

RESEARCH ARTICLE

Optimization of compound-specific chlorine stable isotope analysis of chloroform using the Taguchi design of experiments

Berhane Abrha Asfaw¹  | Kaori Sakaguchi-Söder^{1,2}  | Anat Bernstein³  |
Hagar Siebner³ | Christoph Schüth¹

¹Institute of Applied Geosciences, Technische Universität Darmstadt, Schnittspahn Straße 9, Darmstadt, 64287, Germany

²Institut IWAR, Technische Universität Darmstadt, Franziska-Braun Straße 7, Darmstadt, 64287, Germany

³Department of Environmental Hydrology and Microbiology, The Zuckerberg Institute for Water Research, Ben-Gurion University of the Negev, Sde Boker Campus, Beer-Sheva, 84990, Israel

Correspondence

Berhane Abrha Asfaw, Institute of Applied Geosciences, Technische Universität Darmstadt, Schnittspahn Straße 9, 64287 Darmstadt, Germany.
Email: berhane@geo.tu-darmstadt.de

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Rationale: Chloroform, a probable human carcinogen, is commonly detected in various concentration levels in many surface water and groundwater sources. Compound-specific chlorine stable isotope analysis (CI-CSIA) is significant in investigating the fate of chlorinated contaminants in the environment. Analytical conditions should, however, be thoroughly examined for any isotopic fractionation. In this study, we simultaneously optimize three analytical parameters for a robust online CI-CSIA of chloroform using the Taguchi design of experiments.

Methods: For CI-CSIA, a purge-and-trap autosampler coupled to a gas chromatograph in tandem with a quadrupole mass spectrometer, with electron ionization in selected ion monitoring (SIM) mode, was used. Using the Taguchi method, the dominant parameter affecting the results of CI-CSIA for chloroform was identified through concurrent investigation of the signal-to-noise ratios (S/N) of three parameters, each at three levels: purging time (5, 10, 15 min), transfer time (80, 120, 160 s), and dwell time (20, 60, 100 ms). Moreover, the optimum combination of the levels was identified.

Results: The purging time, with a maximum S/N, resulted in the highest influence on the isotope ratios determined. It was further refined through additional experiments to sufficiently extract chloroform from the aqueous phase. Accordingly, 8 min of purging time, 120 s transfer time and 100 ms dwell time were the optimum conditions for CI-CSIA of chloroform. Post-optimization, a precision of ± 0.28 ‰ was achieved for 8.4 nmol of chloroform (equivalent to 0.89 μg or approx. 25 nmol Cl-mass on column).

Conclusions: A simple online method for CI-CSIA of chloroform was optimized with the Taguchi design of experiments. The Taguchi method was very useful for the optimization of the analytical conditions. However, the purging conditions should be fine-tuned and selected so that sufficient extraction of a target compound is confirmed to acquire a stable and higher precision of the method.

[Correction added on 25 September 2020, after first online publication: ⁸⁵ was omitted from Equation 1 and was restored.]

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1 | INTRODUCTION

Chloroform (CHCl₃), a toxic chlorinated hydrocarbon, is detected at various concentrations in tap water, surface water reservoirs (natural and manmade), and some aquifers. In addition to the natural formation of chloroform,^{1,2} chlorination of water supplies may result in the formation of trihalomethanes (THMs), as the chlorine reacts with organic matter,^{3,4} with chloroform being one of the main products.⁵ Moreover, its wide manufacture and use as an industrial solvent, and its commercial use contribute to its common occurrence and detection. In groundwater systems, chloroform could result from various sources including improper handling and disposal, spills and leakage, and recharge of chlorinated water such as treated wastewater or water from public water supplies.⁵ The Environmental Protection Agency in the United States of America (US EPA) has classed chloroform as a probable human carcinogen (Group B2).⁶

Compound-specific isotope analysis (CSIA) has been found to be very useful to investigate contamination sources, degradation pathways and fate of organic contaminants in a variety of environmental systems.^{7–16} For instance, Sturchio et al¹⁷ evaluated the natural attenuation of trichloroethylene (TCE) in an aquifer. Hunkeler et al⁹ and Breider et al¹⁸ demonstrated the separation of natural from anthropogenic origins of chloroform using carbon (C)-CSIA and chlorine (Cl)-CSIA, respectively. Dual isotope data of C- and Cl-CSIA reported by Palau et al¹⁹ have shown a typical fractionation pattern for 1,2-dichloroethane (1,2-DCA) during reductive biodegradation in the field to distinguish aerobic from anaerobic conditions, and the same technique was used for pathway identification,²⁰ confirming CSIA as a powerful tool in environmental forensics.

