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IMPROVEMENT IN THE DETERMINATION OF TRACES OF NITRATE AND NITRITE IN NATURAL MINERAL WATERS BY ION CHROMATOGRAPHY

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This preliminary study refers at an improvement in the determination of the nitrate and nitrite anions at trace level in mineral waters with different compositions, in an incipient correlation with their geological provenience. A suppressed elution system was used and the reliability of the optimized analytical method was checked and confirmed in terms of accuracy, precision and total uncertainty. The method can be successfully applied on potable and mineral water samples for the anions analysis in different ranges on concentrations: 100-10000 µg L^{-1} *for* Cl^- *and* SO_4^{2-} *and 1-100 µg* L^{-1} *for* F^-, NO_2^-, Br^- , NO_3^- *and* PO_4^{3-}.

Keywords: ion chromatography, mineral waters, nitrate, nitrite

1. Introduction

Natural mineral water, microbiologically pure originates in underground water table from spring tapped at one or more natural or bore exits, [1] and it has distinguishing and constant chemical composition. The list recognized by the member States of the European Union includes more than 2000 natural mineral water sources. [2] Legal limits are fixed for elemental composition and indicated in. [3]

In the last years several studies have focused on the elemental composition of bottled mineral water by determining it or derived from the compositional information given on bottle's label.[4]

From a hydrogeological point of view the natural mineral water resources are found in carbonate aquifers in the mountain areas, where dolomite occurs with occasional intercalations of volcanic deposits. Among the parameters which characterize the composition of the natural mineral waters, the total mineralization is one of the most important. It is reflected by the total solid residue and the HCO_3^- concentration. Based on this parameter, the natural mineral waters can be

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classified into: very low, low, intermediate and high mineral concentrations [5], the low mineral concentration being predominant in the consumer preference. This is an important parameter which affects the chromatographic analysis of anions in mineral waters, another one being the concentration of dissolved carbon dioxide, which must be removed before analysis.

Nitrogen is one of the most commonly occurring elements in nature which forms inorganic ionic species, mainly nitrate, nitrite and ammonium ions. Sources of nitrogen in mineral waters vary, ranging from fertilizers to sewage treatment plants or septic systems to ground water, air and rainfall. NH_4^+ is released from decomposing organic material under anoxic conditions. If NH_4^+ comes into contact with oxygen, it is converted to NO_2^- which is oxidized to NO_3^-. An important source of nitrates in the organism is drinking water, an estimated daily dose of nitrates reaching approximately 100 mg, of which only 10 % comes from water.[6] The admissible concentration of nitrate in drinking water in the majority of countries is 50 mg L^{-1}. There are some European countries, such as Austria, Germany and Italy where the maximum allowable limit of nitrate (as nitrate) in waters suitable for infant food is fixed at 10 mg L^{-1}. [7] Nitrates do not have direct carcinogenic effects on humans, but carcinogenic tumors are related to the formation of N-nitroso compounds and to methemoglobinemia in infants below 3 months old related to the transformation of nitrates in nitrites in humans.[8] Nitrates and nitrites on baby food are strictly required,[9, 10] fast, sensitive and reliable methods of quantification being necessary. Nitrate ions are determined in drinking water, in underground and surface waters by several chemical methods of analysis like: colorimetric [11], potentiometric [12], spectrophotometric (UV/VIS, IR)[13], and chromatographic methods.[14,15]

Ion chromatography is one of the most used methods for the determination of anions and cations, because it offers the possibility of the ions determination in a short time, in small volumes of sample, with good sensitivity and reproducibility. The problems related to an efficient separation find solutions through changing the composition of the eluent, the flow of the eluent, types of columns and types of detectors. The use of a classical conductivity detector, a mixture of Na_2CO_3 and $NaHCO_3$, as an eluent and a flow of the mobile phase of 0.7 mL/min offer the optimal conditions of operation. The US Environmental Protection Agency (EPA) recommends the methods of ion chromatography for the determination of anions. [16] All the analysis and assessments were performed in accordance with the directives on natural mineral waters 2009/54/EC and 2003/40/EC.

