Full length article

A simple method for direct mercury analysis in dried blood spots (DBS) samples for human biomonitoring studies

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A B S T R A C T

Human exposure to mercury can have serious health effects, especially in vulnerable groups such as children and fetuses. The use of dried blood spot (DBS) samples to collect capillary blood greatly facilitates sample collection and fieldwork, being a less invasive alternative to blood collection by venipuncture, needing a small volume of sample, and does not require specialized medical staff. Moreover, DBS sampling reduces logistical and financial barriers related to transport and storage of blood samples. We propose here a novel method to analyze total mercury in DBS samples in a Direct Mercury Analyzer (DMA) that allow the control of the volume of the DBS samples. This method has shown good results in terms of precision (<6% error), accuracy (<10% coefficient of variation) and recovery (75–106%). The applicability of the method in human biomonitoring (HBM) was demonstrated in a pilot study involving 41 adults aged 18–65. Mercury concentrations of DBS samples from capillary blood collected by finger prick (real DBS samples) were determined in the DMA and compared with those determined in whole blood (venous blood) by ICP-MS, the method usually used in HBM. The sampling procedure was also validated by comparison of real DBS samples and DBS generated artificially in the laboratory by depositing venous samples in cellulose cards (laboratory DBS). There were no statistically significant differences in the results obtained using both methodologies (DMA: Geometric Mean (confidence interval 95%) = 3.87 (3.12–4.79) μg/L; ICP-MS: Geometric Mean (confidence interval 95%) = 3.46 (2.80–4.27) μg/L). The proposed method is an excellent alternative to be applied in clinical settings as screening methodology for assessing mercury exposure in vulnerable groups, such us pregnant woman, babies and children.

1. Introduction

Mercury is a global environmental pollutant with high toxicity for human and animal life (EEA, 2018; UNEP, 2017). The World Health Organization (WHO) included mercury among the top 10 chemicals of "major public health concern" (WHO, 2013a), and international efforts are focused on to reduce human health and environmental risk from mercury releases (UNEP, 2017; Minamata Convention on Mercury, 2013).

Mercury toxicity and adverse health effects e.g. cardiovascular, reproductive, renal and central nervous systems, have been well described in numerous studies (Yorifuji et al., 2013; Bernhoft, 2012; Kurland et al., 1960). Methylmercury is the chemical species of mercury of greatest human health concern (UNEP, 1990) since it is well known that it affects the fetus' neurodevelopment (Bjørklund et al., 2017; Grandjean et al., 2014).

Human biomonitoring (HBM) programs and studies usually include mercury among the chemicals analysed. The major source of methylmercury exposure in the general population is the diet, mainly through seafood consumption (Castaño et al, 2015; WHO, 2013b; MacIntosh et al., 1996) that can contain high levels of methylmercury. The source of exposure to inorganic and elemental mercury includes occupational settings (dentistry, gold mining) or the contact with products containing this metal (dental amalgams, fluorescent bulbs, etc.) (Basu et al., 2018; Ha et al., 2017; UNEP/WHO, 2008).

Blood, urine and hair are the matrices commonly used to assess exposure to mercury, providing different information about the mercury chemical species and time of exposure (Esteban-López et al., 2022). The measurement of total mercury in blood reflects exposure to all chemical forms, organic, inorganic and elemental mercury, and provides information on recent exposure (1–2 moths) (Clarkson and Magos, 2006).

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Blood sampling is traditionally done by collecting venous blood by venipuncture. This procedure is quite invasive, usually it requires a clinical setting as well as trained health professionals and special transport and preservation conditions. Furthermore, the limitation in the volume can be a problem in some populations (e.g. newborns and children). Dried blood spot (DBS) samples are a promising alternative to conventional blood samples in HBM, facilitating the logistics in the fieldwork as they are easy to collect, preserve, transport and store. DBS samples are commonly and routinely used in the clinical setting, for example in newborn screening to identify inborn metabolism errors (Mei et al., 2001; Guthrie and Susi, 1963) or in therapeutic drug monitoring (Enderle et al., 2016) and they have become increasingly popular in HBM studies (Nyanza et al., 2019a, Nyanza et al., 2019b; Funk et al., 2015; Vacchina et al., 2014; Funk et al., 2013; Langer et al., 2011; Chaudhuri et al., 2009; Hsieh et al., 2009), including mercury exposure assessment (Santa-Ríos et al., 2021; Schweizer et al., 2021; Nyanza et al., 2020; Nelson et al., 2016).