Online stable chlorine isotope measurements, with the use of molecular and/or fragment ions of compounds formed in the ion source, have formerly been performed using quadrupole mass spectrometry (qMS), isotope ratio mass spectrometry (IRMS), and other online instrumental techniques, on several compounds.^{21–24} The qMS method was first developed by Sakaguchi-Söder et al.²² Round robin tests of gas chromatography (GC)/IRMS and GC/qMS for Cl-CSIA on TCE by Bernstein et al,²⁵ and on chloroform and carbon tetrachloride by Heckel et al,²⁶ have shown that the methods agree very well in terms of precision and accuracy. The use of the GC/qMS technique is therefore preferable, as it is comparatively simple, widely applicable for a range of chlorinated compounds, and does not require upstream conversion.

In both the IRMS and the qMS techniques, the environmental concentrations of target compounds are often too low for CSIA measurements. As a result, online sample enrichment techniques, such as solid-phase microextraction (SPME) and purge and trap (P&T), for C-CSIA using GC/IRMS have successfully been demonstrated.^{9,27–29} The P&T method was found to be more suitable, as it resulted in less isotopic fractionation.²⁸ Various optimization methods for carbon isotope analyses (C-CSIA) are documented.^{9,30,31} For instance, the effect of different purging times on carbon isotope ratios was demonstrated with the use of a continuous-flow P&T system³⁰ and a P&T system with a liquid autosampler.²⁸

With respect to sample enrichment for online Cl-CSIA using qMS, headspace injection techniques are commonly used.^{18,19,23,26} However, it was shown that the isotope ratios and the precision may vary, due to various analytical conditions.^{21,26,32} As a result, different optimization approaches for Cl-CSIA using a variety of instrumental configurations, peripherals, and compounds have been published.^{21,23,32–37} For example, Heckel et al²⁶ and Jin et al²³ demonstrated for Cl-CSIA that the dwell time has to be considered. In addition to the dwell time, for the measurement of Cl-CSIA from aqueous samples, the purging time and the transfer time could play a role in the precise determination of isotope ratios, but they have not been tested in detail yet.

Analyzing the effect of each analytical parameter separately may not result in the highest overall precision when combined. Therefore, methods are needed for the simultaneous optimization of various parameters to determine their best combination. In the engineering sciences, the Taguchi design of experiments method is widely applied to optimize the performance of processes or the quality of a product with reduced costs.^{38–40} With the orthogonal array of the Taguchi method, multiple parameters and multiple levels are run independently at the same time and evaluated subsequently, thereby reducing the number of experiments needed to aim at a certain target.^{38,41}

In this study, we applied the Taguchi design of experiments method to systematically optimize and significantly improve the determination of compound-specific stable chlorine isotope ratios, with chloroform as a model compound, using a simple online P&T-GC/qMS technique. We specifically looked at the three parameters – purging time, transfer time, and dwell time – to select their best combination for a stable and precise isotope analysis.

2 | MATERIALS AND METHODS

2.1 | Chemicals and instrumentation

A pure chloroform standard (PHR1552 3 × 1.2 mL, LRAA4468, CAS #67-66-3; Sigma-Aldrich, Steinheim, Germany) was diluted using methanol (>99.9%; Carl Roth GmbH, Karlsruhe, Germany) to prepare stock solutions.

A P&T system (PTA 3000; IMT, Vohenstrauß, Germany) was used to extract the chloroform from an aqueous phase. The P&T system was connected to a 6890N gas chromatograph coupled to a 5973N quadrupole mass spectrometer (both from Agilent, Santa Clara, CA, USA), with a transfer line heated at 200°C. Using this P&T-GC/qMS system, the Cl-CSIA analyses were performed online.

