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Validation of Ion Chromatographic method for simultaneous quantification of Mg^{2+} , K^+ , NH_4^+ and Na^+ ions in food salt

Helsinki Metropolia University of Applied Sciences

Bachelor of Engineering

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Salt is a vital substance for all of us. Usually salt contains sodium, which can be very dangerous if consumed in high amounts. Because of this issue, some salt companies have been developing alternative salts with less sodium.

The aim of this project was to ascertain if the Metrohm Ion Chromatographic method and the device itself are adequate for the determination of ammonium, potassium and magnesium ions in salt samples provided by Smart Salt Inc. Another aim was to determine basic data for the foundation of method validation. Ion chromatography is a simple and fast method for separation and determination of ions based on their charge, and it is suitable for fast analyze and especially for environmental analysis.

Smart salt Inc. has invented a co-crystallized triple salt of magnesium-, potassium- and ammonium chloride. The triple salt is blended with sodium and potassium chloride in various ratios and used in food products to effect a sodium reduction of up to 60%.

The reason for analyzing these positive ions is to verify that all the molar ratios remain the same after the manufacturing process of the product.

Five standard solutions of four cations (Mg^{2+} , K^+ , NH_4^+ and Na^+ ions) are used to specify each peak of the wanted ions. The linearity test after calibration had a satisfactory correlation coefficient of $R^2 > 0.999$.

The parameters which have been determined for method validation are linearity, accuracy, precision, injection repeatability, and t-test for error significance.

It was concluded on the basis of the results that the method used for validation exhibits evidence for systematic error at the 95 % confidence level. This systematic error is probably due to the calibration period of 6 months between Smart Salt analysis and the *analyst samples* analysis. Therefore, this method cannot be used. The second calibration yielded more accurate results.

Keywords

ion chromatography, Smart Salt Inc., molar ratios, validation, repeatability, accuracy, precision

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Abbreviations

IC	Ion Chromatography
FDA	Food and Drug Administration
GRAS	Generally Recognized As Safe
IP	Intellectual Property
MNK	Magnesium, Ammonium and Potassium
PEEK	Polyether ether ketone
SD	Standard deviation
RSD	Relative standard deviation
R	Correlation coefficient

Introduction

1.1 Aim of this project

Salt is a vital substance for all of us. Usually salt contains sodium, which can be very dangerous if consumed in high amounts. Because of this issue, salt companies have been developing salt recipes with less sodium. Smart Salt Inc. is one of these companies.

Smart Salt Inc. manufactures food salt using magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), various ratios of ammonium chloride (NH_4Cl), potassium chloride (KCl) and sodium chloride (NaCl).

The purpose of this thesis can be divided in two parts. The main aim was to investigate if Metrohm method and Ion Chromatographic device are adequate for detecting and quantifying four positive ions (Mg^{2+} , K^+ , NH_4^+ and Na^+) in salt recipes prepared by Smart Salt Inc. The reason of analysing these cations is that the manufacturer can verify that the molar ratios established for each salt recipe remain the same after the manufacturing process.

The second aim was to validate the method by determining parameters such as linearity, accuracy, precision, injection repeatability and error significance.

1.2 Smart Salt Inc.

Smart Salt, Inc. was founded in year 2007 in California, USA, by Juhani and Tapio Mäki to capitalize on a new salt alternative. One of the reasons to put up a company in US was to get FDA GRAS approval and other food grade certifications for the product. These have already been accomplished. The product has also got EU-approval.

Smart Salt's new salt substitution product is based on a Finnish invention made by Juhani Mäki as a result of a decade of research work. The key of the invention is a co-crystallized triple salt of magnesium-, potassium- and ammonium chloride. The triple

salt, called “magnesa”, is blended with sodium and potassium chloride in various ratios and used in food products to effect a sodium reduction of up to 60%. The new sodium replacement system solves the problems related to existing low sodium salts and has the potential to revolutionize the alternative salt market category. The salt has a very good mineral balance of magnesium, potassium and sodium. It allows the use of magnesium chloride, a mineral with fully documented health benefits but typically not used due to its hygroscopic nature (i.e. a property that certain materials have to absorb water.)

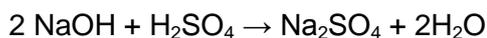
The Company has an extensive IP portfolio of patents and pending patents in over 40 countries. The Company has registered the trademark Smart Salt in strategically important countries. The Company started its operations in Finland in February 2010 by founding Smart Salt Oy [17].

2 Background and theory

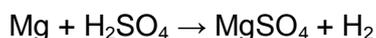
2.1 Chemistry of salt

Salt is an ionic compound which dissociates in water into a cation other than H^+ and an anion other than OH^- . They are typically the product of a chemical reaction between:

A base and an acid to form a salt and water, e.g.:



A metal and an acid to form a salt and hydrogen, e.g.:



An oxide acid and a basic oxide to form a salt, e.g.:



In general, the salts form crystals (Figure 1). These are often soluble in water, where both ions are separated. The salts generally have a high melting point, low or high

hardness and low compressibility. If molten or dissolved in water, salts conduct electricity because they dissociate into their constituent ions, passing these to function as electrolytes.

The most popularly known salt is the sodium chloride, a widely used ingredient of food commonly known as salt or table salt.

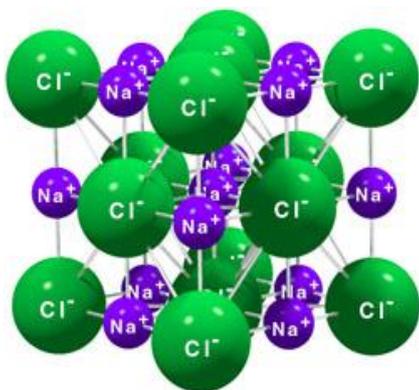


Figure 1. NaCl crystal lattice. [1]

2.2 Ion Chromatography

The analyses were performed on a Metrohm 761 Compact Ion Chromatograph under non-suppressed ion exchange mode for inorganic cations [2].

This instrument was launched in 1997 and was the first ion chromatograph with all functional components contained in a single box. With all capillaries for the flow path preinstalled, the 761 Compact IC did not require any set-up on the user's part. Although it does not use the latest technology available nowadays, it is still suitable for fast analysis and especially for environmental analysis [3].

The IC instrument was purchased about ten years ago by Helsinki Metropolia University of Applied Sciences for educational purposes. The same apparatus was used during the whole experiment but some essential parts were reordered, such as the columns and syringe filter. Before attempting the experiment, column regeneration was performed.

According to the Metrohm manual Metrohm 761 [2], the ion chromatography system for cation analyses, typically consists of a liquid eluent, a high-pressure pump, a sample injector, a guard and separator column, and a conductivity detector coupled to a recorder (Figure 2).

The stages of the ion chromatographic analysis process are shown in Figure 2 and can be summarised as follows:

1. The eluent works as an ion extraction liquid to separate the sample ions as well as carries the sample through the ion chromatography system. [5]
2. The liquid sample is injected manually into a sample loop. Once injected into the loop, the Metrohm 761 Compact injects automatically the sample into the eluent stream [2].
3. The ion guard column (precolumn) is to protect the separator column from particles and contaminants, extending its working life [3].

In the separation column the sample ions are separated. The stationary phase detains the ions until they are separated [3].

4. By using conductivity detector the ions are detected by concentration changes, which lead to changes in the conductivity when passing through the detector [5].
5. The detector is connected to a recorder. When sample ions are passed through the detector, the conductivity will rise and show a peak in the chromatogram [5].

