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METHOD ARTICLE

REVISED Standard operating procedure for the analysis of major ions in hydrothermal fluids by ion chromatography

[version 3; peer review: 2 approved, 1 approved with reservations]

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Abstract

This standard operating procedure (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 for cations Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, $NH4^{+}$ and anions Cl^{-}, Br^{-}, $NO3^{-}$, $NO2^{-}$, $SO4^{2-}$, $PO4^{3-}$.

Plain language summary

This manuscript details the standard operating procedure used for the determination of major cations and anions in hydrothermal fluids, which are characterized by a high variability of physico-chemical parameters. Due to the high variability of our samples, we customized the method to compensate for the variability on the physico-chemical parameters. The results are useful to interpret the geochemical processes and for understanding how these processes influence microbial diversity in geothermal systems.

Open Peer Review

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Keywords
major ions, hydrothermal fluids, ion chromatography, Standard Operating Procedure, aqueous geochemistry

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REVISED **Amendments from Version 2**

- We have corrected a confusing sentences (pag 7).
- We have added a brief description of sample preservation and citation that support this sentence (pag 7).
- We have explained the conditioning of the membrane filter in the text (pag 7).
- We have added the loop injection volume (pag 8).
- We have added a brief sentence mentioning the replicates (pag 10).
- We have added a brief sentence mentioning the standard addition method (pag 7).
- but we have added a brief sentence mentioning that In environmental samples it is rare that the results falls in the region between LOD and LOQ (pag 9).
- We have added a brief sentence mentioning the QC failure in QC section (pag 7).
- We have added a brief sentence mentioning the calibration standard point (pag 6).

Any further responses from the reviewers can be found at the end of the article

Introduction

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-} and PO_4^{3-}) in hydrothermal fluids using ion chromatography. This analysis can provide different information, in fact it is well known that these elements are required for the nutrition and metabolism of microbes, in particular for growth, oxidative metabolism and active transport. Some of these species can be used by microorganisms for redox reactions (Hay Mele *et al.*, 2023; Moore *et al.*, 2017), while others can inform on the nature and origins of fluids under investigation (Giggenbach, 1988). Microbial diversity is primarily driven by physical-chemical conditions, such as temperature, pH, redox state, and availability of diverse electron donors and electron acceptors (Delgado-Baquerizo *et al.*, 2018; Merino *et al.*, 2019). 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 (Fullerton *et al.*, 2021; Rogers *et al.*, 2023). 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 substrates for microbial life (Falkowski *et al.*, 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 of geochemical processes that could have important implications for 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 procedure (SOP) developed to routinely analyze such a wide range of different fluid compositions.

Methods

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 simultaneously.

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 (1.925.0020) equipped with a conductivity detector (1.850.9010). 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 in the field using 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 $r^2 \geq 0.999$ 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 (1.925.0020 Metrohm, Switzerland)
- 863 Compact IC Autosampler (1.863.0010 Metrohm, Switzerland)
- Metrosep A Supp 5 column (6.1006.530 Metrohm, Switzerland)
- Metrosep C4 column (6.1050.430 Metrohm, Switzerland)
- Metrosep A Supp 5 Guard/4.0 (6.1006.500 Metrohm, Switzerland)
- Metrosep C4 Guard/4.0 (6.1050.500 Metrohm, Switzerland)
- Vent Filter MPK01 (TANKMPK01 Merck, USA)
- MagIC Net 3.3 software (Metrohm, Switzerland)
- Analytical balance capable of 0.0001 g sensitivity (MS105DU Mettler Toledo, USA)
- Class A volumetric flasks (VFL3-010-002, VFL3-100-002 Labbox)
- Pipettes for reagent and standard preparation
- PP syringe filters 0.22 µm (TS-900-063 Perlabo)

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 a 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 to 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-} and PO_4^{3-} 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. For more accurate results, if a wide calibration range is required, it is advisable to utilize low, medium and high range of calibration points.