Purging of chloroform in the aqueous phase was carried out at 40°C with a purging flow of 20 mL/min (helium). The trap was filled with Tenax as the adsorbent (M16T 08; IMT, Moosbach, Germany) and operated at –35°C. When purging was completed, the trap was rapidly heated to 190°C. The desorbed chloroform was transferred splitless to the GC/qMS system for Cl-CSIA. Between measurements, the trap was conditioned at 190°C for 23 min to avoid memory effect caused by possible residuals on the trap. For the conditioning, nitrogen gas was used.

The gas chromatograph was equipped with a DB 624 capillary column (60 m × 0.25 mm, 1.4 μm film thickness; Agilent J&W, Santa Clara, CA, USA). The temperature program started at 40°C which was held for 2 min. The temperature was then raised to 90°C at 8°C/min, held for 4 min, raised to 125°C at 6°C/min, raised to 220°C at 15°C/min, and finally held for 5 min. The ion source was operated at 70 eV using electron ionization (EI) and the mass spectrometer was set in the selected ion monitoring (SIM) mode. The masses to be analyzed for the CI-CSIA of chloroform were *m/z* 83 and 85.

2.2 | Mathematical equation and peak area correction

For chloroform, the targeted masses of the CHCl₂⁺ fragment ion (*m/z* 85 and 83; ¹²C¹H³⁵Cl³⁷Cl⁺ and ¹²C¹H³⁵Cl₂⁺, respectively) were chosen. These are the two highest peaks in terms of their relative abundance, enabling low detection limits, and they were also used by others.^{26,18} The chlorine isotope ratio *R*_{Cl} was determined using the following equation:

$$R_{Cl} = \frac{{}^{37}\text{Cl}}{{}^{35}\text{Cl}} = \frac{{}^{85}\text{I}}{83\text{I}} \quad (1)$$

where ⁸⁵I and ⁸³I are the peak signal intensities of the fragment ions at *m/z* 85 and 83, respectively, reflecting the ratio of the heavy to light isotopes.

Isotope analyses typically show a mass, i.e. peak area (PA), dependency that has to be corrected.^{42,43} We used an in-house standard of chloroform at three different concentrations (59.6, 104.5, and 149 μg/L) that were run in each sequence. In this concentration range, there was an evident linear relationship between the chlorine isotope ratios of the standards and the peak area of the *m/z* 83 fragment ion (Figure S1, supporting information). We used the slope of this relationship for peak area correction and the final calculation of the isotopic composition as in Sakaguchi-Söder.⁴²

2.3 | Taguchi experimental design and evaluation method

The analytical conditions of the P&T-GC/qMS system, namely, purging time, transfer time and dwell time, were optimized using an L₉ orthogonal array of the Taguchi experimental design.⁴⁴ For each sequence, a total of nine experiments, with four replicates, were designed under nine systematic but non-identical combinations of three parameters (P1–3) and three levels per parameter i.e., P1: Purging time (5, 10, 15 min), P2: Transfer time (80, 120, 160 s) and P3: Dwell time (20, 60, 100 ms). The selected parameters and levels are summarized in Table 1, and Table 2 shows the layout of L₉ array for this study.

A total of 36 identical samples were prepared in 20-mL headspace vials, containing 10 mL Millipore water (Simplicity® UV, Lot

TABLE 1 Parameters and levels selected for optimizing the CI-CSIA method for chloroform with Taguchi-method L₉ (3 levels × 3 parameters) using P&T-GC/qMS

		Level		
		1	2	3
Parameters	P1: Purge time [min]	5	10	15
	P2: Transfer time [s]	80	120	160
	P3: Dwell time [ms]	20	60	100

TABLE 2 Layout of the parameters and corresponding signal-to-noise ratios (S/N) following the Taguchi experimental design (L₉). The nine experiments were run in one sequence and the sequence was replicated four times. PT = purging time, TT = transfer time, DT = dwell time

Experiment no.	P1: PT [min]	P2: TT [s]	P3: DT [ms]	S/N
1	5	80	20	S/N ₁
2	5	120	60	S/N ₂
3	5	160	100	S/N ₃
4	10	80	60	S/N ₄
5	10	120	100	S/N ₅
6	10	160	20	S/N ₆
7	15	80	100	S/N ₇
8	15	120	20	S/N ₈
9	15	160	60	S/N ₉

