Validation of Ion Chromatographic Method for Determination of Standard Inorganic Anions in Treated and Untreated Drinking Water

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Abstract. An ion chromatographic method for determination of fluoride, chloride, nitrate and sulphate in untreated and treated drinking waters was described. An automated 850 IC Professional, Metrohm system equipped with conductivity detector and Metrosep A Supp 7-250 (250 x 4 mm) column was used. The validation of the method was performed for simultaneous determination of all studied analytes and the results have showed that the validated method fits the requirements of the current water legislation. The main analytical characteristics were estimated for each of studied analytes: limits of detection, limits of quantification, working and linear ranges, repeatability and intermediate precision, recovery. The trueness of the method was estimated by analysis of certified reference material for soft drinking water. Recovery test was performed on spiked drinking water samples. An uncertainty was estimated. The method was applied for analysis of drinking waters before and after chlorination.

1. Introduction
During the last decades ion chromatography (IC) have gain much attention due its unique capability to detect anions in different matrices in very precise and accurate manner offering low detection limits, low sample and reagent consumption, as well as short analysis time [1, 2]. Nowadays the main challenges in environmental analysis by ion chromatography are related to new analytes separation and determination at trace levels. Due to the intensive technological development new molecules appeared in the environment, which imposes determination of new analytes in common matrices or standard anions in new matrices. Novel stationary phases with appropriate selectivity and high separation efficiency have been proposing din attempt to respond to this challenge [3, 4]. The environmental and health authorities, as well as modern industrial technologies impose stringer and stringer demands to the water quality and methods for determination at trace levels are highly demanded [5-9]. Ion chromatography appears to be widely studied method for anion determination which successfully addressed this issue [1, 2, 7-12].

It should be emphasised the need of validation of analytical methods in order to ensure quality of the results and their fit for purpose according to the demands of customers. A line of studies on optimisation and validation of ion chromatographic methods for anions determination have been
recently published [7-13]. The validation procedure was profoundly described in the international legislation to ensure comparable laboratory results [14-17]. The validation should prove that the results obtained by given laboratory were not significantly different from the reference results [16, 17]. Validation of a method for chloride, nitrate and sulphate ions determination in drinking water was reported [12]; all contributions involved in the analysis were considered in estimation of the uncertainty. A validation of an ion chromatographic methods for determination of fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulphate anions in wet depositions and other meteoric water sources with precision less than 2 % was described [11]. A sensitive and reliable ion chromatographic method with limit of detection at low ppb level was validated for determination of corrosive anions (fluoride, chloride, nitrate, phosphate and sulphate) in power plants water [7]. Single-run determination of several anions using different separation columns was proposed and the results from method validation in environmental samples were reported [8,13]. A thorough review on ion chromatographic methods for ox halide ions determination has been recently published [1], the results obtained with different columns, eluents and suppression modes were compared. As can be seen from the literature survey the most intensively studied ion chromatographic determination is based on conductivity detection using different suppressors.

This paper presents the results from a validation study of ion chromatographic method with conductivity detection after chemical and carbon dioxide suppression for determination of standard anions in treated and untreated drinking water.

2. Experimental

An ion chromatographic, metal-free system (IC-850 Professional model, Metrohm AG, Switzerland) controlled by Metrodata MagIC Net™ software and equipped with 858 Professional Sample Processor, sample filtration system with a 0.2 µm regenerated cellulose membrane, six channel injection valve, low pulsation high-pressure pump, chemical suppression and CO2 suppression, eluent degasser and conductivity detector was used. The separation was performed on a Metrosep A Supp 7-250 column (250 x 4 mm, polyvinyl alcohol with quaternary ammonium groups, 5 µm particle size), Metrosep RP 2 Guard/3.5 (polymer with pore size 0.2 µm). In order to keep the baseline signal as low as 0.99 µS/cm, 0.1 M sulphuric acid solution was used for regenerated chemical suppression. The optimized chromatographic parameters are presented in Table 1.

