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ARTICLE TYPE

Quick and robust method for trace determination of MeHg in rice and rice products without derivatisation

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Abstract

Recent studies of Methylmercury (MeHg) in rice have shown that rice grown on mercury contaminated soil contributes to the human MeHg intake similar to a fish diet. Trace levels of MeHg in biological samples are often determined via a complex multi-stage process following EPA method 1630. We developed a simple and cost effective method suited for food quality monitoring based on a simple
10 sample preparation procedure and the subsequent analysis of the sample by online preconcentration - high performance liquid chromatography-cold vapor atomic fluorescence spectrometry (SPE-HPLC-CV-AFS). The reliability of this method for MeHg in rice and rice products in the low ppb range was investigated for 4 different rice product samples. At present, no CRM for MeHg in rice or rice products is available. Therefore we cross-validated our method against standard addition and species-specific isotope dilution gas chromatography inductively coupled plasma mass spectrometry (SSID-GC-ICP-MS), which showed no significant difference versus the
15 external calibration with SPE-HPLC-CV-AFS. Potential species interconversion during sample preparation and measurement was ruled out by using a spike of isotopically enriched inorganic mercury. The preconcentration HPLC-CV-AFS developed in our work has proven to be a robust, fast, cost efficient, competitive and reliable method for MeHg speciation in rice and rice products with a limit of detection of 0.12 µg kg^{-1} and a reproducibility comparable to the SS-ID-GC-ICPMS method which is sufficient for the determination of MeHg concentration in the four market rice samples. The concentrations of MeHg ranged from 1.6 to 2.7 µg kg^{-1}.

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Introduction

Rice is a staple food and provides 20 % of the world's dietary energy supply.[1] Rice grains can however accumulate methylmercury (MeHg) from paddy fields, and Feng et al. could
25 show that rice grown in mercury contaminated areas has a similar mercury contribution as a moderate fish diet.[2] The precise and accurate determination of trace amounts of MeHg in rice requires a method that fully liberates the MeHg bound in the rice grain, while conserving the analyte speciation.
30 MeHg concentrations are usually in the low ppb range, and only in contaminated areas can MeHg amount to > 100 µg kg^{-1}, therefore, a speciation method must be sensitive and selective.[3] An established method for MeHg analysis in rice is described by Liang et al.[4] The protocol involves the digestion of rice (up to 0.5
35 g) in KOH/methanol, acidification, extraction into dichloromethane and back-extraction into water. Subsequently, MeHg is ethylated by adding sodium tetraethylborate and the ethylated MeHg purged onto Tenax traps and finally thermally desorbed for analysis via GC-AFS, following the US-EPA
40 method 1630.[5-7] Horvat et al. used a leaching process with KBr/H$_2$SO$_4$ for the extraction of MeHg from rice followed by an extraction of the MeHgBr into toluene. A back-extraction of MeHgBr into aqueous L-cysteine solution and a final extraction into benzene

45 concludes the preparation. Analysis is done by direct injection of the benzene solution into GC-ECD (gas chromatography with electron capture detector).[8]

These established methods all require a sequence of several time-consuming steps and make the mercury speciation analysis of rice
50 rather cumbersome.

MeHg is a well-known neurotoxin, and limits for MeHg consumption were defined, e.g. by the European Food Safety Authority (EFSA): in 2012, a tolerable weekly intake (TWI) of
55 1.3 µg kg^{-1} per body weight was established for MeHg, while the TWI for inorganic mercury was set to 4 µg kg^{-1} per body weight.[9] This mirrors the growing interest to determine the Hg speciation in other food commodities, especially rice: China was the first country to set a limit for Hg concentration in rice of 20 µg kg^{-1},
60 and other countries may follow this example and thus Hg analysis in rice may soon be imperative for the import/export of rice.[10] In this study, we focused on the development of a trace level (< 5 µg kg^{-1} MeHg) method, which is robust, cost effective, reliable and accurate as well as easy to use for food safety monitoring.
65 This method will be fit to be used on a large scale, e.g. in food basket studies of MeHg in rice and rice products. The basis for this method is a HPLC-CV-AFS approach that we described in earlier studies[11,12], which has proven to be robust, simple and cost-effective for water, sediment, and biological tissues (e.g.

