sodium fluoride were not detectable by this method. On the other hand sodium chloride, potassium chloride and magnesium chloride were all detected and gave a peak with a similar R_t around 10.55 min. These results verified that the peak obtained corresponded to chloride anions (Cl^-) and not sodium cations (Na^+) and therefore that this methodology could be applied to the efficient quantification of chloride salts in the presence of sugars, alcohols and acetic acid. The results also revealed that other inorganic salts like sodium sulphate and sodium bromide can be detected with a similar R_t to chloride salts. Di-saccharides (maltose, sucrose and lactose) eluted at a R_t around 12.5 min followed by mono-saccharides and sugar-alcohols (glucose, galactose, xylose, fructose, mannitol, sorbitol and arabinose) which eluted at a R_t between 14.45 and 16.7 min. Low carbon organic acids and alcohols eluted after 20 min. This method can distinguish between different organic acids (citric acid, succinic acid, lactic acid acetic acid) as they eluted at different R_t. But, this method cannot distinguish between these organic acids and their sodium salts (sodium citrate, sodium succinate and sodium lactate) – if the acid and its salt are present together in the same sample – as these salts elute at a R_t similar to their organic acids. However, this finding gives other evidence that this method does not depend on the presence of sodium cations in the compound.

Although this methodology cannot distinguish between chloride salts present in one sample, it would prove useful for the determination of total chloride salts. The current colorimetric methods for measuring NaCl, such as Volhard, Mohr and Chloride analyser, are also not NaCl specific but they measure all chloride ions t present in the sample under investigation (Stankey et al., 2015).

The results obtained as regards the unambiguous determination of chloride were expected for the kind of analytical column used during this work. Note the dimensions of the column: 300 mm length and 7.7 mm I.D., as well as the size particle (as great as 8 μm). These dimensions favour the development of low backpressures and consequently the elution of non-polarizable analytes like those included in this paper, as a function of properties others than polarizability. The results confirm conclusions recently published for chromatographic and solid phase fractionations by Andrade-Eiroa et al. (2014) (Andrade-Eiroa et al., 2014). On the other hand, small cations cannot be retained most likely due to two reasons: a) most of them are too small and b) the column is positively charged and the cations are strongly repelled (Andrade-Eiroa et al., 2011).

Although the manufacturer claims that the retention mechanism of the column is a mixture between size exclusion and anion-exchange, the results do not support this statement. As a matter of fact, and due to the pH of the mobile phase (about 2.3), the target analytes are most likely positively charged (a higher positive charge of a molecule correlates with presence of OH groups) and consequently are repelled by the positively charged column. As charge on the molecule increases, the more significant the repulsion force of the column and the smaller the retention time. This might explain the short retention times and the elution order: glucose (6 —OH groups), glycerol (3 —OH groups), acetic acid and finally ethanol (1 —OH group).

Table 2
Intra variation of four separate assays[a] – accuracy, precision, and linearity of the standard curve samples.

Nominal concentration (g/L)		Intra-run of each assay							
		Calculated concentration (g/L) ($n = 3$)				Ave.	SD (g/L)	CV (%)[b]	DEV (%)[c]
		1	2	3	4				
High concentrations	**40**	40.49	39.98	39.77	39.80	40.01	0.33	0.83	−0.03
	20	20.49	20.69	20.53	19.72	20.36	0.44	2.14	−1.78
	10	9.50	10.42	10.24	10.15	10.08	0.40	3.95	−0.77
	5	5.42	4.94	5.52	5.18	5.27	0.26	4.87	−5.34
	R^2	**0.9992**	**0.9995**	**0.9998**	**0.9999**	**0.9999**	**0.00031**	**0.0305**	**0.01**
Low concentrations	**2.5**	2.55	2.54	2.48	2.56	2.53	0.03	1.30	−1.21
	1	1.08	1.11	1.09	1.1	1.10	0.01	1.18	−9.50
	0.5	0.48	0.51	0.52	0.49	0.50	0.02	3.79	−0.40
	0.25[d]	0.25	0.24	0.21	0.25	0.24	0.02	7.46	4.64
	R^2	**0.999**	**1**	**0.9994**	**0.999**	**0.9999**	**0.000473**	**0.047263**	**0.01**

[a] A linear curve was fitted to the data for response of NaCl versus theoretical concentration as described in Section 3. The calculated concentration was derived from reading the response for the standard sample against calibration curve. Each entry (assays 1–4) corresponds to the average value of triplicate analysis.
[b] CV (coefficient of variation, precision) = calculation according to Eq. (1).
[c] Accuracy (DEV%) = the deviation of the calculated concentration from the nominal value. Calculated according to Eq. (2).
[d] (LOQ) limit of quantification.