Sample collection, preservation and storage

In the field, samples are collected and filtered through a 0.22 µm membrane filter in clean plastic tubes. Both membrane filters and plastic tubes are conditioned with the sample 3 times before collection. If samples are not analysed immediately, they can be stored at 4 °C until they are ready to be prepared for analysis. Samples preservation and holding times depend on the type of cation or anion to be analysed (Pfaff, 1996). In some cases, preservation solutions specific for each ion can be used, for example, in case of chloride, ethylenediamine (EDA) can be added (Jackson, 2000).

Quality control of the analytical setup

The full analytical setup consists of a series of quality control (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. QC failure can occur due to various reasons such as instrumental issues (e.g., column degradation, detector problem), method problems, sample preparation errors (incorrect or degradation of samples) and environmental factors (temperature fluctuations and air quality). Therefore, to resolve QC failures, it is important to check all these steps. Normally a recovery between 80 % and 120 % is acceptable.

- 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.
- If the sample contains components that result in a matrix effect which may reduce the signal of the analyte, it is advisable to use the standard addition method, which involves adding a known amount of the standard to the sample to monitor recovery.

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 switching between 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. Allow 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 (in our system the injection volume is 10 µl) and dilution applied to the sample.
- Organize the autosampler according to the analytical setup described in Figure 1. Mark in the software the position of the wash solution. Select "Start" to run the analysis.

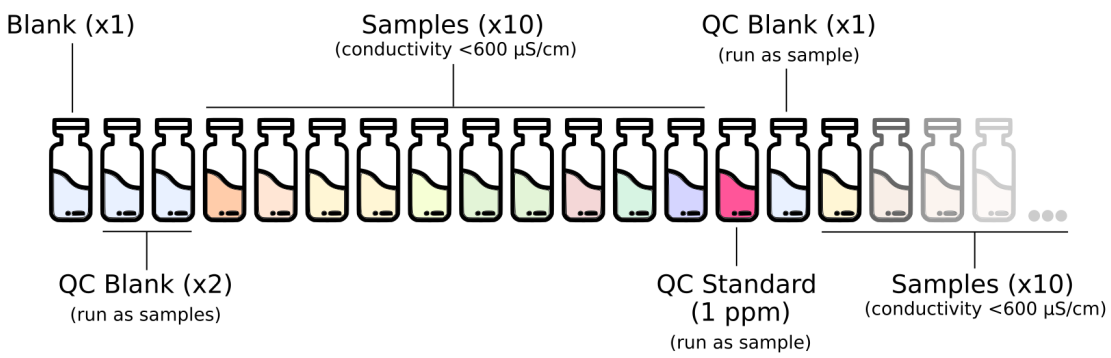

Figure 1. Analytical setup for the routine analysis of anions and cations in deeply-sourced seeps fluid samples.

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).

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 & Brouder, 2010). The LOD and LOQ values obtained for our IC SOP are given in Table 1.

If the sample falls in the region between LOD and LOQ, the signal is detectable but not necessarily quantifiable.

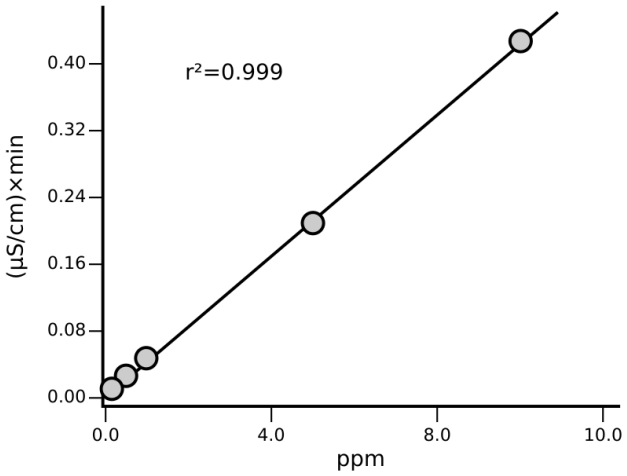

Figure 2. Example of calibration curves for Na^+ with a final concentration of 0.1, 0.2, 0.5, 1, 5, 10 mg/L determined following the described SOP.

Table 1. LOD and LOQ for the IC SOP in the Giovannelli Lab at the University of Naples Federico II, Italy.

