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4 **Determination of Cu in blood *via* direct analysis of dried blood spots**
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6 **using high-resolution continuum source graphite furnace atomic**
7 **absorption spectrometry**
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48 **Abstract**
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52 The performance of state-of-the-art high-resolution continuum source graphite furnace
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54 atomic absorption spectrometry (HR CS GFAAS) instrumentation and four novel
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56 devices to produce dried blood spots of perfectly defined volumes are evaluated with
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3 the aim of developing a simple, direct method for the determination of Cu in blood
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5 samples.
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9 In all cases, it is feasible to obtain accurate quantitative information using any of the
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11 four devices tested (Mitra, HemaXis DB10, Capitainer qDBS and HemaPEN) *via*
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13 simple external calibration with aqueous standards. One of the main differences in the
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15 performance of such devices is related with the blanks obtained, such that HemaXis
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17 DB10 and HemaPEN are preferred when abnormally low Cu levels ($500 \mu\text{g L}^{-1}$ or
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19 lower), associated with some diseases, need to be determined. The results prove that
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21 accurate values with RSD values below 10% can be achieved for these devices even
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23 for such Cu levels, while for Capitainer qDBS and, to a higher extent, for Mitra, the
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25 blank variations will ultimately increase the uncertainty.
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31 It is important to stress that analysis of four real samples using both venipuncture and
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33 all the DBS specimens showed a very good agreement. Thus, the approach proposed
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35 could be readily applied, such that patients with disorders requiring Cu control can
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37 prepare their own samples and submit them *via* postal mail to the lab for HR CS
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39 GFAAS direct and fast analysis.
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1. Introduction

Copper (Cu) is an essential transition metal for biological processes. It is one of the most abundant trace element in the human body and it is found in many cells and tissues. Cu participates in many enzymatic activities and is present in proteins involved in oxidative stress and metabolic homeostasis.¹ Problems in Cu homeostasis are linked to numerous diseases, such as Wilson disease, Menkes disease, biliary cirrhosis, hemochromatosis, Alzheimer disease, diabetes, angiogenesis and cancer, or cardiovascular disease.²⁻⁴ Thus, the importance of Cu blood level determination. Cu blood level can be used for the diagnosis of some of these disorders, often in conjunction with other tests, and it can also be used as a marker of the disease state.⁴ Finally, Cu determination can also be used in order to better understand the metabolism of the diseases that are related with this element.

Conventionally, blood analysis to determine Cu levels is carried out after venipuncture in a clinical setting, with the traditional inconveniences that such approach represents: i) the procedure is invasive, and not suitable for newborns; ii) patients need to travel to these centers, which is not easy for people with reduced mobility and/or who live far away from them; iii) samples are relatively unstable and need specific preservation (-80 °C fridges); iv) all the remains and residues produced are biohazardous and should be disposed of according to strict procedures.

In order to overcome these disadvantages, the development of alternative sampling methods for biological fluids in general, and for blood in particular, is continuously under investigation since 1913, when Ivar Bang⁵ published the first analysis of blood deposited onto a filter paper and proved the utility of DBSs (dried blood spots) for glucose determinations. However, the DBS working strategy was not fully implemented

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3 until 1963, when R. Guthrie and A. Susi published their work on the determination of
4 phenylalanine in blood of newborns deposited onto DBSs for diagnosis of
5 phenylketonuria.⁶ From this moment on, DBSs have been employed in neonatal
6 screening programs throughout the world, and the investigation associated with DBSs
7 have grown exponentially.⁷⁻⁹

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10 One of the main advantages of DBSs is the easier sampling, which may enable the
11 own patient or his/her relatives to prepare them with minimal training.⁷ Besides, they
12 require only a few microliters of sample volume (μL), which is excellent for studies with
13 newborns or model rodents, among other situations where just some μL can be
14 obtained. Normally, blood collection is carried out *via* finger puncture (or heel puncture
15 in the case of newborns). In addition, and due to its dried nature, DBSs can be stored
16 at room temperature, which facilitates both preservation and transport, enabling
17 shipping through regular post. These advantages make biobanking easy, which serves
18 as a basis for retrospective research. Moreover, these benefits might also translate
19 into an increased participation in epidemiological studies requiring volunteers.^{10,11}