Seaweed IAEA-140, dogfish muscle DORM-3, dogfish liver DOLT-2 and DOLT-4 and Lobster hepatopancreas TORT-2). However, this method needed to be modified for the analysis of rice, mainly concerning the sample preparation. The low concentrations made it necessary to use relatively high sample amounts around 300 mg, which yielded a highly viscous digest, which could not be injected into the AFS system. A second extraction step was therefore inserted as described below in the procedures. Furthermore, the sample contained a high amount of organic matrix, causing extensive foaming in the gas-liquid separator (GLS). This was suppressed by using a mechanical device within the GLS, in the form of a plastic ring around the glass tube through which the purge gas is injected; the device can be seen in section A of the supporting information, Figure S1. The main obstacle is however the validation of this method; while numerous certified reference materials (CRM) are available for a variety of matrices, there is none for rice, rice products or other cereals. Therefore, it is not possible to determine the accuracy of the analytical method for rice. Here, we cross-validated the SPE-HPLC-CV-AFS method with SS-ID-GC-ICPMS, and used standard addition as well as Hg^{2+} isotope spikes into the rice matrix to determine any artificial formation or disintegration of MeHg during sample preparation and analysis.

Experimental

Standards, Reagents and Samples

10,000 mg L^{-1} MeHg (as Hg) stock solution was prepared by dissolving methylmercury chloride (Sigma Aldrich, UK) in methanol. Further dilutions were carried out in 0.3 M HCl to a intermediate standard of 1 mg L^{-1}. This intermediate standard was diluted daily to a 1 µg L^{-1} MeHg solution, from which the external calibration standards (2.5, 5, 10 and 20 ng L^{-1}) were prepared in 0.12 M HCl (AnalR grade, VWR, UK).

Isotopically enriched $Me^{201}Hg$ was prepared according to literature from ^{201}HgO [13] and stock solutions of 10 mg L^{-1} were stored at -20 °C prior to use. This solution was further diluted for species specific isotope dilution.

^{199}Hg solution was prepared by dissolving ^{199}HgO (Oak Ridge National Lab, USA) in 1.5 M hydrochloric acid and further dilutions were carried out in 1.5 M HCl for the experiments.

10 mg L^{-1} inorganic mercury standard was purchased from AccuStandard® (AccuTraceTM Reference Standard, USA). Dilutions were carried out in 0.3 M HCl to a stock standard of 1 mg L^{-1}. From this solution, a daily intermediate standard of 10 µg L^{-1} was prepared which was the basis of the calibration standards of 10, 20, 40 and 60 ng L^{-1} for the T-Hg analysis.

Double distilled water was produced by an Aquatron water still A4000D (Bibby Scientific Limited, Stone, UK).

Methanol (AnalR grade, VWR, UK) and ammonium pyrrolidine dithiocarbamate (~99%, Sigma Aldrich, UK) are used for the preparation of the mobile phase for HPLC separations. 0.01 M Titrisol® bromine solution (Merck, Darmstadt, Germany) and 1.2 M HCl (AnalR grade, VWR, UK) were used as oxidants and 2% (m/v) tin(II)chloride (tin(II)chloride dihydrate (98%; Alpha Aesear, UK) and 1.2 M HCl (v/v) (AnalR grade, VWR, UK) in

double-distilled water for the reductant solution. The preconcentration material is a mixture of thiol and thiourea bound to silica, and is commercially available (PS Analytical, UK; PSA L820K005); preconcentration material is replaced after 100 runs.

Tetramethylammonium hydroxide as a 25 % (m/v) solution (99.9999% (metals basis), Alfa Aesear, UK) is used for digestion of the rice sample and 37 % (v/v) HCl (AnalR grade, VWR, UK) is used for the second digestion step.

0.5 M acetic acid-acetate buffer at pH 3.9 is prepared from acetic acid (100%, HiPerSolv® Chromanorm, VWR, UK) and the pH adjusted with NaOH (Laboratory reagent grade, Fluka Analytical, UK). Sodium tetrapropylborate (Chemos, Germany) is used for propylation of the mercury species and 2,2,4-trimethylpentane (Chromasolv® Plus, for HPLC, ≥ 99.5%, Sigma Aldrich, UK) is used for extraction after propylation.