The aim of this study was to improve the separation of the of the anions NO_3^- and NO_2^- at trace level in mineral waters with different compositions using the IC standardized method SR EN ISO 10304-1:2009. A suppressed elution system was used and the reliability of the optimized analytical method was checked and confirmed in terms of accuracy, precision and total uncertainty. The second aim was to understand at a preliminary level the ionic composition of natural mineral waters in connection with some geological features. The authors proposed in the past several potentiometric method for the analysis of inorganic species in aqueous solutions, the results being compared with those obtained by ion chromatography. [[17],[18]]

2. Experimental part

Instrumentation

Ion chromatograph: 850 Professional IC AnCat-MCS using Metrohm intelligent Partial Poop (MiPT) technique with conductivity detector; Anion separator column-Metrosep C4-250/4.0; Anion guard column-Metrosep RP 2 Guard/3.5; Anion suppressor device SMARTPACK anion self – regenerating suppressor with external ultrapure water source; Detector: 850 Professional conductivity detector (0-15000 µS/cm); Professional drift < 0.2 nS cm/h; Professional sample processor; Metrohm patented Dosino technology

Reagents

Reagent water: ultrapure water (UPW) from Millipore Direct Q3 with UV lamp: resistivity > 18.2 MΩ cm (25^{o}C); flow = 0.5 L/min; total organic carbon TOC < 5 ppb; particles not larger than 0.22 µm.
Eluent solution: 3.2 mM Na_2CO_3 solution and 1.0 mM $NaHCO_{3.}$
Supressor regeneration and rinsing solution: Metrohm suppressor module(MSM) for chemical suppression during anion analysis using 100 mM H_2SO_4 in ultrapure water (UPW) resistivity > 18.2 MΩ cm (25 ^{o}C) and Metrohm CO_2 suppressor (MCS).

Stock standard anion solutions: 1000 mg L^{-1} are purchased as certified solutions or prepared from ACS reagent grade, potassium or sodium salts.

Methods: IC standardized method SR EN ISO 10304-1: 2009 for the determination of the anions in water.

Analysis

All bottles were soaked in Milli-Q water, placed in an ultrasonic bath and rinsed three times with Milli-Q water before use. An aliquot (20 mL) of the samples was transferred into propylene vials and degassed for 30 minutes before analysis. The instrument was calibrated weekly against external standard solution a regression fit value of 0.995 being required for each element. Preparation and analysis of samples, standards and blanks were carried out in triplicate. The method detection limit (MDL) for the anions was calculated three times the standard deviation of the signal of the blank sample analyzed 10 times.[19] The anions fluoride, chloride, nitrite, nitrate and sulfate were analyzed by ion chromatography (IC).

Mineral water samples sparkled and flat were purchased at the local supermarket. The flat mineral water samples were used without previous treatment, being directly transferred from the original bottles to the autosampler cups. The sparkled mineral water samples were analyzed after degassing by ultrasonic treatment for 30 minutes for the removal of CO_2.

Validation procedure for the optimized IC method of analysis

The validation procedure used certified reference materials (CRM), inter-comparison samples and routine samples doped with a known concentration of the element, or of the compound of interest using a CRM. The samples used were mineral bottled water with different matrixes. The validation will be applicable to a working range of concentrations between 1 and 100 µg L^{-1} for fluoride, nitrite, bromide, nitrate and phosphate and between 100 and 10 000 µg L^{-1} for chloride and sulfate anions, in respect with the usual matrix of natural mineral waters. Three different levels of concentrations were selected in order to cover the lower standard range and the higher one established in mineral waters legislation. The determined parameters of the method were: range of measurement, calibration-linearity, method detection level, the level of quantitation, method precision and accuracy and measurement uncertainty.

3. Results and discussion

In our study a suppressed elution system was tested for the determination of anions. The suppressed methods provide good sensitivity and improved detection limits in comparison with the non-suppressed ones. For improving the detection limit of NO_3^- anion from 0.1 mg L^{-1} to 0.01 mg L^{-1} and the separation between nitrite and nitrate, the Metrosep A Supp 5-250/4.0 column was used. The mixture of eluents 3.2 mM Na_2CO_3 and 1 mM $NaHCO_3$ at a flow of 0.7 mL min^{-1} achieved sufficient resolution for a recording time of 20 minutes. An isocratic elution of all the mentioned anions was possible at a pressure of 7.95 MPa. The Metrosep column (250 mm instead of 150 mm) improved the measurements.