Mercury analysis can be done by different analytical techniques, although inductively coupled plasma mass-spectrometry (ICP-MS) has traditionally been preferred (Suvarapu and Baek, 2015) because it allows the analysis of a high number of samples in a reduced time, and potentially multiple elements can be analysed simultaneously in a single run at very low concentrations (ppb-ppt). However, it requires sophisticated facilities, well trained staff, and in some cases, sample pre-treatment which leads to an increase in the analysis time. An alternative method for selective mercury analysis is the thermal decomposition, amalgamation and atomic absorption spectrometry (TDA-AAS). This is a sensitive method that allows the direct analysis of total mercury in different environmental and biological matrices (Schweizer et al., 2021; Soares et al., 2015; Torres et al., 2015; Lasrado et al., 2005; Cizdziel and Gertenberger, 2004) without sample pre-treatment reducing the time of analysis.

The analysis of total mercury in DBS samples by TDA-AAS can be an alternative to assess mercury exposure in clinical settings or to perform HBM studies in remote locations with limited resources or focus on sensitive populations (such as newborns and children). This work proposes a method for the analysis of total mercury in DBS using TDA-AAS in a direct mercury analyser (DMA) as a tool for prevention in public health.

2. Material and methods

2.1. Method development

Mercury measurement in DBS samples was carried out by TDA-AAS using a Direct Mercury Analyzer DMA-80 Tricell (Milestone, Inc., Shelton, CT). The optimal analysis conditions, drying/decomposition (time and temperature), were tested to evaluate completed combustion and memory effect. To select the initial instrument's parameters the guide provided by the instrument's manufacturer and EPA method 7473 (EPA, 1998) were consulted.

The optimal volume of the DBS samples to obtain mercury signal above the background of the DMA instrument, was determined by depositing 30 and 50 µL of blood samples at increased mercury concentration onto Whatman 903 protein saver cellulose cards (GE Healthcare, Dassel). The optimal volume was subsequently used for the validation of the method and the subsequent pilot study.

Calibration curves were prepared by spiking blank blood (defibrinated sheep blood, ThermoFischer Scientific, Inc.) with increasing concentrations of mercury certified solution of 10 µg/mL in 5% HNO_3 (PerkinElmer, Inc.).

These ranged from 0.15 to 20 µg/L, to cover the range of blood mercury concentrations reported in the general population in previous studies (Castaño et al., 2019; Basu et al., 2018; Bellanger et al., 2013). A controlled volume of 50 µL of each concentration was deposited in Whatman cellulose cards using calibrated microcapillary tubes (50 µL ±

0.25%, Hirschman, Fisher Scientific).

For ICP-MS calibration, increased mercury concentrations spiked in blank blood matrix in the range of 0.025–25 µg/L were used.

2.2. Method validation

To validate the proposed method for total mercury measurement in DBS using the DMA, different quality parameters were determined: detection limit, quantification limits, linearity range, precision, accuracy and recovery.

The potential effect in the results of the background mercury levels present in the Whatman cellulose cards was checked. Blanks, consisting on empty circles of different cards, were analyzed in the DMA. These results were used for calculating the limits of detection and quantification, as follows: $LOD = 3*SD_{blank}$ and $LOQ = 10*SD_{blank}$, with SD_{blank} = standards deviation of blank measurements (n = 30).

The materials used to calculate the quality parameters were a set of four in house control material consisting of defibrinated sheep blood spiked with different mercury concentrations (1.5, 4, 8 and 16 µg/L, from a mercury certified solution at 10 µg/mL); human whole blood surplus material from the Quebec Multielement External Quality Assessment Scheme, QMEQAS, INSPQ (3.53, 7.04, 13.7 and 38.6 µg/L); and two blood certified reference materials (Seronorm® Trace Elements Whole Blood level 1 (1.57 µg/L) and level 2 (16.6 µg/L), Sero AS, Bilingstad, Norway).

2.3. Testing the applicability of the method in a HBM study

The applicability of the method in HBM studies was tested in a pilot study carried out with volunteers from the staff of the National Centre for Environmental Health of the Instituto de Salud Carlos III.

