Comparison of on-site field measured inorganic arsenic in rice with laboratory measurements using a field deployable method: method validation

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ABSTRACT. A commercial arsenic field kit designed to measure inorganic arsenic (iAs) in water was modified into a field deployable method (FDM) to measure iAs in rice. While the method has been validated to give precise and accurate results in the laboratory, its on-site field performance has not been evaluated. This study was designed to test the method on-site in Malawi in order to evaluate its accuracy and precision in determination of iAs on-site by comparing with a validated reference method and giving original data on inorganic arsenic in Malawian rice and rice-based products. The method was validated by using the established laboratory-based HPLC-ICPMS. Statistical tests indicated there were no significant differences between on-site and laboratory iAs measurements determined using the FDM ($p=0.263$, $\acute{\alpha}=0.05$) and between on-site measurements and measurements determined using HPLC-ICP-MS ($p=0.299$, $\acute{\alpha}=0.05$). This method allows quick (within 1 hour) and efficient screening of rice containing iAs concentrations on-site..

KEYWORDS. Rice, arsenic; field deployable method; inorganic arsenic; laboratory; onsite; maximum contaminant limit.

LIST OF COMPOUNDS: Arsenic, (Arsenic-75) (PubChem CID: 5359596); Arsenic(III) (PubChem CID: 104734), Arsenic(V) (PubChem CID: 104737); arsines (PubChem CID: 68978); Dimethylarsinous acid (PubChem CID: 185792); Monomethylarsonous acid (PubChem CID: 161491); Mercury bromide (HgBr2), Mercuric dibromide (PubChem CID: 24612); sodium borohydride (NaBH4) (PubChem CID: 4311764); sulfamic acid (PubChem CID: 5987); and Nitric acid (HNO3) (PubChem CID: 944).

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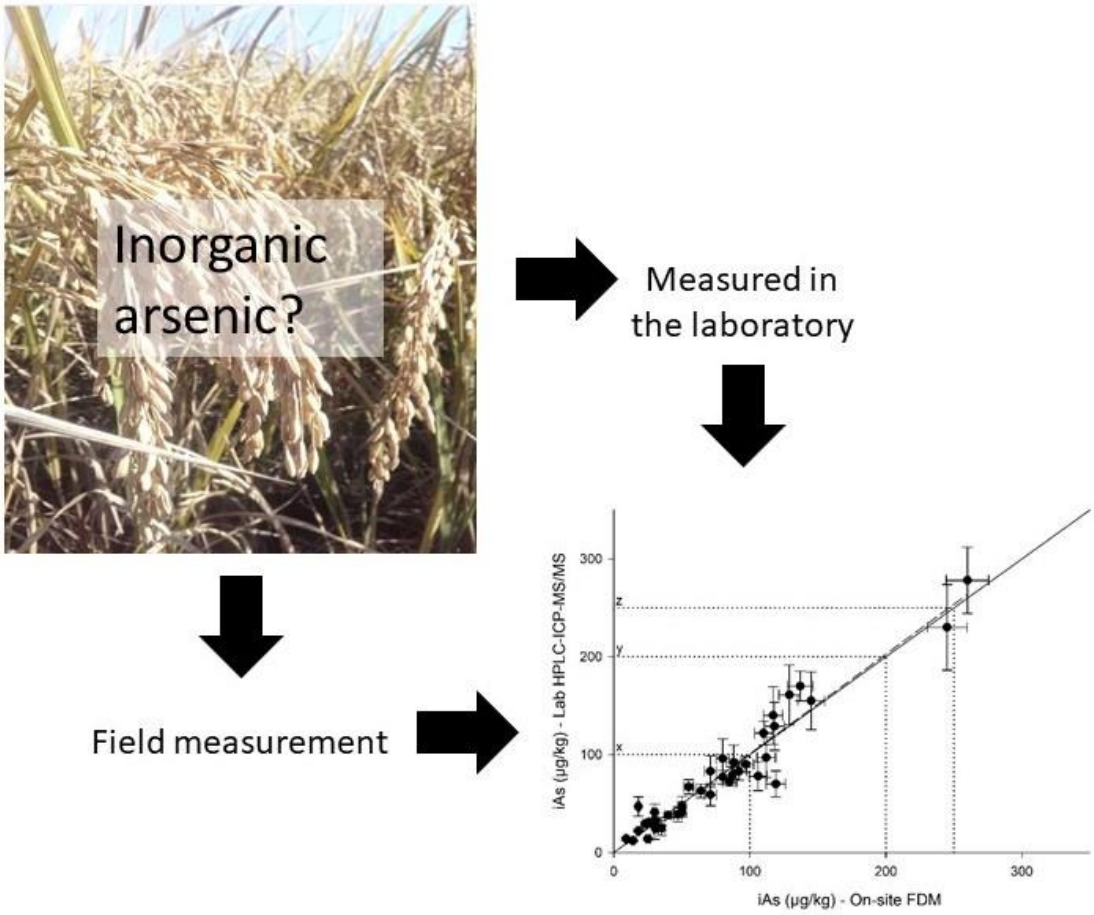


A field deployable technique was tested in Malawi for screening of inorganic arsenic in different rice cultivars cultivated in different areas. Results indicate that there is no bias to results achieved by HPLC-ICP-MS/MS and less than 10% false positives and false negatives to the reference method iAs values at EU maximum contaminable limit for baby food (100 µg/kg) were obtained.