3.3. Validation of the method for the accurate quantification of NaCl

3.3.1. Assay linearity, accuracy, precision, and sensitivity

A set of NaCl standards in the range of 5.00 – 40.00 g/L (high concentrations) were used to build the calibration curves. The data from 4 different sequences of standard NaCl samples run on separate occasions are shown in Table 2. The relationship between the NaCl concentration and the peak areas was described by the linear regression equation: $y = 289.49x + 485.74$ ($n = 12$, $R = 0.999$), in which x is the NaCl concentration in (g/L) and y is the chromatogram peak area of NaCl. The precision and the accuracy of the results were within an acceptable level with CV and DEV values of ≤5.34% for all standards (Table 2).

Similarly, NaCl standards in the range of 2.50–0.25 g/L (low concentrations) were used to build calibration curves that suit samples contain NaCl at concentrations below 5 g/L. The linear regression equation: $y = 409.67x + 24.853$ $R^2 = 0.9996$ ($n = 12$, $R = 0.999$) was used to calculate the NaCl concentrations for 4 different sequences of NaCl standard samples run on separate occasions as shown in Table 2. The precision and the accuracy of the results were within an acceptable level with CV and DEV values ≤7.80% for all samples (Table 2).

Table 3 shows the inter-run average results of all standard curve samples. The accuracy of the assay was demonstrated by DEV values ≤7.80% and by precision CV values less than 9.43% for all samples representing both high and low standard ranges.

The reproducibility of the method was evaluated by analysing the quality control samples of NaCl made up at concentrations of 1.00, 2.00, 3.00, and 7.5.00, 15.00 and 30.00 g/L ($n = 4$). The accuracy and precision of the assay are demonstrated by DEV values ≤8.20% and by CV values ≤6.51% for all samples (Table 4).

3.3.2. LOD and LOQ

The lower LOD was determined as the sample whose S/N was just greater than 3 and corresponded to 0.2 g/L NaCl. On the other

Table 3
Inter-run variation of four separate assays[a] – accuracy, precision, and linearity of the standard curve samples.
[b]CV (coefficient of variation, precision) = calculation according to Eq. (1).
[c]Accuracy (DEV%) = the deviation of the calculated concentration from the nominal value. Calculated according to Eq. (2).
[d](LOQ) limit of quantification.

Nominal concentration (g/L)		Inter-run			
		Mean ($n = 12$)	SD (g/L)	CV (%)[b]	DEV (%)[c]
High concentrations	**40**	40.03	0.88	2.19	−0.06
	20	20.62	0.11	0.53	−3.10
	10	10.07	0.59	5.82	−0.73
	5	5.28	0.30	5.63	−5.62
	R^2	**1.00**	**0.00**	**0.00**	**0.03**
Low concentrations	**2.5**	2.53	0.17	6.74	−1.21
	1	1.1	0.01	−0.32	−9.06
	0.5	0.50	0.04	7.71	−0.40
	0.25[d]	0.24	0.02	7.63	3.12
	R^2	**0.999**	**0.00**	**0.00**	**0.1**

[a]The data are shown as averages, SD, accuracy (percent deviation, DEV%), and CV (precision). Accuracy and precision calculations were carried out by Eqs. (1) and (2), respectively.
[b]CV (coefficient of variation, precision) = calculation according to Eq. (1).
[c]Accuracy (DEV%) = the deviation of the calculated concentration from the nominal value. Calculated according to Eq. (2).
[d](LOQ) limit of quantification.

Table 4
Quality Control Samples[a].

