Standard Operating Procedure for the analysis of major ions in hydrothermal fluids by Ion Chromatography

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ABSTRACT

This SOP describes an ion chromatography (IC) procedure for the major cations and anions in hydrothermal fluids. Hydrothermal fluids are aqueous solutions with a wide range of temperature, salinity, pH and ion species that can be used by microbial metabolism as electron donors and electron acceptors. Due to the high variability of the environmental physical-chemical parameters in these samples, we have developed this protocol taking into account the special features of the matrices analyzed. An Eco IC Metrohm system equipped with a conductivity detector was used. Calibration curves are linear in the 0.1 to 10 mg/L concentration range of cations Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, NH_4^{+} and anions Cl^{-}, Br^{-}, NO_3^{-}, NO_2^{-}, SO_4^{2-} , HPO_4^{2-} .

Keywords: Major Ions, hydrothermal fluids, ion chromatography, Standard Operating Procedure, Aqueous geochemistry

SCOPE AND APPLICATION

This method was developed in the Giovannelli Lab at the Department of Biology of the University of Naples Federico II for the sequential measurement of major cations (Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, NH_4^{+}) and anions (Cl^{-}, Br^{-}, NO_3^{-}, NO_2^{-}, SO_4^{2-} e HPO_4^{2-}) in hydrothermal fluids using ion chromatography. This analysis can provide different information, in fact it is well known that major elements are required for microbes nutrition and metabolism, in particular for growth, oxidative metabolism and active transport. Some of these species can be used by microorganisms for redox reactions (Moore et al. 2017), while others can inform on the

nature and origins of fluids under investigations (Giggenbach 1988). Microbial diversity is primarily driven by the physical-chemical conditions, such as temperature, pH, redox state, and availability of diverse electron donors and electron acceptors (Merino et al. 2019; Delgado-Baquerizo et al. 2018). For example, chemolithotrophic bacteria can obtain the energy required for their growth from oxidation of inorganic compounds like nitrate, sulfide or ammonium (Beller et al. 2006; Bock et al. 1990). Chemolithoautotrophic ecosystems support a set of metabolic processes tightly connected to deep subsurface geological and geochemical parameters (Rogers et al. 2022; Fullerton et al. 2021). Macroelements can be correlated with prokaryotic microbes to understand whether geochemical parameters affect the composition of microbial communities (Liu et al. 2018). Moreover, tectonic activity can act as a source of substrate for microbial life (Falkowski, Fenchel, and Delong 2008; Giovannelli et al. 2022), indeed tectonically controlled geochemistry of hydrothermal fluids resulting from water-rock interaction are correlated with bacterial community composition (Fullerton et al. 2021). Therefore, the abundance of ionic species in fluids can expand our knowledge about geochemical processes that could have important implications for the microbial communities.

Deeply-sourced seeps, encompassing a wide variety of diverse features that include fumarole, mofettes, acid-sulfate springs, deep chloride springs, steam heated waters and alkaline soda springs (Giovannelli et al. 2022), show extremely diverse ionic compositions accompanied by wide variations in temperature, pH, salinity and redox conditions. Our dataset routinely includes samples with pH varying between 0.5 to 11.2, temperatures ranging from 2 °C to 375 °C and salinity from 0 % to 35 %. Here we describe the standard operating procedures (SOP) developed to routinely analyze such a wide range of different fluid compositions.

SUMMARY OF METHOD

Samples are injected through a series of ion exchange columns until they reach the conductivity detector. The first column effectively protects the analytical column from contaminants that may be present in the samples, extend the life of the analytical column and has no effect on chromatographic separation performance. The analytical column separates ions according to their affinity with the selective ion-exchange column using high-purity mobile phase. For anionic chromatography, the suppressor reduces the background conductivity of the eluent and enhances the conductivity of the analytes. Finally, analytes are identified according to their retention time and the quantification is carried out through certified standards. We developed this protocol to work with a single ion chromatograph on which we change the column to analyze cations and anions in different runs, however the described SOP can be used only for cations or anions, or to analyze them in parallel with two ion chromatographs that are setup to run in parallel.

