Talanta 188 (2018) 192–198

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Using standard additions to improve extraction and quantification of inositol hexakisphosphate in sediment samples by ion chromatography electrospray ionization mass spectrometry

Constantin Recknagel[a,*], Pernilla Thelin[b], Marion Abraham[a], Detlef Schulz-Bull[a], Per J.R. Sjöberg[b]

[a] Leibniz Institute for Baltic Sea Research Warnemuende, Department of Marine Chemistry, Seestrasse 15, 18119 Rostock, Germany
[b] Department of Chemistry – BMC, Uppsala University, P.O. Box 599, S-75124 Uppsala, Sweden

ARTICLE INFO

Abbreviations:
InsPnInositol phosphates
TOCTotal organic carbon
TPTotal phosphorus
TOPTotal organic phosphorus
DWDry weight

Keywords:
Inositol phosphates
IC-ESI-MS
Standard addition
Sediment

ABSTRACT

Several key aspects for the analysis of inositol hexakisphosphate ($InsP_6$) have been investigated in order to establish a suitable method for the study of sediment samples from different aquatic systems. Apparent matrix effects for the ion chromatography electrospray ionization tandem mass spectrometric detection (IC-ESI-MS/MS) method were accounted for with a standard addition approach, which also compensated for variation in extraction efficiency. Several parameters of the extraction method were optimized to improve the extraction efficiency for different sediment types. We observed an improvement in the extraction efficiency between 18% and 720%. Finally, the method was used to gain first insights into the relevance of $InsP_6$ in two aquatic systems located at the German Baltic coastal area. $InsP_6$ was detected in several sediment samples with concentrations between 2.3 and 15.2 µg $InsP_6$-P/g dry weight (DW).

1. Introduction

Phosphorus (P) is a key nutrient in the aquatic environment during eutrophication. Inorganic P is easily bioavailable, however, organic forms of P have been shown to contribute to eutrophication as well [1]. There are a wide number of different organic P-compounds (OP), and they are normally determined as total organic P (TOP) [2]. Due to the fact that different OP-compounds show different bioavailability, it seems to be necessary to determine the different OP-compounds separately.

One important group of OP-compounds are the inositol phosphates (InsP). The most abundant natural representative of this compound group is *myo*-inositol hexakisphosphate (*myo*-$InsP_6$), also referred as phytic acid [3]. *Myo*-$InsP_6$ was identified to be a key component of the total organic P in most lake sediments [3]. However, the fraction of *myo*-$InsP_6$ on the total P in sediments varies considerably between evaluated aquatic systems. In earlier studies, it ranged from less than 1% [4] up to more than 50% [5].

The method mostly used for determination of inositol phosphates in sediments and soils is based on ^{31}P NMR [6–10]. Other methods are based on liquid chromatography using different separation modes such as size exclusion chromatography [11], ion-exchange chromatography [12–18] or reversed-phase chromatography, mostly with the ion-pairing mode [19–22]. Several detector strategies are utilized such as post column derivatization enabling colometric or fluorimetric detection [23,24]. Recent methods use a mass spectrometer for detection [12–14,19,21].

In a previous study [13] an LC-MS method was presented to separate and quantify different inositol phosphate isomers present in environmental samples. The method had a simple sample preparation protocol with a single NaOH-EDTA extraction step with subsequent LC-MS/MS analysis. Good separation, reliable quantitative performance, and good extraction recovery and precision were achieved for the studied samples. However, since the introduction of this method, more samples have been analyzed, and some concerns regarding matrix effects have arisen. To overcome those, the use of SPE was tested in our former studies with unsatisfactory results though [13]. Additionally, matrix effects could also influence the extraction and result in different extraction efficiencies. Therefore, it is important to use a calibration method which corrects for matrix effects during the whole procedure. Using an internal standard seems to be problematic. There is currently no isotopically-labelled $InsP_6$ commercially available which would be

* Corresponding author.
E-mail address: constantin.recknagel@io-warnemuende.de (C. Recknagel).

https://doi.org/10.1016/j.talanta.2018.05.072
Received 22 December 2017; Received in revised form 19 May 2018; Accepted 21 May 2018
Available online 22 May 2018
0039-9140/

the optimal internal standard.

Therefore, the aim of this study is to further explore matrix effects during extraction and analysis of $InsP_6$ from environmental samples, to utilize the standard addition method to overcome these matrix related uncertainties and to develop a suitable method for the quantification of $InsP_6$. Our study is based on an approach for analyzing polychlorinated biphenyls (PCB) presented by Mechlińska et al. in which the standard was added to the sediment before extraction in a larger volume to ensure its homogeneous distribution in the sediment sample [25].

Finally, our proposed method was tested on sediment samples from two estuarine systems which locate close to the German Baltic Sea Coast in the federal state of Mecklenburg-West Pomerania to gain first insights into the relevance of $InsP_6$ in these systems.

2. Experimental

2.1. Chemicals and reagents

Ammonium carbonate ($(NH_4)_2CO_3$) and 2-propanol were of analytical grade and purchased from Sigma-Aldrich (Germany). Milli-Q®-water was used for preparing aqueous solutions as well as for preparing the eluents for the HPLC. A stock solution of $InsP_6$ with a concentration of 2.54 mM was prepared by dissolving an appropriate amount of *myo*-inositol hexakis(dihydrogen phosphate) dipotassium salt (Sigma-Aldrich) in Milli-Q®-water. The concentration of $InsP_6$ in this InsP-salt was determined by calibrating the system with an in-house standard as the used salt consists of $InsP_6$ and $InsP_5$ (c = 0.64 mM, approx. 20%, Fig. S1 in the supplementary material). Details regarding the preparation and characterization of this in-house standard can be found in our previous work [13]. Working solutions were prepared from the stock solution by dilution with Milli-Q®-water. The extraction solvents were prepared by dissolving an appropriate amount of sodium hydroxide (NaOH, p.a. EKA Bohus-Sweden) and ethylenediaminetetraacetic acid disodium salt (EDTA disodium salt, Merck) in Milli-Q®-water.