Digestion for total Hg is performed with 70 % nitric acid (PrimarPlus-Trace analysis grade, Fisher Scientific, UK).

Iso-DiscTM PTFE 25-4 Filters (25 mm × 0.45 µm, Sigma Aldrich, UK) are used for filtration of the digested rice samples.

For consistency, all MeHg concentrations are reported as µg kg^{-1} Hg.

Instrumentation

GC-ICP-MS. A gas chromatograph (Agilent 6980 GC, Agilent Technologies, USA) is coupled with an in-house built heated transfer-line to an ICP-MS (Agilent 7500c ICP-MS, Agilent Technologies, USA), allowing the introduction of a liquid internal standard mixed to the GC effluent via a cyclonic spray chamber. The detailed set-up of the coupling is described elsewhere.[11]

SPE-HPLC-CV-AFS. The HPLC-AFS system used in this study has been described in detail in recent publications by Brombach et al.[11, 12] Briefly, the HPLC-preconcentration system can be described as an online solid phase extraction (SPE) consists of a 6-port valve where the sample loop is replaced by an HPLC column (Empty HPLC column, 2.1 × 30 mm, 2 µm frits, (Thames Restek, UK)) filled with a thiol/thiourea silica material (PSA L820K005; particle size 40-63 µm). 35 mL of the acidified sample is pumped across the preconcentration column with a HPLC pump at a speed of 5 mL/min (HPLC 1: Spectra-Physics Analyical P100, UK) and the mercury species are retained on the preconcentration material. The 6-port valve is switched and a mobile phase (1.5 mM ammonium pyrrolidine dithiocarbamate in 75 % (v/v) methanol), pumped by a second HPLC pump (HPLC 2: Kontron 420, Kontron Instruments, UK), elutes the mercury species and separates them on a C8 column (Eclipse XDB C8 (4.6 × 150 mm, 5 µm), Agilent, USA). During elution, the valve is turned again and the next sample can be loaded onto the preconcentration column; thus the sample throughput is four per hour. The post column treatment consists of the addition of bromine as oxidant and UV-light to support the conversion of organic mercury to divalent mercury. Divalent mercury is reduced with acidic tin(II)chloride, and Hg^0 separated from the solution in the gas-liquid-separator. A PSA Millenium Merlin atomic fluorescence spectrometric detector (P.S. Analytical Ltd.,

Orpington, UK) is used for Hg quantification. A modification of the gas-liquid-separator (see **Figure S1** in the supporting information) is necessary in order to get a clean chromatogram (Figure 1).
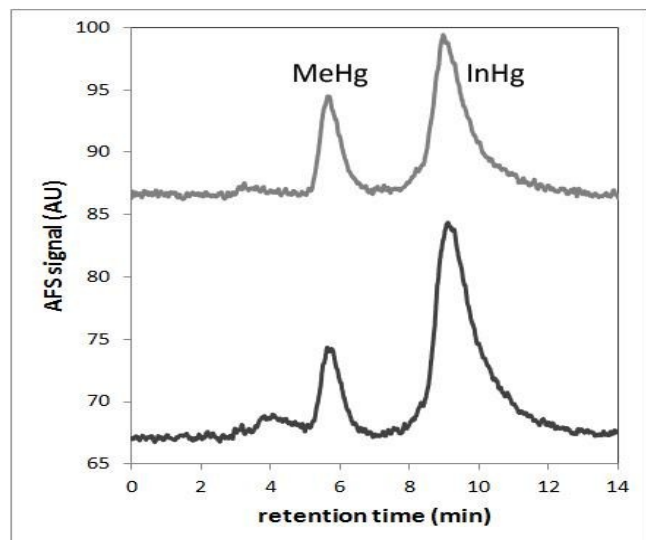

Figure 1: HPLC-CV-AFS chromatogram for rice 2 containing 1.56 µg g^{-1} MeHg (dark grey) and 5 ng L^{-1} MeHg standard (light grey, shifted by 20 units), corresponding to 0.197 ng MeHg (as Hg) for the rice sample and 0.175 ng MeHg (as Hg) in the standard.