As previously mentioned, hydrothermal fluids are aqueous solutions with a wide range of temperature, salinity and pH that requires specific adjustments compared to the routine

analysis of drinking water or seawater for which these parameters vary less on a per sample basis. Given the presence of a conductivity detector and the ionic exchange that takes place in the column, care must be taken to keep the sample conductivity near or under 600 µS/cm. This extends column lifetime and increases the signal to noise ratio. Additionally, the presence of some ionic species in excess of others in the sample might interfere with peak detection. For example, chloride and sodium can hide the peaks of nitrite and ammonium, and the presence of high concentrations of organics can sometimes overprint the entire chromatogram significantly, increasing the detection limits for the specific sample. These issues can generally be resolved using sample pretreatment such as offline silver ion exchange columns for chloride, or solid phase extraction C18 columns to selectively remove organics. Additionally, ions exist in solution in different forms depending on the pH of the solution, so the pH of the samples may affect ion exchange within the column. This can be avoided by keeping the ratio between the volume of the sample and the volume of eluent very small (usually 1:1000) and pre-diluting the sample (at least 1:10 dilution for our specific case) before injection and always below 600 µS/cm.

STANDARD OPERATING PRACTICE FOR DEEPLY-SOURCED SEEPS FLUIDS

The method was developed using a Metrohm ECO-IC ion chromatograph equipped with a conductivity detector. In order to reduce the total dissolved solids, all samples are filtered in the field through a 0.22 µm filter. The samples are then stored at 4 °C in the dark until analysis. Once in the lab, samples are diluted to reduce the specific conductivity to below 600 µS/cm (measured with a HANNA instrument multiprobe, HI98194). All dilutions are made with type I water (18 MΩ/cm), which is also used as blank. Anions are run using a 3.2 mM Na_2CO_3 + 1 mM $NaHCO_3$ mobile phase with a Metrosep A Supp 5 column (Metrohm) equipped with a 0.15 M ortho-phosphoric acid suppressor with a flow of 0.7 ml/min for 30 min. Cations are run using 2.5 mM HNO_3 + 0.5 mM $(COOH)_2 \cdot 2H_2O$ mobile phase with Metrosep C4 column (Metrohm) with a flow of 0.9 ml/min for 35 min. Data acquisition and analysis is carried out through MagIC Net 3.3 software provided with the instrument. Calibration curves are designed using certified external standards (CPAchem) for each of the anions and cations analyzed. Calibration curves for the ion species of interest are run in the range of 0.1 and 10 ppm with $R \geq 0.999$ at least once a month, and a 1 ppm multistandard containing all the ions of interest is run every 10 samples to check for recovery and peak drift.

APPARATUS AND EQUIPMENT

- Eco IC ion chromatography (Metrohm, Switzerland)
- 863 Compact IC Autosampler (Metrohm, Switzerland)
- Metrosep A Supp 5 column (Metrohm, Switzerland)
- Metrosep C4 column (Metrohm, Switzerland)
- Metrosep A Supp 5 Guard/4.0 (Metrohm, Switzerland)
- Metrosep C4 Guard/4.0 (Metrohm, Switzerland)
- Vent Filter MPK01 (Merck, USA)
- MagIC Net 3.3 software (Metrohm, Switzerland)
- Analytical balance capable of 0.0001 g sensitivity (Mettler Toledo, USA)
- Class A volumetric flasks
- Pipettes for reagent and standard preparation
- Filters 0.22 μm

REAGENTS AND STANDARDS

STOCK ELUENT SOLUTION

- Anions eluent stock solution. A fresh batch of eluent solution is prepared before each run. Dissolve 0.678 g of Na_2CO_3 extra pure (99 % grade) and 0.168 g of $NaHCO_3$ extra pure (99 % grade) in 80 ml type I water in a 100 ml volumetric flask. When dissolution is complete, bring up to 100 ml with type I water. Final concentration: 3.2 mM Na_2CO_3 + 1 mM $NaHCO_3$.
- Cations eluent stock solution. A fresh batch of eluent solution is prepared before each run. Dissolve 0.63 g of $(COOH)_2 \cdot 2H_2O$ extra pure (99 % grade) in 80 ml type I water in a 100 ml volumetric flask and add 1.67 ml of 67 % HNO_3. When dissolution is complete, bring up to 100 ml with type I water. Final concentration: 2.5 mM HNO_3 + 0.5 mM $(COOH)_2 \cdot 2H_2O$.