	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
PO_4^{3-}	0.0592	0.6168	0.1796	1.8712

Therefore, the analytical method must be optimized regarding instrument calibration or sample preparation. However, in the case of environmental samples, it is rare for the result to fall within the region between LOD and LOQ due to the presence of moderate to high concentrations of major ions.

Results

Use case

The proposed procedure was applied to investigate the concentration of major inorganic cations and anions in hydrothermal fluids of diverse geothermal systems, and has been successfully used in the past to investigate how the geochemical composition of the geothermal fluids influences microbial diversity (Fullerton *et al.*, 2021). Figure 3 presents a typical chromatogram obtained after applying this procedure for separating the investigated ions in geothermal fluids.

The chromatogram presented is from a sample (KR1) taken during the ICE21 expedition in Krysuvik (Iceland), a very dynamic and diverse geothermal activity region. The basic environmental parameters for this sample are reported in Table 2. Replicates are normally used in sample preparation to assess the variability of the method caused by inherent errors in the sample preparation step. The number of replicates should be at least 2 but we recommended 3. The concentrations of aqueous anions (Cl^-, SO_4^{2-}, and HCO_3^-) obtained

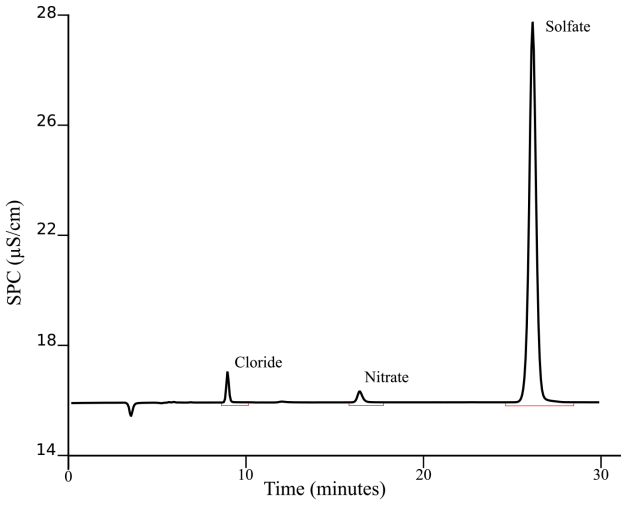

Figure 3. Example of chromatogram obtained from KR1 sample following the described SOP.

are plotted using Giggenbach's ternary diagram (Giggenbach, 1988), allowing us to distinguish four different types of waters: volcanic waters, steam-heated waters, mature waters, and peripheral waters (Figure 4).

A case study application of the methods was carried out using hydrothermal fluids collected throughout the Campania region (Italy). The ternary distribution of aqueous anions shows fluids from all four different types of waters. A single sample (PI) is classified as volcanic waters, and a single sample (VR) is classified as steam-heated water in spite of the low temperature of the site (12 °C at the time of sampling), which suggest a deep source for the volatile in the area that cool and mix with shallow waters during ascent. Both samples reflect water significantly affected by deep heat sources rich in sulfates. Most of the fluids are found in the peripheral water, enriched in HCO_3^-, due to a higher influence of meteoric waters. The remaining samples are found in the mature waters region, which are thought to represent well-equilibrated fluids from deep geothermal wells.