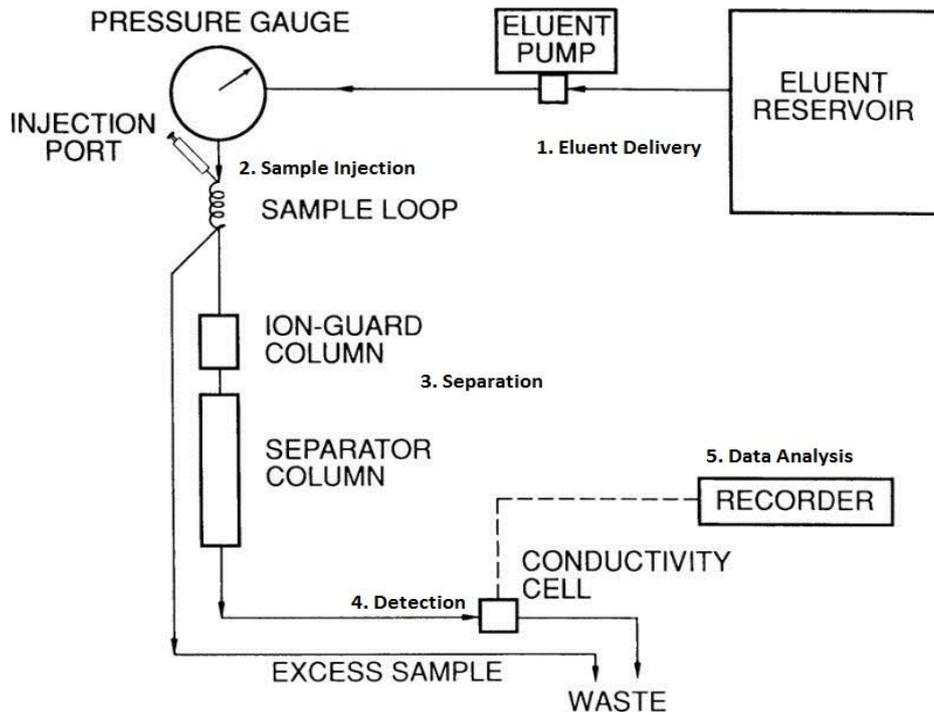


Figure 2. Schematic diagram of the non-suppressed conductivity ion chromatograph [4].

2.2.1 Columns

2.2.1.1 Guard Column

To protect the separation column, a guard column is often used. The guard column is attached immediately in front of the separation column. It acts as a filter and provides some separation as well. The main role of the guard column is to remove particles and contaminants, increasing the lifetime of the separation column. It has a low price comparing to the separator column and therefore, it can be periodically replaced [3].

2.2.1.2 Separator Column

The sample ions are separated in the separator column. The column packing material contains charged functional groups (i.e. ion exchange groups) that perform the actual separation.

In the stationary phase the ions are detained until they separate. The time for the ions to separate in the stationary phase depends on the composition of the sample. Differ-

ent ions have different affinity for the pack material. The mobility of the ions depends on their mass and charge. High charge leads to a low separation and a longer retention time. Large mass leads to a low mobility and a longer retention time (Figure 3).

There is a variety of separator columns available; the shorter the separator column is, the faster the analysis is. The size of the column is chosen depending on the ions to be analyzed.

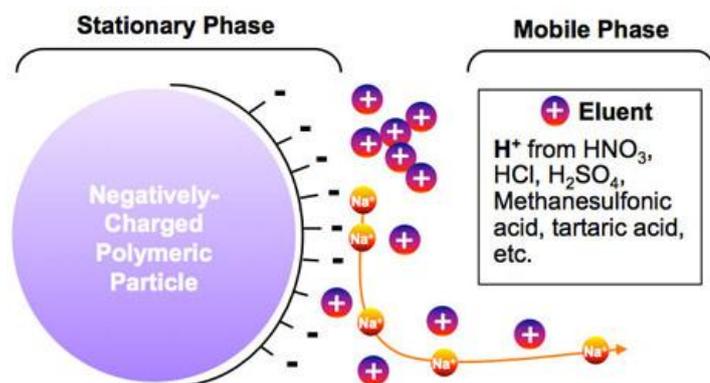


Figure 3. Illustration of how the ions are separated [5].

The interaction between the stationary and mobile phase are essential for separating the ions. This process happens in the separator column [5].

2.2.2 Eluent

The eluent is a liquid that helps to separate the sample ions and transports the sample through the system. The eluent is a solution of salt (or several salts) in water, which also acts as a buffer, providing a stable pH. The eluents used for non-suppressed cation exchange are weak acids with a complexing agent such as dipicolinic. They can be prepared manually or be purchased. There are several eluent compositions with different chemicals and concentrations available. The eluent is chosen depending on the kind of sample ions that will be separated and the type of separation column that will be used.

2.2.3 Detector

The identification and quantification of ions take place in the detector. Since all ions are capable to conduct current, the most common type of detector used is the conductivity detector.

Once the sample ions are totally separated in the separator column, they go into the conductivity detector. In the detector, the eluate passes through a flow cell with two electrodes, which have a voltage between them. In the cell the capability of the solution to conduct electrons increases. The increase of the current is proportional to the increase of conductivity.

The distance between the electrodes is called d , and the area of the electrodes is called A . Detector's cell constant (K) is denoted by d/A . The conductance (G) between the electrodes is continuously measured and depends on the concentration and size of the ion charges. The resistance (R) and conductance are inverses [6].

The electrolytic conductivity (k) of a solution, which is a measure of its ability to conduct electricity, is calculated from the conductance. Therefore, when the sample ions leave the detector, the conductivity rises and generates a peak in the chromatogram.

Conductance can be expressed as

$$G = \frac{1}{R} \quad [\text{S or } \Omega^{-1}]$$

The electrolytic conductivity can be calculated using the following formula:

$$k = K * G = \left(\frac{d}{A}\right) * G \quad [\text{S cm}^{-1}]$$

2.2.4 Software

Data acquisition was carried out using 761 Compact IC 1.1 software. This program runs under Windows 95, Windows 98 and Windows NT operating systems [2].

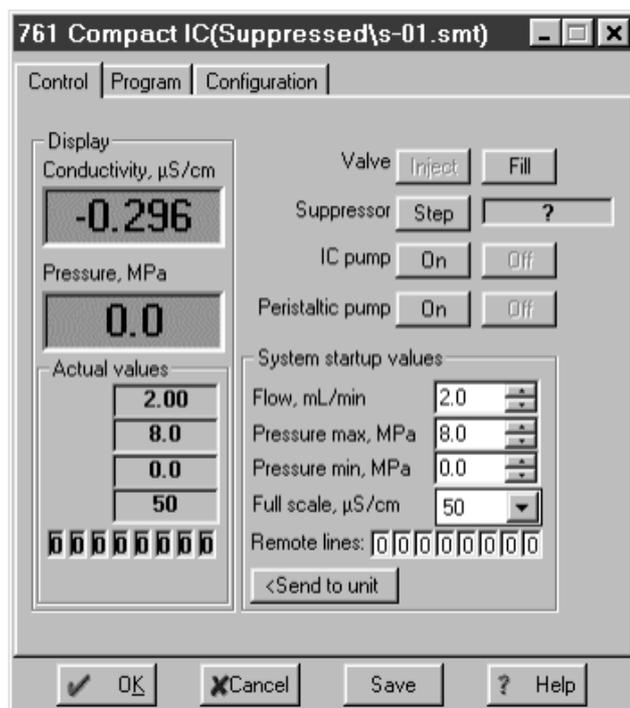


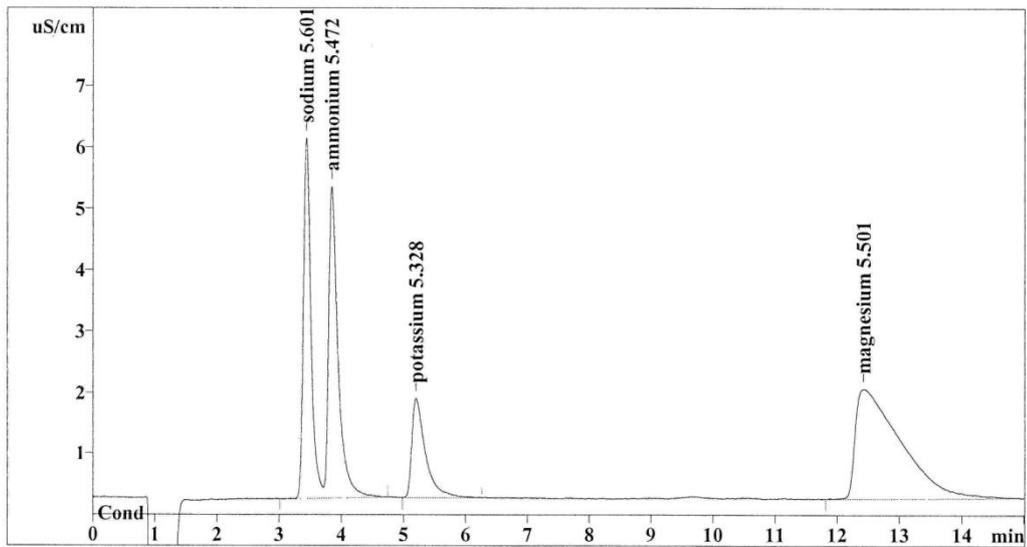
Figure 4. Illustration of how the ions are separated [5].