No. FOAA76868; Millipore S.A.S., Molsheim, France) with a concentration of 100 μg/L chloroform. The vials were closed using Alu-crimp top caps with Teflon-coated butyl rubber septa (Wicom GmbH, Heppenheim, Germany). Samples were randomly selected for each L₉ experiment. An L₉ array was conducted in one sequence and replicated four times to determine the chlorine isotope ratios of the chloroform and their reproducibility. Blank water and blank air vials were run after every three measurements for quality assurance. There was no contribution to the targeted masses of *m/z* 83 and 85, from the background or sample preparation procedures, confirming that there was neither interference nor carryover from sample to sample during the measurements.

To evaluate the results of the Taguchi experiments, first the signal-to-noise (S/N) ratios have to be determined based on the nominal-the-best criteria,⁴⁰ as shown in Equation (2):

$$S/N_i = 10 \log \left(\frac{\mu_i^2}{\sigma_i^2} \right) \quad (2)$$

where *i* is the experiment number 1 to 9; S/N_{*i*} is the signal-to-noise ratio, μ_{*i*} is the mean isotope ratio (R), and σ_{*i*} is the standard deviation of the isotope ratio of the *i*th experiment. The S/N values were further evaluated to determine the mean of S/N per parameter per level. For each parameter, the level with the largest mean of S/N is considered to be the most favorable one. Furthermore, the parameter with the

largest range in S/N for the three levels is considered to be the most sensitive parameter, with highest rank, in this L_9 test.

3 | RESULTS AND DISCUSSION

3.1 | Results of Taguchi experiments

The isotope ratios (R) determined for the nine experiments, L_9 , with four repetitions, are presented in Table 3. For each of the nine experiments, a S/N was determined using Equation (2). The arithmetic means of the S/N, for each level in each parameter, were calculated based on the L_9 layout from Table 3 and are summarized in Table 4. As described in section 2, the range was calculated for the evaluation of the degree of influence that the selected parameters have on the precision of determining the isotope ratios.

Having a S/N of 1.60, the smallest range among the three parameters, it is evident that the transfer time has the lowest influence on the precision of the method. The isotope ratios of chloroform showed very similar results for transfer times of 80 and 120 s (see also Table S1, supporting information).

Furthermore, the influence of the selected levels of the dwell time on the precision of the method was limited, with a S/N range of 2.23 (Table 4). The S/N for a dwell time of 100 ms was the largest

among the levels within the parameter, indicating the optimal condition. The smallest relative standard deviation (RSD) in the isotope ratio was achieved under the same dwell time (Table S2, supporting information). Others have also observed some influence of dwell time on isotope ratios, but the results are very dependent on the instruments, analytical methods and analytes. For example, Aeppli et al²¹ reported that a dwell time of between 50 and 100 ms makes no significant difference in the isotope ratios for perchloroethylene (PCE) when using GC/qMS. Heckel et al²⁶ examined the use of different groups of ions and GC/qMS instrument configurations for an optimized CI-CSIA of chloroform and carbon tetrachloride. Of the 50, 70 and 100 ms dwell times that they investigated, using the two most abundant ions (m/z 85 and 83) for CI-CSIA of chloroform, they reported that 70 ms gave the best precision, whereas Breider et al¹⁸ selected 50 ms as the optimal dwell time for that compound.

As indicated in Table 4, the range of S/N for purging time was 4.52, by far the largest range, indicating that purging time has the highest effect on the precision of the method. Based on our results, a purging time of 5 min was optimum with the smallest RSD in isotope ratio (Table S3, supporting information). The influence of purging time on isotope analysis has been studied by several researchers. For instance, for stable carbon isotopes, Jochmann et al³⁰ investigated the effect of different purging times on extraction efficiency for various organic compounds using a continuous-flow P&T system coupled to

TABLE 3 Taguchi design of experiments (L_9) and the resulting chlorine isotope ratios and signal-to-noise ratios (S/N). PT = purging time, TT = transfer time, DT = dwell time