Nominal concentration (g/L)	Average (n = 4)	SD	CV (%)	DEV (%)
30	29.50	0.83	2.82	1.68
15	15.86	0.18	1.14	−5.75
7.5	8.07	0.53	6.51	−7.64
3	2.93	0.16	5.34	2.21
2	2.14	0.09	4.21	−7.08
1	1.08	0.06	5.55	−8.20

[a] The data are shown as averages, SD, accuracy (percent deviation, DEV%), and CV (precision). Accuracy and precision calculations were carried out by Eqs. (1) and (2), respectively.

hand, the lowest LOQ was estimated at 0.25 g/L NaCl which displayed an S/N ratio equal to 10. The accuracy (DEV%) and precision (CV) values were within 10% of the nominal concentration values (Table 2).

3.4. Application of the HPLC assay to the quantification of chloride salts in food and beverages

3.4.1. Analysis of NaCl content using the HPLC method and comparison with three existing NaCl determination methods

Table 5 shows the results of NaCl content in 15 food and drink samples were measured using the HPLC method developed in this study, as compared with three existing methods for sodium chloride quantification. The expected salt content in 3 types of energy drinks was around 2 g/L. The results shown in Table 5 revealed that the majority of the salts in these drinks were sodium-based salts. Hence, only FP could provide the labelled salt content in those sample. Results obtained by our HPLC method were close to those results obtained by the IC in the case of 8 samples including; milk, whey (a) and (b), feta cheese, cheddar cheese, pickle solution (a) and (b) and Peri-Peri sauce. Also, the results from HPLC were close to the results obtained by ATAGO meter in the case of 6 samples (5, 6, 7, 10, 13 and 14) and close to the results obtained by FP in the case of 5 samples (6, 7, 10, 11 and 15).

As expected, the results from IC were closer to that obtained from the HPLC method reported in this study, as both methods are Cl^- based methods. However, looking at the expected salts content and the results from the other methods, the HPLC method recorded better results comparing with IC in the case of 5 products (samples; 4, 5, 6, 10 and 15). ATAGO meter is a simple method and can provide a rapid measurement of the salt content in a sample but the results maybe not very accurate as the conductivity is usually affected by other components in the solution.

3.4.2. Analysis of food sample using the HPLC method

Table 6 shows sugars, organic acids and ethanol content in salty food samples using the HPLC method developed in this study. The method was able to quantify different types of sugars (sucrose, glucose, lactose and galactose) that exist in the samples under investigation. The results obtained for the sugars were similar to the total sugar content showed on the labels of these samples. The separation of the sugar peaks was clear and away for the peak of Cl^- salt. This was one of the major objectives of this study because

Table 5
Determination of salt content in food samples using HPLC method with comparison with 3 other methods.

No	Sample	Salt content on the label	HPLC	Ion Chromatography (IC)		Flame Photometer (FP)		ATAGO Meter
			NaCl	Cl^-	as NaCl	Na+	as NaCl	NaCl
		g/L (samples 1 to 10) or g/kg (samples 11 to 15)						
1	Energy Drink (a)	2	0.56 ±0.01	0.0077	0.013	0.85 ±0.04	2.15	0.80 ±0.01
2	Energy Drink (b)	2	0.31 ±0.01	0.0075	0.012	0.66 ±0.05	1.67	0.80 ±0.00
3	Energy Drink (c)	2.3	0.31 ±0.00	0.0078	0.013	0.85 ±0.04	2.15	1.23 ±0.02
4	Lime soda	0.3	0.29 ±0.01	0.0043	0.007	0.32 ±0.06	0.81	0.47 ±0.01
5	Tomato Juice	5.5	5.73 ±0.2	2.3775	3.920	1.19 ±0.04	3.03	6.33 ±0.04
6	pickle solution (a)	NA	40.98 ±0.22	21.6337	35.665	14.57 ±1.06	37.03	39.00 ±0.17
7	pickle solution (b)	NA	38.58 ±0.65	22.2017	36.602	15.37 ±0.39	39.06	38.33 ±0.06
8	Milk	1.5	1.77 ±0.12	0.9704	1.600	0.41 ±0.01	1.05	2.63 ±0.01
9	Whey (a)[a]	NA	2.32 ±0.01	1.1046	1.821	0.42 ±0.03	1.07	3.53 ±0.01
10	Whey (b)[a]	NA	36.73 ±0.62	26.8964	44.341	13.97 ±0.53	35.50	36.00 ±0.00
11	Feta Cheese	25	17.55 ±0.23	9.5582	15.758	6.40 ±0.51	16.27	26.00 ±0.00
12	Cheddar Cheese	18	22.77 ±0.02	13.5634	22.361	7.30 ±0.25	18.56	30.00 ±0.00
13	Humus	8.1	11.35 ±0.01	4.5170	7.447	3.33 ±0.39	8.47	13.00 ±0.00
14	Peri-Peri Sauce	15	16.90 ±0.01	9.9333	16.376	5.27 ±0.15	13.39	17.67 ±0.01
15	Tomato Sauce	7.2	8.71 ±0.01	3.8774	6.392	2.23 ±0.15	5.68	12.00 ±0.00