Microwave digestion

A Mars 5 microwave (CEM Corporation, USA) was used for the extraction/digestion of the samples. A closed vessel digest using PTFE vessels (XP-1500 plus, CEM Corporation, USA) was used for T-Hg analysis, and an open vessel digest extraction was performed in glass vials (22 mL, Supelco, USA) for mercury speciation.

Centrifugation of samples was achieved by using a Micro Centaur centrifuge (MSE, UK) at 13226g (13000 rpm) for 10 min, or an ALC 4218 centrifuge (ALC International S.R.L., Italy) at 1650g (3500 rpm) for 10 min.

Procedures

Sample preparation. Rice samples were ground into a fine powder with a Coffee grinder (F203 Grinder, Krups, Germany) and stored in closed glass vessels at room temperature.
Rice flour standard reference material 1568a (NIST, USA) is specified for 5.8±0.5 µg kg^{-1} T-Hg and was used for quality assurance of the T-Hg analysis.

Sample preparation for T-Hg analysis. 0.5 g rice was accurately weighed and pre-digested overnight with 7.5 mL 70 % HNO$_3$ in PTFE vessels for pressurised microwave digestion and then microwaved for 1h at 140 °C. 2.5 mL of the digest was taken and remaining nitrous oxides removed and diluted to 20 mL. T-Hg in the digested samples was analysed using CV-AFS (PSA

Millennium Merlin, PSA 10.025, PS Analytical, UK). 2 % (m/v) SnCl$_2$ in 1.2 M HCl was used as a reductant with a flow-rate of 5 mL min^{-1} and a sample flow-rate of 10 mL min^{-1}.

Sample preparation for MeHg analysis by SPE-HPLC-CV-AFS. Approx. 0.3 g rice was microwave extracted with 3 mL TMAH (25 % (m/v)) for 20 min at 55 °C and 20 min at 60 °C @ 1600W in open vessels. The digest was allowed to cool down and 2 mL HCl (37%) carefully added. After shaking, the digest was heated again in the microwave for 20 min at 55 °C and 20 min at 60 °C. The suspension was centrifuged at 13,226g (13,000 rpm) for 10 mins and the supernatant filtered through 0.45 µm filter discs. Approximately 60% of the clear digest solution was aliquoted into a clean vessel, and was topped up to 50 mL, of which 35 mL were analyzed by SPE-HPLC-CV-AFS. All dilution factors were calculated by weight.

Sample preparation for MeHg analysis by SS-ID-ICP-MS. Approx. 0.6 g rice was extracted with 6 mL 25 % (m/v) TMAH in the microwave (open vessel) for 20 min at 55 °C and 20 min at 60 °C. 1.380 mL HCl (37 %) was added to the cooled digest to adjust the pH to a range between 4 and 6, and re-digested in the microwave for 20 min at 55 °C and 20 min at 60 °C. The suspension was centrifuged at 13,226 g (13,000 rpm) for 10 min, and the supernatant spiked with Me^{201}Hg$^+$ and left standing for 10 min for equilibration, buffered with 5 mL 0.5 M acetic acid / sodium acetate (pH 3.9) and overlaid with 1 mL 2,2,4-trimethylpentane. 1 mL 1 % (m/v) sodium tetra(n-propyl)borate was then added to the solution and the mixture shaken for 10 min to extract the derivatized mercury species into the organic layer. The mixture was centrifuged at 1650g (3500 rpm) to improve phase separation and approximately 0.5 mL of the organic layer was recovered and transferred into autosampler vials. The organic solvent was reduced with a stream of air to approx. 0.02 - 0.05 mL, and 2 µL injected into the GC-ICP-MS system.

Samples

For the method development and validation, four different rice samples were used which were two rice grain samples (sample 1 and 4) and two baby rice samples (sample 2 and 3), purchased in local shops in Aberdeen, UK.

Validation approach of the SPE-HPLC-CV-AFS method for MeHg in rice samples

Validation of a new method is usually depending on certified reference materials, which can give reassurance that the values obtained are accurate and sufficiently precise for the purpose. For MeHg determination in rice, there is however no CRM available. Therefore, we used different orthogonal analytical strategies and methods, which can in turn be used to cross-validate the results obtained with the SPE-HPLC-CV-AFS mercury speciation method.