The study complied with the Spanish Legislation regarding human research and was approved by the Research Ethics Committee of the Instituto de Salud Carlos III.

2.3.1. Sample collection

Blood samples were collected in January-February 2021 from 41 adults aged 18 to 65.

DBS samples were collected following the SOP elaborated (Suplementary material) and based on the Bond and Richards-Kortum (2015) and EQA-WHO protocol (WHO, 2005). Briefly, one finger (middle or ring finger) was warmed and pricked with a contact-activated lancet (BD Microtainer, High flow, 1.5 mm blade, Franklin Lakes, NJ). The first drop of blood was removed with a wipe to avoid possible contamination. Then, blood was collected using a 50 µL microcapillary tube and deposited into a dotted circle of a cellulose card. At least three DBS samples (real DBS) were collected from each participant in one cellulose card. The cellulose cards were left to dry at room temperature and then packed into a ziplock plastic bag with silica gel to control humidity and stored at room temperature until analysis.

Blood samples were collected by venipuncture using NH trace element sodium heparin tubes (6 mL) (Vacuette® Greiner BioOne, Monroe, NC) and kept at 4 °C. Once in the laboratory, four subsamples taken from the Vacuette® tube were collected with clean capillary tubes and deposited in the cellulose card to create DBS (laboratory DBS) for analysis in the DMA. The remaining volume was aliquoted into 0.5 mL volume PP tubes and stored at −20 °C until analysis by ICP-MS.

2.3.2. Sample analysis

Mercury analysis both from real and laboratory DBS samples was conducted by TDA-AAS in the DMA applying the method developed.

The DBS samples were carefully cut including the dotted area of the circles using porcelain scissors. The sample was directly deposited in the nickel boat and analysed in the DMA. Internal quality controls were measured routinely at the beginning and the end of the run, and each 10 samples. These quality controls were DBS samples generated by

depositing certified reference material (Seronorm® Trace Elements Whole Blood, level 1 and 2) just before the analysis. Field blanks and laboratory blanks (empty circles from Whatman cellulose cards) were also included every ten samples. The laboratory blanks together with the self-cleaning protocol performed by the DMA when the levels of mercury reach 50 ng, allowed the control of possible contamination of the system and from one sample to other.

Mercury concentrations in blood samples were measured by inductively coupled plasma mass spectrometer, ICP-MS (Elan DRC-e, Perkin Elmer) in clean-room facilities according to the method described in Castaño et al. (2019). In brief, blood samples were mixed gently for homogenization and diluted 1:50 in an aqueous solution containing 10 ppb of rhodium as internal standard, triton X-100 (0.05%, v/v), EDTA (0.05%, v/v), propanol (10%, v/v), 10 µg/L $H(AuCl_4)$ and tetramethy-lammonium hydroxide (TMAH) (1%, v/v). Internal quality controls were applied routinely (Seronorm® Trace Elements Whole Blood, level 1 and 2), at the beginning and the end of the run, and each 10 samples.

2.4. Statistical analysis

Statistical analysis was performed with R 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria). The data were logarithmically transformed and arithmetic mean, geometric mean, quartiles, minimum, and maximum were calculated. Differences in means and standard deviation were tested by T-test and F-test, respectively, and Pearson correlation was also evaluated across all pairs of mercury blood analysis assayed. Deming regression models were used to compare the results obtained in the two methods. In addition, a Bland-Altmant plot analysis (Bland and Altman, 1986) was elaborated to investigate the agreement between both methods.

3. Results and discussion

3.1. Method development and validation

The optimal instrument operating conditions were: drying at 200 °C for 60 sec, 60 sec ramp to 650 °C, and finally decomposition at 650 °C for 90 sec.

Both volumes tested, 30 and 50 µL, provided mercury signal above the background of the instrument. However, 50 µL was the volume used for the validation of the method, and the subsequent pilot study, for better results in case of samples with low mercury concentrations.

The calibration curve linearity was evaluated by a square fit model using the DMA 80-Tricell internal software. Linear regression from pooled data of 15 calibration curves was y = 0.1088x-0.0002 (CI 95% Slope: 0.102, 0.116; Intercept: −0.001, 0.0006) and coefficient determination (R^2) was 0.998 (CI 95%: 0.996, 1.0).