1. INTRODUCTION

Arsenic (As) is a toxic trace element widely present in the natural environment. Elevated concentrations of As have been found in crops such as rice (Mandal & Suzuki, 2002; Meharg et al., 2009; Rosas-Castor, Guzmán-Mar, Hernández-Ramírez, Garza-González, & Hinojosa-Reyes, 2014). Rice is cultivated on 159 million ha and it is estimated that, for 3 billion people, 35-60% of their dietary calorie intake is through rice

consumption (Fageria, 2007; GRISP, 2012; Vasudevan, Mathad, Doddagoudar, & Shakuntala, 2014). The toxicity of As is dependent on the chemical form present (Gong, Lu, Ma, Watt, & Le, 2002; Juskelis, Li, Nelson, & Cappozzo, 2013; Syu, Huang, Jiang, Lee, & Lee, 2015; Zwicker, Zwicker, Laoharojanaphand, & Chatt, 2011). Inorganic arsenic species (iAs) are classified as a class I carcinogen (IARC, 2004; Munera-Picazo et al., 2014; Weinber, 2004), and are more toxic and carcinogenic than organic species (Ammann, 2011; Henke, 2009). Ingestion of rice and rice products is reported to be a major dietary uptake of iAs for humans, especially among infants and young children who are at high risk of ingesting elevated levels of iAs due to high consumption of rice products per kg body weight (Munera-Picazo et al., 2014). In January 2016, The European Union (EU) legislated a maximum contaminant limit (MCL) of 0.250 mg/kg iAs for husked rice, 0.200 mg/kg iAs in rice and 0.100 mg/kg iAs in rice destined to produce baby food (Signes-Pastor et al., 2017; The Commission of the European Communities, 2015) (**Table S1**) in order to protect infants, young children and the general population from ingesting elevated iAs levels through rice consumption.

The EU legislation restricts importation of rice and rice products violating the legislated limits into European Union member countries (The Commission of the European Communities, 2015). Thus, it became a requirement that rice and rice products imported into EU be certified to meeting the legislated limits. Not only has the EU set iAs MCL for rice, but other regulatory bodies have also set regional or country based MCL that range from 0.100 mg/kg (EU) to 0.300 mg/kg for iAs and up to 0.700 mg/kg for total arsenic (tAs) (**Table S1**). A market survey on rice products destined for babies and infants, bought after the introduction of the MCL in the EU, found that almost 50% of the products did not comply with the legislation (Signes-Pastor et al., 2017).

To date, a number of robust analytical methods for detecting and quantifying tAs and iAs in rice have been developed and reported (Feldmann, Raab, & Krupp, 2017; Hung, Nekrassova, & Compton, 2004; Kinniburgh & Kosmus, 2002). The established methods all use HPLC coupled to Inductively Coupled

Plasma Mass Spectrometry (ICP-MS) and give reliable results but suffer from high costs and lack of availability in many routine analytical laboratories, therefore other cheaper methods have been developed for the detection of iAs in rice such as hydride generation (HG) coupled to AFS (Chen, Ma, & Chen, 2014) or ICP-MS (Chen et al., 2014; Petursdottir et al., 2014). Although these robust analytical instruments are valid and reliable, they are laboratory based and too bulky to transport to field for on-site analyses (Bralatei, Lacan, Krupp, & Feldmann, 2015; Sankararamakrishnan, Chauhan, Nickson, Tripathi, & Iyengar, 2008). Therefore, there is a need to develop less expensive, portable and robust field methods to use for screening it's the iAs content in rice on-site in low income rice producing countries that are challenged in accessing robust laboratory based analytical instruments. In view of this challenge, Bralatei et al. (2015) modified a commercial arsenic field kit designed to measure iAs in water into a field deployable method (FDM) to allow determination of iAs in rice on-site. The method developed by Bralatei et al. (2015) employs the Gutzeit reaction in which the sample containing As(III) and/or As(V) reacts with sodium borohydride under acidic conditions and converts both species of iAs to arsine gas (AsH_3) (**Equation 1**) (Bralatei et al., 2017, 2015; Hung et al., 2004).

$$2H_3AsO_4 + 2H_3O^+ + 2NaBH_4 \rightarrow 2AsH_3 + 2B(OH)_3 + 4H_2O + 2Na^+. \qquad \textbf{Equation 1}$$

During the reaction, arsine gas formed evolves and reacts with a mercuric bromide impregnated filter lid to form a colored Lewis acid/base arsenic mercury product ($H_2As{-}HgBr$), while methylated arsines do not form any complex with the mercury bromide (**Equation 2**) (Bralatei et al., 2015; Fransisca et al., 2015; Kinniburgh & Kosmus, 2002). The intensity of the orange/yellow color is proportional to the concentration of iAs in the sample solution.(Bralatei et al., 2015; Sankararamakrishnan et al., 2008).

$$AsH_3 + HgBr_2 \rightarrow H_2As{-}HgBr + HBr. \qquad \textbf{Equation 2}$$

The FDM method was previously evaluated to give a quick, accurate and precise determination of iAs in rice samples when used in controlled conditions within a laboratory (Bralatei et al., 2015). High recoveries within the acceptable range of the reference method (HPLC-ICP-MS) and detection limits for iAs sufficient

to detect the EU MCL for baby rice were reported (Bralatei et al., 2015). Although the FDM was designed for field determination of iAs in rice, on-site field performance of the method has not been tested and evaluated to date. In this study, the FDM method was used on-site in Malawi, a country which does not have any lab based facilities for detecting iAs in rice, in order to evaluate whether the FDM can deliver fast screening data of high quality and without bias in the field. The iAs contents of field collected rice samples from Malawi were compared to international guideline values.