Firstly, we present data obtained with SPE-HPLC-CV-AFS using an external calibration approach. For one sample, a standard addition approach (spiking into the original sample) was used to determine any matrix effects or possible MeHg degradation that may occur. Secondly, we used an orthogonal analytical method based on species specific isotope dilution, using GC-ICPMS analysis (SS-ID-GC-ICPMS). These results should be able to detect any bias compared to the new method. Thirdly, on one sample, we used an isotopically enriched inorganic Hg spike ($^{199}Hg^{2+}$) at 20-fold excess to determine any artificial MeHg formation from inorganic Hg in the sample. These different approaches are described and evaluated below. Finally, four different rice samples were submitted to each HPLC-CV-AFS and SS-IDMS-GC-ICPMS for comparison. In addition, T-Hg was determined for all samples. Below, the different analytical approaches along with their results are shown and discussed.

Results and Discussion

Development of a new sample preparation approach for MeHg analysis in rice matrix

Sample preparation for MeHg in biological matrices, especially fish, is often accomplished using an alkaline digestion with TMAH.[14] This procedure has proven to destroy the organic tissue while keeping the MeHg species intact. Rice is however a matrix with very dense organic matter, and with our initial approach of using TMAH it was found that this digestion alone did not destroy the matrix sufficiently. When digesting rice grain in a proportion of 1:10 (0.3 g rice to 3 mL TMAH), the digest obtained was very viscous and not amenable to direct analysis via HPLC-CV-AFS. We added a second digestion step which involved the addition of HCl, as described above in the sample preparation paragraph which allowed a leaching of the mercury species from the organic digest. The HCl leachates can then directly be injected into the SPE-HPLC-CV-AFS system for analysis after filtration and dilution.

Determination of T-Hg in Rice

T-Hg was determined with CV-AFS after closed vessel microwave digestion, and measured using straightforward CV-AFS. Additional to the Hg speciation approaches, T-Hg was determined in each sample to assess the overall mass balance. This was typically done by subtraction of the MeHg concentration from the T-Hg value. Validation of the T-Hg measurement was performed using rice flour standard reference material 1568a (NIST, USA), specified for 5.8 ± 0.5 μg kg^{-1} T-Hg. T-Hg was determined with a recovery of 98.3 ± 8.8 % in the standard reference material. T-Hg was measured for all samples prior to MeHg speciation. The concentration of T-Hg varied between 1.7-3.3 μg kg^{-1}. The three replicates from the four rice samples varied between 2.0-14.7 % for the relative standard deviation (RSD).

Direct MeHg analysis in rice with SPE-HPLC-CV-AFS using an external calibration

The external calibration for MeHg was produced in a concentration range of 0 to 20 ng L^{-1}, with 2.5 ng L^{-1} as the lowest standard. Linear correlation factors of typically $R^2 > 0.998$ are obtained with a preconcentration volume of 35 mL, resulting in a limit of detection of 0.4 ng L^{-1}, or 0.014 ng MeHg absolut. (**Figure S2a** in the supporting information shows overlayed chromatograms for the calibration of MeHg from 0 to 20 ng L^{-1}, while **Figure S2b** shows a typical linear calibration). The results for the four rice samples are shown in **table 1** and the concentrations vary between 1.56 and 2.69 μg kg^{-1} MeHg in the market rice samples. The relative standard deviation for three replicates varied between 5.2-16.7 % and seem to be independent of the MeHg concentration, which may be attributed to the concentrations being close to the limit of quantification.

