

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

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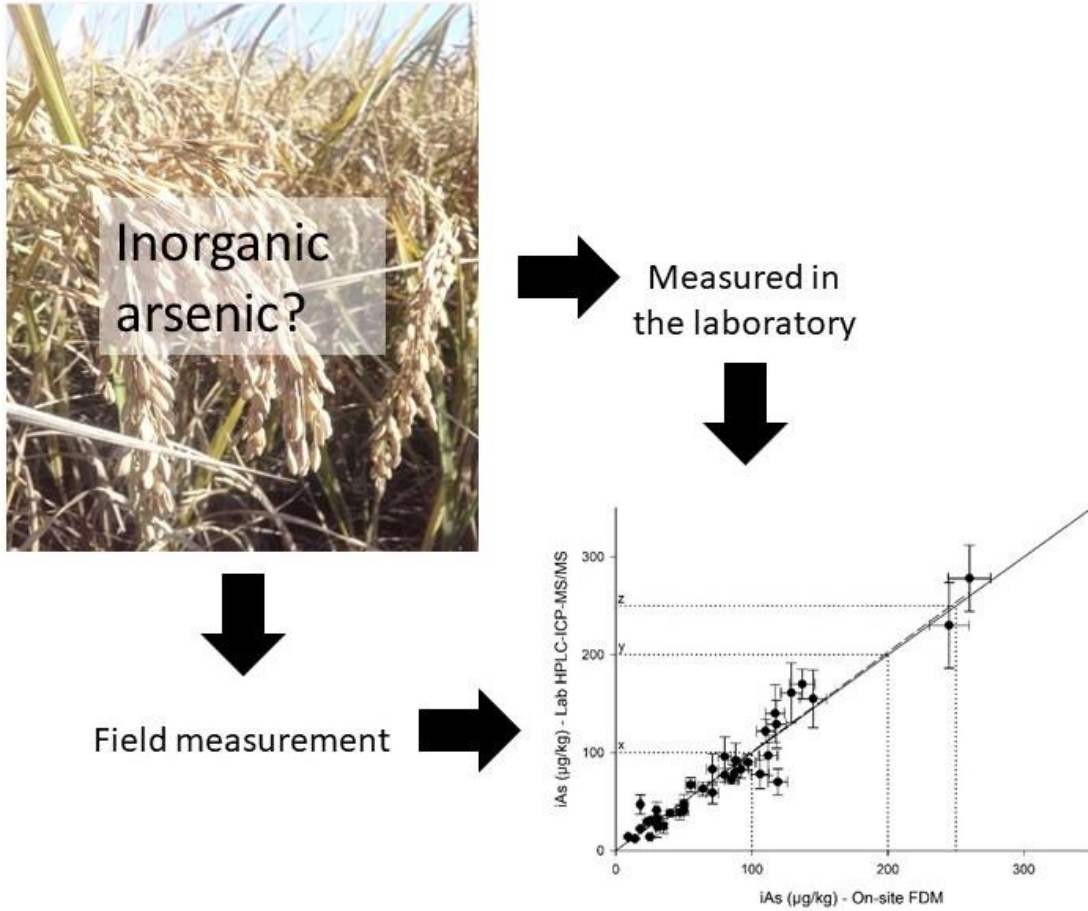
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11 **ABSTRACT.** A commercial arsenic field kit designed to measure inorganic arsenic (iAs) in water
12 was modified into a field deployable method (FDM) to measure iAs in rice. While the method has
13 been validated to give precise and accurate results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

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

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25 **LIST OF COMPOUNDS:** Arsenic, (Arsenic-75) (PubChem CID: 5359596); Arsenic(III) (PubChem CID:
26 104734), Arsenic(V) (PubChem CID: 104737); arsines (PubChem CID: 68978); Dimethylarsinous acid
27 (PubChem CID: 185792); Monomethylarsonous acid (PubChem CID: 161491); Mercury bromide (HgBr₂),
28 Mercuric dibromide (PubChem CID: 24612); sodium borohydride (NaBH₄) (PubChem CID: 4311764);
29 sulfamic acid (PubChem CID: 5987); and Nitric acid (HNO₃) (PubChem CID: 944).