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

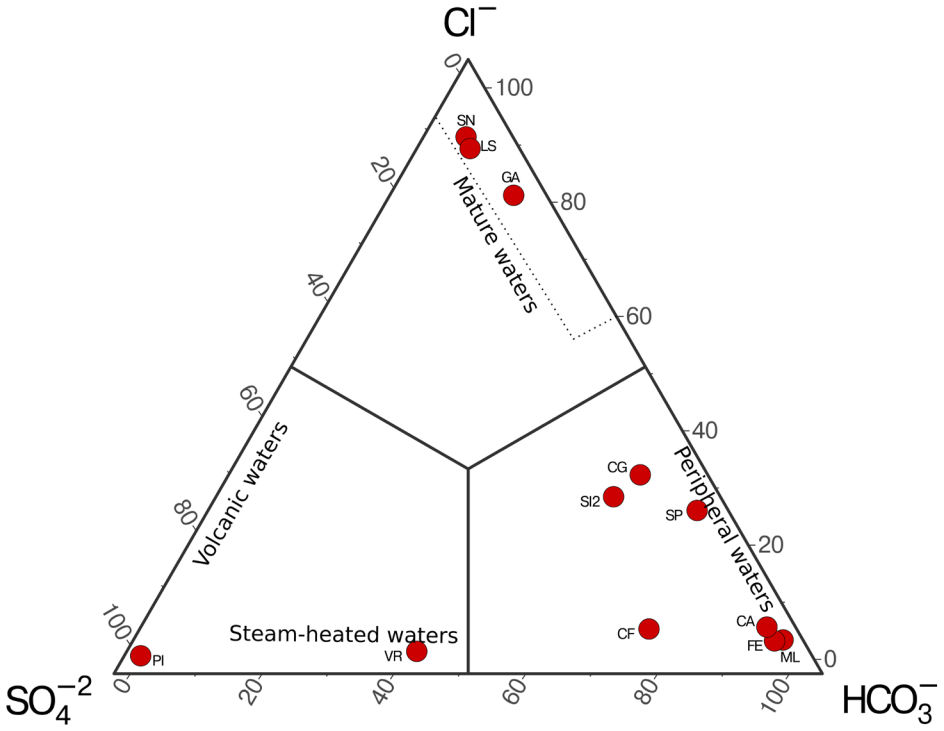

Figure 4. Example of ternary plot for the classification of hydrothermal fluids from the Campania Region (Italy, unpublished data) based on anion concentrations following Giggenbach (1988).

Data and software availability

Zenodo. giovannellilab/GiovannelliLab_SOPs: Standard Operating Procedure for the analysis of trace elements in hydrothermal fluids by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). 10.5281/zenodo.7680349 (Giovannelli, 2023)

This project contains the following underlying data:

- Table1.csv (Contains the data for Table 1 of the manuscript.)
- Table2.csv (Contains the data for Table 2 of the manuscript.)
- SOP_IC_example_data.csv (Contains the data used in the Case Study in the Campania Region used to plot Figure 4)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Author contributions

All authors contributed equally to this manuscript.

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References

Beller HR, Chain PSG, Letain TE, *et al.*: **The genome sequence of the obligately chemolithoautotrophic, facultatively anaerobic bacterium *Thiobacillus denitrificans.*** *J Bacteriol.* 2006; **188**(4): 1473–88.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Bock E, Koops HP, Möller UC, *et al.*: **A new facultatively nitrite oxidizing bacterium, *Nitrobacter vulgaris* sp. nov.** *Arch Microbiol.* 1990; **153**(2): 105–110.
Publisher Full Text

Delgado-Baquerizo M, Oliverio AM, Brewer TE, *et al.*: **A global atlas of the dominant bacteria found in soil.** *Science.* 2018; **359**(6373): 320–325.
PubMed Abstract | **Publisher Full Text**

Falkowski PG, Fenchel T, Delong EF: **The microbial engines that drive Earth's biogeochemical cycles.** *Science.* 2008; **320**(5879): 1034–9.
PubMed Abstract | **Publisher Full Text**

Fullerton KM, Schrenk MO, Yücel M, *et al.*: **Effect of tectonic processes on biosphere-geosphere feedbacks across a convergent margin.** *Nat Geosci.* 2021; **14**(5): 301–306.
Publisher Full Text

Giggenbach WF: **Geothermal solute equilibria. Derivation of Na-K-Mg-Ca geoindicators.** *Geochim Cosmochim Acta.* 1988; **52**(12): 2749–2765.
Publisher Full Text

Giovannelli D: **giovannellilab/GiovannelliLab_SOPs: Standard Operating Procedure for the analysis of trace elements in hydrothermal fluids by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).** (v2.4). *Zenodo*. 2023.
http://www.doi.org/10.5281/zenodo.7680377