The linearity parameters obtained in this work show good agreement ($R^2 = 0.998$) in the range of mercury concentrations tested (0.15 to 20 µg/L). This range covers total mercury levels expected in the general population, which are generally < 5 µg/L (Basu et al., 2018; Bellanger et al., 2013). However, higher values have been reported in populations with high seafood consumption, e.g. geometric mean of 6.35 µg/L as reported by Castaño et al. (2019) or a pooled central median of 8.6 µg/L (Interquartile range, IQR: 2.9–21.2 µg/L) across the cross-sectional studies reviewed by Basu et al. (2018). Higher mercury concentrations have also been reported in groups exposed to inorganic and elemental mercury including occupational exposure e.g. dental and artisanal gold mining workers (pooled central median of 6.9 µg/L (IQR: 2.8–12.3 µg/L) (Basu et al., 2018). In addition, mercury concentrations in children have been reported in the range of 0.21 to 10 µg/L, also covered by the range of concentrations tested in the present study (Basu et al., 2018).

The mercury background concentration present in cellulose cards used as blanks was in the range of 0.00065–0.00084 ng, that correspond to 0.013 to 0.0168 µg/L assuming 50 µL of volume, much lower than the levels found in blood samples. Therefore, it was decided not to include a blank correction in the measurements. This is consistent with a previous study, where the use of a blank correction was not recommended at individual sample level (Funk et al., 2015).

The calculated LOD was 0.10 µg/L of mercury for a volume of 50 µL. These results are in line with recent studies using blood microsampling (DBS or VAMS (volumetric absorptive microsampling)) and total mercury analysis in the DMA, e.g. Schweizer et al. (2021) obtained a LOD value of 0.14 µg/L in a study using DBS samples estimating a volume of 60 µL (Table S1) from 44 non-exposed volunteers, and Koutsimpani-Wagner et al. (2022) reported a LOD value of 0.10 µg/L using double VAMS sampling devices (estimated volume 46 µL) (Table S1). The LODs reported for measurements on DBS using ICP-MS (Table S1), vary from 10 times lower (0.012 µg/L; Nyanza et al., 2019a) to 7 times higher (0.7 µg/L; Nelson et al., 2016) than the one achieved here.

Regarding the LOQ a value of 0.40 µg/L was obtained. This allows its application as a screening tool in most general population studies in which reported ranges are from 0.65 to 3.4 µg/L from national HBM studies (Basu et al., 2018). A challenge for the applicability of this method however could be the determination of blood mercury in newborns, babies and children, in which the levels can be approximately 2-fold lower than in adults as reported in different national HBM studies (Basu et al., 2018). According to Basu et al. (2017), estimated DBS total mercury in newborns range from 0.3 to 0.5 µg/L, reaching levels of 1.8 µg/L in the case of children. However, the accuracy of these

Table 1
Quality control parameters for mercury spiked blood samples, certified reference materials and samples from surplus of interlaboratory assays (QMEQAS) deposited in Whatman cellulose cards analysed in the DMA.

Spiked samples concentration µg/L	Measurements obtained (SD) µg/L	Accuracy (Error, %)	Precision (CV, %)	% recovery
1.5	1.13 (0.06)	24.5	5.56	75.4
4	3.26 (0.12)	18.4	3.69	81.6
8	7.65 (0.17)	4.37	2.26	95.6
16	15.6 (0.40)	2.68	2.58	97.3
Certified Reference Material Concentration (Nominal value, µg/L)	**Measurements obtained (SD) µg/L**	**Accuracy (Error, %)**	**Precision (CV, %)**	**% recovery**
Seronorm L1 (1.57)	1.66 (0.08)	6.05	4.77	106.0
Seronorm L2 (16.6)	17.5 (0.29)	5.70	1.67	105.7
QMEQAS material concentration (Assigned value, µg/L)	**Measurements obtained (SD) µg/L**	**Accuracy (Error, %)**	**Precision (CV, %)**	**% recovery**
3.53	3.42 (0.37)	3.07	4.77	96.9
7.04	6.46 (0.40)	8.30	4.79	91.7
13.7	13.2 (0.44)	3.33	3.84	96.7
38.6	34.9 (1.21)	9.52	1.46	90.5

Table 2
Results of the pilot study to test the applicability of the analysis of total mercury in DBS samples.