Hypothesis 1: Accuracy and precision of the on-site FDM iAs results *are not different from laboratory FDM and reference method* iAs results.

Hypothesis 2: In reference to international guideline values, the number of false-positives and false-negatives results is low for both FDM and laboratory based analytical instruments results Thus, iAs values obtained on-site using the FDM are reproducible with laboratory based analytical instruments.

2. EXPERIMENTS AND METHODS

2.1. **Samples and sampling.** The rationale for the sampling design was to cover all rice producing districts of Malawi, the most abundant cultivars and all rice products used in the country. Thirty-three rice samples (whole grain, rice bran, rice husks, unpolished rice and polished rice) of different rice cultivars that included Kilombero, Faya, TCG-10, Nunkile and Nerica were analysed on-site at various rice farms, rice irrigation schemes and research stations located in 10 rice growing districts in Malawi (**Figure 1**). The sampling sites were mainly along the lake shores of Lake Malawi, Lake Chilwa and Lake Malombe and along the Shire River Valley (**Figure 1**). Two rice fields per rice scheme or research station were randomly selected for on-site analyses. Composite rice samples (50 to 150 g) were collected from 5 different randomly selected points in each field. Replicate samples, collected for laboratory analyses, were labelled and packed in zipp-log bags with details of site location, rice material, rice cultivar and date of sampling.

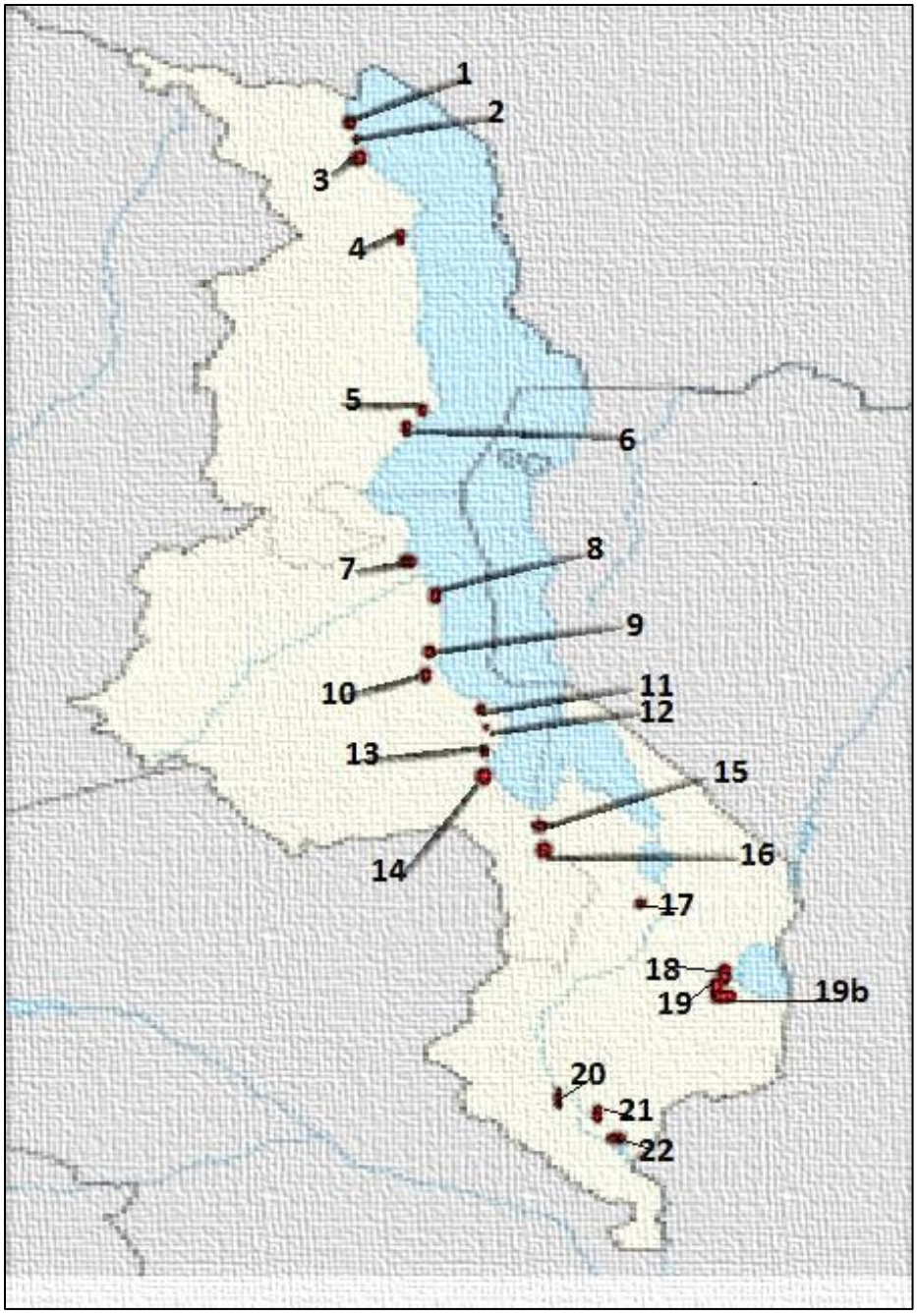


Figure 1. Map of Malawi showing sampling locations where on-site iAs determination was conducted. Sites are Lufiriya rice scheme (1), Baka research station (2), Hara rice irrigation scheme (3), Chiweta (North Rumphi) (4), Limphasa irrigation scheme (5), Nkondezi research station (6), Liwaradzi (7), Dwangwa (8), Nkhunga and Mtupi (9), Chimphangwi (10), Lifuwu research station (11), Maganga/Sengedzi river (12), Ndindi (13), Chipoka (14), Bwanje irrigation scheme (15), Bwanje scheme (research) (16), Balaka–

Liwawadzi river (17), Domasi irrigation scheme (18), Khanda irrigation scheme (19), Likangala rice scheme (19b), Kasinthula research station (20), Nazolo irrigation scheme (21) and Nkhate irrigation scheme (22).