Giovannelli D, Barry PH, de Moor JM, *et al.*: **Sampling across large-scale geological gradients to study geosphere-biosphere interactions.** *Front Microbiol.* 2022; **13**: 998133.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Hay Mele B, Monticelli M, Leone S, *et al.*: **Oxidoreductases and metal cofactors in the functioning of the earth.** *Essays Biochem.* 2023; **67**(4): 653–670.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Indrayanto G: **Validation of chromatographic methods of analysis: application for drugs that derived from herbs.** In: *Profiles Drug Subst Excip Relat Methodol.* Elsevier, 2018; **43**: 359–392.
PubMed Abstract | **Publisher Full Text**

Jackson PE: **Ion chromatography in environmental analysis.** In: *Encyclopedia of Analytical Chemistry*. edited by Robert A. Meyers, 1st ed. Wiley, 2000.
Publisher Full Text

Liu K, Ding X, Tang X, *et al.*: **Macro and microelements drive diversity and composition of prokaryotic and fungal communities in hypersaline sediments and Saline-Alkaline soils.** *Front Microbiol.* 2018; **9**: 352.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Merino N, Aronson HS, Bojanova DP, *et al.*: **Living at the extremes: extremophiles and the limits of life in a planetary context.** *Front Microbiol.* 2019; **10**: 780.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Moore EK, Jelen BI, Giovannelli D, *et al.*: **Metal availability and the expanding network of microbial metabolisms in the Archaean eon.** *Nat Geosci.* 2017; **10**(9): 629–636.
Publisher Full Text

Pfaff JD: **Determination of Inorganic Anions by Ion Chromatography.** In: *Methods for the Determination of Metals in Environmental Samples*. Elsevier. 1996; 388–417.
Publisher Full Text

Rogers TJ, Buongiorno J, Jessen GL, *et al.*: **Chemolithoautotroph distributions across the subsurface of a convergent margin.** *ISME J.* 2023; **17**(1): 140–150.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Tietje C, Brouder A: **International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use.** In: *Handbook of Transnational Economic Governance Regimes.* Brill Nijhoff, 2010; 1041–1053.
Publisher Full Text

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Reviewer Report 10 September 2024

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✓ **Juris Burlakovs**
Riga Technical University, Riga, Latvia

the SOP for analysis of major ions is comprehensive and relevant with approved techniques and fully repeatable, results and conclusions reasonable
the Introductory part reflects the problem and is aiming the pathway to the determination of problem and potential solutions
methodology is sound
the innovative pattern is obvious and is helping provide new insights in elaboration of analytical techniques

Is the rationale for developing the new method (or application) clearly explained?
Yes

Is the description of the method technically sound?
Yes

Are sufficient details provided to allow replication of the method development and its use by others?
Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: geochemistry, geology, environmental studies

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 04 September 2024

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Dzenita Avdibegovic
KU Leuven, Leuven, Flanders, Belgium

The authors implemented necessary changes.
The authors have responded and revised the SOP according to my comments.
The SOP can be accepted.

Is the rationale for developing the new method (or application) clearly explained?
Yes

Is the description of the method technically sound?
Yes

Are sufficient details provided to allow replication of the method development and its use by others?
Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?
Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 08 August 2024

https://doi.org/10.21956/openreseurope.19527.r42558

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Łukasz Nuckowski
Faculty of Oceanography and Geography, University of Gdansk (Ringgold ID: 49646), Gdańsk, Poland

1) The authors haven't shown LOD and LOQ values properly. Please express them with the same number of significant figures, not decimal places.
2) The calibration curve needs improvement. The calibration points should be distributed evenly.

Is the rationale for developing the new method (or application) clearly explained?
Yes

Is the description of the method technically sound?
Yes

Are sufficient details provided to allow replication of the method development and its use by others?
Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Chromatgoraphy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 09 Aug 2024

Donato Giovannelli

Dear Reviewer, thank you for the suggestions.

1. I am unsure why we should drop significant digits for some of our detection limits to show the same number of significant figure for all the elements. There is no current

standard that requires to drop significant digits to unify a table. So respectfully disagree on this request, and this change does not affect the validity of the SOP presented.
2. Similarly calibration points across a calibration curve do not need to be uniformly spaced, and their distribution depends from several factors. The important aspect is covering the entire range of necessary dilutions while keeping a strong accuracy.

