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

Berhane Abrha Asfaw1\* , Kaori Sakaguchi-Söder1,2, Anat Bernstein3, Hagar Siebner3, Christoph Schüth1

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

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

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

\*Correspondence to: Berhane Abrha, Institute of Applied Geosciences,Technische Universität Darmstadt, Schnittspahn Straße 9, 64287 Darmstadt, Germany.

E-mail: berhane@geo.tu-darmstadt.de

**Supplementary Materials**

C:\Users\Berhane\Desktop\Fig S2.emf

Figure S1. A plot of chlorine isotope ratio (R, raw value\*1000) against peak area of *m/z* 83 (in 10 million) of three different concentrations of an in-house standard of chloroform that were measured in each sequence .The slope of the linear regression of each sequence was used to correct the raw-R values of nine experiments in each sequence.

**Table S1.** Statistical analysis of chlorine isotope ratios (Rcorr\* x 1000) under different transfer times in Taguchi L9 experiments

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | Chlorine isotope ratio (Rcorr\* x 1000) | | | | |
| Transfer time (sec) | n | Mean | Minimum | Maximum | SD | RSD [‰] |
| 80 | 12 | 324.87 | 323.64 | 326.38 | 0.759 | 2.3 |
| 120 | 12 | 324.71 | 323.29 | 325.98 | 0.729 | 2.2 |
| 160 | 12 | 324.33 | 321.67 | 325.97 | 1.08 | 3.3 |

Rcorr\* Raw R-values were corrected for a given peak area based on slopes in Figure S1

**Table S2.** Statistical analysis of isotope ratios under different dwell times in Taguchi L9 experiments

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | Chlorine isotope ratio (Rcorr\* x 1000) | | | | |
| Dwell time (ms) | n | Mean | Minimum | Maximum | SD | RSD [‰] |
| 20 | 12 | 324.09 | 321.67 | 325.56 | 0.985 | 3.0 |
| 60 | 12 | 324.81 | 323.63 | 325.99 | 0.728 | 2.2 |
| 100 | 12 | 325.00 | 324.17 | 326.38 | 0.661 | 2.0 |

Rcorr\* Raw R-values were corrected for a given peak area based on slopes in Figure S1

**Table S3.** Statistical analysis of isotope ratios under different purging times in Taguchi L9 experiments

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | Chlorine isotope ratio (Rcorr\* x 1000) | | | | |
| Purging time (minute) | n | Mean | Minimum | Maximum | SD | RSD [‰] |
| 20 | 12 | 324.61 | 323.64 | 325.50 | 0.521 | 1.6 |
| 60 | 12 | 324.50 | 321.67 | 325.99 | 1.15 | 3.6 |
| 100 | 12 | 324.78 | 323.29 | 326.38 | 0.892 | 2.7 |

Rcorr\* Raw R-values were corrected for a given peak area based on slopes in Figure S1

C:\Users\Berhane\Desktop\Fig S2.emf

**Figure S2**. Peak area (PA) at *m/z* 83 of chloroform, under different purging conditions (n=3). Peak area at 5 minutes purging time is significantly lower than at 10 or 15 minutes, indicating lower extraction efficiency.

**Table S4.** Analyses of variance (ANOVA) of results between the various purging times and their effect on the Peak area (PA) and the PA corrected isotope ratios. There is no significant difference in PA and isotope ratio for purging times between 7 and 10 minutes.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Peak area versus purging time | | | | Rcorr versus purging time | | | |
|  | 5 vs7 | 7 vs 8 | 7 vs 9 | 7 vs 10 | 5 vs. 7 | 7 vs 8 | 7 vs 9 | 7 vs 10 |
| F-test | 0.579 | 0.338 | 0.548 | 0.521 | 0.690 | 0.689 | 0.689 | 0.790 |
| T-test | 0.048 | 0.434 | 0.118 | 0.133 | 0.025 | 0.765 | 0.946 | 0.749 |
| Statistically Significant? | yes | no | no | no | yes | no | no | no |