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22 Traditionally, DBSs are prepared after the deposition of a volume in the range of 50 -
23 100 μL of blood onto a standardized clinical filter paper, which should absorb a
24 constant amount of blood per cm^2 . Later, after drying, a portion of the DBS (circles of
25 3.2 mm of diameter is often the standard) is punched out and taken for analysis. With
26 this approach, obtaining fully quantitative results is challenging, unless the volume
27 deposited onto the paper is measured (*e.g.*, with some pipette or calibrated capillary)
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29 Even then, the volume deposited per area for real samples depends on some
30 physicochemical factors, such as the viscosity, which is ultimately determined by the
31 hematocrit level.¹²⁻¹⁶ It has been demonstrated that the hematocrit level is the
32 parameter that has more influence in the spread of blood on DBS materials; in fact
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3 there is an inverse relation between blood hematocrit level and spot area.^{12,17}
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5 Moreover, the distribution of the analyte across the DBS is typically not constant, as
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7 chromatographic effects appear when depositing any liquid onto a hydrophilic surface
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9 (“coffee ring effect” or “volcano effect”),^{14,18,19} which poses an issue of representativity
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11 if only a portion of the DBS is subjected to analysis.¹³
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15 Different strategies have been proposed in order to solve the hematocrit¹⁵ and coffee
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17 ring effect problems,²⁰ but they often required extra measurements or more procedural
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19 steps that could be difficult to implement in a routine analysis. Analysis of the whole
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21 DBS is always a good option, provided that its size is manageable.
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25 In the last years, this situation has started to change with the development of new
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27 specimens to produce DBSs with a reproducible and known volume,²¹ regardless of
28
29 the hematocrit level. Such specimens differ in nature but have several aspects in
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31 common, such as the fact that they rely on smaller volumes (typically, 10 – 30 μ L
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33 range) than the DBSs traditionally used in hospitals, among other reasons because
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35 they are designed with the patient in mind. These are intended not only for newborns,
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37 but for other situations where adults need frequent controls (*e.g.*, for monitoring the
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39 evolution of a chronic condition or for screening a large population from an area of
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41 interest) and the auto sampling of larger volumes from a single finger puncture is not
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43 so easy or comfortable. The idea behind these devices is to carry out the analysis of
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45 each DBS produced as a whole, without further subsampling.
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51 In this work, four different specimens that were considered as particularly promising
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53 have been selected and tested. The first one called Mitra was introduced in 2014.²²
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55 This material is based on the absorption of a fixed volume of sample (10, 20 or 30 μ L)
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57 onto a porous hydrophilic tip by wicking. The second device is a microfluidic-based
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3 volumetric sampling system denominated HemaXis DB10 that was introduced in
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5 2015.²³ The sample is taken through a capillary (10 μ L) and afterwards, by closing the
6
7 card, it is deposited onto a clinical filter paper. The third device is known as Capitainer
8
9 qDBS, and is an updated version of the Capitainer B, the latter being released in
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11 2018.²⁴ This system makes use of microfluidics to produce volume defined DBSs on
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13 conventional DBS filter paper, as explained elsewhere by Lenk *et al.*²⁵ In particular,
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15 this device produces a DBS with 10 μ L of blood. The last device tested is the
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17 HemaPEN (QCPEN), and its last iteration was reported in literature also in 2018.²⁶ It
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19 is based on the volumetric application of blood onto pre-cut filter paper discs. This
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21 device is equipped with four integrated 2.74 μ L microcapillaries, which are filled when
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23 they are put into contact with a droplet of blood. By applying pressure, such blood is
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25 deposited onto the four pre-cut filter papers, thus producing the DBSs. A more detailed
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27 description of each one of these microsampling devices is presented in Sections 2.2
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29 and 2.3.
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36 Use of these devices clearly helps in solving a key analytical challenge, as the exact
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38 blood volume used to produce the DBS can be known. Another problem is that, by
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40 using DBSs, a liquid sample is transformed into a solid sample, and most of the
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42 analytical techniques typically used for Cu determination are originally designed to
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44 work with solutions, such as flame atomic absorption spectrometry (FAAS),²⁷
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46 inductively coupled plasma optical emission spectrometry (ICP-OES),²⁸ and
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48 inductively coupled plasma mass spectrometry (ICP-MS).²⁹ This entails that the DBS
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50 would have to be digested or subjected to an extraction procedure to release Cu from
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52 the filter paper, with the subsequent dilution and/or contamination or recovery issues.³⁰
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55 The use of direct solid analysis may be an alternative to solve these problems and
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57 increase sample throughput. In cases where a single element is to be monitored, high-
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3 resolution continuum source graphite furnace atomic absorption spectrometer (HR CS
4 GFAAS) can offer a robust performance, providing low limits of detection, good signal
5 stability and potential to detect and correct for spectral overlaps,^{31–35} ideally relying on
6 external calibration with aqueous standard solutions only. Such technique has been
7 seldom evaluated in the past for this type of samples (only for Pb,³⁶ and for Fe and
8 Zn,³⁷ in addition to an earlier work that used line source GFAAS³⁸). Moreover, in those
9 cases, “traditional” DBSs, where a larger amount of blood is deposited and later some
10 areas are punched out, were deployed, which can translate into some of the problems
11 discussed before (e.g., heterogeneous analyte distribution).
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25 This work aims at the evaluation of four novel DBS specimens in the context of Cu
26 determination in blood, in order to develop a simple and accurate methodology based
27 on the direct analysis of Cu using solid sampling HR CS GFAAS, while also assessing
28 whether the results obtained *via* DBS monitoring are comparable to those that can be
29 achieved by means of venipuncture.
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37 2. Experimental

38 2.1 Instrumentation

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41 All the measurements for Cu determination were carried out with a HR CS GFAAS
42 spectrometer, ContrAA 800G (Analytik Jena, Jena, Germany). This instrument is
43 equipped with a xenon short-arc lamp as a continuous radiation source, a high-
44 resolution double monochromator, and a CCD array detector. This detector possesses
45 588 pixels, of which 200 are used for monitoring the spectral window. The system is
46 equipped with a SSA 600 fully automated solid sampler with liquid dosing unit.^{34,39}
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2.2 Reagents and materials

A Cu mono-elemental solution of 1 g L⁻¹ (Merck, Darmstadt, Germany) was used for the preparation of the standards, by dilution in 1% v v⁻¹ HNO₃. Pd, Rh, Pt, Ir and Ru solutions of 1 g L⁻¹ (Merck) were tested as chemical modifiers. Ultrapure water (resistivity ≥ 18 MΩ cm) was deionized by a Milli-Q purifying system (Millipore, Bedford, USA). HNO₃ 65% and HCl 30% Suprapur were purchased from Merck.

Seronorm™ Trace Elements Whole Blood reference material (RM) Level 1 (L-1, Lot: 1406263), Level 2 (L-2, Lot: 1406264) and Level 3 (L-3, Lot: 1509408) produced by SERO AS (Billingstad, Norway) were utilized as reference materials to evaluate the analytical method. For simplicity, these materials will be referred to as blood RM L-1, L-2 and L-3, respectively. These RMs were available in lyophilized form and were reconstituted in 3 mL of ultrapure water, following the manufacturer instructions.

Four different DBS devices were tested: (i) Mitra; (ii) HemaXis DB10; (iii) Capitainer qDBS; and (iv) HemaPEN (QCPEN).