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

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38 1. INTRODUCTION

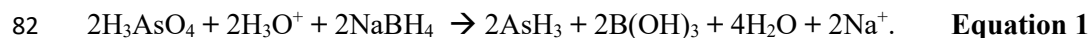
39 Arsenic (As) is a toxic trace element widely present in the natural environment. Elevated concentrations of
 40 As have been found in crops such as rice (Mandal & Suzuki, 2002; Meharg et al., 2009; Rosas-Castor,
 41 Guzmán-Mar, Hernández-Ramírez, Garza-González, & Hinojosa-Reyes, 2014). Rice is cultivated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

43 consumption (Fageria, 2007; GRISP, 2012; Vasudevan, Mathad, Doddagoudar, & Shakuntala, 2014). The
44 toxicity of As is dependent on the chemical form present (Gong, Lu, Ma, Watt, & Le, 2002; Juskelis, Li,
45 Nelson, & Cappozzo, 2013; Syu, Huang, Jiang, Lee, & Lee, 2015; Zwicker, Zwicker, Laoharojanaphand,
46 & Chatt, 2011). Inorganic arsenic species (iAs) are classified as a class I carcinogen (IARC, 2004; Munera-
47 Picazo et al., 2014; Weinber, 2004), and are more toxic and carcinogenic than organic species (Ammann,
48 2011; Henke, 2009). Ingestion of rice and rice products is reported to be a major dietary uptake of iAs for
49 humans, especially among infants and young children who are at high risk of ingesting elevated levels of
50 iAs due to high consumption of rice products per kg body weight (Munera-Picazo et al., 2014). In January
51 2016, The European Union (EU) legislated a maximum contaminant limit (MCL) of 0.250 mg/kg iAs for
52 husked rice, 0.200 mg/kg iAs in rice and 0.100 mg/kg iAs in rice destined to produce baby food (Signes-
53 Pastor et al., 2017; The Commission of the European Communities, 2015) (**Table S1**) in order to protect
54 infants, young children and the general population from ingesting elevated iAs levels through rice
55 consumption.

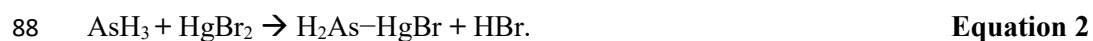
56 The EU legislation restricts importation of rice and rice products violating the legislated limits into
57 European Union member countries (The Commission of the European Communities, 2015). Thus, it
58 became a requirement that rice and rice products imported into EU be certified to meeting the legislated
59 limits. Not only has the EU set iAs MCL for rice, but other regulatory bodies have also set regional or
60 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
61 total arsenic (tAs) (**Table S1**). A market survey on rice products destined for babies and infants, bought
62 after the introduction of the MCL in the EU, found that almost 50% of the products did not comply with
63 the legislation (Signes-Pastor et al., 2017).

64
65 To date, a number of robust analytical methods for detecting and quantifying tAs and iAs in rice have been
66 developed and reported (Feldmann, Raab, & Krupp, 2017; Hung, Nekrassova, & Compton, 2004;
67 Kinniburgh & Kosmus, 2002). The established methods all use HPLC coupled to Inductively Coupled

68 Plasma Mass Spectrometry (ICP-MS) and give reliable results but suffer from high costs and lack of
69 availability in many routine analytical laboratories, therefore other cheaper methods have been developed
70 for the detection of iAs in rice such as hydride generation (HG) coupled to AFS (Chen, Ma, & Chen, 2014)
71 or ICP-MS (Chen et al., 2014; Petursdottir et al., 2014). Although these robust analytical instruments are
72 valid and reliable, they are laboratory based and too bulky to transport to field for on-site analyses (Bralatei,
73 Lacan, Krupp, & Feldmann, 2015; Sankararamakrishnan, Chauhan, Nickson, Tripathi, & Iyengar, 2008).
74 Therefore, there is a need to develop less expensive, portable and robust field methods to use for screening
75 it's the iAs content in rice on-site in low income rice producing countries that are challenged in accessing
76 robust laboratory based analytical instruments. In view of this challenge, Bralatei et al. (2015) modified a
77 commercial arsenic field kit designed to measure iAs in water into a field deployable method (FDM) to
78 allow determination of iAs in rice on-site. The method developed by Bralatei et al. (2015) employs the
79 Gutzeit reaction in which the sample containing As(III) and/or As(V) reacts with sodium borohydride under
80 acidic conditions and converts both species of iAs to arsine gas (AsH₃) (**Equation 1**) (Bralatei et al., 2017,
81 2015; Hung et al., 2004).