This program contains all the information regarding instrument parameters, such as pressure of the pump, flow and conductivity (Figure 4).

The software creates a table with the main parameters. These parameters are adjusted according to the method to be used.

Data Analyses works as follows:

- The conductivity cell transmits the signal to a data collection system.
- The data collection system identifies the ions based on retention time, and quantifies each ion by integrating the peak height or area. The data is quantitated by comparing the chromatogram with sample peaks to those produced from a standard solution. The results are displayed as a chromatogram and the concentrations of ionic analytes can be determined (Figure 5).



Quantitation method: Custom

No	Retention min	Height uS/cm	Area uS/cm*sec	Conc. mg/l	Name
1	3.43	5.89	49.958	5.601	sodium
2	3.84	5.09	54.106	5.472	ammonium
3	5.20	1.63	24.908	5.328	potassium
4	12.42	1.81	91.311	5.501	magnesium
4	15.01	14.42	220.284	21.901	

This report has been created by IC Net

Figure 5. Typical cation chromatogram with the main peaks of interest, elution order and their respective retention times.

Figure 5 shows a typical chromatogram (i.e. report template), which consists of a plot of all peaks detected and the peak analysis. The peak analysis provides the characteristics of each peak such as the peak area, peak height, retention time and concentration. These characteristics are shown with help of Figure 6 below.

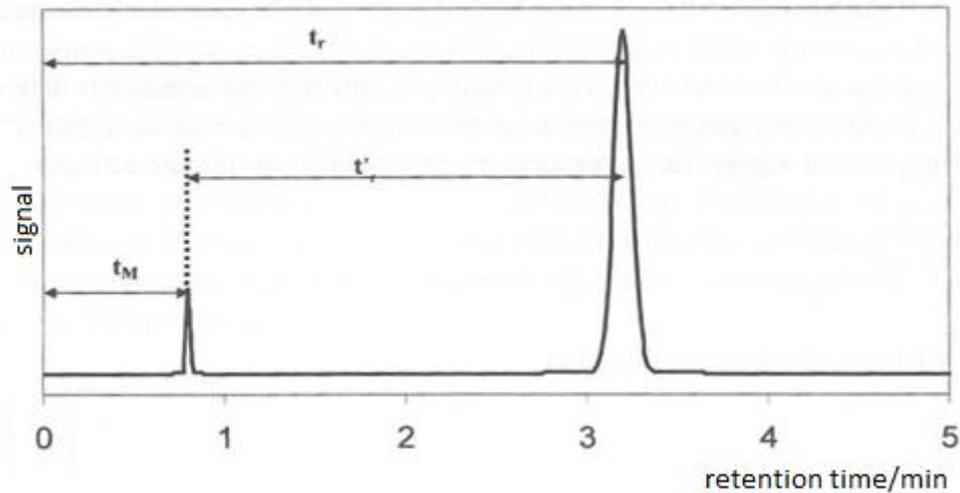


Figure 6. Chromatogram where t_M for the solute peak and t_r for the analyte [7].

- The retention time (t_r) is maybe the most important measurements in ion chromatography. The retention time is the time it takes for the compound to reach the detector, which means, the time for the peak to appear. It is measured in minutes. The time taken for a non-retained compound peak to appear is represented by t_M . t'_r is the actual retention time. The greater the retention time is, the longer the molecule stays in the column, and longer the analysis takes. In another hand, if retention time is too short, the compound leaves quickly the column and it does not have time to have a proper separation [7].
- The height of peak measured by the 761 Compact IC 1.1 software is in micro Siemens per centimeter unit [$\mu\text{S}/\text{cm}$], (see 2.2.5).
- The concentration of each ion is represented in milligrams per liter unit [mg/l].
- The area of the peak measured is in micro Siemens per centimeter times second [$\mu\text{S}/\text{cm}\cdot\text{sec}$].
- The resolution (R_s) of two chromatographic peaks is defined by the height difference between the peaks divided by the width of the peaks measured at 50% of the peak height [7]. This can be notated as follows:

$$R_s = \frac{(t_{r2} - t_{r1})}{\frac{1}{2} * (W_1 + W_2)}$$

- Asymmetry (AS) is determined by measuring the right (a) and left (b) side widths of the peak at a certain ratio, often at 10% of the height (Figure 7) [7].

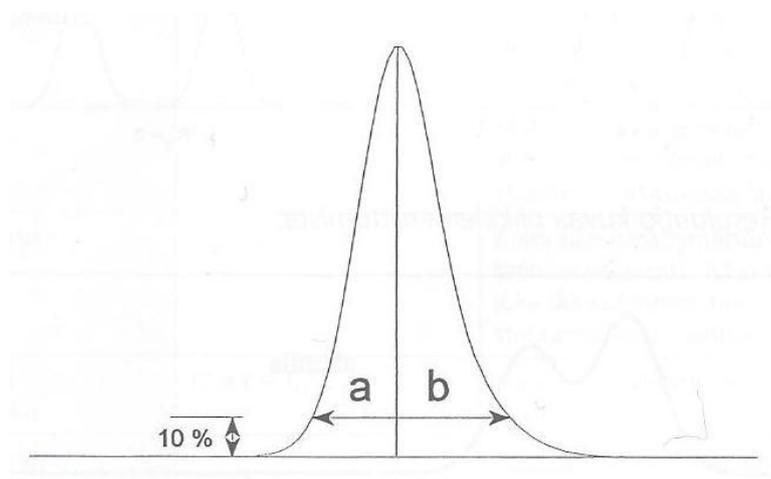


Figure 7. The asymmetry of the chromatography peaks [7].

2.3 Validation of method

Validation is essential to determine whether developed methods are fully appropriate or not for the goals designed, in order to obtain reliable results that can be satisfactorily interpreted. Thus, it enables knowledge of the limitations and reliability in measurements performed in analyses.

Depending on the purpose of the method, some of the parameters may not be evaluated. The accuracy and precision of the method are very important parameters and always studied, regardless of the method purpose, except methods for qualitative goals.

It should be noted that the method can be considered valid, even if some parameters do not fall within the limits established in the literature, but which are thoroughly known and, therefore, appropriate to the objectives of the study to be performed.

2.4 Validation parameters

2.4.1 Deviations (errors)

- **Systematic errors:** Systematic error is the difference between the true value and the conventional average number of measurements. It affects the accuracy. This error can be caused by various reasons such as wear of the measurement system, settings, measuring method, environmental conditions, etc [8].
- **Random errors:** Random error is the difference between a measurement result and average of a number of measurements. It affects the repeatability of the analysis. The factors that contribute to the appearance of random error may be due to vibration, internal instability, dilutions and weighing of the sample [8].
- The average result (\bar{x}) is calculated by summing the individual results and dividing this sum by the number (n) of individual values [8].

$$\bar{x} = \frac{\sum_{i=1}^n x_i}{n} \quad (1)$$

- The standard deviation (SD) is one of the most widely used measures of variation for a data group. The advantage of presenting the variance is to allow a direct interpretation of the variation of the data set, because the standard deviation is expressed in the same unit as the variable (cm, kg, atm, etc). The standard deviations can be understood as an average of the absolute values of the deviations, i.e. deviations considered all with positive sign, the average obtained, however, by a rather elaborate process: calculating the square of each deviation, the average of these squares is obtained, and then the square root of the average of the squared deviations [8].

$$SD = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}} \quad (2)$$

- The relative standard deviation (*RSD*) is often more convenient. It is expressed in percent and is obtained by multiplying the standard deviation by 100 and dividing this product by the average [8].

$$RSD = SD \cdot 100 / \text{mean value} \quad (3)$$

2.4.2 Linearity test

The linear range of detectability is dependent on the compound analyzed and detector used. The linearity test is done to ensure that linear range of is not being violated (e.g. ion contents extrapolate beyond the range). The correlation coefficient (R^2), tells how well the experimental point fits a straight line [9].