2.2 External calibration of FDM and quality assurance. Accuracy of FDM in both laboratory and on-site analyses was checked using As(V) standards. Calibration standards of As(V) solutions were prepared by diluting 1000 mg/L of As(V) in 1% (v/v) HNO_3 solutions. Recoveries of As(V) standards were computed as percentage of determined value to theoretical value of the standard. Limit of detection was calculated as LOD= X+3*SD (where X is the mean blank value in mg/kg of As(V) solutions and SD is the standard deviation of blanks iAs concentration). Recoveries were evaluated daily to check accuracy of the method. Computed recoveries are reported in **Table S2**.

2.3 **On-site and Laboratory sample extraction and determination of iAs using the FDM.** In the field, air dried rice materials were ground using a coffee grinder. Rice husk and rice bran were mainly collected from rice mills in rice irrigation schemes or research stations. Approximately 5.0 g of homogenized ground and air dried rice material (whole rice grain (WGR), rice husk (RHU), rice bran (RBR), polished rice (POR) and unpolished (brown) rice (UPR)), scooped using a graduated spoon (on-site) and accurately measured using an analytical balance (in the laboratory), were mixed with 50 ml of 1% nitric acid (HNO_3) in a 250 ml beaker and extracted by boiling the mixture at around 90-100 °C temperature for 20 min using a gas stove (on-site) and electric stove with adjustable control knob (in the laboratory); thereafter extracts were cooled for 3-5 min at ambient room temperature and a further 5-10 min in a water bath (tap water). Loss of heat by convection may have occurred during on-site analytes extraction since boiling was done in an open space which may have an effect on the uniform analyte extraction. The entire sample extract was then transferred into an Erlenmeyer volumetric reaction flask which was tightly closed with a tri-filter bung device fitted with detector slips, immediately after adding 0.050 to 0.100 ml (2-3 drops) of antifoam, 0.150 g (one sachet) of sulfamic acid (Palintest, U.K.), and 0.500 g (one tablet) of sodium borohydride ($NaBH_4$)

147 (Palintest, U.K.). After 20 minutes the iAs concentration in the sample extract was determined using the
148 colour change of the detector strip (Palintest, U.K.) by comparing it to a calibrated chart. Alternatively, the
149 concentration of sample solution was determined using the arsenator photometer (Palintest, U.K.) as
150 described by Bralatei et al. (2017, 2015). One analyses was complete within around one hour.

151

152 2.4. **Sample extraction and determination of iAs using HPLC-ICP-MS/MS.** Exactly 0.200 g of each
153 rice material sample (air dried: 7.5±4.8 % mean moisture content) was mixed with 10 ml of extracting
154 reagent (1% (v/v) HNO_3 and 2% (v/v) H_2O_2) and then extracted using an open vessel MARS5 microwave
155 digestion system. Then, the samples were cooled at room temperature, and centrifuged two times before
156 analyses. Inorganic arsenic (sum of As(III) and As(V)) and DMA in rice and DMA standards were
157 determined using high performance liquid chromatography (HPLC)-ICP-MS/MS. To ensure accuracy of
158 the generated data, a standard reference material (SRM) NIST 1568a Rice Flour and blanks were analysed
159 alongside the samples. Arsenic speciation analysis of rice samples was conducted using a HPLC (1290
160 series, Agilent Technologies) coupled to a ICP-MS/MS (8800 series, Agilent Technologies). A Hamilton
161 PRP-X100 (10 µm, 250 x 4.1mm) anion exchange column was used for the separation of the As species.
162 Ammonium carbonate buffer (3 g/L, pH=9.2) was used as eluent (flow rate: 1 ml/min). The sample injection
163 volume was 80 µl. For the ICP-MS/MS, reaction cell gas flow rate was 0.24 ml oxygen/min, rhodium (Rh)
164 was used as the internal standard (ISTD), and mass to charge (m/z) ratio of m/z =91 for analyte (As) and
165 m/z =103 for ISTD (Rh) were selected for detection. Upon obtaining chromatograms of DMA standards
166 (0.1, 0.5, 1.0, 5.0, 20, 50 μg/kg), peaks were integrated using Origin 6.1 software and quantified using
167 external calibration.