Mitra devices (Lot: 70615A-P5L18-1951) were acquired from Neoteryx (Torrance, USA). The absorptive part of the Mitra device (see **Figure 1**) is a hydrophilic polymer with an absorption volume of 11 ± 0.35 μL for water and 10.5 μL (uncertainty not provided by the manufacturer) for blood. These are the values provided by the manufacturer, although similar values were obtained in an independent previous work.⁴⁰ A value of 11 μL was used for calculations concerning the reference materials (closer to water in terms of viscosity) while 10.5 μL were used for calculations concerning the real blood samples and the LOD determinations. HemaXis DB10 devices (Lot: 2019/002/285161) were obtained from DBS System SA (Gland, Switzerland). They incorporate a microfluidic chip of 10.0 ± 0.5 μL (see **Figure 2**).

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3 Capitainer qDBS specimens (Lot: 800207) were purchased from CAPITAINER AB
4 (Stockholm, Sweden). This device (see **Figure 3**) possesses a capillary microchannel
5 of 10 μL (with a reproducibility better than 0.5 μL as one standard deviation). QCPEN
6 (Lot: 44U-317685A) were supplied by Trajan Scientific Europe Ltd (Milton Keynes,
7 United Kingdom). This is actually a simplified version (inner part) of the HemaPEN
8 specimen (see **Figure 4A**), which is produced for work in clinical labs (thus the label
9 QC), while the complete HemaPEN version is designed with the patients in mind, and
10 it is easier to use. However, the operating principle is the same. The QCPEN is shown
11 in **Figure 4B**. It is composed of four microcapillaries of $2.74 \pm 0.14 \mu\text{L}$. This device will
12 be referred as HemaPEN from now on, for simplicity. In addition to the QCPEN
13 devices, both Perkin Elmer 226 and Whatman 903 filter paper discs were provided by
14 the manufacturer for testing.

2.3 Samples and sample preparation

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35 Blood samples from 4 volunteers (adults) were obtained from the Hospital Universitario
36 Miguel Servet (Zaragoza, Spain). For comparison purposes, during the same session,
37 the blood was extracted by both the conventional method (venipuncture) and by
38 puncturing the finger with a small lancet to prepare the DBSs with the different devices.
39 The principles outlined in the declaration of Helsinki regarding all the experimental
40 research involving humans or animals were followed.

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42 DBSs were prepared as recommended by the manufacturer with blood RMs and with
43 the real blood samples, as detailed below. After the deposition of either blood RMs or
44 real blood samples, all the devices were dried at room temperature during at least 4
45 hours, except for the HemaPEN, which was dried for at least 2 hours.

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3 Mitra DBSs (see **Figure 1**) were prepared by introducing the absorptive part (the tip)
4 into the reconstituted RMs for 5 seconds. For real blood samples, the tip of the device
5 was put into contact with the blood droplet until it appeared to be full (visually).
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11 HemaXis DB10 (see **Figure 2**) preparation with the reference material was carried out
12 with the help of a micropipette with which a 12 μL droplet (a volume higher than the
13 volume of the microfluidic chip) was created and put into contact with the microfluidic
14 chip inlet. For real blood samples, the microfluidic chip was put into contact with the
15 blood droplet until it was full. In both cases, when the microfluidic chip was full, the
16 HemaXis DB10 was closed, thus resulting in the blood being deposited onto the filter
17 paper card (Whatman 903), producing the blood spots of 10 μL . Once dried, each DBS
18 was cut from the whole card with ceramic scissors before analysis.
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31 Capitainer qDBS (see **Figure 3**) preparation with the RMs was carried out by
32 depositing 15 μL of blood (a volume higher than the volume of the microchannel) of
33 blood onto the inlet port with a micropipette. For real blood samples, a droplet of blood
34 punctured directly from the finger was deposited onto the inlet port. In both cases, after
35 deposition, a microchannel is filled with a constant volume of 10 μL . After that, a thin
36 film at the inlet is dissolved by any excess of blood, and such excess of blood is
37 absorbed by a paper matrix separating it from the microchannel. Finally, the blood
38 filling the microchannel is transported by capillarity, dissolving another thin film and
39 being deposited onto the pre-perforated filter discs (Ahlstrom grade 222) that are
40 located on the other side of the device, forming DBSs of 10 μL .
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55 For the HemaPEN (see **Figure 4**), DBSs were prepared by introducing the four
56 capillaries of the QCPEN (HemaPEN) into the reconstituted RMs until it was observed
57 that the capillaries were full. For real samples, the preparation was carried out taking
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3 the blood directly from the finger with the QCPEN. For that purpose, the four capillaries
4 of the QCPEN were put into contact with the droplet of blood and filled directly. Next,
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6 in both cases, the capillaries were put into contact with the filter paper (Whatman 903)
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10 by pressing the system, as recommended by the manufacturer.
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13 2.4 Measurement protocol 14 15