Except IC, data were presented as a mean value of 3 replicates ±SD.
[a]Whey (a) was obtained from out of date milk sample (8), while whey (b) was obtained by adding 35 g/L NaCl, 11 g/L lactic acid and 11 g/L acetic acid in milk sample (8) and incubated at 35 °C for 3 days.

Table 6
HPLC analysis of food products from retail market.

No	Sample	Organic compounds									
		Sugars						Organic acids			Ethanol
		Sucrose	Lactose	Fructose	Glucose	Galactose	Total	Citric acid	Lactic Acid	Acetic acid	
		g/L (samples 1 to 10) or g/kg (samples 11 to 15)									
1	Energy Drink (a)	57.90 ±3.68	0.00	15.79 ±0.66	25.43 ±4.16	0.00	99.12	9.69 ±1.31	0.00	0.00	0.00
2	Energy Drink (b)	58.60 ±1.66	0.00	10.16 ±0.51	32.33 ±3.46	0.00	101.09	10.93 ±1.53	0.00	0.00	0.24 ±0.01
3	Energy Drink (c)	0.00	0.00	0.00	0.00	0.00	0.00	9.26 ±1.30	0.00	0.00	0.00
4	Lime soda	0.00	0.00	0.00	0.00	0.00	0.00	3.42 ±0.19	0.00	0.00	0.34 ±0.01
5	Tomato Juice	1.18 ±0.04	0.00	12.93 ±0.47	12.88 ±0.49	0.00	26.99	4.38 ±0.56	0.00	0.00	0.00
6	pickle solution (a)	0.00	0.00	5.10 ±0.34	3.40 ±0.31	0.00	8.50	3.50 ±0.04	6.68 ±0.06	10.61 ±0.09	0.36 ±0.00
7	pickle solution (b)	0.00	0.00	0.00	4.50 ±0.11	0.00	4.50	0.29 ±0.00	2.23 ±0.07	1.59 ±0.05	0.74 ±0.02
8	Milk	0.00	60.91 ±3.24	0.00	0.00	0.00	60.91	0.00	0.00	0.35 ±0.04	0.00
9	Whey (a)[a]	0.00	58.14 ±0.86	0.00	0.00	0.29 ±0.01	58.43	0.00	0.42 ±0.00	0.55 ±0.01	0.00
10	Whey (b)[a]	0.00	42.72 ±0.75	0.00	0.00	0.00	42.72	0.17 ±0.00	9.99 ±0.23	12.00 ±0.18	0.00
11	Feta Cheese	0.00	6.27 ±0.02	0.00	0.00	0.00	6.27	2.31 ±0.06	18.19 ±0.27	0.00	0.00
12	Cheddar Cheese	0.00	0.00	0.00	0.00	0.00	0.00	2.60 ±0.01	18.80 ±0.03	0.00	0.00
13	Humus	7.48 ±0.68	0.00	2.27 ±0.06	1.93 ±0.35	0.00	11.68	11.91 ±1.04	0.00	0.00	0.00
14	Peri-Peri Sauce	0.00	0.00	6.33 ±0.11	4.78 ±0.05	0.00	11.11	5.56 ±0.15	0.00	33.19 ±0.69	0.00
15	Tomato Sauce	15.51 ±0.42	0.00	32.17 ±0.86	29.28 ±0.79	0.00	76.96	11.78 ±0.18	0.00	0.00	0.00