168

169 **3.0 RESULTS AND DISCUSSIONS**

170 3.1. **Analytes recovery analysis of Field Deployable Method (FDM) iAs values.** The limit of detection
171 (LOD) of 47 µg/kg was comparable to the LOD value of 50 µg/kg reported by Bralatei et al., (2015). The

accuracy of the method in both laboratory and onsite analyses was determined using As(V) standards (5, 10, 20, 25, 50, 75 and 100 µg/kg) with theoretical concentrations of 5.0 ± 1.2, 10.2 ± 1.0, 20.1 ± 0.5, 25.8± 1.5, 50.4 ± 1.6, 75.8± 0.2 and 100.2 ± 2.4 µg/kg. Percentage recoveries for each As(V) standard ranged from 79.8 to 127% for on-site measurements; and 82.0 to 117.1% for laboratory analyses (**Table 1**) which are comparable to recoveries of 72–120% reported by Williams, West, Koch, Reimer, & Snow (2009) and 81–150% reported by (Safarzadeh-Amiri et al., (2011) and Sankararamakrishnan et al. (2008). Average variability of the determined concentration for As(V) standards was low (11.5% for on-site analyses and 7.8% for laboratory analyses) and indicating excellent agreement with theoretical values. *T*-test p-values (**Table 2**) showed that laboratory analyses of As(V) standards were not significantly different from on-site analyses ($p=0.984$; significant at $\alpha=0.05$). Mean theoretical As(V) concentrations were correlated with mean measured laboratory As(V) values of standards. Slope and R^2 values were very close to 1 (**Figure S1**) indicating minimal biasness and strong correlation of the data sets respectively.

Calculated concentrations of iAs in rice were corrected in the field by assuming 10 % moisture content since MCL is given in dry matter. Mean moisture content of rice materials were determined later in laboratory to be 7.5 ± 4.8% (**Table S3**), hence the expected error is minimal.

Reference material (NIST 1568a Rice Flour) was analysed for tAs and a concentration of 285±50 µg/kg As was obtained. The tAs was in excellent agreement with certified tAs value (290±30 µg/kg As). Furthermore, iAs (sum of As(V) and As(III)), DMA and MMA were also determined and 104±15 µg/kg iAs, 165±17 µg/kg DMA and <15±2.5 µg/kg MMA were obtained. The obtained amounts of As species were within the previously reported ranges (Heitkemper, Vela, Stewart, & Westphal, 2001; Narukawa & Chiba, 2010). Narukawa & Chiba (2010) reported iAs, DMA and MMA values of 98±2, 175±2 and 13±1 µg/kg respectively whereas Juskelis, Banaszewski, & Cappozzo, n.d.; Juskelis et al. (2013) reported 100±20, 171±34 and 11±2 µg/kg which are both comparable to values obtained in this study, hence acceptable.

Table 1. Comparison of theoretical concentrations versus mean experimental concentrations of As(V) standards determined using FDM on-site and laboratory. Mean concentrations are reported as µg/kg ± SD (n=6).

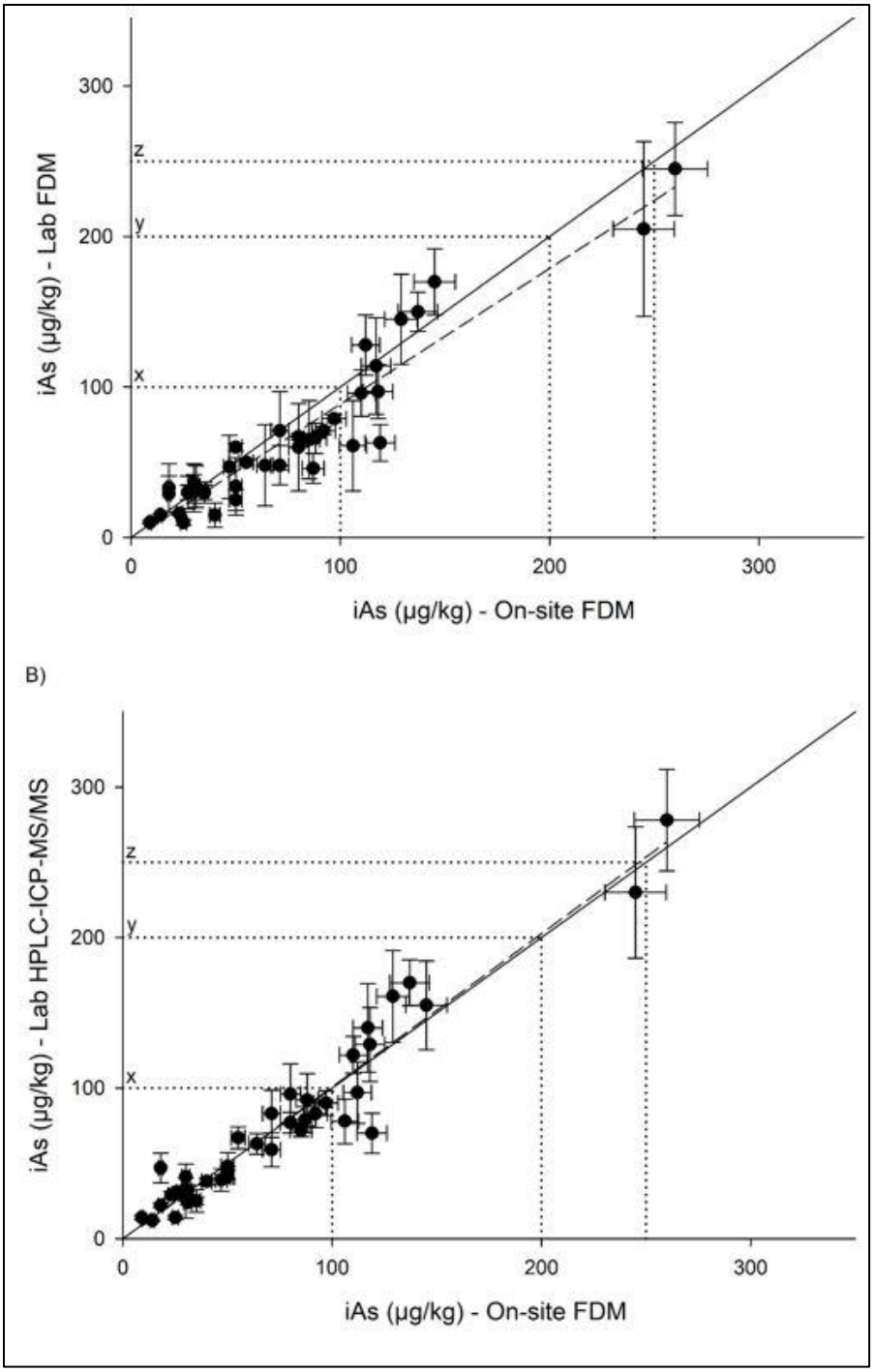
Mean theoretical of As(V) concentration µg/kg	Experimental values (µg/kg)			
	On-site iAs(V) µg/kg	Recovery %	Lab iAs(V)) µg/kg	Recovery %
5.0±1.2	5.7±1.0	114.0%	4.1±2.2	82.0%
10.2±1.0	9.9±0.6	106.9%	10.6±0.5	103.9%
20.4±0.5	16.2±2.8	79.8%	24.2 ±1.8	116.2%
25.8±1.5	-	-	26.7±2.1	103.5%
50.4±1.6	64.1±4.6	127.4%	50.5±2.2	102.2%
75.8±0.2	-	-	69.5±1.3	91.7%
100.2± 2.4	99.0±4.4	98.8%	102.6±14	102.4%
Average variability	10.5%		13.4%	