Exp#	PT [min]	TT [s]	DT [ms]	Cl isotope ratio Rcorr* ×1000				S/N
				Sequence 1	Sequence 2	Sequence 3	Sequence 4	
1	5	80	20	324.93	323.64	324.59	324.34	55.492
2	5	120	60	325.50	324.14	324.63	324.46	54.944
3	5	160	100	325.38	324.17	324.86	324.65	56.237
4	10	80	60	325.99	324.11	325.02	324.96	52.501
5	10	120	100	325.98	324.27	325.01	324.84	53.211
6	10	160	20	323.43	321.67	324.40	324.39	48.038
7	15	80	100	326.38	324.35	325.00	325.07	51.657
8	15	120	20	325.56	323.29	324.44	324.37	50.891
9	15	160	60	325.97	323.63	324.68	324.67	50.569

Rcorr* Raw R-values were corrected for a given peak area based on slopes in Figure S1 (supporting information).

TABLE 4 Summary of means of S/N per level per parameter. Each mean was determined from the S/N (Table 3) based on the L_9 array. Using three means of S/N per parameter, the ranges are calculated and ranks assigned. PT = purging time, TT = transfer time, DT = dwell time

Level	Parameter			Mean of S/N per parameter per level		
	PT (min)	TT (s)	DT (ms)	PT (min)	TT (s)	DT (ms)
1	5	80	20	55.56	53.22	51.47
2	10	120	60	51.25	53.02	52.67
3	15	160	100	51.04	51.61	53.70
Range				4.52	1.60	2.23
Rank				1	3	2

IRMS. They showed that longer purging times resulted in better extraction efficiencies and that isotope ratios are affected mainly by too short purging times. Zwank et al²⁸ as well as Beneteau et al⁴⁵ reported increased extraction efficiencies for longer purging time.

3.2 | Further optimization of purging time

In our Taguchi approach we focused on the highest precision of the isotope results by optimizing transfer time, dwell time, and purging time. However, we observed in our experiments that, although a purging time of 5 min had the highest precision, the extraction efficiency was lowest compared with purging times of 10 and 15 min, which have similar efficiencies (Figure S2, supporting information). Low extraction efficiencies could influence isotope ratios.^{28,30,45}

We therefore conducted additional experiments with purging times of 5, 7, 8, 9 and 10 min, each in triplicate ($\text{CHCl}_3 = 100 \mu\text{g/L}$), wherein samples were randomly sequenced to refine the method. In these measurements, a transfer time of 120 s and dwell time of 100 ms were fixed. The results are presented in Figure 1.

Analyses of variance (ANOVA) on the peak areas, isotope ratios and the corresponding purging times were carried out. The results (Table S4, supporting information) support the hypothesis that there is a significant difference in the peak area (PA) between purging times of 5 and 7 min, but no further significant increase in PA was observed for the longer purging times. To further evaluate the influence of the selected purging times on isotope ratios, the chlorine isotope ratios (R) and RSDs were calculated, and are presented in Figure 2. Similar to the results shown in Figure 1, the R-values at a purging time of 5 min are systematically lower than the others, whereas the isotope ratios did not significantly change as a result of the increase of purging time from 7 to 10 min. As a peak area correction was performed, it can be concluded that the change in isotope ratios between 5 min purging time and the longer purging times was related to insufficient extraction efficiency. This result indicates that a certain degree of isotopic fractionation occurs in the purging process if the extraction of chloroform from water is not completed.

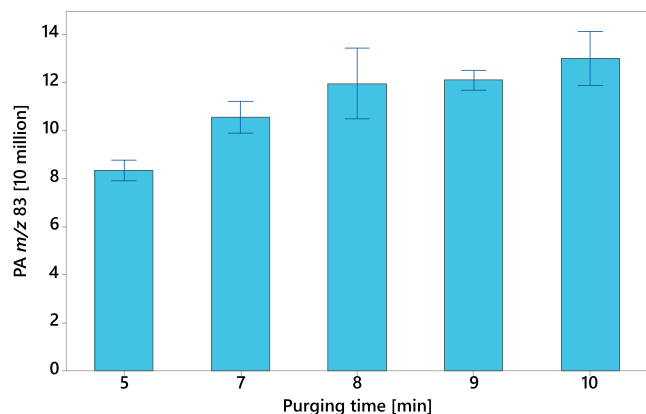


FIGURE 1 The mean of the peak area of the fragment ion m/z 83 ($n = 3$, at each purging time) versus the purging time (min) [Color figure can be viewed at [wileyonlinelibrary.com](#)]