83 During the reaction, arsine gas formed evolves and reacts with a mercuric bromide impregnated filter lid to
84 form a colored Lewis acid/base arsenic mercury product (H₂As–HgBr), while methylated arsines do not
85 form any complex with the mercury bromide (**Equation 2**) (Bralatei et al., 2015; Fransisca et al., 2015;
86 Kinniburgh & Kosmus, 2002). The intensity of the orange/yellow color is proportional to the concentration
87 of iAs in the sample solution.(Bralatei et al., 2015; Sankararamakrishnan et al., 2008).



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

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

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

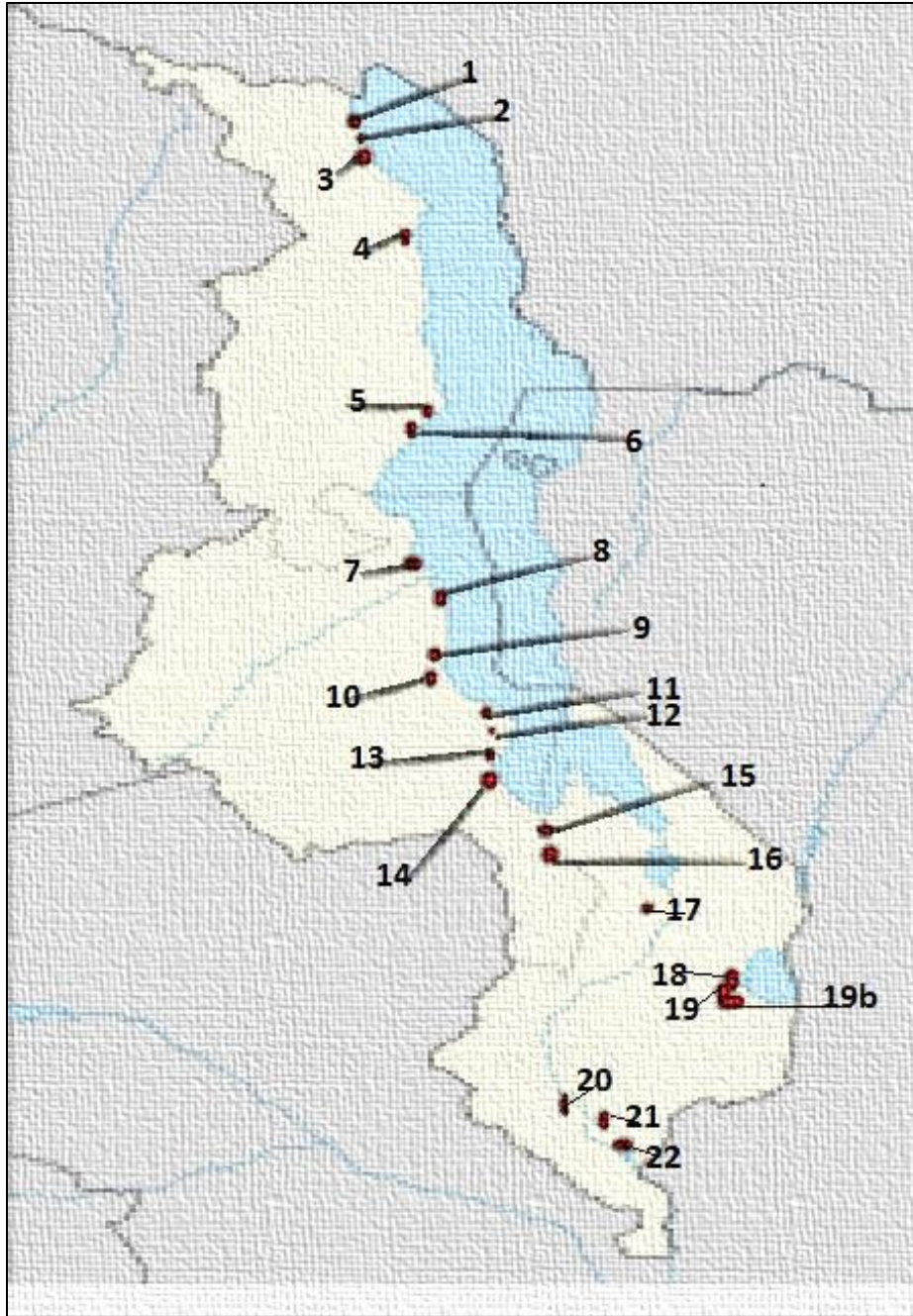
100 *Hypothesis 2:* In reference to international guideline values, the number of false-positives and false-
101 negatives results is low for both FDM and laboratory based analytical instruments results. Thus, iAs values
102 obtained on-site using the FDM are reproducible with laboratory based analytical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