2.4.3 Precision

Precision means the ability of a measuring instrument provide indications close together when measuring the same measurand under the same conditions. It defines how an instrument can play a value obtained in a measurement, even though it is not correct. The precision is defined by the standard deviation of a series of measurements of the same sample or the same point. The greater the deviation, the lower is the precision. The precision is related to the random uncertainty of the measurement and to the quality of the instrument. It can be determined by injection repeatability [10].

2.4.3.1 Injection repeatability

Injection repeatability expresses the precision under the same operating conditions over a short interval of time and with the same solution.

2.4.4 Method accuracy

Accuracy is the ability of an instrument to give responses close to the true value of the measurand. It is the ability of the measuring instrument must provide a correct result. An accurate equipment is one that after a series of measurements gives an average value that is close to true value, even if the standard deviation is high, which means low precision. Accuracy is related to the systematic uncertainties of the measurement.

Accuracy was investigated to observe the closeness of agreement between the true value and the value found of the ions content in the samples [6].

2.4.5 *t* test

Measurement results likely follow a normal distribution if the number of measurements is the high. In analytical chemistry usually there are only few analysis results. Therefore, the *t* test (*Student t test*) is more suitable for small number of samples. This test was performed for detection of systematic error (bias) and its significance.

$$t = \frac{\bar{x} - \mu_0}{\frac{s}{\sqrt{n}}} \quad (4)$$

where μ_0 is the true value and s is the standard deviation (*SD*).

3 Method and material

3.1 Chemicals

The chemicals used in this project can be listed as follows:

- 1) Magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$)
- 2) Potassium chloride (KCl)
- 3) Ammonium chloride (NH_4Cl)
- 4) Sodium chloride (NaCl)
- 5) Tartaric acid ($\text{C}_4\text{H}_6\text{O}_6$),
- 6) Dipicolinic acid ($\text{C}_7\text{H}_5\text{NO}_4$)
- 7) Milli-Q water (resistance 18.2 M Ω cm)
- 8) Tap water

3.2 Standard

Standard solutions with several concentrations and four chloride salts containing Na^+ , K^+ , NH_4^+ and Mg^{2+} ions were prepared (see 3.5.2)

3.3 Eluent

Eluent suitable for detection of Na^+ , K^+ , NH_4^+ and Mg^{2+} ions according to Metrohm Ion Chromatography manual [5] was prepared (see section 3.5.1).

3.4 Apparatus

3.4.1 Instruments and equipment

Instruments and equipment used in this project can be listed as follows:

- 1) Injection syringe
- 2) Syringe filter
- 3) Column and guard column
- 4) Detector and pump
- 5) Software system

3.4.2 Column and instrument parameters

The parameters below were set as fixed parameters. They were the same during the whole project, and therefore called basic parameters (Table 1).

Table 1. Parameters used for the analyses.

Guard column	Metrosep C 4 Guard/4.0
Separation column	Metrosep C 2 100
Injection volume	20 μl
Syringe filter	25 mm filter with 0.45 μ nylon membrane
Flow rate	1.0 ml/min
Peak measurement	concentration, area, height, retention time

Specifications for both guard column and separator column are listed below (Table 2).

Table 2. Column specifications.

	Metrosep C 2 100	Metrosep C 4 Guard/4.0
Column dimensions (mm)	4.0 x 100	5 x 4.0
Max. pressure (MPa)	25	-
Maximum flow (ml/min)	5	-
Standard flow (ml/min)	1.0	-
Housing material	PEEK	PEEK
Carrier material	Silica gel with carboxyl groups	Silica gel with carboxyl groups
Particle size (μm)	7	5
pH range	2....7	2....7

3.5 Analysis Procedure

3.5.1 Preparation of eluent

The eluent was prepared from freshly high purity water (i.e. Milli-Q water) with low conductivity. Milli-Q water should not be stored for long time, neither in glass vessels, nor in plastic bottles, since the conductivity increases fast, causing a decrease in the eluent quality. Therefore, it was always taken from the high purity water generator.

The eluent was also degassed before use to avoid air bubbles in the pumps and to lower the noise in the detector. The eluent was degassed in an ultrasonic bath.

The eluent adequate for this type of analysis (i.e. non-suppressed cation analysis) was prepared according to the Metrohm manual [5] as described below.

- 167 mg dipicolinic acid and 600 mg tartaric acid were dissolved in 100 ml high purity water in a 1000 ml Erlenmeyer flask.
- The solution was warmed up until the chemicals were totally dissolved.
- The Erlenmeyer flask was filled up to 1000 ml with high purity water and then vacuum filtered.

- The eluent was transferred to a plastic bottle suitable for IC device and placed into an ultrasonic bath for 15 min.

3.5.2 Preparation of stock solutions and standard solutions

Stock solutions with cation concentration of 1 M were prepared from four chloride salts provided by the laboratory of the Helsinki Metropolia University of Applied Sciences. In order to avoid errors, the stock solutions were prepared separately, which means, one bottle for each chloride salt. Standard solutions of four cations (Na^+ , NH_4^+ , K^+ and Mg^{2+}) were prepared from the stock solutions by dilution. Sodium chloride was included in the standard solution to verify if the ammonium peak and sodium peak are not overlapping since their retention time are very close to each other. The concentrations chosen for the standard solutions are shown on Table 3 below:

Table 3. Concentrations of the standard solutions [mg/l].

	Mg^{2+}	K^+	NH_4^+	Na^+
Standard 1	5	5	5	5
Standard 2	10	10	10	10
Standard 3	20	20	20	20
Standard 4	30	30	30	30
Standard 5	50	50	50	50

3.5.3 Calibration

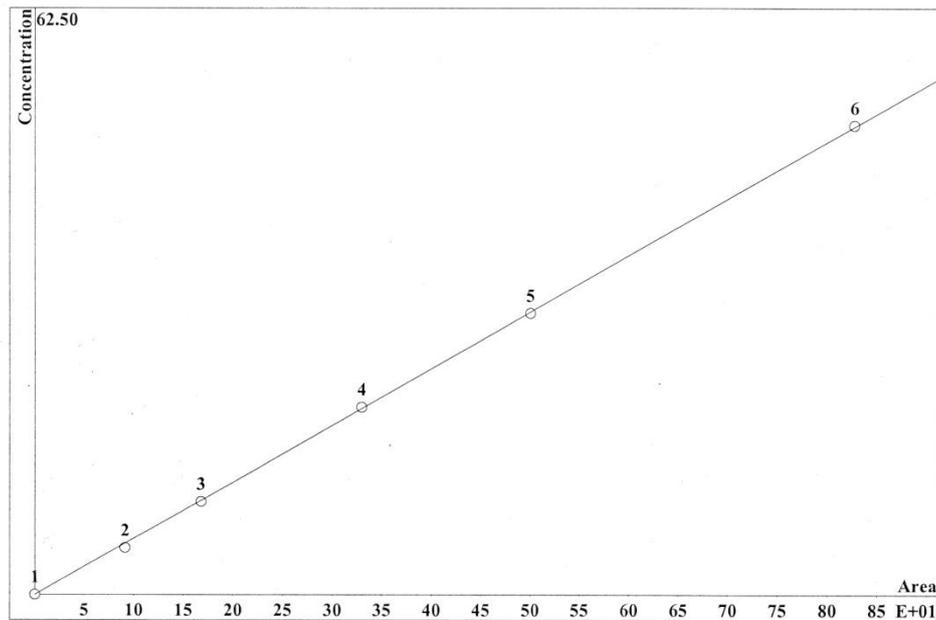
Calibration is done by running the standards one by one, from the smallest to largest concentration. Each standard run takes about 15 minutes due to the short column used (4.0 x 100 mm).