3.2. **Comparison of iAs values determined using FDM on-site and in the laboratory and using HPLC-ICP-MS/MS (laboratory).** Linear regression analysis and paired sample *t*-tests were computed in order to evaluate whether on-site iAs values correlate to and/or are statistically different from iAs values determined under controlled conditions in the laboratory using the same method (FDM) and/or HPLC-ICP-MS/MS. Results showed no statistical difference between FDM field measured and FDM laboratory measured iAs values in the same sample (**Table 2**). There was also no significant statistical difference between the field measured and HPLC-ICP-MS/MS laboratory measured iAs in the same samples (**Table 2**). As shown in **Table 2** and **Figure 2**, the slopes of linear regression equations were close to 1 and the correlation coefficients of each pair of comparison was very close to the 1:1 line and p-values>0.05, results do not only indicate a strong correlation but also congruence of the data set.

Table 2. Comparison of iAs values 33 rice material samples tested by FDM on-site and laboratory by HPLC-ICP-MS/MS (laboratory) indicating p-values, slope, and y-intercept and Pearson correlation coefficient

Parameter	*T*-test p-value[d]	rho[c]	Linear regression equation	R^2
HPLC-ICP-MS/MS vs LAB FDM	0.966	0.935	y = 0.99x - 0.0045	0.88
HPLC-ICP-MS/MS vs on-site FDM	0.299	0.935	y = 1.04x + 0.0003	0.89
LAB FDM vs on-site FDM	0.263	0.957	y = 0.98x + 0.0095	0.89

[c] Pearson correlation coefficient; [d] significant at 0.001



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216 **Figure 2:** Linear regression analyses of HPLC-ICP-MS/MS iAs values versus FDM on-site and lab iAs
217 values in rice samples; (A) Comparison of FDM on-site iAs values vs and FDM lab iAs values; (B)
218 Comparison of HPLC-ICP-MS/MS iAs values vs and FDM on-site iAs values; Red vertical and horizontal
219 lines X, Y and Z indicate maximum contaminable limits at 100, 200 and 250 µg /kg for rice intended for

baby rice products, for rice and husked rice respectively. Solid black lines indicate 1:1 ratio lines whereas the dotted ones indicate linear regression lines. Error bars are standard deviation (SD).

Recovery efficiency tests indicated that 27 out of 33 on-site iAs values (82%) were within ±22% of the HPLC-ICP-MS/MS results which is acceptable and within the range of recoveries for rice (89.5% to 116.3%) reported by Bralatei et al. (2015) and 29 out of 33 (88%) on-site iAs values were within ±17% of Lab iAs values which is also acceptable. The overall on-site relative standard deviation (RSD) of both on-site vs HPLC-ICP-MS/MS and on-site vs LAB FDM iAs values was found to be ±14% (**Table S2**) slightly higher than the RSD reported by Bralatei et al. (Bralatei et al., 2015) for LAB FDM vs HPLC-ICP-MS/MS (±12 %). Nevertheless, on-site iAs values above or below HPLC-ICP-MS values (18%); and above or below Lab iAs values (12%) were not significantly different, indicating good precision. Higher on-site variability could be attributed to variable estimation of sample masses due to variable densities of rice grain, rice bran and rice husks (though not significant), non-uniform on-site analyte extraction (boiling may not be uniform in open space), and variable sample moisture content for samples analysed on-site (**Table S3**). However, sample moisture content in laboratory analyses was checked and determined to range from 2.0% to 20.7% which may have negligible effect on final iAs concentration in rice when a nominal +/- 10% moisture content was used for correction. As shown in **table S**3, iAs concentration values corrected with real moisture content were not significant different from those corrected with nominal 10% moisture content ($p>0.877$ significant at 0.001).