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104 **2. EXPERIMENTS AND METHODS**

105 **2.1. Samples and sampling.** The rationale for the sampling design was to cover all rice producing districts
106 of Malawi, the most abundant cultivars and all rice products used in the country. Thirty-three rice samples
107 (whole grain, rice bran, rice husks, unpolished rice and polished rice) of different rice cultivars that included
108 Kilombero, Faya, TCG-10, Nunkile and Nerica were analysed on-site at various rice farms, rice irrigation
109 schemes and research stations located in 10 rice growing districts in Malawi (**Figure 1**). The sampling sites
110 were mainly along the lake shores of Lake Malawi, Lake Chilwa and Lake Malombe and along the Shire
111 River Valley (**Figure 1**). Two rice fields per rice scheme or research station were randomly selected for on-
112 site analyses. Composite rice samples (50 to 150 g) were collected from 5 different randomly selected points
113 in each field. Replicate samples, collected for laboratory analyses, were labelled and packed in zipp-log
114 bags with details of site location, rice material, rice cultivar and date of sampling.

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117 **Figure 1.** Map of Malawi showing sampling locations where on-site iAs determination was conducted.

118 Sites are Lufiriya rice scheme (1), Baka research station (2), Hara rice irrigation scheme (3), Chiweta (North

119 Rumphi) (4), Limphasa irrigation scheme (5), Nkondezi research station (6), Liwaradzi (7), Dwangwa (8),

120 Nkhunga and Mtupi (9), Chimphangwi (10), Lifuwu research station (11), Maganga/Sengedzi river (12),

121 Ndindi (13), Chipoka (14), Bwanje irrigation scheme (15), Bwanje scheme (research) (16), Balaka–

122 Liwawadzi river (17), Domasi irrigation scheme (18), Khanda irrigation scheme (19), Likangala rice
123 scheme (19b), Kasinthula research station (20), Nazolo irrigation scheme (21) and Nkhate irrigation scheme
124 (22).

125 **2.2 External calibration of FDM and quality assurance.** Accuracy of FDM in both laboratory and on-
126 site analyses was checked using As(V) standards. Calibration standards of As(V) solutions were prepared
127 by diluting 1000 mg/L of As(V) in 1% (v/v) HNO₃ solutions. Recoveries of As(V) standards were computed
128 as percentage of determined value to theoretical value of the standard. Limit of detection was calculated as
129 $LOD = X + 3 * SD$ (where X is the mean blank value in mg/kg of As(V) solutions and SD is the standard
130 deviation of blanks iAs concentration). Recoveries were evaluated daily to check accuracy of the method.
131 Computed recoveries are reported in **Table S2**.

132
133 **2.3 On-site and Laboratory sample extraction and determination of iAs using the FDM.** In the field,
134 air dried rice materials were ground using a coffee grinder. Rice husk and rice bran were mainly collected
135 from rice mills in rice irrigation schemes or research stations. Approximately 5.0 g of homogenized ground
136 and air dried rice material (whole rice grain (WGR), rice husk (RHU), rice bran (RBR), polished rice (POR)
137 and unpolished (brown) rice (UPR)), scooped using a graduated spoon (on-site) and accurately measured
138 using an analytical balance (in the laboratory), were mixed with 50 ml of 1% nitric acid (HNO₃) in a 250
139 ml beaker and extracted by boiling the mixture at around 90-100 °C temperature for 20 min using a gas
140 stove (on-site) and electric stove with adjustable control knob (in the laboratory); thereafter extracts were
141 cooled for 3-5 min at ambient room temperature and a further 5-10 min in a water bath (tap water). Loss of
142 heat by convection may have occurred during on-site analytes extraction since boiling was done in an open
143 space which may have an effect on the uniform analyte extraction. The entire sample extract was then
144 transferred into an Erlenmeyer volumetric reaction flask which was tightly closed with a tri-filter bung
145 device fitted with detector slips, immediately after adding 0.050 to 0.100 ml (2-3 drops) of antifoam, 0.150
146 g (one sachet) of sulfamic acid (Palintest, U.K.), and 0.500 g (one tablet) of sodium borohydride (NaBH₄)

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.