The Metrohm suppressor module (MSM) used for the chemical suppression during anion analysis was pressure stable, robust and resistant. It consists of three suppressor units in total, regenerated with 0.1 M sulfuric acid and rinsed with ultrapure water. Using a carbonate eluent, the following reaction occurs in the MSM: $R\text{-}SO_3H + NaHCO_3/Na_2CO_3 = R\text{-}SO_3Na + H_2O + CO_2$

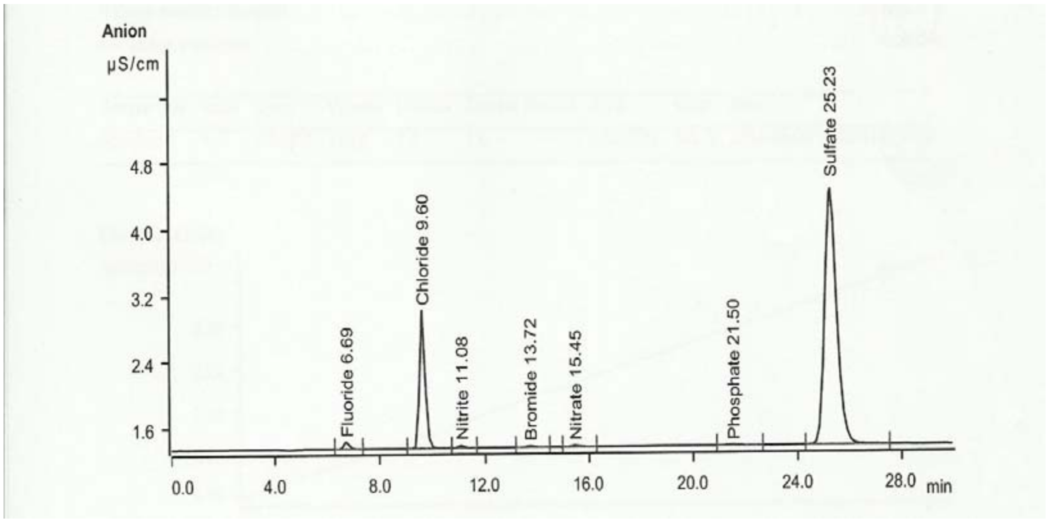

Fig.1. Chromatogram of a standard containing 0.01 mg L^{-1} of the anions: fluoride, chloride, nitrite, nitrate, phosphate and sulfate obtained on Metrosep A Supp 5-250/4.0 column, eluent: 3.2 mM Na_2CO_3 and 1 mM $NaHCO_3$, flow - 0.7 mL min^{-1}

It was observed that the total time needed for the separation is approximately 18 minutes, using a Metrosep A Supp 5-150/4.0 column. For a length of the column of 250 mm, it can be observed (from Fig. 1) that the total time needed for the separation is approximately 26 minutes, but the detection limit of the method and the differences between the retention times of nitrite and nitrate are improved.

Table 1

Equations of the calibration curves, relative standard deviations and correlation coefficients for the anions: fluoride, chloride, nitrite, nitrate, phosphate and sulfate. Analytical conditions: Metrosep A Supp 5-250/4.0 column, eluent: 3.2 mM Na_2CO_3 and 1 mM $NaHCO_3$, flow - 0.7 mL min^{-1}

Anion	Retention time, min	Function, on Metrosep A Supp 5-250/4.0 column	Correlation coefficient	RSD, %
fluoride	6.693	$y = -2.09371x10^{-3}+0.0164607x+8.30169x10^{-5}x^2$	0.999985	0.4698
chloride	9.597	$y = -0.110065+0.0131663x+4.39382x^2$	0.999998	0.3062
nitrite	11.082	$y =-7.05699x10^{-5}+6.22572x10^{3}x+2.29263x10^{-5}x^2$	0.999995	0.3371
bromide	13.722	$y = 1.48001x10^{-4}+4.04074x10^{-3}x+7.53939x10^{-6}x^2$	0.999998	0.2333
nitrate	15.452	$y = 1.85857x10^{-4}+5.01555x10^{-3}x+1.15782x10^{-6}x^2$	0.999999	0.1650
phosphate	21.495	$y =-4.77868x10^{-5}+2.68596x10^{-3}x+4.73948x10^{-6}x^2$	0.999997	0.3280
sulfate	25.227	$y = -0.277475+9.93729x10^{-3}x+1.73806x10^{-8}x^2$	0.999996	0.3078

As it can be observed from Table 1, the values of relative standard deviation (RSD %) are larger than 0.3 % for fluoride, chloride, nitrate, phosphate and sulfate. It can also be observed that the values of RSD% are bigger than 0.3 % for fluoride and nitrite, the length of the column having an influence over the value of this factor for higher concentrations. It has to be mentioned that in our samples there are very low concentrations of NO_3^- and NO_2^- next to high concentrations of SO_4^{2-} and Cl^- anions.