16 For the analysis, the sample has to be deposited onto the platform to be introduced
17 into the graphite furnace. In all cases, the sample deposition and the addition of the
18 chemical modifier was carried out manually. In the case of the liquid blood, 10 μL of
19 blood were deposited onto the platform with a positive displacement micropipette and
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21 10 μL of 1 g L^{-1} Pt was added as chemical modifier.
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29 In the case of the Mitra device, only the absorptive part (the tip) was deposited onto
30 the platform (see **Figure 5a**). Once inside the platform, 10 μL of 1 g L^{-1} Pt solution were
31 added above the Mitra DBS trying to cover the device entirely. For the HemaXis DB10
32 device, the dried DBS was cut with ceramic scissors and was folded in order to be
33 deposited onto the platform (see **Figure 5b**), where previously 10 μL of 1 g L^{-1} Pt had
34 been placed. For Capitainer qDBS, after the drying process, the DBSs were detached
35 from the support with a ceramic knife and cut in half with ceramic scissors; the
36 deposition onto the platform was carried out in four consecutive steps, the first one
37 was the deposition of 5 μL of 1 g L^{-1} Pt followed by the deposition of half of the
38 Capitainer qDBS. This sequence was repeated twice (see **Figure 5c**). In the case of
39 the HemaPEN, the filter papers were removed from it with a metal free micropipette
40 tip, in order to avoid contamination, and the filter paper was deposited onto the platform
41 where previously 10 μL of 1 g L^{-1} Pt had been placed (see **Figure 5d**). In all cases, the
42 introduction of the platform into the graphite furnace was carried out with the
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3 autosampler, controlled by the computer software. After sample atomization, a small
4 amount of ash remains on the platform, which was withdrawn with a flow of
5 compressed air after each replicate.
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11 Two different wavelengths were used for analysis depending on the sensitivity
12 required. For the liquid blood samples and for Mitra, HemaXis DB10 and Capitainer
13 qDBS (approx. volume = 10 μL), the wavelength chosen was 249.21 nm (relative
14 sensitivity of 1.1%, in comparison to the most sensitive line), while for the HemaPEN
15 (approx. volume = 2.74 μL), the wavelength was 222.57 nm (relative sensitivity of 5%
16 of the most sensitive line). The temperature program used is summarized in **Table 1**.
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26 A continuous Ar flow of 2.0 L min^{-1} was used in all the heating steps except during the
27 atomization. The integration time was set to 6 s. Integrated absorbance (peak area) of
28 the 3 central pixels was selected as measurement mode.
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33 For quantitative analysis, external calibration with aqueous standards of adequate Cu
34 levels was performed, introducing between 0.5 and 10 ng of Cu at 222.57 nm and
35 between 5 and 40 ng of Cu at 249.21 nm.
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42 For the evaluation of the accuracy, a liquid whole blood RM was analyzed (L-2) for
43 which three replicates were carried out. Five replicates were measured in the analysis
44 of the DBSs prepared with the different RMs. For DBS analysis, blank correction is
45 mandatory. For this purpose, 10 blanks were measured for HemaXis DB10, Capitainer
46 qDBS and HemaPEN devices, and 15 blanks for the Mitra device. The median of these
47 blank values was subtracted from the signal obtained for every sample.
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56 For analysis of real blood samples, 5 replicates were measured in the case of the liquid
57 blood samples. For the DBSs prepared with such samples the number of replicates
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3 depends on the number of DBSs that each patient was able to produce. For blank
4 correction, 5 blank replicates were measured. The median of these blank values was
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6 subtracted from the signal obtained for every sample.
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11 Concerning the sample throughput, it can be mentioned that the whole measurement
12 of a replicate takes approximately 5 minutes. For every session, it is necessary to
13 calibrate with aqueous standards (e.g., about 1 hour for a blank and 3 standards, if
14 n=3), and measure the blank replicates of the target DBS (e.g., approx. 25 minutes, if
15 n=5). Then, the DBS samples can be analyzed (approx. 25 minutes per sample, if n=5).
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17 No significant memory effects were observed after DBS analyses. In any case, running
18 a measurement with an empty platform for cleaning after obtaining a high signal is
19 always advisable in GFAAS.
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30 3. Results and discussion

31 3.1 Method optimization

32 3.1.1 Wavelength selection

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40 Cu offers several atomic lines, enabling to easily adapt the sensitivity to the demands
41 of every particular application. In this case, the wavelength finally selected for the
42 analyses depended on the amount of blood, and thus of Cu, introduced into the
43 furnace. For the HemaPEN devices, containing only 2.74 μL of blood, the wavelength
44 chosen was 222.570 nm, which shows a relative sensitivity of approx. 5% in
45 comparison to the most sensitive line found at 324.754 nm. On the other hand, for the
46 Mitra, HemaXis DB10 and Capitainer qDBS devices, all containing ca. 10 μL of blood,
47 the atomic line found at 249.215 nm was chosen, which offers a relative sensitivity of
48 roughly 1% compared to the most sensitive line. This fact clearly indicates that the
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3 technique is not lacking sensitivity when attempting this type of analysis, and in fact
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5 more sensitivity could be easily obtained, if required, by monitoring the main atomic
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7 lines for Cu.
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10 11 3.1.2 Modifier selection 12 13