Calibration functions for each ion were established by analyzing the standard solutions at levels dictated by the range of measurement (Table 3). Calibration curves are constructed automatically by the IC, where peak area for each ion is plotted against concentration (Figure 8).

To ensure that the method is working properly, it is recommended to run a standard solution from time to time (i.e. control sample).

CALIBRATION OF COMPONENT magnesium

Method: cat. 100 mm.mtw
Equation: $Q = 1.20489 \cdot A$
RSD: 1.234 %
Correlation coefficient: 0.999940



K3 = 0 K2 = 0 K1 = 1.20489 K0 = 0
Base: Area
Ref.channel: Cond
ISTD:
Formula: $Y = K1 \cdot X$
Weight: 1

Level	Height	Area	Conc.	Vol/Dil	Retention	Used	File
1	0	0	1	0	0	No	
2	1.808	91.31	5	20	11.31	Yes	va101552.chw
3	2.722	167.9	10	20	11.31	Yes	va101609.chw
4	4.245	329.8	20	20	11.31	Yes	va101503.chw
5	5.609	500.7	30	20	11.31	Yes	va101626.chw
6	7.911	827.9	50	20	11.31	Yes	va101534.chw

Figure 8. Typical linear calibration for cation method.

3.5.4 Manufacture of salt samples at Metropolia

Smart Salt Inc. provided all the salt samples for analysis. The salt samples were prepared at the laboratory of the Helsinki Metropolia University of Applied Sciences.

The process steps are described in detail below:

- 1) The dosage of each raw material based on molar ratios or other recipe was calculated.
- 2) The expected tap water for total dissolution (based on solubility data for each component) was calculated.
- 3) All raw materials were poured into the Rota Vapor flask.
- 4) Tap water was added.
- 5) The flask was heated in water bath (100 °C) to aid dissolving; more water was added when needed.
- 6) When everything was dissolved, the flask was moved to the Rota Vapor.
- 7) The liquid content in the flask was evaporated under vacuum (normally as low as possible with the device).
- 8) The condensate production was followed by weighing it.
- 9) When the slurry was rather thick, the Rota Vapor was stopped. The target was to leave about 20- 30% of the water in the crystal slurry. The condensate was sampled.
- 10) The slurry was emptied into a suction funnel with filter paper.
- 11) The slurry was dried under suction for about 1 h.
- 12) The mother liquor was weighed in the suction flask.
- 13) The crystals in the funnel were emptied on a plate and put into oven for drying (min. 4 h at 60 °C).
- 14) The salt was sieved through a 0.8 mm sieve, and all hard lumps were crushed.
- 15) The salt was put back into oven for 2 – 3 h in some cases when it was still moist.
- 16) The salt was re-sieved.
- 17) The salt yield was weighed.

The industrial process is similar to the one performed in the laboratory of the Helsinki Metropolia University of Applied Sciences in several steps. Instead of suction funnel, centrifuging is used. For drying, a fluid bed dryer is superior to oven drying. There are two different processes, one is with removal of mother liquor by centrifuging, and the other is a total crystallization and drying process in an agitated vacuum vessel. The latter one cannot be simulated in RotaVapor, as it is not possible to get out the salt. The RotaVapor does not have an internal agitator, and therefore the salt solidifies on the bottle wall.

The molar ratios of the salt samples provided by Smart Salt Inc. are listed below (Table 4).

Table 4. Molar ratio of each sample.

Sample	Molar ratio MNK*
1	1-0-1
2	1-0.25-0.75
3	1-0.5-0.5
4	1-0.75-0.25
5	1-1-0
6	1-0.5-0.5
7	1-0.5-0.5
8	1-0.25-0.75
reference	1-0.75-0.25
10	1-0.75-3.5
11	1-0.25-4
12	-
13	1-0.25-0.75

*M-N-K: magnesium, ammonium and potassium

The samples 10, 11, 12 and 13 (highlighted in red) are special solutions, which were not prepared at the laboratory of the Helsinki Metropolia University of Applied Sciences.

3.5.5 Preparation of sample solutions

In order to obtain accurate results, samples must be prepared in such a way that the concentration of each cation in question fits in the standard range (Table 3). The salt sample shall also be dry. Therefore, for blended products (i.e. sample 12) it is not recommended to pour the sample from the bag or container, as there is a risk of segregation. So a clean spoon was used to mix the complete sample to a homogenous mix and pick it. The samples were placed in a drying oven at 60 °C for at least two hours. Stock solutions of 10 g/l concentration were prepared for each salt sample. Dilutions from the

stock solutions were made several times until stable peaks were shown in the chromatograms (Table 5).

Table 5. Recommended dilutions for Smart Salt Inc. Concentration of the samples [mg/l]

Sample	Molar ratio MNK	Mg ²⁺	NH ₄ ⁺	K ⁺	Na ⁺
1	1-0-1	300	-	300	-
2	1-0.25-0.75	400	400	400	-
3	1-0.5-0.5	400	400	400	-
4	1-0.75-0.25	400	400	400	-
5	1-1-0	400	400	-	-
6	1-0.5-0.5	400	400	400	-
7	1-0.5-0.5	500	500	500	-
8	1-0.25-0.75	400	400	400	-
reference	1-0.75-0.25	400	400	400	-
10	1-0.75-3.5	300	300	100	-
11	1-0.25-4	600	600	100	-
12	-	500	500	150	150
13	1-0.25-0.75	200	200	200	-

4 Molar ratio analysis

Molar ratio analysis is the main part of the project as it was required by the manufacturer. The target of this analysis was to verify if the molar ratios of each sample remain the same after the manufacturing process (see 3.5.4) of the salt products at the laboratory of the Helsinki Metropolia University of Applied Sciences. The molar ratio stability tells how homogeneous the salts are when applying a different manufacturing process.

Since Rota Vapor was used in the process, there were some losses in the salt yield. Therefore it is important to know if the components of the salt are equally lost after the processes.

The analysis procedure is explained in the following sections of this chapter.

4.1 Results from IC analysis

The IC measurements were performed by running the samples randomly, twice each sample.

The results for Mg^{2+} ion concentrations are shown on Table 6 below.

Table 6. Magnesium concentrations obtained by IC.

sample	run 1 [mg/l]	run 2 [mg/l]	average concentration [mg/l]
1	26.545	26.352	26.449
2	36.524	37.231	36.878
3	35.902	35.745	35.824
4	37.883	37.981	37.932
5	38.285	38.199	38.242
6	34.79	35.337	35.064
7	46.442	45.523	45.983
8	37.54	37.442	37.491
reference	37.523	37.088	37.306
10	8.812	8.782	8.797
11	14.95	14.986	14.968
12	5.456	5.471	5.464
13	14.328	14.289	14.309

The results for NH_4^+ ion concentrations are shown on Table 7 below.

Table 7. Ammonium concentrations obtained by IC.

sample	run 1 [mg/l]	run 2 [mg/l]	average concentration [mg/l]
1	-	-	-
2	7.527	7.522	7.525
3	14.492	14.447	14.470
4	20.801	20.879	20.840
5	28.883	29.48	29.182
6	15.773	15.963	15.868
7	17.165	17.111	17.138
8	8.406	8.435	8.421
reference	21.301	21.394	21.348
10	7.263	7.299	7.281
11	5.699	6.578	6.139
12	6.247	6.401	6.324
13	5.241	5.191	5.216

The results for K⁺ ion concentrations are shown on Table 8 below.

Table 8. Potassium concentrations obtained by IC.

sample	run 1 [mg/l]	run 2 [mg/l]	average concentration [mg/l]
1	45.599	45.268	45.434
2	47.217	46.33	46.774
3	29.431	30.458	29.945
4	15.133	14.734	14.934
5	-	-	-
6	34.53	34.422	34.476
7	34.538	34.243	34.391
8	50.831	49.654	50.243
reference	19.544	20.655	20.100
10	37.485	37.187	112.008
11	40.188	40.201	241.167
12	23.546	24.79	80.479
13	37.937	37.533	37.735

The results for Na⁺ ion concentrations are shown on Table 9 below.