The dissolved carbon dioxide was removed by degassing the samples for 30 minutes before analysis using an ultrasonic treatment. In this way, the penetration effect of dissolved carbon dioxide to the resin stationary phase was minimized. Another problem caused by the presence of carbon dioxide in the samples was the low conductometric sensitivity due to the low limiting equivalent ionic conductance of dissolved carbon dioxide. Several methods to improve the detector response were reported such as: the two-dimensional conductivity detection system with ion-exchange enhancement by Dasgupta[20]or indirect UV detection by Voloshik et al.[21] Tanaka[22] proposed a method in which the dissolved carbon dioxide was converted into a highly ionized compound (KOH) by ion-exchange resin columns. In our method, the Metrohm CO_2 suppressor (MCS) removes CO_2 from the eluent flow. This reduces the background conductivity, improves the detection sensitivity and minimizes the injection and the carbonate peak. The MCS was used for removing CO_2 from the eluent flow before detection. CO_2 can reach the eluent flow through the sample itself, or it can arise from through the suppression reaction in the MSM. The CO_2 peak is effectively minimized through the CO_2 suppression, based on the gas permeability of the membrane inside the degassing cell of the suppressor, where the CO_2 is removed by an adsorptive cartridge. The pressure and the concentration difference in the degassing cell in comparison with the inside of the capillary causes the CO_2 to diffuse out of the eluent flow.

The detection limit of our method was determined for each ion based on the standard deviations of the replicate measurements, as well as the limits of quantitation. It was observed that by increasing the length of the column from 150 mm to 250 mm the sensitivity of the method did not decrease, but the separation of the peaks was improved. The calibration curve in suppressed ion chromatography has an inherent deviation from the linearity. This can be due to the following reasons: the calibration curve is assumed to have a linear relationship between area (conductivity) and concentration. The measured conductivity is a function of the specific conductivities of the respective ions. These specific conductivities are themselves a function of the concentration (the Kohlrausch's law). Therefore, there is no pure linear relationship between measured area and the ion concentration. After chemical suppression there is a certain amount of the remaining dissolved CO_2 in the eluent. During the peak the dissociation equilibrium is changed slightly. The baseline divides from a straight line which cannot be recognized by the integrating software. These two points yield in slightly non-linear calibration curves in suppressed ion chromatography. Quadratic regression (curve fit) in many cases improve the quality of the results.

Table 2

Parameters of the validation method for nitrate and nitrite anions

Ion	Parameter	Low level, 1 µg L^{-1}	Medium level, 10 µg L^{-1}	High level, 100 µg L^{-1}
NO_2^-	Repeatability	2.00	1.92	2.44
	Recovery, %	109.8	95.60	91.40
	Limit of detection, µg L^{-1}	1.06	0.37	3.54
	Limit of quantification, µg L^{-1}	1.22	1.24	11.8
	RSD % of the calibration curve	0.68	0.65	0.68
	Total uncertainty %		15	
NO_3^-	Repeatability	2.67	3.89	0.89
	Recovery, %	115.9	99.84	84.13
	Limit of detection, µg L^{-1}	1.09	0.72	1.92
	Limit of quantification, µg L^{-1}	1.32	2.40	6.46
	RSD % of the calibration curve	0.82	0.80	0.80
	Total uncertainty %		10	

The accuracy and the precision over three ranges of concentrations were estimated and there are presented in Table 2. The precision of the method was determined in terms of repeatability. Repeatability data were obtained by replicate analysis of ten volumes of a standard solution at three concentration levels in the same day. The improved limit of detection for NO_2^- and NO_3^- anion can be correlated to the length of the column and to the eluent composition. In the case of sparkling mineral waters, the degasing treatment prior to the measurement was very important. Total uncertainty combines the uncertainty of the concentration of the standard solution, the uncertainty of the calibration curve and the uncertainty of the conductivity measurement according to the detector specifications. A total uncertainty of 15 % is an accepted value for lower values of the concentration. Gradient elution was found to be inadequate as the suppressor was not able to lower the baseline conductivity in our conditions. Contamination caused by the dissolution of several anions from the laboratory has already been studied.[23] The contamination was avoided by preparing all the samples at the hood and introducing them in capped vials. All the glasses were carefully rinsed with UPW water, generally sub-detection limit contamination being obtained.

All the investigated mineral waters are classified as low and medium mineral content waters, their water chemistry reflecting the geological features of the regions of provenience in an important manner. Types of natural mineral waters, classificatory diagrams, well-identified groups related to their geographic distribution of major anions and cations are presented in. [24] E Dinelli in [25] present a summary classification in seven groups: Ca-HCO_3 group, Ca-Mg-HCO_3 group, Ca-Na-Mg-HCO_3, Na-Cl-HCO_3, Na-Ca-HCO_3-Cl, Ca-Na-Mg-HCO_3-Cl-SO_4 type and Ca-Mg-SO_4.