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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 $\mu\text{g}/\text{kg}$), 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 $\mu\text{g}/\text{kg}$ was comparable to the LOD value of 50 $\mu\text{g}/\text{kg}$ reported by Bralatei et al., (2015). The

172 accuracy of the method in both laboratory and onsite analyses was determined using As(V) standards (5,
173 10, 20, 25, 50, 75 and 100 $\mu\text{g}/\text{kg}$) with theoretical concentrations of 5.0 ± 1.2 , 10.2 ± 1.0 , 20.1 ± 0.5 , $25.8 \pm$
174 1.5 , 50.4 ± 1.6 , 75.8 ± 0.2 and 100.2 ± 2.4 $\mu\text{g}/\text{kg}$. Percentage recoveries for each As(V) standard ranged
175 from 79.8 to 127% for on-site measurements; and 82.0 to 117.1% for laboratory analyses (**Table 1**) which
176 are comparable to recoveries of 72–120% reported by Williams, West, Koch, Reimer, & Snow (2009) and
177 81–150% reported by (Safarzadeh-Amiri et al., (2011) and Sankararamakrishnan et al. (2008). Average
178 variability of the determined concentration for As(V) standards was low (11.5% for on-site analyses and
179 7.8% for laboratory analyses) and indicating excellent agreement with theoretical values. *T*-test *p*-values
180 (**Table 2**) showed that laboratory analyses of As(V) standards were not significantly different from on-site
181 analyses ($p=0.984$; significant at $\alpha=0.05$). Mean theoretical As(V) concentrations were correlated with
182 mean measured laboratory As(V) values of standards. Slope and R^2 values were very close to 1 (**Figure**
183 **S1**) indicating minimal biasness and strong correlation of the data sets respectively.

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

187 Reference material (NIST 1568a Rice Flour) was analysed for tAs and a concentration of 285 ± 50 $\mu\text{g}/\text{kg}$ As
188 was obtained. The tAs was in excellent agreement with certified tAs value (290 ± 30 $\mu\text{g}/\text{kg}$ As). Furthermore,
189 iAs (sum of As(V) and As(III)), DMA and MMA were also determined and 104 ± 15 $\mu\text{g}/\text{kg}$ iAs, 165 ± 17
190 $\mu\text{g}/\text{kg}$ DMA and $<15 \pm 2.5$ $\mu\text{g}/\text{kg}$ MMA were obtained. The obtained amounts of As species were within the
191 previously reported ranges (Heitkemper, Vela, Stewart, & Westphal, 2001; Narukawa & Chiba, 2010).
192 Narukawa & Chiba (2010) reported iAs, DMA and MMA values of 98 ± 2 , 175 ± 2 and 13 ± 1 $\mu\text{g}/\text{kg}$
193 respectively whereas Juskelis, Banaszewski, & Cappozzo, n.d.; Juskelis et al. (2013) reported 100 ± 20 ,
194 171 ± 34 and 11 ± 2 $\mu\text{g}/\text{kg}$ which are both comparable to values obtained in this study, hence acceptable.

195 **Table 1.** Comparison of theoretical concentrations versus mean experimental concentrations of As(V)
 196 standards determined using FDM on-site and laboratory. Mean concentrations are reported as $\mu\text{g}/\text{kg} \pm \text{SD}$
 197 (n=6).