Method	Geometric mean µg/L (CI 95%)	Range µg/L	N < LOQ
ICP-MS	3.46 (2.80–4.27)	0.060–11.29	1
DMA	3.87 (3.12–4.79)	0.218–13.32	1

$LOQ_{DMA} = 0.40$ µg/L, $LOQ_{ICP-MS} = 0.10$ µg/L.

measurement was not guaranteed as they were based on estimations on the volume spotted onto the DBS. Therefore the method developed here is also suitable for measuring total mercury levels in these relevant population groups.

In addition, the method is applicable in the estimation of the fetal exposure to mercury, which is frequently carried out by measuring mercury levels in cord blood. The levels reported in this matrix range from the central median concentration of 1.4 µg/L described in cross-sectional studies to 3.6–8.2 µg/L in other HBM studies worldwide (Basu et al., 2017) being even higher, 5–10 µg/L, in birth cohorts (Basu et al., 2018).

The interest in monitoring the mercury levels in these vulnerable groups highlights the need for methods that can circumvent their inherent sampling and detection issues. The method described here addresses this need.

The quality parameters obtained through the method validation meet the requirements previously fixed in the laboratory (Table 1), based on the guideline on bioanalytical method validation (EMA, 2009) and Guidance for Methods Development and Methods Validation for the RCRA Program (EPA, 2012).

Thus, an accepted level of accuracy was achieved for reference material and surplus material from QMEQAS. In the case of spiked samples, the accuracy quality control parameters were acceptable for higher mercury concentrations up to 4 µg/L.

The coefficient of variation (CV) for most of the assayed samples was below the 15% previously established, which indicates a good precision

of the method. Precision of human whole blood surplus material from QMEQAS was in the range 1.46–4.79%, close to the CV obtained in the certified material assayed (1.67–4.77%). In the case of in-house control material, the CV values obtained were slightly higher (2.26–5.66%).

The recovery percentage in all the samples assayed were in the range of 75–106%, which are within the acceptable recovery criteria established previously in our laboratory.

The absence of agreement in accuracy in mercury-spiked samples at concentrations lower than 4 µg/L could be due to errors during the sample preparation since samples were elaborated by serial dilution from mercury reference material of 10 mg/L and so, the dilution error could have accumulated along the dilutions. This explanation is reinforced by the fact that the parameters of accuracy and recovery in certified reference material are acceptable, and this material include concentration below 4 µg/L.

3.2. Comparison of the proposed method with ICP-MS analysis

Table 2 shows the main results obtained in the HBM pilot study samples analysed by the two methods. All DBS samples analysed in the DMA, except one, had mercury concentrations above the LOQ (0.40 µg/L). The blood sample of this participant was also below the LOQ when the analysis was done by ICP-MS (0.10 µg/L).

The results obtained in both cases, using ICP-MS and TDA-AAS, are similar but there is a trend of ICP-MS to underestimate mercury. This systematic underestimation has been observed also when comparing these two analytical techniques for mercury analysis in hair (Domanico et al. 2017).

The statistical analysis showed that there were no significant differences between the results obtained with both methods, real DBS samples analysed in the DMA and blood samples analysed by ICP-MS (Student test log data, 0.05; p-value = 0.4588).