Data were presented as a mean value of 3 replicates ±SD.
[a] Whey (a) was obtained from out of date milk sample (8), while whey (b) was obtained by adding 40 g/L NaCl, 10 g/L lactic acid and 10 g/L acetic acid in the milk sample (sample 8) and incubated at 35 °C for 3 days.

obtaining accurate quantification for sugars, especially glucose and sucrose, in samples contain Cl^- salts was reported difficult due to similar retention times for Cl^- and sugars (Sims, 1995)

Citric acid was found in most of the products with around 10 g/L or g/kg in 5 samples including; energy drinks (a, b and c), humus and tomato sauce. Acetic acid was detected in 6 samples and recorded at 10.61 g/L in pickle solution (a) and at 33.19 g/kg in Peri-Peri sauce. The presence of high amounts of acetic acid in Peri-Peri sauce was expected as vinegar is one of the main components in its recipe. Lactic acid was detected in 6 samples (6, 7, 9, 10, 11 and 12) and recorded at about 18 g/kg in both cheese samples. The presence of lactic acid in cheese is normal due to the fermentation of lactose during cheese maturation (Olson, 1990). Lactic acid in cheese was reported at 1.5 to 2.0% by many researchers (Blake et al., 2005; Macedo and Malcata, 1997).

Ethanol was detected in small amounts (0.24 ± 0.01–0.74 ± 0.02 g/L) in 4 samples including pickle solutions (a) and (b), energy drink (b), and lime soda. Few studies have reported the presence of ethanol in non-alcoholic foods and beverages (Goldberger et al., 1996; Logan and Distefano, 1998; Lutmer et al., 2009).

4. Concluding remarks

A simple, rapid and reproducible chromatographic methodology has been developed and successfully applied for the determination of chloride in the presence of sugars from food and beverage samples. The results obtained by this method were compared with those obtained using a salt meter (ATAGO), flame photometer and ion chromatography. The results suggested that our new method can be applied to a wide variety of food products such as milk, cheese, whey, pickles, sauces and juices as well as samples from fermentations using seawater. For the first time, the simultaneous determination of Cl^-, sugars, organic acids and ethanol in food and beverages has been achieved in a rapid HPLC assay. The efficient separation of the aforementioned compounds was achieved by using an HPLC system equipped with a Hi-Plex H column and RI detector. The column was capable of fractionating the compounds in approximately 32 min.

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References

AOAC, 1993. Methods of Analysis for Nutrition Labelling. Association of Official Analytical Chemists, Arlington, VA.
Andrade-Eiroa, A., Le-Cong, T., Nguyen, M.-L., Dagau, P., 2011. Reverse-high performance liquid chromatography mechanism explained by polarization of stationary phase. CheM 1, 62–79.
Andrade-Eiroa, A., Shahla, R., Romanias, M.N., Dagaut, P., 2014. An alternative to trial and error methodology in solid phase extraction: an original automated solid phase extraction procedure for analysing PAHs and PAH-derivatives in soot. RSC Adv. 4 (63), 33636–33644.
Blake, A.J., Powers, J.R., Luedecke, L.O., Clark, S., 2005. Enhanced lactose cheese milk does not guarantee calcium lactate crystals in finished cheddar cheese. J. Dairy Sci. 88 (7), 2302–2311.