WORKING ELUENT SOLUTION

- Anions eluent working solution. Take 50 ml of eluent concentrate into a 1 L

volumetric flask and transfer into a glass bottle. Before use, degas the solution using vacuum until no more bubbles can be detected.

- Cations eluent working solution. Take 10 ml of eluent concentrate into a 1 L volumetric flask and transfer into a glass bottle. Before use, degas the solution with vacuum until no more bubbles can be detected.

SUPPRESSOR REGENERANT SOLUTION

To reduce the background conductivity of the eluent and enhance the conductivity of the analytes, we use ortho-phosphoric acid (high purity). Pipette 5.13 ml of 85% H_3PO_4 in 400 ml type I water in a 500 ml volumetric flask. When dissolution is complete, bring up to 500 ml with type I water. Final concentration 0.15 M.

WASHING SOLUTION

To reduce the organic contamination of the injection system and column we use an ethanol wash solution. Pipette 5 ml of 100 % ethanol in 80 ml type I water in a 100 ml volumetric flask. When dissolution is complete, bring up to 100 ml with type I water and transfer in a beaker.

STOCK STANDARD SOLUTION

Standards are purchased as certified solutions. A fresh stock standard solution is prepared each time calibration curves are run. The 1 ppm standard is also run as a sample for quality check purposes once every 10 samples and it is stored at 4 °C between runs.

- Anions standard stock solution. Pipette 1 ml of the 1000 mg/L of the following single standard Cl^-, Br^-, NO_3^-, NO_2^-, SO_4^{2-} e HPO_4^{2-} in a 10 ml volumetric flask, bring up to volume with type I water.

- Cations standard stock solution. Pipette 1 ml of the 1000 mg/L of the following single standard Ca^{2+}, Na^+, K^+, Mg_2^+, NH_4^+ in a 10 ml volumetric flask, bring up to volume with type I water.

Anions and cations standards are prepared by dilution of a mixed standard with a final concentration of 0.1, 0.2, 0.5, 1, 5, 10 mg/L of each element.

SAMPLE COLLECTION, PRESERVATION AND STORAGE

In the field, samples are collected and filtered through a 0.22 µm membrane filter in clean plastic tubes that are conditioned with the sample 3 times before collection. If samples are not processed immediately, they can be stored at 4 °C.

QUALITY CONTROL OF THE ANALYTICAL SET UP

The full analytical setup consists of a series of QC blanks, samples and QC standard run as described in Figure 1. The QC blanks are used to verify the absence of ions carryover in the column and confirm low background levels while the QC standard is used to check for peak drift and consistent quantification of the standards.

- A full calibration is performed at least once a month, after column maintenance/replacement and in case of failed QC step while using the routine 1 ppm standard.
- Blanks composed of type I water (18 MΩ/cm) are run at the beginning of every run.
- Two QC blanks (type I water) are run as samples at the beginning of each run and once every 10 samples after the routine QC standard.
- The 1 ppm calibration standard solution is run as a sample for routine QC verification once every 10 samples.