Standard addition approach for MeHg analysis in Rice. 300 mg of baby-food rice sample 2 was spiked with 50, 100 and 150 μL of a 10 μg L^{-1} MeHg standard solution and digested according to the protocol for analysis via preconcentration HPLC-CV-AFS. The measured peak areas for the different spiked samples were plotted against the added concentration of MeHg and the concentration of MeHg in solution calculated. The concentration of MeHg in the original sample was calculated by applying the dilution factors. A good linearity with a correlation coefficient of 0.9996 could be achieved (see Figure 2). The concentration determined with the external calibration is 1.56 ± 0.16 μg kg^{-1} (n=3 error of 10.6 % RSD from three biological replicates) for MeHg, compared to a value of 1.67 ± 0.08 μg kg^{-1} (n=3 for each spiked concentration, 4.8 % RSD) with standard addition (section C in the supporting information shows the calculation of SD for the standard addition experiment). The results for rice sample 2 determined with standard addition and external calibration overlap within the range of the standard deviation, therefore we can exclude any significant matrix effect during the digestion procedure (t-test, p>0.05).
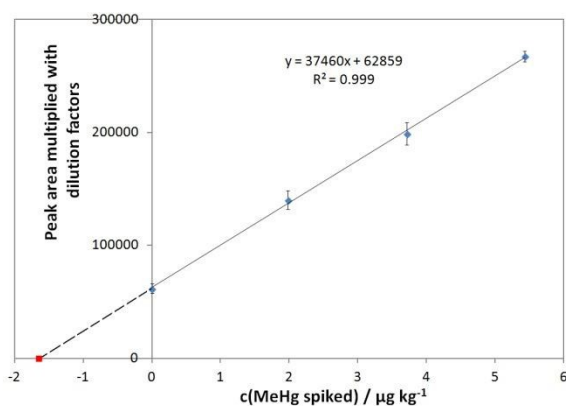

Figure 2: Standard addition for rice 2; peak areas were multiplied with dilution factors to show concentrations as μg kg^{-1} original rice sample. (error bars = 1 SD, n=3 analytical replicates). The dashed line is an extrapolation of the linear trendline for the data

points and the red point marks the concentration of MeHg in rice 2.

SS-ID-GC-ICPMS approach for MeHg analysis in Rice

SS-ID-GC-ICPMS was used as the *gold standard* method for the speciation of mercury, but proved to be less sensitive than the AFS method, with peaks hardly distinguishable in the GC-ICPMS chromatogram. Doubling of sample mass and reduction of the organic phase (approx. 500 µL 2,2,4-trimethylpentane) to ~ 20 - 50 µL of which only 2 µL were injected into a GC-ICP-MS yielded substantial peaks for both MeHg (as MeHgPr) and Hg^{2+} (as Pr_2Hg), as shown in **Figure S3.** The detection limits are around 1 µg L^{-1}, however for the precise peak integration and isotope ratio determination, a higher concentration is often required. The absolute amounts injected into the GC-ICPMS were rather close to the LOD, which is reflected in relatively high SDs for MeHg determination of a minimum of 7.7 % RSD. The results for the four rice samples determined by GC-ICPMS are quoted in **table 2**. The precision for the four rice samples expressed in one SD (n=3) varied from 7.7 – 15.9% RSD, which may reflect the heterogeneous nature of MeHg in the rice, since those rice samples with lower RSD gave also lower RSD for the SPE-HPLC-CV-AFS method and for T-Hg.

In order to evaluate possible degradation of MeHg during the digestion step, for one rice sample (rice 1) the isotopically enriched MeHg spike was added prior digestion. The result obtained by spiking into the sample gave a MeHg concentration of 2.73 ± 0.24 µg kg^{-1}, compared to 2.61 ± 0.25 µg kg^{-1} when the spike was added into the extract, which is not significantly different. Subsequently, all samples were therefore spiked after digestion.

SS-ID-GC-ICP-MS requires a substantial amount of sample preparation after digestion. This involves that the sample is digested, spiked with isotopically enriched MeHg, centrifuged and filtered, buffered, pH adjusted to 3.9, overlayed with isooctane (2,2,4-trimethylpentane), propylation reagent added and shaken manually for 10 mins. After the isolation of the organic layer, a time-consuming step of solvent evaporation is necessary to ensure that the isotope signals are sufficiently high to perform reproducible and precise peak integration. In contrast, online SPE-HPLC-CV-AFS requires only digestion, centrifugation, filtering and dilution prior to analysis and no derivatisation is necessary.

Comparison of results obtained for the different quantification approaches

Table 1 shows a comparison of five rice samples which were analysed with SPE-HPLC-CV-AFS and SSID-GC-ICPMS. The third column shows the T-Hg values with T-Hg usually being higher than MeHg, however in sample 3, MeHg was determined to be higher than T-Hg. The excess is approx. 10 %, which lies within the determination uncertainty. MeHg concentrations have been reported as high as 95% of T-Hg. Here, the rice samples vary between 66 and 110 % of MeHg. The higher MeHg %age in samples 2 and 3 may be associated to additional processing, as these samples are baby-rice products. These processes may

involve washing or cooking in which some Hg^{2+} might be lost opposed to MeHg which might be bound differently, e.g. in proteins. Thus, inorganic Hg might be lost during this process, leading to higher MeHg %ages. Earlier (unpublished) work in our group suggested that Hg^{2+} can be partly removed by washing; this will be further investigated.