The impact of variable density and accuracy of scooping spoon in determining 5 g of bran and husks was also checked conducting verification tests of the scooping spoon (**Figure S3**). As shown, scooped masses were not significantly different from analytical weights ($p>0.93$, significant at 0.05). However, an exceptionally low value of mass was obtained in sample 15, though it did not significantly impair scooping the intended average mass of 5 g (**Figure S3**).

3.3. Variation of iAs values determined by on-site FDM. The field deployable method was successfully used in determination of iAs in rice materials on-site in Malawi. Inorganic As values obtained by both FDM and HPLC-ICP-MS/MS in the laboratory were comparable to FDM on-site iAs values; hence a comparison of on-site iAs measurements of various analysed rice materials was made (**Figure 3**). One-way analyses of variance (ANOVA) statistical test was conducted to evaluate significant differences in the concentration of iAs in the different rice samples. Bran and husk had the highest concentrations and were not significantly different from each other, while the lowest concentrations were observed in POR and UPR, which had significantly lower iAs concentrations than WRG, bran and husk at the 95% confidence level (**Figure 3**). Similar trends were observed by Seyfferth, Webb, Andrews, & Fendorf (2011) and Rahman & Hasegawa (2011). Mean iAs value determined in UPR using FDM (mean: 52±18 µg/kg compared well to mean iAs of 60 µg/kg reported by Joy et al. (2016) for unpolished (brown) Malawian rice. The mean iAs concentration in polished Malawian rice (mean: 31±12 µg/kg; range 9 – 54 µg/kg) is amongst the lowest ever reported concentrations for iAs in rice worldwide (Meharg et al., 2009; P. N. Williams et al., 2005).

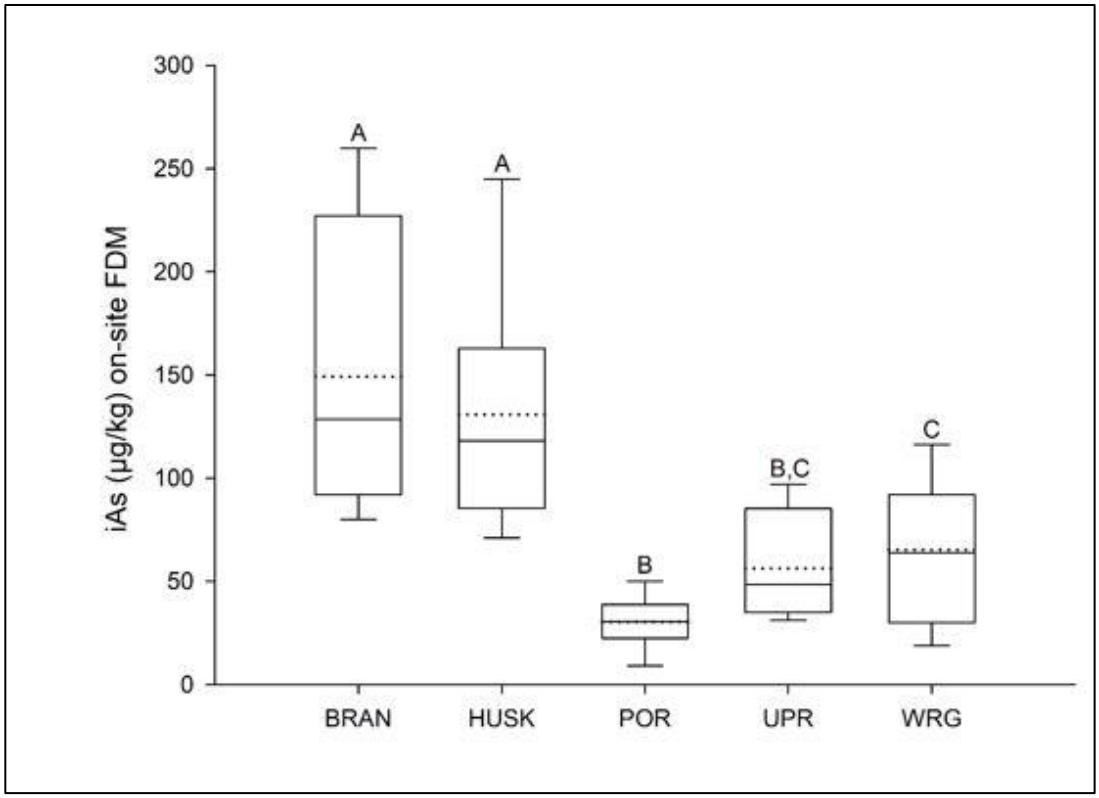

Figure 3. Inorganic As content of various rice materials as determined by field deployable method on-site. Rice material that share common letters (A, B and C) are not significantly different from each other. The boxes represent first and third quartile range iAs values; the solid line across a box represents the median value; the dotted line across a box represents the mean values and whiskers represent minimum and maximum values.