After the method validation, it was possible to associate an uncertainty value for each mercury blood concentration assessed by each method. Fig 1 represents the mercury concentrations obtained in the
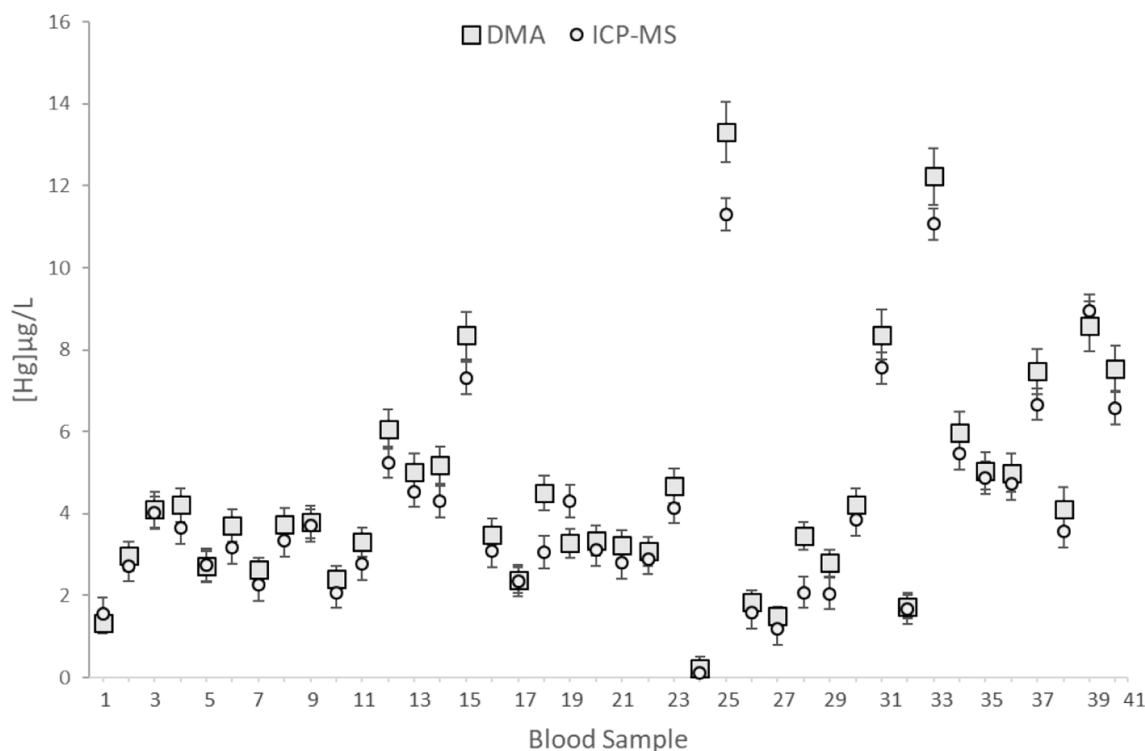

Fig. 1. Comparison of mercury concentrations analysed using the proposed method and ICP-MS. Dots and squares depict mercury concentrations for the two methods with the corresponding associated uncertainty. For each subject, real DBS were measured by DMA and venous blood by ICP-MS.
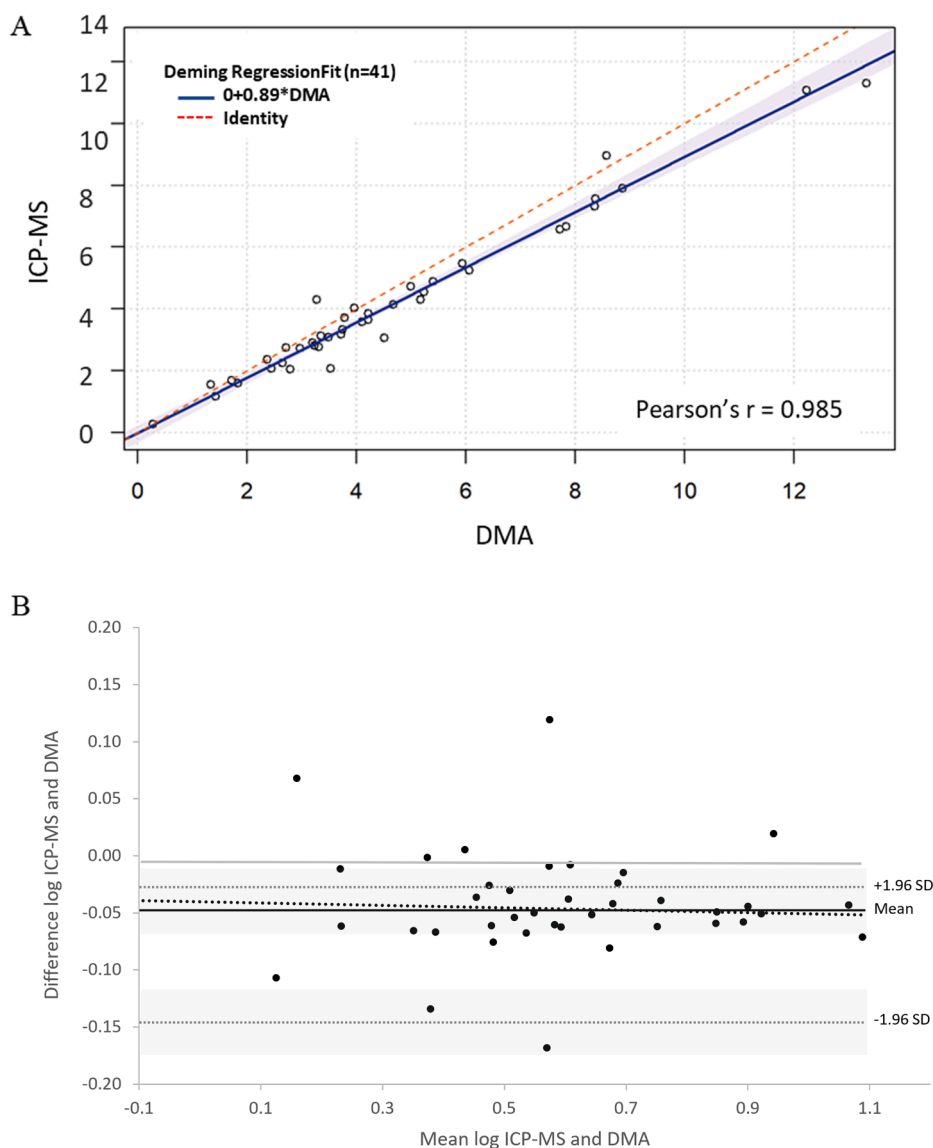
Fig. 2. (A) Regression plot between mercury analysis in blood by ICP-MS (µg/L) and in the DMA in DBS (real samples) (µg/L). Confidence interval 95% of the values is represented by a shaded area. (B) Bland and Altman plot of differences of measures between DMA (real sample) and ICP-MS. Solid line represents mean bias, doted gray line CI 95% (±1.96SD = standard deviation) with limits of agreement (grey light rectangle), doted black line represents the linear regression line.