Table 9. Sodium concentrations obtained by IC.

sample	run 1 [mg/l]	run 2 [mg/l]	average concentration [mg/l]
12	35.375	35.645	118.248

As it can be seen in Table 6 - 9, the ions concentrations obtained by Ion Chromatography had a satisfactory precision. Meaning that the method is able to identify and quantify the ions present in the samples.

4.2 Calculations of molar ratios

4.2.1 Finding the ion concentration

In order to find the molar ratios, the ion concentration of each ion in the samples must be calculated. The ion chromatographic results were not given with their respective

dilution rates, and therefore the actual average concentration had to be calculated according to the formulas below.

$$\text{actual average concentration [mg/l]} = (\text{average of run1 and run2 [mg/l]} * \text{dilution rate})$$

$$\text{ion concentration [mol/l]} = \text{average [mg/l]} / \text{ion molar weight [mg/mol]}$$

Calculation of ion concentration for magnesium is shown on Table 10 below.

Table 10. Ion concentration is calculated for magnesium with molar weight of 24305.1 mg/mol.

sample	dilution rate	run 1	run 2	average [mg/l]	ion concentration [mol/l]
1	33.33	26.545	26.352	881.529	0.0363
2	25	36.524	37.231	921.938	0.0379
3	25	35.902	35.745	895.588	0.0368
4	25	37.883	37.981	948.300	0.0390
5	25	38.285	38.199	956.050	0.0393
6	25	34.79	35.337	876.588	0.0361
7	20	46.442	45.523	919.650	0.0378
8	25	37.54	37.442	937.275	0.0386
reference	25	37.523	37.088	932.638	0.0384
10	33.33	8.812	8.782	293.204	0.0121
11	16.67	14.95	14.986	249.517	0.0103
12	20	5.456	5.471	109.270	0.0045
13	50	14.328	14.289	715.425	0.0294

Calculation of ion concentration for ammonium is shown on Table 11 below.

Table 11. Ion concentration is calculated for ammonium with molar weight of 18038.5 mg/mol.

sample	dilution rate	run1	run2	average [mg/l]	ion concentration [mol/l]
1	-	-	-	-	-
2	25	7.527	7.522	188.113	0.0104
3	25	14.492	14.447	361.738	0.0201
4	25	20.801	20.879	521.000	0.0289
5	25	28.883	29.48	729.538	0.0404
6	25	15.773	15.963	396.700	0.0220
7	20	17.165	17.111	342.760	0.0190
8	25	8.406	8.435	210.513	0.0117
ref	25	21.301	21.394	533.688	0.0296
10	33.33	7.263	7.299	242.676	0.0135
11	16.67	5.699	6.578	102.329	0.0057
12	20	6,247	6.401	126.480	0.0070
13	50	5.241	5.191	260.800	0.0145

Calculation of ion concentration for ammonium is shown on Table 12 below.

Table 12. Ion concentration is calculated for potassium with molar weight of 39098.5 mg/mol.

sample	dilution rate	run1	run2	average [mg/l]	ion concentration [mol/l]
1	33.33	45.599	45.268	1514.299	0.0387
2	25	47.217	46.33	1169.338	0.0299
3	25	29.431	30.458	748.613	0.0191
4	25	15.133	14.734	373.338	0.0095
5	-	-	-	-	-
6	25	34.53	34.422	861.900	0.0220
7	20	34.538	34.243	687.810	0.0176
8	25	50.831	49.654	1256.063	0.0321
ref	25	19.544	20.655	502.488	0.0129
10	100	37.485	37.187	3733.600	0.0955
11	100	40.188	40.201	4019.450	0.1028
12	66.67	23.546	24.79	1611.281	0.0412
13	50	37.937	37.533	1886.750	0.0483

Calculation of ion concentration for sodium is shown on Table 13 below.

Table 13. Ion concentration is calculated for sodium, with molar weight of 22989.7 mg/mol.

sample	dilution rate	average [mg/l]	ion concentration [mol/l]
12	66.67	2367.452	0.1030

4.2.2 Finding the molar ratios

Calculation of molar ratio (NH_4^+ and K^+) was done proportionally to magnesium molar ratio since it is always 1. Therefore, the general formula is as follows.

$$\text{molar ratio} = \text{ion concentration [mol/l] of } \text{NH}_4^+ \text{ or } \text{K}^+ / \text{ion concentration [mol/l] of } \text{Mg}^{2+}$$

Example:

Salt sample 2

$$\text{NH}_4^+: 0.0104 \text{ [mol/l]} / 0.0379 \text{ [mol/l]} = 0.27$$

$$\text{K}^+: 0.0299 \text{ [mol/l]} / 0.0379 \text{ [mol/l]} = 0.79$$

The molar ratios obtained are shown on the Table 14 below.

Table 14. Comparison between original molar rates and analyzed molar rates.

Sample	original molar rate (NH ₄ ⁺)	analysed molar rate (NH ₄ ⁺)	original molar rate (K ⁺)	analysed molar rate (K ⁺)
1	0	-	1	1.07
2	0.25	0.27	0.75	0.79
3	0.5	0.54	0.5	0.52
4	0.75	0.74	0.25	0.24
5	1	1.03	0	-
6	0.5	0.61	0.5	0.61
7	0.5	0.50	0.5	0.46
8	0.25	0.30	0.75	0.83
reference	0.75	0.77	0.25	0.33
10	0.75	1.12	3.5	7.92
11	0.25	0.55	4	10.02
12	-	1.56	-	9.16
13	0.25	0.49	0.75	1.64

It can be noticed in Table 14, the analyzed molar ratios from Sample 1 to reference sample are quite accurate, meaning that the losses in salt during the process did not affect their homogeneity.

Samples 10, 11 and 13, which were not produced at the laboratory of the Helsinki Metropolia University of Applied Sciences, had their analyzed molar ratios about twice as large as the original molar ratios. The molar ratio of Sample 12 was not provided by the manufacturer, and it was not produced at the laboratory of the Helsinki Metropolia University of Applied Sciences either. Since they are special solutions, the reason for their molar ratios being so different could not be investigated by the analyst.

5 Determination and results of validation parameters

5.1 Procedure

Since the exact concentrations in Smart Salt Inc. samples are not known, it was necessary to prepare a few salt samples (*Analyst samples*) with known and molar ratios in order to be possible to determine the validation parameters. The stock solutions were prepared from three chloride salts ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, KCl and NH_4Cl) and high purity water. Molar ratios 0, 0.5 and 1 were chosen since they are more common in Smart Salt Inc. products. The molar ratio of magnesium was fixed to be 1 as in Smart Salt Inc. samples. A special sample with potassium molar ratio of 3.5 was prepared as in sample 10 (Table 4). Sodium ion was not included since it is not present in many salt samples. A matrix with all possible molar ratio combinations was made, giving a total of nine samples (Table 15).

Table 15. Molar ratios of the samples prepared by the analyst.

Analyst sample	Mg^{2+}	NH_4^+	K^+
1	1	0	0
2	1	0.5	0
3	1	0	0.5
4	1	0.5	0.5
5	1	1	1
6	1	0	1
7	1	1	0
8	1	0	0.5
9	1	1	3.5

The amounts (in grams) of chloride salts needed for preparation of stock solutions were calculated based on 1 g of magnesium, since its molar ratio never changes.

Calculation examples of how chemical amounts for preparation of samples were obtained are shown below.