14 The study of the chemical modifiers was carried out with the Mitra device, a relatively
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16 large and thick matrix of unknown polymeric composition, which could be *a priori* more
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18 difficult to be efficiently removed than the others, which are all practically identical
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20 (except for the size) and based on cellulose. Mitra devices were prepared with blood
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22 RM L-2. For these tests, pyrolysis and atomization temperatures were fixed at 1300
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24 and 2400 °C, respectively.
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28 Pd, Rh, Pt, Ir and Ru were tested as modifiers. The DBSs prepared with the Mitra
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30 device were deposited onto the platform together with 10 µL of 1 g L⁻¹ solution of each
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32 modifier, separately. Moreover, in the case of Ir, this element was also tested as
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34 permanent modifier.
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39 It was observed that, when Pd and Rh modifiers were used, the Mitra device did not
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41 decompose completely, obstructing the radiation from the source due to its size (see
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43 **Figure 6a**) and making analysis hardly feasible. However, with Pt, Ru and Ir, the Mitra-
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45 based DBSs decomposed better, leaving only a small amount of ash after each
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47 measurement. Such residue can be easily taken away with compressed air. **Figure 6b**
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49 shows a picture of the Mitra DBS after sample analysis using Pt as chemical modifier.
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54 However, it appears that the different behavior of these modifiers in terms of sample
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56 decomposition may have more to do with their matrix than with the nature of each
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58 element. Pd standard solutions are prepared in 15% HNO₃ and Rh in 2-3% HNO₃,
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3 while Pt, Ru and Ir are prepared in 7% HCl. Further experiments were carried out
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5 adding just 15% HNO₃, which was not sufficient for efficient matrix removal, while using
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7 7% HCl produced similar results as those reported for Pt solutions. It can also be
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9 mentioned that, if only concentrated HNO₃ (65 %) is added, then the matrix is efficiently
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11 removed, probably because its oxidant effect is more pronounced than with a diluted
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13 solution. In fact, in the absence of any modifier, the device also decomposes, although
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15 the Cu signal is not so stable. Therefore, the presence of diluted HNO₃ seems to be
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17 detrimental for the decomposition of this polymeric material. Introduction of air during
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19 the pyrolysis (by adding an ashing step at 600 °C) was also tested without satisfactory
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21 results. Thus, addition of one of these modifiers (Pt, Ru and Ir) was further studied.
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27 It was possible to observe that, when adding Pt, the areas and the peak profiles were
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29 rather constant, while that was not the case for Ru and even less for Ir. In fact, for Ir, a
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31 decreasing trend in terms of peak areas was observed. This may be explained as an
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33 amount of Ir can be deposited onto the platform, acting as permanent modifier. To
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35 corroborate this, the platform was prepared with Ir as permanent modifier. It was
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37 possible to observed that the Mitra was decomposed effectively under these
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39 conditions, but providing only half of the Cu signal than with Pt. Finally, the Pt solution
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41 was chosen as chemical modifier for the rest of the analyses carried out in this work,
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43 as it offers the best sensitivity and stability of all the modifiers tested.
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49 Once this modifier solution was selected, it was also tested with the other DBS devices
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51 and with a whole blood RM L-2 analyzed directly. This approach offers sufficient matrix
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53 removal (see **Figure 6** for the minor residues observed for the DBS devices), good
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55 sensitivity and stable peak profiles in all cases.
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3.1.3 Optimization of the temperature program

The optimization of the temperature program was carried out using two different materials. Mitra and HemaXis DB10 devices were selected: the first shows a different matrix (polymeric), while the second is similar to the rest of the other devices (cellulose), and their corresponding DBSs were produced after absorption of blood RM L-2. Also, Cu aqueous standards were investigated for comparison, as the ultimate goal was to carry out external calibration with them.

For Mitra, the atomization temperature was optimized between 2000 and 2500 °C, while keeping the pyrolysis temperature constant at 1300 °C. The results obtained are shown in **Figure 7**. Except for 2100 °C, peak areas and uncertainties are similar for all the temperatures tested. Finally, a value of 2400 °C was chosen to obtain a well-defined, almost gaussian peak profile (see **Figure 8a**). The pyrolysis temperature was then optimized from 1000 until 1500 °C. The results are also shown in **Figure 7**. Obviously, the use of temperatures below 1200 °C is unsuitable as it results in higher signal variation. When attempting direct solid sampling it can be expected that, below a certain pyrolysis temperature, proper matrix decomposition is not fully achieved. A value of 1300 °C was finally selected since, at this temperature, almost all the matrix was removed (except for minimal ash remains, as discussed in the previous section), without losing analyte. The final temperature program chosen for the Mitra device is shown in **Table 1**.

For HemaXis DB10 devices, the atomization temperature was optimized by selecting a constant pyrolysis temperature at 1300 °C. The atomization temperature varied between 1700 and 2500 °C. The results obtained are shown in **Figure 7**. In view of the results, there are no significant differences for atomization temperatures between 2000

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3 and 2300 °C. A final value of 2300 °C was chosen as atomization temperature as a
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5 profile better defined than that obtained at 2200 °C is observed (see **Figure 8b**). The
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7 signal appears a bit earlier than for the Mitra, which indicates that Cu is less stabilized
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9 by the modifier in this case.
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13 It cannot be expected that all the devices provide exactly the same interaction between
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15 the analyte and the modifier. The way in which the modifier was added was designed
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17 to maximize such interaction, and that is discussed in detail in section 2.4. In the case
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19 of Mitra, the modifier is deposited on top of it and covers it well. In fact, a fraction may
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21 even be absorbed by the device although this is not observed visually. Overall, it seems
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23 that a more intimate contact can be achieved for this device, resulting in delayed
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25 atomization. The HemaXis DB10 has to be rolled over to fit onto the platform (see
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27 **Figure 5b**). If the modifier is added on top, the material expands, and it is difficult to
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29 introduce it into the furnace. So, for this device, the modifier has to be deposited onto
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31 the platform, before placing the DBS.
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37 In any case, a temperature of 2300 °C was chosen. Use of a temperature of 2400 °C
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39 seems also possible, thus unifying the conditions for both devices. However, in order
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41 to prolong the duration of the graphite parts, it is always beneficial to use a temperature
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43 as low as possible, so it was preferred to opt for 2300 °C.
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47 The pyrolysis temperature was also optimized between 700 and 1600 °C and the
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49 results are shown in **Figure 7** (blue trace). Again, selection of a temperature of 1300
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51 °C guarantees that most of the matrix is removed leaving only a small residual ash,
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53 with no loss of analyte and good precision.
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57 Once the temperature program was optimized for the HemaXis DB10, the other
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59 devices (Capitainer qDBS and HemaPEN) were submitted to the same program to
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3 check its feasibility, and the peak profiles obtained are shown in **Figure 9**. The time-
4 resolved spectra are similar to that obtained with the HemaXis DB10 device at 2300
5 °C (**Figure 8b**); although for HemaPEN a bit more tailing is observed. This could also
6 indicate more interaction with Pt. Overall, Cu determination in these devices can be
7 done applying the same conditions.
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12 Finally, the same optimization was performed using Cu standard solutions of similar
13 concentration than that of the blood RM L-2 ($1.34 \pm 0.27 \text{ mg L}^{-1}$). The measurements
14 were carried out by depositing 10 μL of the liquid standard (1.45 mg L^{-1}) and 10 μL of
15 1 g L^{-1} Pt. The atomization temperature was first optimized between 2000 and 2500
16 °C, while the pyrolysis temperature was set at 1300 °C. The results obtained are shown
17 in **Figure 7**.
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30 As could be expected, the Cu absorption peak appears a bit delayed compared to the
31 peaks obtained for the DBSs, since the interaction between Cu and Pt is potentially
32 more effective if both are added as solutions. In any case, there are no significant
33 differences between 2300 and 2400 °C in terms of peak definition and peak area
34 (**Figure 8c**). Therefore, and in order to maintain the same conditions used with the
35 DBS devices, a temperature of 2400 °C was chosen when measuring solutions for
36 calibration when targeting Mitra-based DBSs, and of 2300 °C for the rest of them.
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47 A pyrolysis temperature optimization was also carried out between 1000 and 1500 °C,
48 with the atomization temperature set at 2400 °C. The results are also displayed in
49 **Figure 7**. No differences in terms of signal intensities and uncertainties were observed
50 in the range between 1200 and 1500 °C. A temperature of 1300 °C was chosen for
51 pyrolysis in all cases.
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3 Finally, and most important, the signals for aqueous standard solutions and for DBS
4 samples showed similar sensitivities, confirming the possibility to carry out the direct
5 determination of Cu in DBSs using external calibration with such aqueous standards.
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10 11 3.2 Figures of merit 12