A paired T-test of the results for preconcentration HPLC-CV-AFS vs SSIDMS-GC-ICP-MS yielded p=0.5581 (n=4), showing that the two methods give data which are not significantly different.

Table 1: Concentration of MeHg and T-Hg determined in four different rice samples; MeHg was determined with two orthogonal analytical methods (n=3, error as one SD and expressed in % RSD).

Rice sample	c(MeHg) / µg kg^{-1} (SPE-HPLC-CV-AFS)	c(MeHg) / µg kg^{-1} (SSID-GC-ICPMS)	c(T-Hg)/ µg kg^{-1} (CV-AFS)
1	2.69 ± 0.26 (9.5 %)	2.61 ± 0.25 (9.7 %)	3.26 ± 0.20 (6.3 %)
2	1.56 ± 0.17 (10.6 %)	1.62 ± 0.26 (15.9 %)	1.70 ± 0.22 (13.2 %)
3	2.09 ± 0.11 (5.2 %)	2.14 ± 0.17 (7.7 %)	1.90 ± 0.04 (2.0 %)
4	1.58 ± 0.26 (16.7 %)	1.64 ± 0.18 (10.7 %)	2.39 ± 0.45 (14.7 %)

Enriched isotope spiking for determination of MeHg artifact formation during the digestion process

It is a well established fact that some sample digestion and preparation methods induce artificial MeHg formation from inorganic mercury,[15, 16] leading to an overestimation of MeHg in the sample. This was first discovered in the late 1990s for water vapor distillation applied to sediments and soil samples, which are usually very low in MeHg with a ratio of ~ 1:100 compared to inorganic Hg.[16] This effect was attributed to organic matter in the soil, leading to abiotical MeHg formation during sample preparation. The rice matrix is of course 100% organic, and TMAH cannot break the matrix down completely, as described above.

Therefore we used an enriched isotope spike of $^{199}Hg^{2+}$ to reveal any artificial MeHg formation by comparison of the natural Hg isotope ratios with the isotope ratio determined on the MeHg signal determined with GC-ICPMS. For this, $^{199}Hg^{2+}$ enriched at 98 % was spiked into the sample in 20-fold excess of the total mercury concentrations, and the resulting MeHg isotope ratios were calculated from the MeHg peak.

Table 2: Rice sample 1 spiked with $^{199}Hg^{2+}$; ^{199}Hg spike concentration at 20 fold excess over T-Hg.

	Hg Ratio for MeHg	
	(n=3)	natural ratio
^{199}Hg / ^{200}Hg	0.748 ± 0.042	0.727
^{199}Hg / ^{201}Hg	1.280 ± 0.108	1.272
^{199}Hg / ^{202}Hg	0.572 ± 0.038	0.563
^{200}Hg / ^{201}Hg	1.687 ± 0.149	1.75
^{200}Hg / ^{202}Hg	0.753 ± 0.050	0.775
^{201}Hg / ^{202}Hg	0.442 ± 0.037	0.443

Table 2 shows the isotope ratios of the MeHg peak from the rice sample spiked with inorganic ^{199}Hg. The hypothesis is that due to the excessive Hg^{2+} spike, the isotope ratios involving ^{199}Hg would dramatically change from the natural isotope ratios. This however cannot be seen, as the resulting isotope ratios do not differ significantly from the natural ones. As a comparison also the isotope ratios of Hg which should not be changed have been listed to show the analytical error. Hence, artificial MeHg formation is not observed during the digestion procedure of the rice samples, and neither during derivatisation and measurement, which confirms the occurrence of the neurotoxin MeHg in market rice including baby rice samples in the µg kg⁻¹ range.