same individual using both methods, DMA *vs* ICP-MS, and the associated uncertainty for each assessment. The uncertainty for each value overlaps in the majority of the results (92.5%), demonstrating a good agreement between the two methods.

Evaluating the correlation DMA *vs* ICP-MS, a good correlation was observed (Pearson's coefficient 0.985, p < 0.001). The relationship between both groups of samples was evaluated by Deming linear regression analysis. The models parameters indicate a no constant bias (Intercept: 0.00085 µg/L, CI 95%: −0.3164 – 0.2207) and slight deviations related to the concentration of mercury (negative proportional bias; slope: 0.894; CI 95%: 0.850–0.966). The regression curve was represented in Fig. 2(A).

The Bland and Altman analysis was used to quantify the agreement between the two quantitative measurements, in order to demonstrate whether a method may be safely substituted for another. The results are presented in Fig. 2(B). The mean bias of the difference between log data of mercury concentrations in the DBS samples measured in the DMA and log data of venous blood mercury concentration determined by ICP-MS was −0.046 (CI 95%: 0.029 – 0.064) respect to an ideal zero agreement.

As the difference was not zero, this means that on average, the measure of mercury levels in blood in the DMA was 4.6% higher than by ICP-MS.

Drawing a regression line of the differences between both mercury data obtained by DMA and ICP-MS in Bland and Altman analysis can help in detecting proportional differences respect to the concentration of mercury. The regression parameters (Slope: 0.0081; CI 95%: −0.041, 0.057) indicate that there is no proportional bias. Therefore, the two methods for mercury blood assessment could be used interchangeably.