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14 After method optimization, the main figures of merit were calculated, and the results
15 are shown in **Table 2**. The instrumental limits of detection (LOD), in ng, were calculated
16 as 3 times the standard deviation of 10 blanks divided by the sensitivity, respectively.
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18 In this case, 2.74 μL (for the HemaPEN method, $\lambda = 222.570 \text{ nm}$) or 10 μL (for the rest
19 of the methods, $\lambda = 249.2 \text{ nm}$) of 1% v v⁻¹ HNO₃ plus 10 μL of 1 g L⁻¹ Pt solution were
20 measured as blanks. For expressing the LOD values as concentrations, the limits were
21 divided by the DBS volumes, achieving LOD values of 0.02 mg L⁻¹ of Cu in blood for
22 all DBS.
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33 The method LODs were also calculated as 3 times the standard deviation of 10 blanks
34 divided by the sensitivity, respectively. In this case, the blanks were the different
35 materials used to produce the DBS but without blood, and also adding 10 μL of Pt 1 g
36 L⁻¹. LODs were expressed as blood concentration considering the volumes absorbed
37 by each device. The manufacturer of the Mitra device provides two slightly different
38 absorption volumes for water solutions and for blood. For the calculation of these limits,
39 the volume for real blood (10.5 μL) was used. In the case of the Mitra device, 15 blanks
40 were monitored instead of 10, as such device shows a higher variation of the blank
41 signals and obtaining a representative value is important for blank subtraction.
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55 As it is possible to appreciate in **Table 2**, the method LODs are significantly higher than
56 the instrumental limits. Overall, we believe that these method limits are overestimated.
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58 Most of these DBS specimens have been originally designed with other applications in
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3 mind and they are not always as clean as ideally required for trace element analysis.
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5 This fact agrees with previous reports.⁴¹
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9 Thus, the reason behind such high method LODs is probably related to the presence
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11 of Cu in the DBS devices, which distribution is furthermore not homogeneous. It has to
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13 be remembered that the definition of limit of detection is based on a blank that is
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15 “defined as the signal resulting from a sample which is identical, in principle, to the
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17 sample of interest, except that the substance sought is absent (or small compared to).
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19 The blank thus includes the effects of interfering species.”⁴²
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24 We do not have such pure blanks in this case, so the traditional approach for LOD
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26 calculation may not apply herein. The problem here is not to be able to detect Cu
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28 (sensitivity is more than enough and baselines are stable), but to estimate properly and
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30 subtract the contribution of the blanks to the signal. If that is done, it is possible to carry
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32 out the determination of Cu in blood samples with good accuracy and sufficient
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34 precision at concentrations lower than those than can be inferred from the method
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36 LODs (e.g., calculating the limits of quantification as 3.33 times such LODs), as will be
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38 demonstrated in section 3.3.
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43 In any case, this is a relevant aspect. **Table 3** shows the Cu concentration determined
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45 for the different materials tested in this work following the method described in section
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47 2.4. and considering the volumes absorbed by each device for calculation of the
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49 concentrations. As can be seen, relatively high Cu concentration levels are observed
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51 in most devices, and especially in the Mitra device. Moreover, confidence intervals are
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53 also large, reflecting the heterogeneous distribution of Cu in each individual device.
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55 High blank levels for some elements (including Cu) have already been reported for this
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3 device.^{43,44} It has to be indicated again that the Mitra is the only one prepared from a
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5 polymeric material.
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9 Cleaning the absorptive materials before sample deposition is an option to further
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11 purify them.^{9,45,46} This option was explored for the Mitra devices by introducing them in
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13 EDTA solutions, rinsing with water and drying them subsequently. However, the
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15 decrease in Cu concentration was not significant, and it was observed that, after this
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17 procedure, the devices tended to absorb more liquid than the values provided by the
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19 manufacturers. This fact is problematic, as the absorbed volume should remain
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21 constant and has to be known in advance. As a result, such approach was not explored
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23 further. It is our understanding that some strategies to decrease these blanks are
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25 currently under investigation by the manufacturer of this device.
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31 The rest of devices all use similar cotton-based fibers. The different clinical filter papers
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33 are listed in **Table 3** as well. Capitainer qDBS offers blanks that are higher than the
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35 others. It can be mentioned that the paper used for such device (Ahlstrom 222) is a bit
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37 thicker than the others. In the case of HemaPEN, the device may be equipped with two
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39 different filter paper types (Perkin Elmer 226 or Whatman 903), which actually offer
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41 similar blanks.
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46 Finally, best blanks were obtained for the HemaXis DB10 device. Since the filter paper
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48 used in this device is the same as that used for one series of HemaPEN and actually
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50 the amount of filter paper is smaller in the case of HemaPEN, it is, *a priori*, a bit
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52 surprising to see this difference. However, the paper for HemaXis is probably
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54 manipulated to a lower extent, as it does not need to be precut, unlike the case for
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56 Capitainer qDBS and HemaPEN. Thus, the difference could be related with the way
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3 the paper is handled and protected, or it could be an artifact and the results for other
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5 lots may differ.
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9 In any case, it is important to put these results in perspective. Blood Cu levels for
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11 persons with abnormally low levels (e.g., Wilson's patients), and, also, for newborns
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13 and infants, may be around 0.5 mg L⁻¹ or even below, while for adults without any
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15 disorders it may be in the range 1.0-1.5 mg L⁻¹. Therefore, for detecting abnormal
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17 levels, all of them can perform, but for accurate and precise quantification of Cu in
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19 patients with some Cu-related disorders or in newborns, in principle, better blanks
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21 would be desirable for Mitra and Capitainer qDBS.
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26 In any case, **Table 3** provides a clear reference regarding the Cu concentration that
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28 can be determined for the different DBS devices. Obviously, the closer the expected
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30 values are to these blanks, the higher imprecision that is going to be obtained, as will
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32 be demonstrated in the next section.
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35 36 3.3 Determination of Cu in whole blood reference material using DBSs. Evaluation of 37 38 the accuracy of the method. 39 40