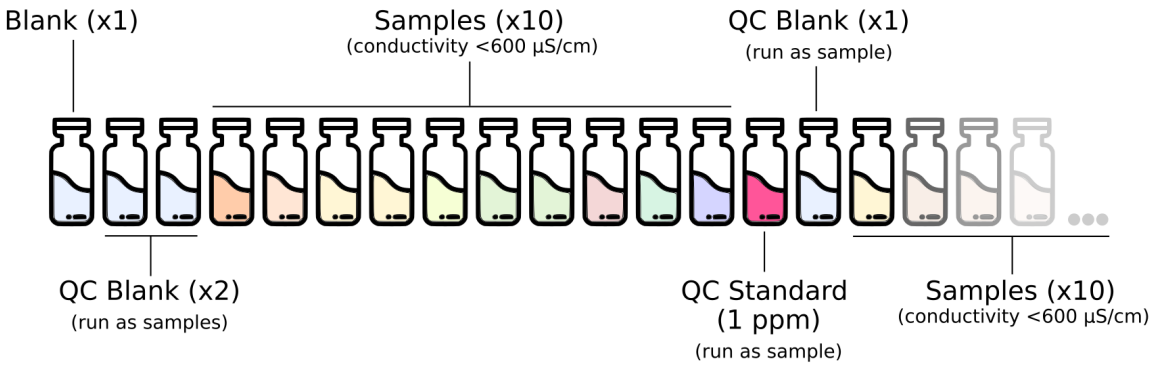

Figure 1. Analytical set up for the routine analysis of anions and cations in deeply-sourced seeps fluid samples.

ANALYTICAL PROCEDURE

- Check whether the amount of eluent is sufficient for the analysis. If not, prepare the solution as described in the “Reagents and Standards” section.
- Check that the installed column is appropriate for the type of analysis. If not, disconnect the column and the guard column. Be sure to cap both ends of the guard and analytical column so they do not dry.
- Be sure that lines to pre-screen the eluent are immersed in the liquid. If switch methods disconnect eluent lines and place the filter in a becker with type I water until the eluent is replaced to prevent the filter from drying out.
- Check that the correct loop is installed.
- Diluted each sample using type I water to a conductivity of 600 μS/cm before the analysis. 10 ml of final diluted sample is sufficient for both cations and anions analysis.
- Open the software and check that the selected method is correct.
- Click “Start HW” in the “Equilibration” windows. Allows the IC to run for 30-45 minutes until the baseline equilibrates, monitor flow, pressure and conductivity (approximately 900 μS/cm for cationic run and 15 μS/cm for anionic run).
- In “Determination series” make a sample list analysis with relative information like expedition, sample origin, sample type, position, injection loop and dilution applied to the sample.
- Organize the autosampler according to the analytical set up described in Figure 1. Mark in the software the position of the wash solution. Select “Start” to run the analysis.

INSTRUMENT CALIBRATION

Calibration is carried out at least once a month, after column maintenance/replacement and in case of failed QC step while using the routine 1 ppm standard. The correlation coefficient resulting from analysis is considered acceptable when $r^2 \geq 0.999$ (Figure 2).

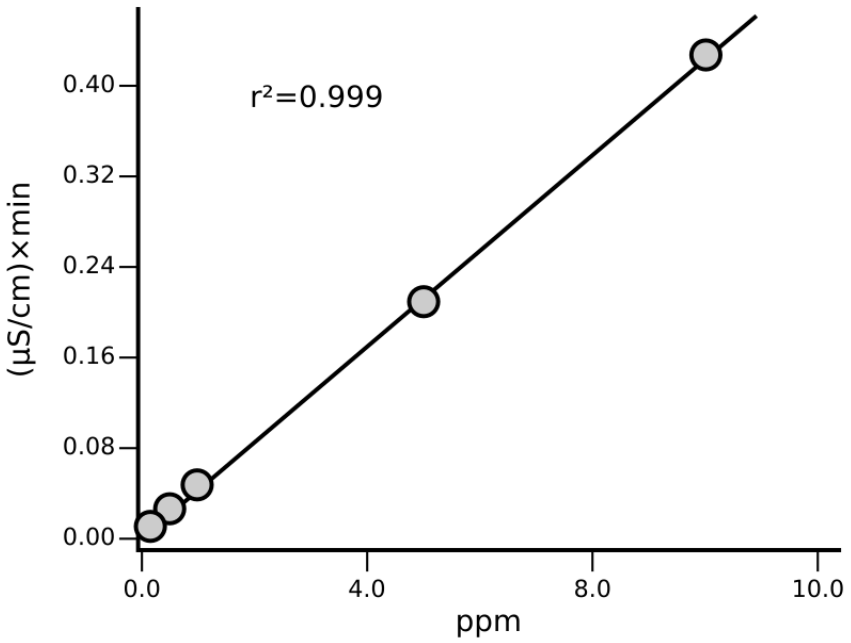

Figure 2. Example of calibration curves for Na^+ with a final concentration of 0.1, 0.2, 0.5, 1, 5, 10 mg/L determined following the described SOP.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

Limit of detection (LOD) is the smallest measure that can be detected with reasonable certainty for an analytical procedure, while the Limit of quantification (LOQ) is the smallest concentration that can be quantified with accuracy and precision. There are several methods to calculate LOD and LOQ. We use the method based on the calibration curves (Indrayanto 2018).