3.4. Screening of rice samples in the field using EU MCL as a yard stick. The accuracy and precision of FDM on-site screening was evaluated with reference values to check compliance of iAs content to EU legislated limits. Evaluation was done based on both wet weight (w/w) basis with the corrected 10 % moisture and dry weight (d/w) basis upon determination of moisture content of each sample (**Table S1** and **Figure S5**). FDM on-site analysis identified 2 (6.1%) samples (for both w/w and d/w) and 11 (33.3%) samples (for w/w) and 10 (30.3%) samples (d/w) with iAs concentration values exceeding the legislated limits of MCL of 200 µg/kg and 100 µg/kg respectively which compared well to those obtained in the

laboratory using FDM (6.1%) at MCL of 200 µg/kg and 24.2 % at MCL of 100 µg/kg) and the reference method (6.1% at MCL of 200 µg/kg and 27.3% at MCL of 100 µg/kg) (**Figure S5**) suggesting high accuracy and precision. Interestingly, neither polished (white) rice nor brown (unpolished) rice contained iAs exceeding the limit of 200 µg/kg while 1 sample of brown (unpolished) rice exceeded the limit of 100 µg/kg. As observed in these results, iAs concentration values determined using FDM in rice materials on a w/w basis did not significantly change after determination and factoring in the effect the of low moisture content (which ranged from 2.0% to 20.7%) (**Table S3**).

Table 3. False positive and negative iAs values obtained by FDM on-site and Laboratory compared to reference method (HPLC-ICP-MS/MS) values.

	FDM On-site iAs value (w/w)			**FDM Lab iAs value (w/w)**		
Deciding MCL value	False Positive	False Negative	True Positive and True Negative	False Positive	False Negative	True Positive and True Negative
At MCL 100 µg/kg	2 out of 33 (6.1%)	1 out of 33 (3.0%)	90.9%	1 out of 33 (3.0%)	1 out of 33 (3.0%)	94.0%
At MCL 200 µg/kg	0	0	100%	0	0	100%
FDM On-site iAs value (d/w)[ð]						
Deciding MCL value	False Positive	False Negative	True Positive and True Negative			
At MCL 100 µg/kg	3 out of 33 (9.1%)	1 out of 33 (3.0%)	87.9%			
At MCL 200 µg/kg	0	0	100%			

ð = iAs concentration corrected with 10% sample moisture content (i.e. d/w).

In these analyses, values obtained using the reference method were regarded as the true values and were compared to FDM values (**Figure S2**). At legislated MCL of 100 µg/kg, 6.1% of FDM on-site iAs values (w/w) were false positive and 3.0% false negative values; whereas 3.0% of FDM Laboratory iAs values were false positive and 3.0% were false negative (**Table 3 and Figure S5**). However, 9.1% on-site iAs values for d/w analyses (corrected with 10% sample moisture content) were false positive and none were false negative values at that limit. Similarly, at legislated 0.200 mg/kg, both on-site and lab analyses indicated a low false positive and low false negative rate (**Table 3**). The observations imply that FDM on-site and laboratory analyses erroneously identified only 6.1% (w/w) and 3.0% (w/w) samples respectively as possessing iAs above MCL 100 µg/kg of rice destined for baby food (false positive) despite possessing safe levels of iAs for baby; and both analyses erroneously identified 3.0% as below that limit (false negative) despite being higher. Both on-site (w/w and d/w) and laboratory false positive and false negative iAs values obtained in this study were low and not significant. However, since the most important and desirable characteristic of a field deployable method is that it should have high probability of giving low

false-positive and false- negative results (Safarzadeh-Amiri et al., 2011), low false positive and false negative values obtained for both samples with low (<100 µg/kg) (9.1% and 3.0%) and high (>100 µg/kg) iAs values (0% and 0%) respectively suggests that accuracy and precision of our the method (FDM) is high. However, despite obtaining low false-positive and false- negative values for rice samples with both low and high iAs values, samples with lower iAs (<100 µg/kg) values exhibited relatively higher false positive and false negative values than those samples with high iAs (>100 µg/kg) values implying that precision could be influenced by the low iAs values (<100 µg/kg) supporting findings reported by Kinniburgh & Kosmus (2002) and Safarzadeh-Amiri et al. (2011). Nevertheless number of false positive and false negative values obtained in this on-site study were not significantly different from laboratory analyses reported by Bralatei et al. (2015) (10% and 7% respectively) for polished rice samples and those reported by Safarzadeh-Amiri et al. (2011) (11% and 2%) for tube-well water. The observation also demonstrates that effect of moisture content, the use of a scoop rather than a balance in the collected samples was low and insignificant to negatively influence accurate and precise screening. Furthermore, cconsidering that the two different methods use different analytical procedures (iAs is chemically mobilized to form AsH_3 in the field kit but arsenic species behave differently in anion exchange chromatography), the strong reproducibility of iAs content in these methods (low false-positive and low false- negative values) indicates that the interferences of redox active elements (Cu, Mn, Fe, and Zn) were minimal and/or not significant.

CONCLUSION

While the present study was designed to validate the use of FDM in screening polished (white) and unpolished (brown) rice to the legislated iAs levels on-site, the study further evaluated the suitability of the method for the determination of iAs in rice bran, whole rice grain and rice husks. It has been shown that the FDM accurately and precisely identified not only white and brown rice but also rice bran, whole rice grain and rice husks with iAs levels above and below the legislated limits of 0.100 mg/kg and 0.200 mg/kg with insignificant false positives (<7%) and false negatives (<3%). The finding indicates the method is capable of producing on-site measurements that are reproducible in laboratory. Thus it can be potentially

used for field screening for compliance of legislated iAs levels in rice in low income countries. However, the main drawback of on-site screening could be greater variability of moisture content of samples (if analyses are done during season of high humidity). Nevertheless, samples could be air dried to uniform moisture prior to analyses which could be used to correct data before comparison with the legislated iAs levels. The method is merited for being simple and quick to use such that one analysis can be completed within one hour. Additionally, the field kit is relatively cheap and easily transported to the sites for field analyses without requiring special equipment.

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