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42 Once the optimization procedure was carried out and the detection limits were
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44 calculated, the accuracy of the method was undertaken. For that purpose, three blood
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46 RMs (L-1, L-2, and L-3) were used to produce DBSs with every device and were
47
48 subsequently analyzed. The blank contribution was subtracted from the signals of the
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50 samples. For this blank correction, 10 blanks were measured for all the devices except
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52 for Mitra, for which 15 blanks were measured. Furthermore, blood RM L-2 was also
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54 analyzed in liquid form.
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3 In the case of the HemaPEN, as discussed before, it is possible to choose the paper
4 found inside the device between Whatman 903 and Perkin Elmer 226 when ordering.
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6 Thus, these two different filter papers were first tested in a simple way: the DBSs were
7 prepared by depositing the RMs directly with a positive displacement micropipette onto
8 them. No significant differences were observed (see Supplementary Information Table
9 S1). Finally, HemaPEN devices containing Whatman 903 were used in the next
10 experiments.
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20 The results obtained for these analyses can be found in **Table 4**, where a good
21 agreement with the reference values is demonstrated, proving that the method
22 performs well for Cu determination in DBSs. The uncertainty, in all cases, seems
23 acceptable for clinical laboratories. There is, in any case, a clear correspondence
24 between the uncertainty and the blank levels discussed in the previous sections, as
25 could be anticipated (e.g., it is higher for Mitra and lower for HemaXis DB10 and
26 HemaPEN, particularly at lower Cu concentration levels). Also, with less contaminated
27 materials, the requirement to measure several blanks for the devices could be
28 minimized.
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41 When considering the origins of the definition of limit of quantification, “minimum
42 working concentration as that for which the relative standard deviation was 10 %”,⁴² it
43 is clear that the levels are above such limit for HemaXis DB10 and HemaPEN. In the
44 case of Capitainer qDBS, RM L-1 is below this limit and, in the case of Mitra, both RM
45 L-1 and RM L-2 are below such limit.
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54 3.4 Cu determination in real blood samples. Venipuncture vs DBSs.

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57 In order to further evaluate the potential of this procedure, real samples were also
58 analyzed. Blood from four volunteers was obtained in the same session both by
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3 venipuncture and from finger puncture using a lancet, which was used for production
4 of DBSs with the different devices tested. Samples were subsequently analyzed and
5 results were compared to check whether there is any influence in the way in which
6 blood is sampled.
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13 For analysis of blood samples in liquid form, five replicates per sample were measured.
14 For DBSs, the number of replicates depended on the number of specimens that the
15 volunteers were able to produce per device tested. Five blanks were measured for
16 each device for blank correction. **Table 5** shows the results with their respective
17 uncertainty values, except for the volunteers 3 and 4 with the Mitra device, for which
18 the uncertainty could not be estimated because only one sample was produced. It is
19 noteworthy that in practice, the patient is not expected to be puncture several times to
20 produce DBSs, but only one. Here it was done for research purposes.
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32 As can be seen, the results obtained *via* direct analysis of DBS agree well with those
33 obtained *via* a standard approach (venipuncture and analysis of the liquid samples).
34 The uncertainties depend again on the type of device (higher for Mitra and Capitainer
35 qDBS) but also the small number of replicates may play a role in some cases.
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42 These results indicate that the way in which the sample is obtained does not show a
43 significant influence in the determination of blood Cu levels. It is necessary to mention
44 that while blood collection and the preparation of the DBSs were carried out almost
45 simultaneously, sample analysis was not. Blood in liquid form was analyzed on the
46 same day of blood collection, in order to avoid any stability issues, while the DBSs
47 were stored at room temperature and were analyzed one month after blood collection.
48 This reinforces the strategy discussed for DBSs before: the possibility to send the
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3 sample by ordinary post to be later analyzed when instrumental/personnel availability
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5 mandates.
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9 Overall, these values can be considered as very encouraging for the use of DBS as a
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11 less invasive sampling methodology for Cu determination in blood. In particular, for
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13 those specimens less affected by blank contamination (HemaXis DB10 and
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15 HemaPEN) the results in terms of accuracy and precision are remarkable.
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18 19 4. Conclusions 20