Sample 1:

Mg = 1g (fixed value)

$$n_{\text{Mg}} = \frac{m_{\text{Mg}}}{M_{\text{Mg}}} = \frac{1 \text{ g}}{24.305 \text{ g/mol}} = 0.0411 \text{ mol}$$

Sample 2:

$n_{\text{NH}_4} = n_{\text{Mg}} * \text{NH}_4$ molar rate

$$n_{\text{NH}_4} = 0.0411 \text{ mol} * 0.5 = 0.0205 \text{ mol}$$

$m_{\text{NH}_4} = n_{\text{NH}_4} * M_{\text{NH}_4}$

$$m_{\text{NH}_4} = 0.0205 \text{ mol} * 18.03 \text{ g/mol} = 0.3705 \text{ g}$$

$$m_{\text{NH}_4\text{Cl}} = \left(\frac{M_{\text{NH}_4\text{Cl}}}{M_{\text{NH}_4}} \right) * m_{\text{NH}_4}$$

$$m_{\text{NH}_4\text{Cl}} = \left(\frac{53.49 \text{ g/mol}}{18.03 \text{ g/mol}} \right) * 0.3705 \text{ g} = 1.0992 \text{ g}$$

Sample 3:

$n_{\text{K}} = n_{\text{Mg}} * \text{K}$ molar rate

$$n_{\text{K}} = 0.0411 \text{ mol} * 0.5 = 0.0205 \text{ mol}$$

$m_{\text{K}} = n_{\text{K}} * M_{\text{K}}$

$$m_{\text{K}} = 0.0205 \text{ mol} * 39.098 \text{ g/mol} = 0.8015 \text{ g}$$

$$m_{\text{KCl}} = \left(\frac{M_{\text{KCl}}}{M_{\text{K}}} \right) * m_{\text{K}}$$

$$m_{\text{KCl}} = \left(\frac{74.533 \text{ g/mol}}{39.098 \text{ g/mol}} \right) * 0.8015 \text{ g} = 1.5279 \text{ g}$$

Table 16 displays the concentration obtained from the calculations.

Table 16. Concentration of mol [g/l].

Analyst sample	Mg ²⁺	NH ₄ ⁺	K ⁺
1	1	-	-
2	1	0.3705	-
3	1	-	0.8015
4	1	0.3705	0.8015
5	1	0.7410	1.6069
6	1	-	1.6069
7	1	0.7410	-
8	1	0.7410	0.8015
9	1	-	5.6223

Several dilutions from stock solutions were tried out until the peaks in the chromatogram were stable. The recommended dilutions and true concentrations of each ion are shown in Table 17 below.

Table 17. Recommended dilutions [mg/l] and true concentrations [mg/l].

Analyst sample	dilution [ml/100ml]	Mg ²⁺ [mg/l]	NH ₄ ⁺ [mg/l]	K ⁺ [mg/l]
1	4	40	-	-
2	4	40	14.820	-
3	4	40	-	32.060
4	4	40	14.820	32.060
5	3	30	22.230	48.207
6	3	30	-	48.207
7	4	40	29.640	-
8	4	40	29.640	32.06
9	0.8	8	-	44.978

True concentrations are calculated by multiplying the concentration of mol (Table 16) by dilution. It should be noticed that the concentration of mol is in [g/l] units, and therefore it must be converted to [mg/l].

5.2 Method testing

5.2.1 First calibration

The same method used for Smart Salt sample analysis was used for method validation. This method was calibrated on October 2011, and *analyst samples* were prepared and analyzed on April 2012.

5.2.1.1 Method Accuracy

A parallel sample (*replicate*) for each of the nine samples was prepared. Both *analyst sample and replicate* were randomly analyzed twice. All samples prepared from high purity water.

The results of both *analyst samples* and their *replicates* are shown in Table 18, 19 and 20 below.

Table 18. IC analysis results for magnesium ions.

Analyst sample #	Analyst sample run1	Analyst sample run2	Replicate run 1	Replicate run 2
1	47.073	47.085	47.089	47.175
2	47.337	52.407	47.403	47.928
3	44.764	47.314	47.279	47.654
4	47.186	47.392	47.162	47.173
5	49.136	47.607	47.325	50.617
6	47.467	47.779	47.753	47.735
7	48.613	47.253	47.230	48.171
8	91.896	91.709	46.997	46.786
9	51.595	49.595	50.315	54.415

The magnesium results of Analyst sample 8 (highlighted in red) were excluded from calculations due to weighing error.

The concentrations of ammonium ion in each replicate are shown in Table 19 below.

Table 19. IC analysis results for ammonium ions [mg/l].

Analyst sample #	Analyst sample run1	Analyst sample run2	Replicate run 1	Replicate run 2
1	-	-	-	-
2	34.272	37.710	34.222	34.568
3	-	-	-	-
4	34.124	34.560	34.102	34.228
5	35.331	33.82	33.997	36.235
6	-	-	-	-
7	35.216	34.058	33.977	34.583
8	33.557	33.340	33.432	33.624
9	-	-	-	-

The concentrations of potassium ion in each replicate are shown in Table 20 below.

Table 20. IC analysis results for potassium ions [mg/l].

Analyst sample #	Analyst sample run1	Analyst sample run2	Replicate run 2	Replicate run 2
1	-	-	-	-
2	-	-	-	-
3	34.727	36.699	36.54	36.531
4	35.847	36.505	36.969	36.976
5	76.841	74.264	72.752	77.745
6	71.589	72.576	71.683	72.724
7	-	-	-	-
8	37.451	36.753	36.398	36.271
9	73.174	72.046	70.976	70.740

In order to be able to compare between the true concentrations and the analyzed concentrations, the dilutions in Analyst sample 5, 6 and 9 were converted to 4. Also, the 0.5 and 3.5 molar ratios must be converted to 1, which is the molar ratio for the magnesium ion.

The comparable true concentrations are show in Table 21 below.

Table 21. True concentrations.

Sample	Molar ratio	Dilution [ml/100ml]	Mg ²⁺ [mg/l]	NH ₄ ⁺ [mg/l]	K ⁺ [mg/l]
1	1	4	40	-	-
2	1	4	40	29.64	-
3	1	4	40	-	64.12
4	1	4	40	29.64	64.12
5	1	4	40	29.64	64.276
6	1	4	40	-	64.276
7	1	4	40	29.64	-
8	1	4	40	29.64	64.12
9	1	4	40	-	64.25

It can be observed in the results (Table18, 19 and 20) that the concentrations of the three ions in general have a good precision. However, all the concentrations are far from known concentrations (i.e. true concentrations).

The error is calculated by subtracting the experimental value (Table 18 - 20) from the true value (Table 21). These errors helped to validate the accuracy of the method.

Errors for the magnesium ion are shown in Table 22 below.

Table 22. Magnesium error [mg/l].

Analyst sample #	Analyst sample run1	Analyst sample run2	Replicate run 1	Replicate run 2
1	7.073	7.085	7.089	7.175
2	7.337	12.407	7.403	7.928
3	4.764	7.314	7.279	7.654
4	7.186	7.392	7.162	7.173
5	9.136	7.607	7.325	10.617
6	7.467	7.779	7.753	7.735
7	8.613	7.253	7.230	8.171
8	51.896	51.709	6.997	6.786
9	11.595	9.595	10.315	14.415

Errors for the ammonium ion are shown in Table 23 below.

Table 23. Ammonium error [mg/l].

Analyst sample #	Analyst sample run1	Analyst sample run2	Replicate run 1	Replicate run 2
1	-	-	-	-
2	4.632	8.07	4.582	4.928
3	-	-	-	-
4	4.484	4.92	4.462	4.588
5	5.691	4.18	4.357	6.595
6	-	-	-	-
7	12.986	11.828	11.747	12.353
8	11.327	11.11	11.202	11.394
9	-	-	-	-

Errors for the potassium ion are shown in Table 24 below.

Table 24. Potassium error [mg/l].

Analyst sample #	Analyst sample run1	Analyst sample run2	Replicate run 1	Replicate run 2
1	-	-	-	-
2	-	-	-	-
3	5.334	9.278	8.96	8.942
4	7.574	8.89	9.818	9.832
5	12.565	9.988	8.476	12.469
6	7.313	7.407	7.407	8.448
7	-	-	-	-
8	10.782	9.386	8.676	8.422
9	8.920	8.792	6.72	6.486

The results for the analyzed samples showed errors in one direction only; therefore it is likely that the results have a systematic error.

5.2.1.2 *t* test

This test was performed to see if there was any evidence for a systematic error in the method at 95 % confidence level. Values for \bar{x} , SD and *t* test were calculated for each ion according to Equation 1, Equation 2 and Equation 4 respectively. The results were compared to *t* values table (see Appendix 1).

The null hypothesis: H_0 : measurements do not deviate (i.e. good results)

The alternative hypothesis: H_a : measurements deviate

The results can be seen in Table 25 below.

Table 25. *t* values calculated for each ion in the samples.