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

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199 **3.2. Comparison of iAs values determined**

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

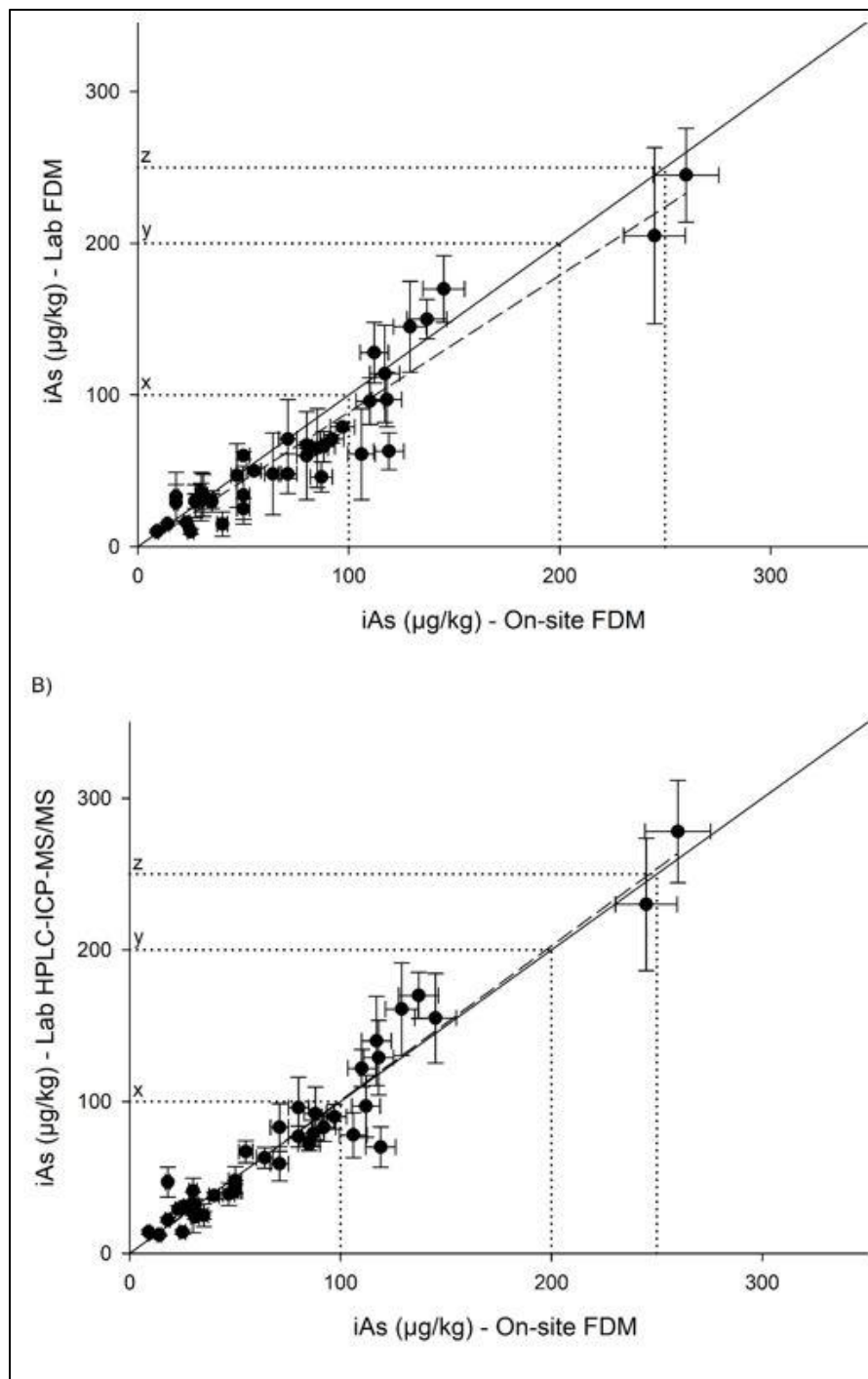
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211 **Table 2.** Comparison of iAs values 33 rice material samples tested by FDM on-site and laboratory by
212 HPLC-ICP-MS/MS (laboratory) indicating p-values, slope, and y-intercept and Pearson correlation
213 coefficient

Parameter	T-test p-value^d	rho^c	Linear regression equation	R²
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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215

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 $\mu\text{g/kg}$ for rice intended for