Romanian natural mineral waters are mostly Ca-HCO_3 type, with low electric conductivity, deriving from sedimentary rocks. Such compositions of water can be found in the Southern part of Romania, in the Eastern Carpathians where carbonate aquifers are common, the dissolution of calcite being the dominant reaction.

Table 3

Comparative data for the determination of anions and cations in some bottled Romanian natural mineral waters

Sample	HCO_3^- mg L^{-1}	Total mineralization, mg L^{-1}	NO_3^-* mg L^{-1}	NO_2^- mg L^{-1}	Cl^- mg L^{-1}	SO_4^{2-} mg L^{-1}	Na mg L^{-1}	Ca mg L^{-1}	Mg mg L^{-1}
Brand 1	219.6	278.8	0.80	< 0.005	0.45	6.76	0.75	48.33	15.20
Brand 2	1281.2	1684	0.034	<0.01	4.9	19.45	4.33	290	82.5
Brand 3			2.3	< 0.005	0.15	7.66	0.98	66.86	2.88
Brand 4	333.4	462.7	4.11		1.08	10.38	2.85	56.73	32.5
Brand 5	72.0		1.80		0.35	2.28	1.2	15.51	4.7
Brand 6	164.7	247.35	2.94		2.96	20.2	0.62	61.53	3.06
Brand 7	48.8	102.4	5.01	< 0.005	4.75	5.80	2.84	9.85	2.55
Brand 8	312	485	2.07	< 0.005	0.80	13.39	1.7	94.5	7.8

From the hydrogeological point of view, the natural mineral water resources are found in carbonate aquifers where dolomite occurs with intercalations of volcanic deposits. The magmatic and metamorphic rocks are sources of mineral waters, because they have low permeability and they can originate springs. Based on the rock types at the aquifer locations, the natural mineral bottled waters are grouped as: plutonic, volcanic, metamorphic, sedimentary, volcanic-metamorphic and volcanic-sedimentary. The first group of natural mineral waters is associated to the mixed volcanic-sedimentary, characterized by high values for elements as Ca^{2+} and HCO_3^-. Few of these waters are naturally sparkling, HCO_3^- rich and with low pH, one being presented in Table 3.

Another group of mineral natural waters presents a slightly higher Mg percentage (Ca-Mg-HCO_3 type), the interaction with mg bearing carbonates being the dominant reaction characterizing the waters. A water type (sometimes naturally sparkling) with Ca-Na-Mg-HCO_3 correlate with volcanic rocks present in the central part of Romania.Ca-Mg-S0_4 waters show interactions with sulfide bearing aquifers, these waters having their origins in sedimentary and metamorphic rocks. Ca-Na-Mg-HCO_3-Cl-SO4 waters characterized by low electric conductivity include waters flowing through metamorphic rocks, with a possible marine water contribution. Tests of the validated method were carried out on several brands of mineral waters using a multi-anion standard solution at different concentration levels between the concentrations of Cl^-, NO_3^- and SO_4^{2-} in the same sample. The results obtained are in good agreement with data found on the labels of the bottles. The method can be successfully applied on potable and mineral water samples for the anions analysis in different ranges on concentrations: 100-10000 µg L^{-1} for Cl^- and SO_4^{2-} and 1-100 µg L^{-1} for F^-, NO_2^-, Br^- , NO_3^- and PO_4^{3-}. Very low concentrations of anions can be determined with the optimized method presented in the manuscript. These low concentrations of some anionic species can be explained by the dolomite aquifer of some mountains area, where CO_2 of post-volcanic origin favors the solubility of the dolomite rocks and it modifies the pressure of the mineral water source. Another explanation can be the lack of sources of pollution with nitrates in the region.

4. Conclusions

This preliminary study tries to establish connections between the bedrock lithology on the composition of some bottled mineral waters in Romania. Standard methods using ion chromatography were applied for inorganic anions analysis in mineral natural waters. The quantification limits for NO_3^- and NO_2^- anions were lowered by a factor of 10 using suppression IC mode, a longer Metrosep column and a degassing treatment before analysis. It can be concluded that the limits of quantification for certain ions can be lowered by using larger injection volumes and reduced contamination, the external one being avoided by using MQ water. However further work is necessary for developing the validated improved method of analysis for traces of NO_3^- and NO_2^- in the presence of high levels of Cl^- and SO_4^{2-} in highly carbonated samples.

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