Table 1. Optimized parameters for ion chromatographic measurement.

| Parameter          | Value                                      |
|--------------------|--------------------------------------------|
| Column             | Metrosep A Supp 7–250/4.0                  |
| Precolumn          | Metrosep RP 2 Guard/3.5                    |
| Eluent             | Na2CO3 (3.6mmol/L)                         |
| Eluent flow rate   | 0.7 mL/min                                 |
| Elution mode       | Isocratic                                  |
| Sample volume      | 20 µL                                      |
| Column temperature | 45 °C                                      |
| Detection          | Suppressed conductivity                    |
| Full-scale range   | 0 – 15000 µS/cm single range              |
| Suppressor         | Metrohm Suppressor Module (MSM)           |
| CO2 Suppressor     | Metrohm CO2 Suppressor (MCS)              |
| Determination mode | Peak area                                  |

Eluent solution was prepared by sodium carbonate superpose Na2CO3(CertiPUR®, Merck, Darmstadt, Germany). Multi ion standard solutions of fluoride, chloride, nitrate and sulphate were prepared by appropriate dilution of their anion standard stock solution (Fluka 1000 mg/L for IC, TraceCERT®, Sigma–Aldrich, Buchs, Switzerland). All solutions were prepared gravimetrically in
Milli-Q water, (Mill-Q Direct 8, Merck Millipore; resistivity >18.2 MΩcm, equipped with a Millipack 0.22µm filter). Certified Reference Material for soft drinking water UK- Anions № ERM® - CA016a, LGC, Teddington, Middlesex was used to confirm the trueness of the method. Sulphuric acid (96% Suprapur®, Merck KGaA, Darmstadt, Germany) was used in conductivity suppression regeneration system after appropriate dilution. Before measurements the samples were filtered by MF-Millipore™ membrane filter with pore size of 0.45 µm (Merck KGaA, Darmstadt, Germany).

3. Results and discussion

The studied anions F-, Cl-, NO₃⁻ and SO₄²⁻ are separated by 3.6 mmol/L Na₂CO₃ eluent under the optimized parameters (Table 1). Examples of the obtained chromatograms are presented on Figure 1. All anions were separated completely in a run with total analysis time of 30 min.

The retention time of the studied anions and their relative standard deviation obtained by spiking untreated water sample are presented in Table 2. The standard deviation is estimated for 5 injections at 5 different concentration levels varying as follows: 0.02-0.18 mg/L F-, 1-5 mg/L NO₃⁻, 4-20 mg/L SO₄²⁻ and 1.5-10 mg/L Cl-. Therelative standard deviation was less than 1 % and it proved that the increased concentration do not affect the peak position. Additionally, the retention time of each anion in the standards and in the sample were compared. The relative difference was not exceeding 2 % and thus fulfilled the tolerance criterion established by the European Commission Decision 2002/657/EC [6]. According to the cited document the retention time of analyte in standard solution must correspond to that in the sample within tolerance of 2.5% [6].

| Anion | Retention time (min) | s (min) | s (%) |
|-------|----------------------|--------|------|
| F⁻    | 6.11                 | 0.0045 | 0.07 |
| Cl⁻   | 9.87                 | 0.0100 | 0.10 |
| NO₃⁻  | 17.92                | 0.0114 | 0.06 |
| SO₄²⁻ | 28.64                | 0.0460 | 0.16 |

3.1. Linearity

Calibration curves were obtained in the concentration interval 0.4 - 12.0 mg/L for F-, Cl-, NO₃⁻ and 0.4 - 10 mg/L for SO₄²⁻. The measurements were run in quadruplicate for 7 concentration level of multi-ion standards and the means were used for calibration curves. The regression analysis and “lack-
of-fit test” were used according to the IUPAC validation guidelines [15]. The results are presented in Table 3. It should be pointed out that the method showed wider working range if we consider that good linearity is observed in the concentration range 0.1-1.0 mg/L of each studied anion (N=7, r=0.9997) and 10-25 mg/L (N=7, r=0.9996). However, in the concentration range 10-25 mg/L a memory effect was observed which needs long washing periods for the signal to reach base line. Hence, a dilution of more concentrated samples was recommended. The quality requirement for the acceptance of the calibration function was: correlation coefficient $r^2 \geq 0.995$ [18].

The linearity in all 3 studied concentration regions were additionally proved by lack-of-fit test. All standard curves fulfilled the criterion $F_{\text{tabulated}} > F_{\text{calculated}}$ and the obtained models were considered as “fit to data” [15].