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

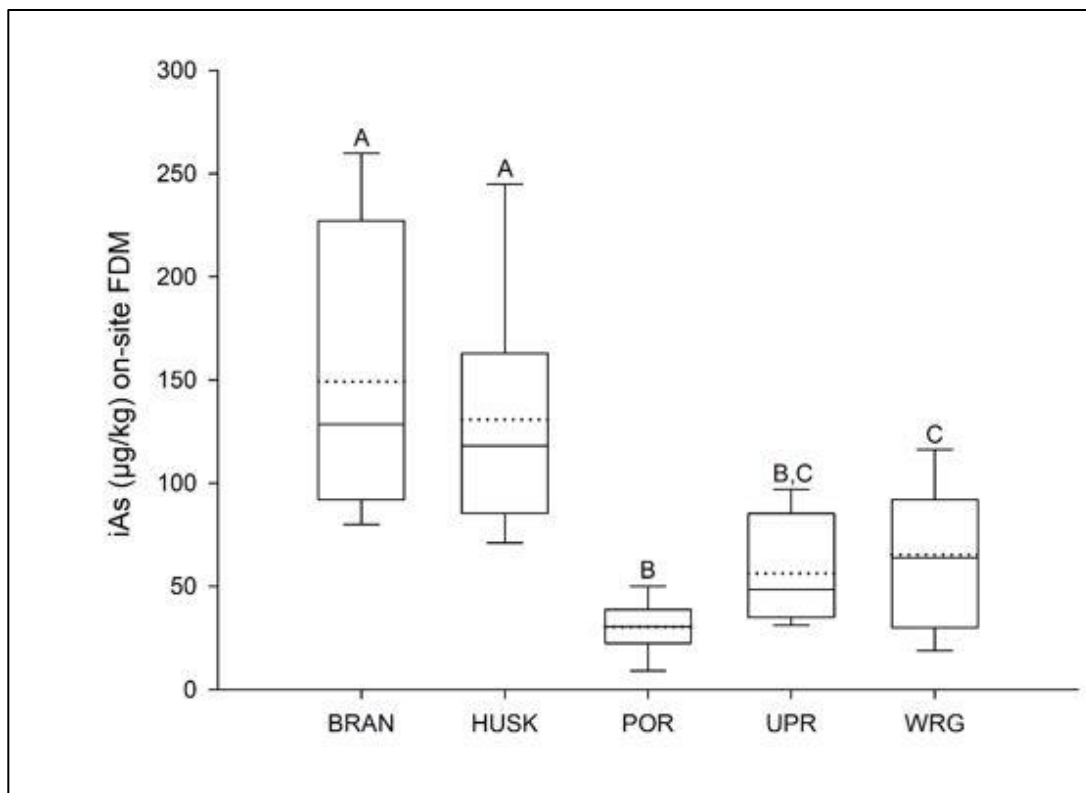
222 Recovery efficiency tests indicated that 27 out of 33 on-site iAs values (82%) were within $\pm 22\%$ of the
223 HPLC-ICP-MS/MS results which is acceptable and within the range of recoveries for rice (89.5% to
224 116.3%) reported by Bralatei et al. (2015) and 29 out of 33 (88%) on-site iAs values were within $\pm 17\%$ of
225 Lab iAs values which is also acceptable. The overall on-site relative standard deviation (RSD) of both on-
226 site vs HPLC-ICP-MS/MS and on-site vs LAB FDM iAs values was found to be $\pm 14\%$ (**Table S2**) slightly
227 higher than the RSD reported by Bralatei et al. (Bralatei et al., 2015) for LAB FDM vs HPLC-ICP-MS/MS
228 ($\pm 12\%$). Nevertheless, on-site iAs values above or below HPLC-ICP-MS values (18%); and above or below
229 Lab iAs values (12%) were not significantly different, indicating good precision. Higher on-site variability
230 could be attributed to variable estimation of sample masses due to variable densities of rice grain, rice bran
231 and rice husks (though not significant), non-uniform on-site analyte extraction (boiling may not be uniform
232 in open space), and variable sample moisture content for samples analysed on-site (**Table S3**). However,
233 sample moisture content in laboratory analyses was checked and determined to range from 2.0% to 20.7%
234 which may have negligible effect on final iAs concentration in rice when a nominal $\pm 10\%$ moisture
235 content was used for correction. As shown in **table S3**, iAs concentration values corrected with real
236 moisture content were not significant different from those corrected with nominal 10% moisture content
237 ($p > 0.877$ significant at 0.001).