Online SPE-HPLC-CV-AFS is a simple and reliable method for the speciation of MeHg in rice and could be used as a standard method in laboratories. The sample preparation is limited to centrifugation, filtering and dilution after digestion, which can be done in an open vessel microwave, saving time and material cost. Regarding overall costs for MeHg analysis, the instrument costs for the preconcentration-HPLC-CV-AFS method are far lower than GC-ICP-MS, which is mainly due to the high cost of ICP-MS compared to CV-AFS. Cost-efficiency is particularly of interest to food agencies, as new regulatory limits are being introduced as of late. China introduced a T-Hg limit for rice import already, set to 20 µg kg⁻¹, and other countries may follow this move.

Already, in 2012, the European Food Safety Authority (EFSA) set a new tolerable weekly intake (TWI) for Methylmercury of 1.3 µg kg⁻¹ bodyweight,[17] which is lower than the established value from 2004 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of 1.6 µg kg⁻¹ bodyweight.[18]

Especially in cultures or ethnic groups with high rice consumption, rice must be considered as a contributor of the overall MeHg burden in our diet, even though on a smaller scale than from seafood products.

Conclusions

We introduced a cross-validated method for fast, robust, cost-efficient and sufficiently precise determination of trace MeHg in rice samples. Four rice samples were analyzed for MeHg with a previously described SPE-HPLC-CV-AFS approach using an external calibration, and compared the results with SS-ID-GC-ICPMS methodology. A t-test showed no significant difference for the results obtained with the two different methods.

The accuracy of the online SPE-HPLC-CV-AFS approach was further evaluated using a standard addition approach, which confirmed the results obtained with the external calibration. This also reassures that the MeHg species is not degraded during the digestion step. Furthermore, enriched isotope spiking with $^{199}Hg^{2+}$ confirmed that there is no artificial formation of MeHg from Hg^{2+}, despite the highly organic matrix. The precision of the method is comparable to the SS-ID-GC-ICPMS for MeHg in rice at the ultra-trace level.

Acknowledgement

Notes and references

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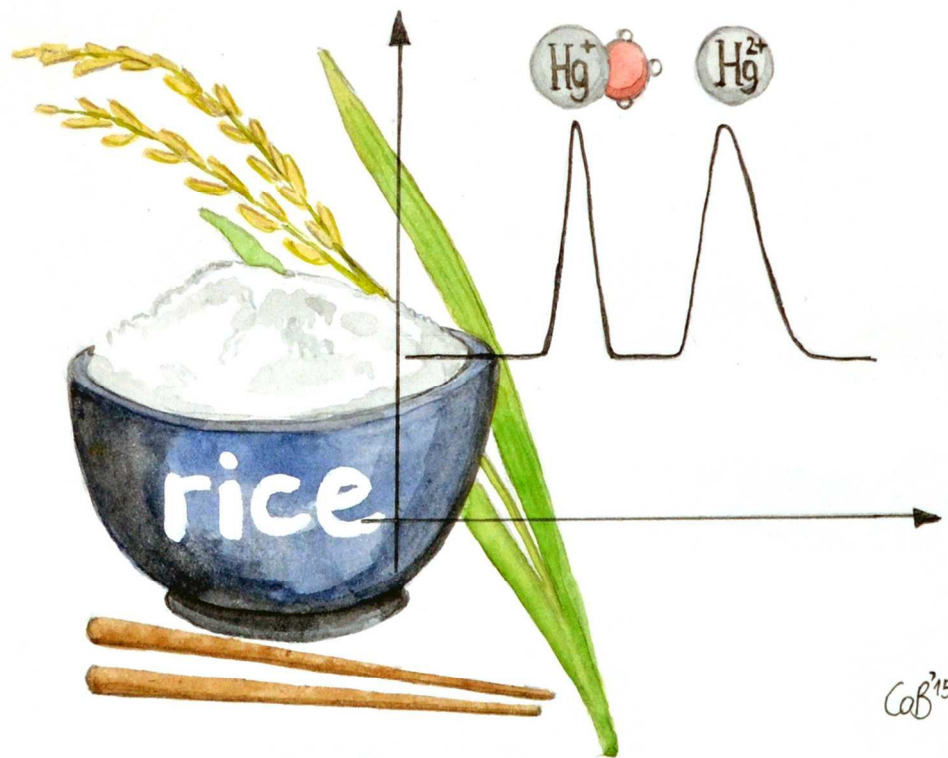
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