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 28 May 2024

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The SOP is relatively clear and concise.
However, I recommend to consider and include the following corrections:

- The authors recommend to store the samples at under the condition that they are "not processed" immediately. The word "processed" is not very clear. Thus, I advise that the authors recommend to store the samples at 4 °C until they are ready to be prepared for analysis.
- The authors should include for how long the samples can be stored at 4 °C, as well as to add recommendations in case samples cannot be analyzed during that time (e.g. in case of equipment failure - is freezing recommended in that case?
- In case the samples are filtered prior to analysis it is advisable that the blanks are filtered as well
- The authors should recommend standards/sample injection volume
- The authors should give recommendations on number of replicates
- The authors should give recommendations on spiked samples
- The authors should advise on data interpretation in case the results fall in the region between LOD and LOQ
- The authors should add a QC sample after blank, thus at the beginning of the sample sequence
- The authors should include the criteria for QC (recovery %) and correction actions in case of QC failure

- The authors should add recommendations to prepare series of calibration standards in the low region, middle region and high region

Is the rationale for developing the new method (or application) clearly explained?
Yes

Is the description of the method technically sound?
Yes

Are sufficient details provided to allow replication of the method development and its use by others?
Partly

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Analytical chemistry, ion exchange, chromatography

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 12 Jun 2024

Donato Giovannelli

We thank the reviewer for the useful comments. We respond point by point below.
1. Thank you for pointing this out! We routinely store at +4˚C in the dark. We have corrected these confusing sentences (pag 7).
2. We have added a brief description of sample preservation and citation that support this sentence (pag 7).
3. The leachable ions are very labile and are essentially removed completely if the filter is pre-washed with water or conditioned with the sample before collection, as we do in the field. That said, filter and reagent blanks are routinely analyzed as part of the QC in the lab. We have now explained the conditioning of the membrane filter in the text (pag 7).
4. We have added the loop injection volume. (pag 8)
5. We have added a brief sentence mentioning the replicates.(pag 10)
6. We have added a brief sentence mentioning the standard addition method. (pag 7)
7. In environmental samples it is rare that the results falls in the region between LOD and LOQ, but we have added a brief sentence mentioning this point (pag 9)

8. Considering that at the end of each set of samples, and thus also before the end of the analysis, we run a standard and a blank, we do not deem it necessary to run another standard before the start of the analysis.
9. We have added a brief sentence mentioning the QC failure in QC section (pag 7)
10. We have added a brief sentence mentioning the calibration standard point. (pag 6)

Competing Interests: No competing interests were disclosed.

Reviewer Report 30 November 2023

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The authors do a lot of work preparing the manuscript. They provided all the data necessary to repeat and apply the described method.
However, I do not see any differences between the given method and the standard method described by IE column producers without any changes.

Moreover, I have a few minor comments on the article:

1) The authors describe that samples were stored at 4 °C in the dark until analysis. Is there any data to prove their stability?

2) Calibration curve in Figure 2. is poor. It shows only 3 regularly distributed calibration points. In this way, the correlation coefficient will always be high.

3) LOD and LOQ are displayed with different numbers of significant places. Their number should be standardized, for example, by using exponential notation.

Is the rationale for developing the new method (or application) clearly explained?
No

Is the description of the method technically sound?

Yes

Are sufficient details provided to allow replication of the method development and its use by others?
Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Chromatgoraphy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 30 Apr 2024

Donato Giovannelli

We want to thank the reviewer for the useful comments. We respond point by point below.
1. We follow the procedure for sample handling and preservation reported in the Standard Methods for the analysis of waters and wastewater Ed. 23, method 4110 B. Filtration is carried out at 0.22 µm instead of 0.45 µm as reported in the 4110 B method to remove microorganisms and stored at +4 °C.
2. The reported calibration in Figure 2 contains 6 points with concentration of 0.1, 0.2, 0.5, 1, 5, 10 mg/L, as visible in the graph and reported in the caption.
3. Al the LOD and LOQ are reported at the 4th significant place.

Competing Interests: No competing interests were disclosed.