Doughty, H.W., 1924. Mohr's method for the determination of silver and halogens in other than neutral solutions. J. Am. Chem. Soc. 46 (12), 2707–2709.
Duarte-Delgado, D., Narváez-Cuenca, C.-E., Restrepo-Sánchez, L.-P., Kushalappa, A., Mosquera-Vásquez, T., 2015. Development and validation of a liquid chromatographic method to quantify sucrose, glucose, and fructose in tubers of *Solanum tuberosum* Group Phureja. J. Chromatogr. B 975, 18–23.
Goldberger, B.A., Cone, E.J., Kadehjian, L., 1996. Unsuspected ethanol ingestion through soft drinks and flavored beverages. J. Anal. Toxicol. 20 (5), 332–333.
Helrich, K., 1990. Official methods of analysis of association of official analytical chemists, 15th ed. Official Methods of Analysis of Association of Official Analytical Chemists, Inc., Arlington, VA pp. 33–34.
Johnson, M.E., Olson, N.F., 1985. A comparison of available methods for determining salt levels in cheese. J. Dairy Sci. 68 (4), 1020–1024.
Leong, W.M., Geier, R., Engstrom, S., Ingham, S., Ingham, B., Smukowski, M., 2014. Growth of *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, and *Staphylococcus aureus* on cheese during extended storage at 25 °C. J. Food Prot. 77 (8), 1275–1288.
Lin, C.S.K., Luque, R., Clark, J.H., Webb, C., Du, C., 2011. A seawater-based biorefining strategy for fermentative production and chemical transformations of succinic acid. Energy Environ. Sci. 4 (4), 1471–1479.
Logan, B.K., Distefano, S., 1998. Ethanol content of various foods and soft drinks and their potential for interference with a breath-alcohol test. J. Anal. Toxicol. 22 (3), 181–183.
Lutmer, B., Zurfluh, C., Long, C., 2009. Potential effect of alcohol content in energy drinks on breath alcohol testing. J. Anal. Toxicol. 33 (3), 167–169.
Macedo, A.C., Malcata, F.X., 1997. Changes of lactose, lactic acid, and acetic acid contents in Serra cheese during ripening. Zeitschrift für Lebensmitteluntersuchung und –Forschung A 204 (6), 453–455.
Marin, R.M., Franchini, K.G., Rocco, S.A., 2007. Analysis of adenosine by RP-HPLC method and its application to the study of adenosine kinase kinetics. J. Sep. Sci. 30 (15), 2473–2479.
Olson, N.F., 1990. The impact of lactic acid bacteria on cheese flavor. FEMS Microbiol. Lett. 87 (1), 131–147.
Rajković, M.B., Sredović, I.D., Miloradović, Z.N., 2010. Comparison of different methods for determination of sodium chloride in cheese. J. Agric. Sci. 55 (1), 65–77.
Schales, O., Schales, S.S., 1941. A simple and accurate method for the determination of chloride in biological fluids. J. Biol. Chem. 140 (3), 879–884.
Shah, V., Midha, K., Findlay, J.A., Hill, H., Hulse, J., McGilveray, I., McKay, G., Miller, K., Patnaik, R., Powell, M., Tonelli, A., Viswanathan, C.T., Yacobi, A., 2000. bioanalytical method validation—a revisit with a decade of progress. Pharm. Res. 17 (12), 1551–1557.
Sims, A., 1995. HPLC analysis of sugars in foods containing salt. J. Agric. Food Chem. 43 (2), 377–380.
Stankey, J.A., Akbulut, C., Romero, J.E., Govindasamy-Lucey, S., 2015. Evaluation of X-ray fluorescence spectroscopy as a method for the rapid and direct determination of sodium in cheese. J. Dairy Sci. 98 (8), 5040–5051.
Wolfbeis, O.S., Hochmuth, P., 1984. A new method for the endpoint determination in argentometry using halide-sensitive fluorescent indicators and fiber optical light guides. Microchim. Acta. 84 (1), 129–138.
Yen, H.-W., Liao, Y.-T., Liu, Y.X., 2016. Cultivation of oleaginous Rhodotorula mucilaginosa in airlift bioreactor by using seawater. J. Biosci. Bioeng. 121 (2), 209–212.
Zaky, A.S., Tucker, G.A., Daw, Z.Y., Du, C., 2014. Marine yeast isolation and industrial application. FEMS Yeast Res. 14 (6), 813–825.
Zaky, A.S., Greetham, D., Louis, E., Tucker, G., Du, C., 2016. A new isolation and evaluation method for marine-derived yeast spp. with potential applications in industrial biotechnology. J. Microbiol. Biotechnol. 26 (11), 1891–1907.
Zhao, C.-M., Wang, W.-X., 2011. Comparison of acute and chronic toxicity of silver nanoparticles and silver nitrate to Daphnia magna. Environ. Toxicol. Chem. 30 (4), 885–892.