Additionally, a cross validation was carried out to verify data obtained from different methods in the same samples. The Guideline in Bioanalytical Method Validation published by the European Medicines Agency (EMA, 2009) indicates that the difference between the two values obtained in different methods should be within 20% of the mean for at least 67% of the samples analyzed to confirm the reliability of the method. In the work presented here, 80% of the calculated differences between ICP-MS and DMA values lie within the criteria mentioned. These data corroborate the reliability of the method proposed for the analysis of mercury concentration in DBS in the DMA respect to the analysis of blood samples by ICP-MS, currently considered as the gold
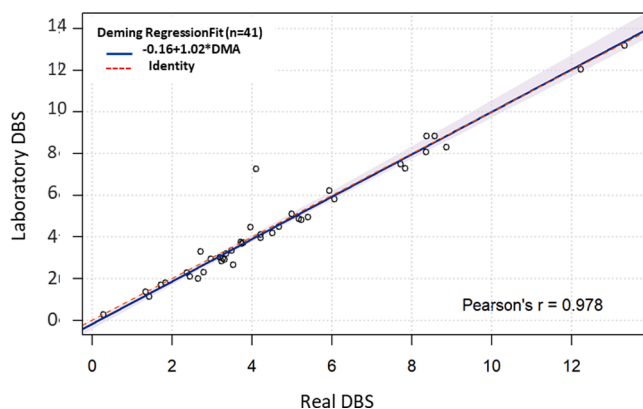
Fig. 3. Regression plot between mercury analyses in blood in the DMA in samples generated in the laboratory (by depositing venous blood samples) and real samples (DBS obtained by finger prick) (μg/L). Confidence interval 95% of the values were represented by shaded area.

standard method, as well as what was observed in the regression analysis. Therefore, this methodology can be a valuable alternative for assessing mercury exposure in HBM studies.

A key point in the method development was the control of the volume of samples deposited in the Whatman cellulose cards. This issue was already highlighted in the recommendations on the validation of bioanalytical methods for DBS (Timmerman et al., 2011). The DMA measures mercury concentrations referred to the amount of blood (mass of sample), therefore the control of the volume is critical. Recent studies used DBS samples for mercury assessment in blood by DMA obtaining satisfactory results (Schweizer et al., 2021; Koutsimpani-Wagner et al., 2022). However, the volume of blood samples deposited in the devices was not controlled, and the results were calculated by estimating the sample volume. In this study we elaborated a standard operating procedure for sampling (Supplementary material) which includes the use of calibrated capillary tubes for collecting the samples to accurately spot them onto the DBS. Additionally, the sampling procedure was tested during the pilot study. No statistical differences were found between the results obtained in DBS prepared in the laboratory using blood samples collected by venipuncture and the real DBS, both blood samples from the same subject (Student test log data, $p = 0.8317$). A high correlation was observed (Pearson's coefficient 0.978, $p < 0.001$) between the two kinds of DBS samples. In the same way, data fit in a Deming regression curve showed a strong linear relationship between the DBS samples with both deposition procedures ($R^2 = 0.978$) (Fig. 3). No constant or proportional bias were found in the resulting regression models parameters (Intercept: -0.156; IC 95%: $-0.406–0.037$; slope : 1.017; IC 95%: $0.981–1.089$).

Based on those results, we can assert that the use of calibrated capillary tubes ensures the control of the blood samples volume, leading to accurate and precise results.

4. Conclusions

The use of DBS for mercury analysis has proven to be useful in HBM studies due to the small volume of sample required, and the easiness in sampling, storage and transportation. In addition, the method developed in our laboratory adds further advantages as it allows for a proper control of the volume by using calibrated capillary tubes and a selective and easy analysis by direct mercury analysis (DMA). The volume control by means of calibrated 50 μL capillary tubes, is a key point in order to guarantee the accuracy and precision of the results. The analysis by DMA is fast and does not require any sample preparation making it less time consuming compared to other techniques, being sensitive, selective, accurate, precise and reliable enough to evaluate the concentration of mercury in blood in the population, even in reduced volumes as in

DBS samples.

The quality parameters obtained in the method development and the LOD/LOQ achieved guarantees its applicability to assess mercury exposure in the general population and can be used in HBM studies being particularly useful as screening method when evaluating exposure in vulnerable groups, such us pregnant women, babies and children, and in studies performed in remote areas with limited resources. Due to the simple sample collection, preparation and the reduced cost of a DMA analysis amongst others, it could be a very useful tool also in clinical settings in countries in which mercury exposure is a public health concern.

CRediT authorship contribution statement

Juana María González-Rubio: Methodology, Formal analysis, Data curation, Writing – original draft. **Noelia Domínguez-Morueco:** Writing – review & editing. **Susana Pedraza-Díaz:** Writing – review & editing. **Ana Cañas Portilla:** Writing – review & editing. **Miguel Ángel Lucena:** Methodology, Formal analysis. **Ana Rodriguez:** Formal analysis. **Argelia Castaño:** Writing – review & editing, Project administration. **Marta Esteban-López:** Methodology, Conceptualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.107958.

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