2.2. Sampling

Sediment samples were collected at different stations from the Darss-Zingst Bodden Chain and the river Warnow in Mecklenburg-West Pomerania, Germany (see Table S1 in the supplementary material). Samples were collected in March 2016 with a bottom sampler according to van Veen (Hydro-Bios, Kiel, Germany) and in May 2016 (Darss-Zingst Bodden Chain) and June 2016 (Warnow) with a sediment corer (Uwitec, Mondsee, Austria). All samples taken with the corer were sliced into pieces of one cm immediately after sampling and stored at −20 °C before freeze-drying. The Oder Bank sample was taken with a multi-corer during cruise MSM50 in January 2016 and treated in the same way as the other sediment cores. For $InsP_6$ analysis the upper 2 cm were used.

2.3. Preparation of spiked sediment samples and extraction of $InsP_6$ from sediment

Two different modes of spiking sediment samples were used depending on whether the extraction efficiency (results presented in Section 3.2) or the method performance (results presented in Section 3.3) was investigated.

In-house reference sediment samples were prepared for evaluation of extraction efficiency by first adding 750 µL Milli-Q®-water containing a known amount of $InsP_6$ to approximately 100 mg of dry sediment, and incubated by shaking overnight in a 2.0 mL micro centrifuge tube (VWR International LLC, Randor, PA) to allow complete adsorption of $InsP_6$. The samples were then extracted using aqueous NaOH containing EDTA with a ratio of 5:1 at different concentrations. 1.5 mL of this extraction solvent was added to the sediment and the mixture was shaken overnight unless otherwise mentioned, using a Multi Reax (Heidolph Instrument, Schwabach, Germany). The samples were centrifuged at 10,000 rpm for 20 min (Spectrafuge 7 M, Labnet International Inc. Edison, NJ) and the solution was transferred to a 1.5 mL glass vial. Standard addition was done by transferring 270 µL of the extract into a 1.5 mL glass vial and spiking with 30 µL of $InsP_6$ in Milli-Q®-water at four different concentration levels. When the extraction was done with NaOH/EDTA (1 M/0.2 M) the solution was diluted 1:1 before analysis.

The performance of the method was tested by standard addition to the sediment before the extraction to correct for incomplete extraction (Section 3.3). Therefore, four samples at approximately 100 mg sediment were weighed into 2.0 mL tubes. The four subsamples were spiked with different $InsP_6$ concentrations, i.e. ~ 0.1 µmol $InsP_6$/g sample as the spiking concentration and 0%, 50%, 100% and 150% of this as standard addition. The samples were shaken overnight to allow complete adsorption. Afterwards the samples were extracted overnight with NaOH/EDTA (1 M/0.2 M). Before instrumental analysis the extracts were diluted 1:1 with Milli-Q®-water.

Samples from the Warnow and the Darss-Zingst Bodden chain were analyzed with a qualitative screening before quantification to check for occurrence of $InsP_6$. The samples were extracted as described before and the extract was then injected directly into the LC-system. All samples where $InsP_6$ was detected were then conducted to further quantitative analysis using the described standard addition method. However, no dilution of the NaOH/EDTA-solution (1 M/0.2 M) was done after extraction as it was shown that there was no negative influence of the high NaOH-concentration on the performance of the column and the mass spectrometer.

2.4. Instrumentation

Samples were analyzed for $InsP_6$ using a 3200 Q-Trap MS/MS system (AB Sciex, Concord, ON, Canada) equipped with a 1260 Infinity LC system (Agilent Technologies, Waldbronn, Germany). The LC-MS/MS method was basically the same as previously reported [13].

Long term stability of the chromatographic column was improved by incorporating an injection program. After elution of $InsP_6$ Milli-Q®-water (10 µL), NaOH-EDTA (50 µL: 0.25 M/0.05 M) and formic acid (50 µL, 0.1%) were injected for cleaning the column. Long term stability for the MS-detection was achieved by reducing the turbo gas flow to 10 psi and turning off the nebulizer gas and high voltage after $InsP_6$ elution.

Evaluation of the data was done with the Analyst® 1.4.2 software (AB Sciex). $InsP_6$ was detected by using the multiple reaction monitoring mode (MRM) as reported previously [13] and all MRM transitions were summed before manual peak integration.

The instrumental method was carefully characterized in terms of linearity and reproducibility. Furthermore, the limit-of-detection (LOD) and the limit-of-quantification (LOQ) were evaluated as described in the supplementary materials. As a result, the method was characterized by a LOD of 0.2 µM $InsP_6$ and a LOQ of 0.6 µM $InsP_6$.

3. Results and discussion

3.1. Method characteristics

The work presented in this paper is based on our previously published work [13]. When the method was applied to a larger set of samples, matrix effects became of concern for certain types of samples. Matrix effects can be caused by a multitude of reasons, and two main strategies are used to reveal if they are present: namely the post-extraction addition technique; and the postcolumn infusion technique [26]. One potential problem could be co-elution of components that in the end influence the detection of the analytes of interest. To investigate this, we adopted the postcolumn infusion technique that was originally presented by Bonfiglio et al. [27]. By continuous infusion of a standard solution containing $InsP_6$ (5 µL/min, 250 µM) and mixing it with the LC-