Table 3. Calibration curves for F$^-$, Cl$^-$, NO$_3^-$ at 0.4-12 mg/L (N=7, P=95%; $F_{\text{tab}} = 2.9582$) and SO$_4^{2-}$ at 0.4-10 mg/L, (N=6, P=95%; $F_{\text{tab}} = 3.2592$).

| Anion   | Slope   | Slope stdev | Intercept | Intercept stdev | $r^2$ | $F_{\text{calc}}$ |
|---------|---------|-------------|-----------|-----------------|-------|-----------------|
| F$^-$   | 0.4579  | 0.0056      | 0.0729    | 0.0372          | 0.9992| 0.1234          |
| Cl$^-$  | 0.3071  | 0.0004      | 0.0303    | 0.0025          | 0.9999| 1.8520          |
| NO$_3^-$| 0.1711  | 0.0004      | 0.0062    | 0.0022          | 0.9999| 0.8158          |
| SO$_4^{2-}$ | 0.2398 | 0.0003      | 0.0039    | 0.0013          | 0.9999| 1.6806          |

3.2. Limits of detection (LOD) and limits of quantification (LOQ)

LOD and LOQ were estimated using two approaches: (1) calculation of LOD and LOQ based on 3.3 or 10 times, respectively, standard deviation of the lower concentration obtained from standard curve. The standard deviation (SD) was calculated in the repeatability conditions [13]. Three spiked reagent blanks at the low concentration levels were prepared for this study, each sample was analyzed in triplicate and the means are used to calculate standard deviation. The standard deviation (in mg/L) was divided by the square root of the number of measurements; (2) calculation based on 3.3 or 10 times the standard deviation of residuals divided by the slope of standard curve [16,17].

In both cases the calibration curves were prepared in the µg/L region: 0.038-0.057 mg/L F$^-$ (N=9, r=0.9962); 0.019-0.057 mg/L Cl$^-$(N=5, r=0.997); 0.0087-0.077 mg/L NO$_3^-$(N=5, r=0.997); 0.0093-0.047 mg/L SO$_4^{2-}$(N=5, r=0.991). The obtained values of LOD and LOQ differ according depending on the calculation strategy. The obtained results are presented in Table 4. For comparison some literature data are included in the Table. As can be seen the ion chromatography with chemical and carbon dioxide suppression offers low detection limits down to µg/L level. The achieved LOD and LOQ are lower than LOD for water samples reported in the literature [11-13, 18]. Zivojnovic et al. [7] reported remarkably low LOD for fluoride and chloride 0.077 and 0.082 µg/L, respectively, in ultrapure water but using1000µL sample volume. Hence, the method LOD and LOQ could be additionally lowered if higher injection volume was used.

Table 4. Limits of detection and limits of quantification of IC method.

| Anion   | LOD (mg/L) | LOQ (mg/L) | LOD (mg/L) | LOQ (mg/L) | LOD (mg/L) | LOQ (mg/L) | LOD (mg/L) | LOQ (mg/L) | LOD (mg/L) | LOQ (mg/L) | LOD (mg/L) | LOQ (mg/L) |
|---------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| F$^-$   | 0.0023     | 0.0007     | 0.0043     | 0.013      | 0.080      | 0.050      | 0.001      | 0.004      | 0.02       | -          | -          |
| Cl$^-$  | 0.0042     | 0.013      | 0.0032     | 0.010      | 0.160      | 0.068      | 0.010      | 0.04       | 0.11       | 0.14       | 0.47       |
| NO$_3^-$| 0.0161     | 0.049      | 0.0062     | 0.019      | 0.180      | 0.015      | 0.006      | 0.02       | 0.04       | 0.22       | 0.72       |
| SO$_4^{2-}$ | 0.0039 | 0.012      | 0.0054     | 0.016      | 0.200      | 0.023      | 0.008      | 0.03       | 0.09       | 0.13       | 0.45       |

*the results are calculated by multiplying the standard deviation of the lower concentration by 3 or 10.*
3.3. Repeatability and intermediate precision

The repeatability and intermediate precision tests were made according to the recommendation of Eurachem Guide 2014 [14]. The repeatability test was made for a short period, by the same user with the same instrument. Measurements were made at 5 concentration levels in 5 replicates. The intermediate precision was estimated by analyzing 5 replicates in the same concentration range for the period of 4 months, each measurement was made in 5 replicates. Relative standard deviations are presented in Table 5.