	Mg^{2+}	NH_4^+	K^+
<i>t</i> calculated	2.956	2.963	2.972

The null hypothesis is rejected if the t value calculated is larger than the t value from table. Comparing the calculated values and table values (Appendix 1), it can be observed that in all cases the null hypothesis is rejected. Therefore, there is evidence for systematic error at the 95 % confidence level.

5.2.1.3 Linearity test

The purpose of this test was to obtain correlation coefficients (R^2) for the ions composing the calibration curve (Mg^{2+} , K^+ , NH_4^+ and Na^+ ions).

The calculation was done by plotting peak areas of all ion standard solutions against their different concentrations. A separate calculation process was not needed because the 761 Compact IC 1.1 software automatically plots and calculates the correlation coefficients after calibration.

A correlation coefficient $R^2 > 0,999$ were obtained for all ions.

5.2.2 Second calibration

The method was calibrated on September 2012 and the same *analyst samples*, which were prepared on April 2012, were analyzed.

5.2.2.1 Injection repeatability

The target of this experiment was to calculate the *RSD* value when a solution was injected 8 times.

A three ion (Mg^{2+} , NH_4^+ and K^+) standard solution of 10 mg/l concentration was prepared. This solution was injected eight times and the *SD* and *RSD* were calculated according to Equation 2 and Equation 3 respectively.

The concentrations obtained from IC analysis are show in Table 26 below.

Table 26. Injection repeatability. Concentrations in [mg/l].

	Mg ²⁺	NH ₄ ⁺	K ⁺
Run 1	11.068	9.975	9.220
Run2	11.028	10.000	9.553
Run 3	11.037	9.962	9.629
Run 4	11.127	10.226	9.737
Run 5	11.151	9.949	9.256
Run 6	11.326	10.136	9.664
Run 7	11.306	10.134	9.226
Run 8	11.154	10.109	9.390

SD and *RSD* were calculated according to Equation 3 and Equation 4 (see section 2.4.1) respectively. The results can be seen in Table 27 below.

Table 27. Values for standard deviation and relative standard deviation.

	Mg ²⁺	NH ₄ ⁺	K ⁺
<i>SD</i>	0.1135	0.1028	0.1958
<i>RSD</i>	1.02 %	1.02%	2.06%

Low *RSDs* indicate good reproducibility and precision of the procedures. *RSD* is recommended to be below 1%. However, according to Metrohm, for this kind of analysis an *RSD* below 5% is acceptable.

5.2.2.2 Sample stability test

Some of the *analyst samples*, which were prepared on April 2012, were analyzed now with the September 2012 calibration (Table 28).

Table 28. Samples analyzed on September 2012.

Analyst sample	Mg ²⁺	NH ₄ ⁺	K ⁺
2	42.114	56.83	-
5	41.576	57.69	62.93
8	-	56.90	63.28

Two months later the same samples were analyzed. The results are displayed in Table 29.

Table 29. Same samples analyzed on November 2012.

Analyst sample	Mg ²⁺	NH ₄ ⁺	K ⁺
2	43.863	57.086	-
5	44.492	56.883	63.464
8	-	56.544	64.034

It can be observed in Table 28 and 29 that the results had a good reproducibility within two months. Magnesium and potassium concentrations are now closer to the true values (Table 21) than before when the experiment was conducted by the old calibration. However, the ammonium concentration increased unexpectedly.

6 Conclusions and recommendations

The main objective of this project was to obtain information on the stability of the ions molar ratios of the Smart Salt Inc. samples after undergoing manufacturing process at the Helsinki Metropolia University of Applied Sciences. The process includes a few steps, which are not conducted in industrial manufacturing process. As mentioned before, the RotaVapor retains part of the salt, making it basically impossible to remove it since it solidifies on the bottle wall.

The results for molar ratio analysis were in general satisfactory, showing that even with loss of salt yield, the molar ratios remained quite close to the original ones. With this, it can be concluded that the homogeneity of the samples was not affected by the unusual

process performed at the laboratory of the Helsinki Metropolia University of Applied Sciences.

Method validation is an essential component of the measure to ensure that accurate and reliable results are produced. The initial plan for the method validation was to prepare several samples (*analyst samples*) with known concentrations and molar ratios, and finally analyze them using the same method in which Smart Salt samples were analyzed.

It turned out that all the ions concentrations obtained with this method had good precision, but they were higher than the actual concentrations (true concentrations). Therefore, it was suspected that the method had a systematic error. The *t* test performed to see if this suspicion was correct showed that there was evidence for systematic error at the 95 % confidence level (usually chosen). The calibration gave a linear correlation of $R^2 > 0.999$ for all ions, which is excellent (a strong positive linear R^2 is close to +1). However, this calibration was done on October 2011, and the *analyst samples* were analyzed on April 2012, which suggests that this systematic error is probably due to the calibration period of 6 months between Smart Salt analysis and the *analyst samples* analysis. Weighing error of chemicals, poor Milli-Q water quality, or even impurities in the flasks may have contributed to inaccurate results. Therefore, to avoid errors, it is recommended to use commercial standard solutions and brand new dishes. However, these would cost the Helsinki Metropolia University of Applied Sciences hundreds of Euros.

Another calibration was done on September 2012. Some of the *analyst samples* (prepared on April 2012) were randomly chosen and analyzed right way after calibration. Injection repeatability test of a standard sample was done two months later, and the calculated *RSD* for each ion showed a good reproducibility according to Metrohm, which recommends an *RSD* below 5% for this type of analysis.

The same samples were analyzed two months later, and the results were compared, showing that the ions concentrations did not have a significant variation within two months. The magnesium and potassium concentrations were observed to be more accurate (i.e. closer to the actual concentrations) than the concentrations obtained by the old calibration, reinforcing the fact that the old calibration was no longer suitable for any kind of analysis. The ammonium concentration increased, suggesting that there

was probably a mistake in the calibration for ammonium. The new calibration also showed that apparently the samples did not suffer any drastic changes over the last 7 months (from the month they were prepared to now).

Some difficulties were faced throughout the project, such as calibrating the method. Since the system calibration do not often recognize all wanted ions at the same time, several calibration trials were done by slightly changes in parameters until all the ions are recognized in the calibration curves. Another issue, which was time-consuming, was the fact that each ion has its own concentration in the samples and that depending on the concentration, the method does not detect or quantify it properly. Therefore, several dilutions of each sample were made until all the ions concentrations fitted into the standard calibration range at the same time in the chromatogram. The time availability was not enough for preparing more samples and doing more analyses, which may have affected affect the accuracy and reliability of the result.

There are a variety of method validation parameters that would be interesting to evaluate. One of these is the *specificity test*, which is performed to show that no peak of other possible impurities interferes with the known peaks. This test could have investigated if the calcium peak would interfere with the magnesium peak and the sodium peak with the ammonium peak since their retention times are very close to each other. Sodium chloride was added to standard solutions to check if the sodium peak interferes with the ammonium peak during the sample analysis. However, it cannot be considered as a test since it was not done separately and deeply investigated. Another parameter is *intermediate precision*, which is tested by repeating the analysis described in the method, by two different analysts in order to observe within-laboratory variations. These two validation parameters require, for example preparation of several samples and dozens of injections, which was not possible within the timeframe allocated for this engineering project.

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Critical values for a 2 tailed t test

		Confidence Interval						
		80%	90%	95%	98%	99%	99.8%	99.9%
		α level two-tailed test						
df		0.2	0.1	0.05	0.02	0.01	0.002	0.001
1		3.078	6.314	12.706	31.821	63.657	318.313	636.589
2		1.886	2.920	4.303	6.965	9.925	22.327	31.598
3		1.638	2.353	3.182	4.541	5.841	10.215	12.924
4		1.533	2.132	2.776	3.747	4.604	7.173	8.610
5		1.476	2.015	2.571	3.365	4.032	5.893	6.869
6		1.440	1.943	2.447	3.143	3.707	5.208	5.959
7		1.415	1.895	2.365	2.998	3.499	4.785	5.408
8		1.397	1.860	2.306	2.896	3.355	4.501	5.041
9		1.383	1.833	2.262	2.821	3.250	4.297	4.781
10		1.372	1.812	2.228	2.764	3.169	4.144	4.587