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

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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 $\mu\text{g}/\text{kg}$) compared well to mean iAs of 60 $\mu\text{g}/\text{kg}$ reported by Joy et al. (2016) for unpolished (brown) Malawian rice. The mean iAs concentration in polished Malawian rice (mean: 31 ± 12 $\mu\text{g}/\text{kg}$; range $9 - 54$ $\mu\text{g}/\text{kg}$) is amongst the lowest ever reported concentrations for iAs in rice worldwide (Meharg et al., 2009; P. N. Williams et al., 2005).



259
 260 **Figure 3.** Inorganic As content of various rice materials as determined by field deployable method on-site.
 261 Rice material that share common letters (A, B and C) are not significantly different from each other. The
 262 boxes represent first and third quartile range iAs values; the solid line across a box represents the median
 263 value; the dotted line across a box represents the mean values and whiskers represent minimum and
 264 maximum values.

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 266 **3.4. Screening of rice samples in the field using EU MCL as a yard stick.** The accuracy and precision
 267 of FDM on-site screening was evaluated with reference values to check compliance of iAs content to EU
 268 legislated limits. Evaluation was done based on both wet weight (w/w) basis with the corrected 10 %
 269 moisture and dry weight (d/w) basis upon determination of moisture content of each sample (**Table S1** and
 270 **Figure S5**). FDM on-site analysis identified 2 (6.1%) samples (for both w/w and d/w) and 11 (33.3%)
 271 samples (for w/w) and 10 (30.3%) samples (d/w) with iAs concentration values exceeding the legislated
 272 limits of MCL of 200 µg/kg and 100 µg/kg respectively which compared well to those obtained in the

273 laboratory using FDM (6.1%) at MCL of 200 $\mu\text{g}/\text{kg}$ and 24.2 % at MCL of 100 $\mu\text{g}/\text{kg}$) and the reference
274 method (6.1% at MCL of 200 $\mu\text{g}/\text{kg}$ and 27.3% at MCL of 100 $\mu\text{g}/\text{kg}$) (**Figure S5**) suggesting high accuracy
275 and precision. Interestingly, neither polished (white) rice nor brown (unpolished) rice contained iAs
276 exceeding the limit of 200 $\mu\text{g}/\text{kg}$ while 1 sample of brown (unpolished) rice exceeded the limit of 100
277 $\mu\text{g}/\text{kg}$. As observed in these results, iAs concentration values determined using FDM in rice materials on a
278 w/w basis did not significantly change after determination and factoring in the effect the of low moisture
279 content (which ranged from 2.0% to 20.7%) (**Table S3**).

280

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

Deciding MCL value	FDM On-site iAs value (w/w)			FDM Lab iAs value (w/w)		
	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%
Deciding MCL value	FDM On-site iAs value (d/w) ^δ					
	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%			

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

284

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

298 false-positive and false- negative results (Safarzadeh-Amiri et al., 2011), low false positive and false
299 negative values obtained for both samples with low (<100 µg/kg) (9.1% and 3.0%) and high (>100 µg/kg)
300 iAs values (0% and 0%) respectively suggests that accuracy and precision of our the method (FDM) is high.
301 However, despite obtaining low false-positive and false- negative values for rice samples with both low
302 and high iAs values, samples with lower iAs (<100 µg/kg) values exhibited relatively higher false positive
303 and false negative values than those samples with high iAs (>100 µg/kg) values implying that precision
304 could be influenced by the low iAs values (<100 µg/kg) supporting findings reported by Kinniburgh &
305 Kosmus (2002) and Safarzadeh-Amiri et al. (2011). Nevertheless number of false positive and false
306 negative values obtained in this on-site study were not significantly different from laboratory analyses
307 reported by Bralatei et al. (2015) (10% and 7% respectively) for polished rice samples and those reported
308 by Safarzadeh-Amiri et al. (2011) (11% and 2%) for tube-well water. The observation also demonstrates
309 that effect of moisture content, the use of a scoop rather than a balance in the collected samples was low
310 and insignificant to negatively influence accurate and precise screening. Furthermore, cconsidering that the
311 two different methods use different analytical procedures (iAs is chemically mobilized to form AsH₃ in the field kit
312 but arsenic species behave differently in anion exchange chromatography), the strong reproducibility of iAs content
313 in these methods (low false-positive and low false- negative values) indicates that the interferences of redox
314 active elements (Cu, Mn, Fe, and Zn) were minimal and/or not significant.

315 **CONCLUSION**

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

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

330

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339

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