Limit of detection (LOD) is expressed as:

$$\mathrm{LOD} = 3.3 * \sigma/S$$

and Limit of quantification (LOQ) is expressed as:

$$\mathrm{LOQ} = 10 * \sigma/S$$

Where σ is the standard deviation of the regression and S is the slope of the calibration curve (Tietje and Brouder 2010). The LOD and LOQ values obtained for our IC SOP are given in Table 1.

Table 1. LOD and LOQ for the IC SOP in the Giovannelli Lab.

	LOD		LOQ	
Analyte	**mg/L**	**µM**	**mg/L**	**µM**
Ca^{2+}	0.0080	0.1996	0.0242	0.6038
Na^{+}	0.0104	0.4523	0.0315	1.3701
K^{+}	0.0099	0.2532	0.0300	0.7672
Mg_2^{+}	0.0056	0.2304	0.0170	0.6994
NH_4^{+}	0.0119	0.6596	0.0362	2.0066
Cl^{-}	0.0037	0.1043	0.0113	0.3187
Br^{-}	0.0566	0.7083	0.1715	2.1463
NO_3^{-}	0.0504	0.8127	0.1529	2.4657
NO_2^{-}	0.0273	0.5933	0.0829	1.8017
SO_4^{2-}	0.0190	0.1977	0.0577	0.6006
HPO_4^{2-}	0.0592	0.6168	0.1796	1.8712

APPLICATION

The proposed procedure was applied to investigate the concentration of major inorganic cations and anions in hydrothermal fluids. Figure 3 presents a typical chromatogram obtained after applicating this procedure for separating the investigated ions. This sample (KR1) was taken during the ICE21 expedition in Krysuvik (Iceland) and the basic environmental parameters for this sample are reported in Table 2. The concentrations of aqueous anions (Cl^{-}, SO_4^{2-}, and HCO^{3-}) obtained can be plotted in Giggenbach's ternary diagram (Giggenbach 1988) allowing us to distinguish four different types of water: volcanic waters, steam-heated waters, mature waters, and peripheral waters. Figure 4 represents an example of such a plot, obtained after analyzing hydrothermal fluids collected throughout the Campania region (Italy).