|             | Repeatability (RSD %) | Intermediate precision (RSD %) |
|-------------|-----------------------|--------------------------------|
| Concentration (mg/L) | 0.2 | 0.8 | 1.0 | 8.0 | 0.2 | 0.8 | 1.0 | 8.0 |
| F^+         | 0.83 | 0.17 | 0.48 | 0.21 | 1.81 | 0.35 | 1.58 | 0.15 |
| Cl^-        | 0.79 | 0.50 | 0.43 | 0.11 | 1.68 | 0.58 | 0.51 | 0.12 |
| NO_3^-      | 1.45 | 0.90 | 0.30 | 0.20 | 2.83 | 1.66 | 0.44 | 0.22 |
| SO_4^{2-}   | 1.10 | 0.33 | 0.44 | 0.17 | 2.35 | 0.49 | 0.60 | 0.17 |

3.4. Trueness

The trueness of the method was estimated by analysis of certified reference material for soft drinking water (UK-Anions № ERM® - CA016a) in four replicates with external calibration. Zeta-score was used as an estimate of the method trueness and performance indicator. Zeta-score was calculated as:

\[ \zeta = \frac{X_{lab} - X_{ref}}{\sqrt{u_{lab}^2 + u_{ref}^2}} \]  

where \( X_{lab} \) is the mean value obtained in the laboratory; \( X_{ref} \) is the actual value of CRM; \( u_{lab} \) is the uncertainty of the result obtained by the laboratory; \( u_{ref} \) uncertainty of the reference value from CRM. The obtained zeta-score was less than 2.0 and the method performance was considered as “satisfactory” [18, 19]. The recovery of analytes in CRM was below the acceptance limits recommended in the Council Directive 98/83/EC [5]. The results are presented in Table 6.

|             | Certified Value (mg/L) | Uncertainty (mg/L) \( k=2 \) | Measured Value (mg/L) | Uncertainty (mg/L) \( k=2 \) | Recovery (%) | Trueness |
|-------------|------------------------|-------------------------------|------------------------|-------------------------------|--------------|----------|
| F^+         | 1.5                    | ± 0.1                         | 1.52                   | ± 0.16                        | 101.0        | 0.08     |
| Cl^-        | 250                    | ± 7                           | 247.3                  | ± 3.2                         | 98.9         | 0.36     |
| NO_3^-      | 48                     | ± 3                           | 49.0                   | ± 4.4                         | 102.1        | 0.20     |
| SO_4^{2-}   | 254                    | ± 10                          | 248.6                  | ± 2.5                         | 97.9         | 0.52     |

Additionally, a recovery of spiked treated and untreated drinking water samples was estimated at 3 concentration levels of standard additions. The results are presented in Table 7. As an acceptance criterion, a tolerance of ±10% of actual value was implemented [5, 18]. To study matrix influence multiple standard addition method was used. The slope of standard and multiple addition curves in treated and untreated water samples coincided well and the ratio of the slopes was in the range 99-102

Table 6. Recovery test results from CRM soft drinking water.
%. As can be seen from the results the validated method was not influenced by the matrix composition.

Table 7. Recovery test results from spiked water samples.

| Concentration of spike (mg/L) | Recovery in untreated drinking water (%) | Recovery in drinking water after chlorination (%) |
|-------------------------------|------------------------------------------|-------------------------------------------------|
| F⁻                           | 0.02                                     | 105.5                                           | 98.7                                           |
|                              | 0.04                                     | 98.3                                            | 99.8                                           |
|                              | 0.08                                     | 104.1                                           | 99.3                                           |
|                              | 1.25                                     | 111.5                                           | 114.9                                          |
| Cl⁻                           | 2.5                                      | 105.8                                           | 108.0                                          |
|                              | 5.0                                      | 103.6                                           | 104.4                                          |
|                              | 0.75                                     | 112.1                                           | 113.7                                          |
| NO₃⁻                         | 1.5                                      | 104.9                                           | 106.7                                          |
|                              | 3.0                                      | 103.3                                           | 104.4                                          |
|                              | 3.0                                      | 109.0                                           | 110.2                                          |
| SO₄²⁻                         | 6.0                                      | 104.6                                           | 105.6                                          |
|                              | 12.0                                     | 102.8                                           | 103.7                                          |

3.5. Uncertainty

The uncertainty was estimated according to the procedure recommended by EURACHEM/CITAC Guide [20]. The expanded uncertainty calculated using a coverage factor of 2 (k=2) and 95% confidence level, was presented with: U = 2u_c, where u_c was a combined uncertainty. Three sources of uncertainty were included in the uncertainty budget. Linear least squares fitting procedure was applied to estimate the uncertainty of concentration of each of studied anions from the standard curve [20]. Standard stock solution was used in the estimation. The results are presented in Table 8.