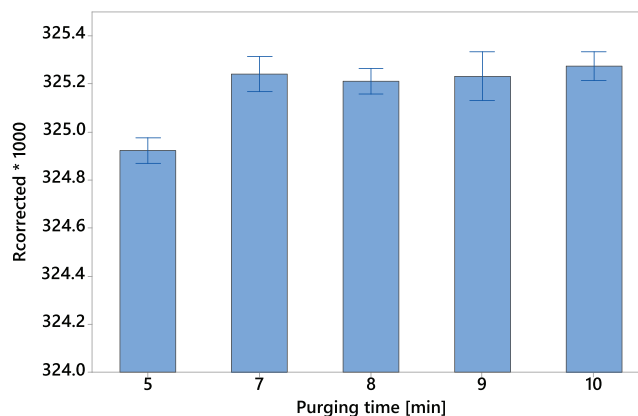


FIGURE 2 Isotope ratios (PA corrected) against the purging time [min] ($n = 3$, at each purging time). There is no significant difference for isotope ratios for purging times between 7 and 10 min. At a purging time of 8 min the highest precision ($\pm 0.28 \%$) was achieved for $100 \mu\text{g/L}$ of chloroform [Color figure can be viewed at [wileyonlinelibrary.com](#)]

The RSD of R-values among the 7 to 10 min was compared for the selection of the best purging time. As a result, a purging time of 8 min ($\text{RSD} \pm 0.28 \%$, $n = 3$) was selected as the optimum condition both to sufficiently extract chloroform from the aqueous phase and to achieve the most reproducible result for Cl-CSIA of chloroform. However, even for longer purging times, no trend in increased standard deviation was observed. Combining all the measured isotope ratios obtained between 7 and 10 min results in a precision of $\pm 0.41 \%$ ($n = 12$) indicating that a longer purging time does not influence the isotope ratio.

About 25 nmol of Cl-on column was analyzed in this investigation. The precision of 0.28 %, achieved after the optimization, is comparable with the precisions obtained using Cl-CSIA such as in Renpenning et al³² ($< 0.3 \%$) using GC/IRMS with high-temperature conversion (HTC) for selected chlorinated hydrocarbons ($< 15 \text{ nmol}$) and Heckel et al²⁶ ($\approx 0.35 \%$) using GC/qMS for carbon tetrachloride and chloroform (aqueous concentration 1–5 mg/L).

4 | CONCLUSIONS

We have applied the Taguchi design of experiments method to optimize the analytical conditions for online Cl-CSIA, using chloroform as a model compound. The Taguchi method significantly reduces the number of experiments needed to design a stable Cl-CSIA method through concurrent tests and analyses of multiple parameters and levels. With the Taguchi method, the contribution and extent of each parameter and level to the precision of the isotope ratio were examined and ranked based on the signal-to-noise ratios.

The purging time, having the highest influence on the precision of the isotope ratio, was further refined through additional experiments in order to ensure that chloroform was sufficiently extracted from the aqueous phase. As a result, 8 min of purging time, 120 s of transfer

time and 100 ms of dwell time were found to be the optimum conditions for CI-CSIA of chloroform, and a precision of 0.28 ‰ was achieved. This shows that a direct and accurate determination of the stable isotope ratios of chloroform from aqueous samples, using GC/qMS, without upstream offline chemical conversion or other online conversion techniques is possible.

The Taguchi experimental design proved to be very useful for the optimization of analytical conditions for stable isotope analyses. In principle, it can be applied to various compounds and analytical methods by selecting appropriate parameters and conditions. However, additional fine-tuning may be necessary for specific purposes.

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PEER REVIEW

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ORCID

Berhane Abrha Asfaw  <https://orcid.org/0000-0001-6036-4840>

Kaori Sakaguchi-Söder  <https://orcid.org/0000-0001-6927-661X>

Anat Bernstein  <https://orcid.org/0000-0001-9758-3254>

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SUPPORTING INFORMATION

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