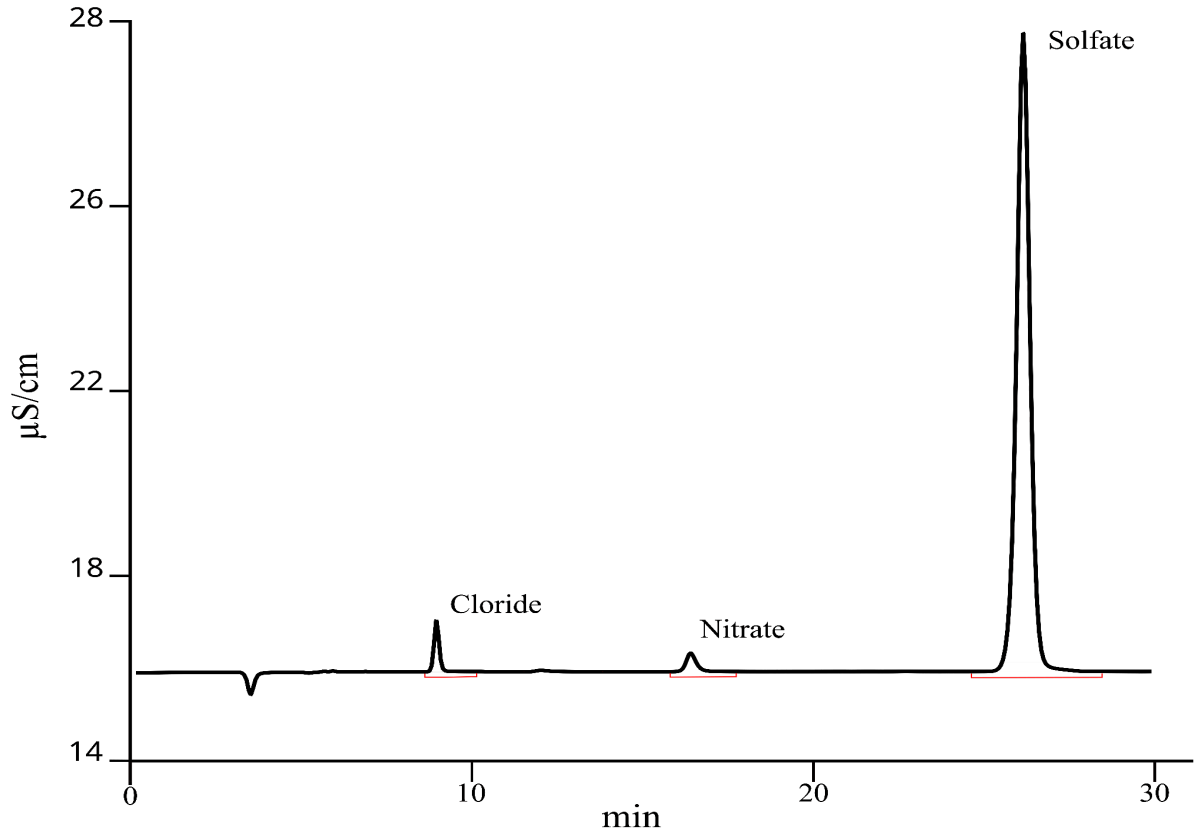

Figure 3. Example of chromatogram obtained from KR1 sample following the described SOP.

Table 2. Location (GPS coordinates), and physico-chemical parameters measured on the sampled locations.

Station	Latitude (°N)	Longitude (°E)	Altitude (m)	Temperature (°C)	PH	DO (%)	Spc (µS/cm)	Sal (%)
KR1	63.89546	-22.056914	246	87.5	3.1	91	2.543	0.53

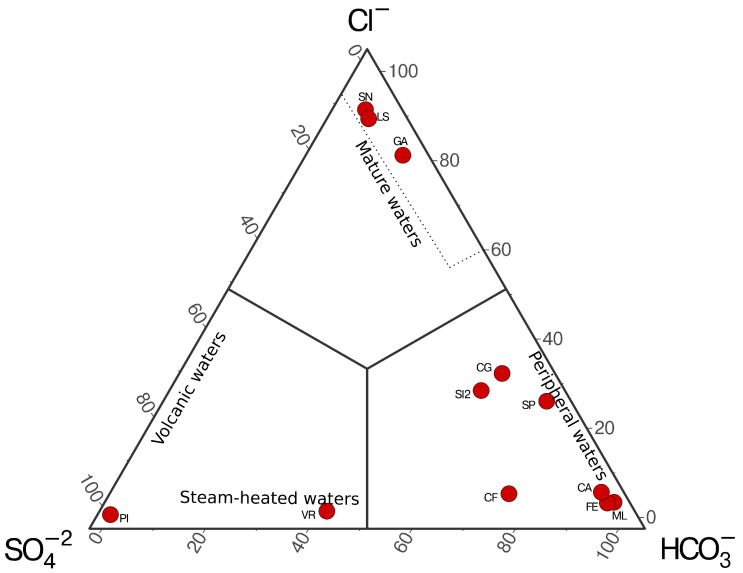

Figure 4. Example of ternary plot for the classification of hydrothermal fluids from the Campania Region (Italy, unpublished data) based on anion concentrations following Giggenbach (1988).

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Author Contributions

All authors contributed equally to this manuscript.

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Competing interests

The authors declare no competing interests.

Data and software availability

No data was produced for this manuscript. The software used is propriety of the rightful owner..

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