Table 8. Uncertainty determination of F⁻, Cl⁻, NO₃⁻ and SO₄²⁻ in CRM-No ERM - CA016a.

| Anion | Sources of uncertainty | Value x | Standard Uncertainty u(x) | Relative Standard Uncertainty u(x)/x | Measured Value (mg/L) | Combined Uncertainty u_c | Expanded Uncertainty U |
|-------|------------------------|---------|----------------------------|--------------------------------------|-----------------------|--------------------------|-------------------------|
| F⁻    | Linear least squares calibration | 0.075 mg/L  | 0.004 mg/L                | 0.0533                               |                       |                          |                         |
|       | Balance (Dilution factor) | 2.040 6 g | 0.0004 g                  | 0.0002                               | 1.52                  | 0.0809 mg/L             | 0.16 mg/L               |
|       | Concentration of the standard stock solution | 1001 mg/L | 4.0 mg/L                  | 0.002                                |                       |                          |                         |
| Cl⁻   | Linear least squares calibration | 5.016 mg/L  | 0.031 mg/L                | 0.0062                               | 1.618 mg/L            | 3.24 mg/L               |                         |
Table 8. Uncertainty determination of F⁻, Cl⁻, NO₃⁻ and SO₄²⁻ in CRM-No ERM-CA016a.

| Anion      | Sources of uncertainty | Value x | Standard Uncertainty u(x) | Relative Uncertainty u(x)/x | Measured Value (mg/L) | Combined Uncertainty uc | Expanded Uncertainty U |
|------------|------------------------|---------|---------------------------|-----------------------------|----------------------|-------------------------|------------------------|
|            | Balance (Dilution factor) | 0.518   | 0.0004 g                  | 0.0008                      | 247.3                |                         |                        |
|            | Concentration of the standard stock solution | 1001 mg/L | 4.0 mg/L | 0.002 |
| NO₃⁻       | Linear least squares calibration | 0.994   | 0.045 mg/L                | 0.0453                      | 2.222 mg/L | 4.44 mg/L |
|            | Balance (Dilution factor) | 0.520   | 0.0004 g                  | 0.0008                      | 49.0 | 49.0 |
|            | Concentration of the standard stock solution | 1001 mg/L | 4.0 mg/L | 0.002 |
| SO₄²⁻      | Linear least squares calibration | 5.041   | 0.023 mg/L                | 0.0046                      | 1.253 mg/L | 2.51 mg/L |
|            | Balance (Dilution factor) | 0.518   | 0.0004 g                  | 0.0008                      | 248.6 | 248.6 |
|            | Concentration of the standard stock solution | 1001 mg/L | 4.0 mg/L | 0.002 |

3.6. Drinking water analysis
The validated method was applied for analysis of treated and untreated drinking water. The results are presented in Table 9 and a chromatogram on Figure 2. The obtained concentrations are far below the recommended chemical and indicator values in drinking water [5].

Table 9. Results from analysis of drinking water by validated IC method (n=3, P=95%).

| Concentration (mg/L) | Recommended values [5] | Drinking untreated water | Drinking water after chlorination |
|----------------------|------------------------|-------------------------|---------------------------------|
| F⁻                   | 1.5                    | 0.037 ± 0.004           | 0.037 ± 0.004                   |
| Cl⁻                  | 250                    | 2.33 ± 0.04             | 2.80 ± 0.04                     |
| NO₃⁻                 | 50                     | 1.57 ± 0.08             | 1.53 ± 0.08                     |
| SO₄²⁻                | 250                    | 6.05 ± 0.03             | 6.06 ± 0.03                     |
4. Conclusions

An ion chromatographic method was validated for determination of fluoride, chloride, nitrate and sulphate ions in drinking water before and after chlorination. The method characteristics were evaluated, the trueness was confirmed by analyzing certified reference material and the uncertainty budget was built up. The obtained values for method characteristics satisfied the requirements of water authorities. The validated method was accepted in the routine work of the Department of Chemical Measurements and Ionizing Radiation of the Bulgarian Institute of Metrology, Bulgaria.

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