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22 This paper has evaluated the performance of different devices aiming at direct
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24 determination of Cu in blood *via* HR CS GFAAS analysis of DBS. Overall, all the
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26 devices enable such determination to be carried out in a simple way relying on external
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28 calibration with aqueous standards under optimized conditions and using 1 g L⁻¹ Pt in
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30 7% HCl as chemical modifier.
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34 The different devices show, however, different strengths and issues. Mitra devices are
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36 easy to use and show suitable dimensions for direct analysis with the HR CS GFAAS
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38 technique, but Cu blanks are very high and variable, thus hampering determination of
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40 Cu for persons with abnormally low levels and requiring the monitoring of a
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42 considerable number of blanks for proper blank correction. Capitainer qDBS shows a
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44 similar but smaller problem in this regard. It is a device easy to use by the patient, but
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46 it is a bit large for the application intended herein, requiring cutting the DBSs before
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48 deposition onto the platform.
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53 The best blanks are obtained for HemaXis DB10. It is a flexible specimen, as the filter
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55 paper card used for producing DBS can be easily replaced by the user for other types
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57 of materials. Still, it is also a bit large for this application requiring further manipulation
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3 (cutting it and rolling it) before deposition onto the platform. It was considered also a
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5 bit more difficult for sampling by the volunteers.
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9 Finally, the HemaPEN device offers an ideal size (filter papers of 3.2 mm diameter,
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11 which is one of the most common standards for this type of sample) for the graphite
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13 platform and the blanks seemed sufficiently low and stable. In its commercial version,
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15 it is probably also simpler to use by the patients, although the specimen tested in this
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17 work was a QCPEN device that is designed for method development. It produces DBSs
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19 with smaller volumes (2.74 μL instead of approx. 10 μL for the rest), but that is not a
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21 problem for this application. In fact, sensitivity is more than enough when trying direct
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23 analysis by HR CS GFAAS, such that less sensitive Cu lines need to be monitored.
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25 Another advantage of this device consists in obtaining 4 separate discs from only one
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27 puncture.
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33 The values presented support the use of DBS as a minimally invasive approach that
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35 can provide blood Cu levels in good agreement with those achieved after venipuncture,
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37 and thus an appealing possibility for the control of chronic patients or patients
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39 subjected to some treatment related with Cu metabolism.
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45
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47
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49
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56 Conflicts of interest

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58
59 There are no conflicts of interest to declare
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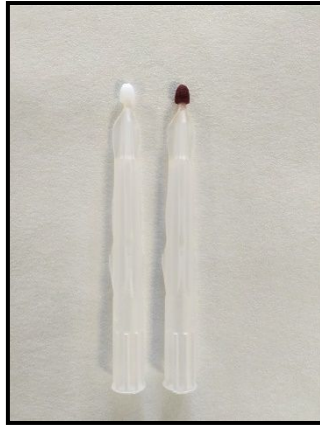


Figure 1. Mitra device without blood (left) and after collection of approximately 10 μL of blood (right)

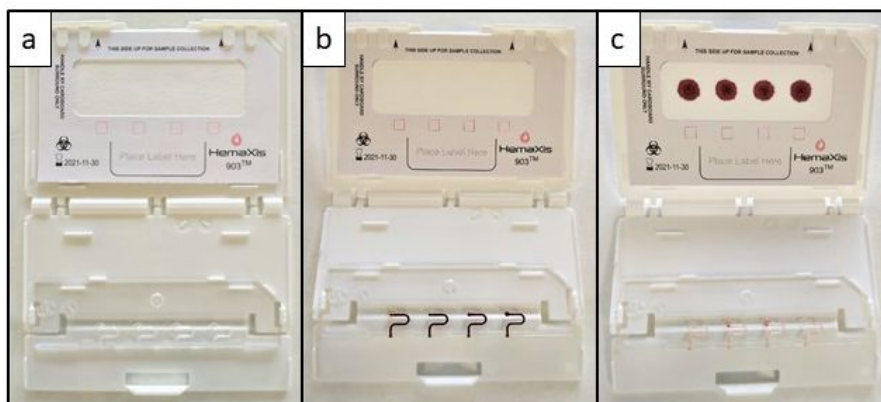


Figure 2. HemaXis DB10 device: (a) empty; (b) microfluidic chips filled with blood (approximately 10 μ L each); (c) after the device is closed and blood is deposited onto the filter paper for DBS production.

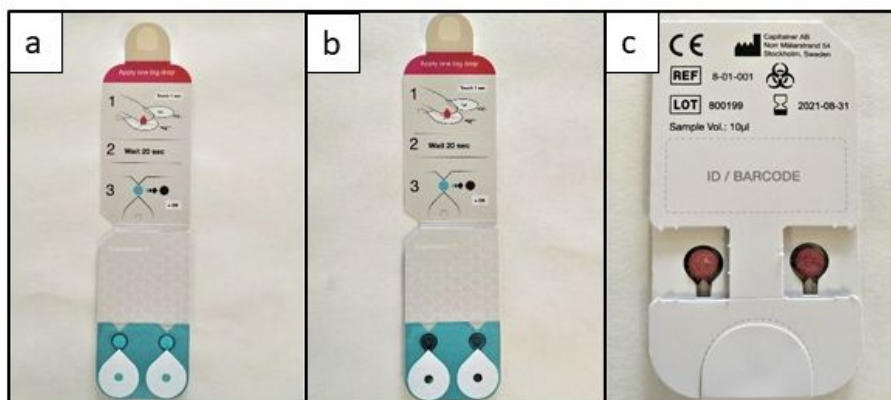


Figure 3. Capitainer qDBS device: (a) empty; (b) after blood deposition onto the indicated area; (c) DBSs produced after deposition of blood (approximately 10 μ L) onto the filter paper found in the back of the device.

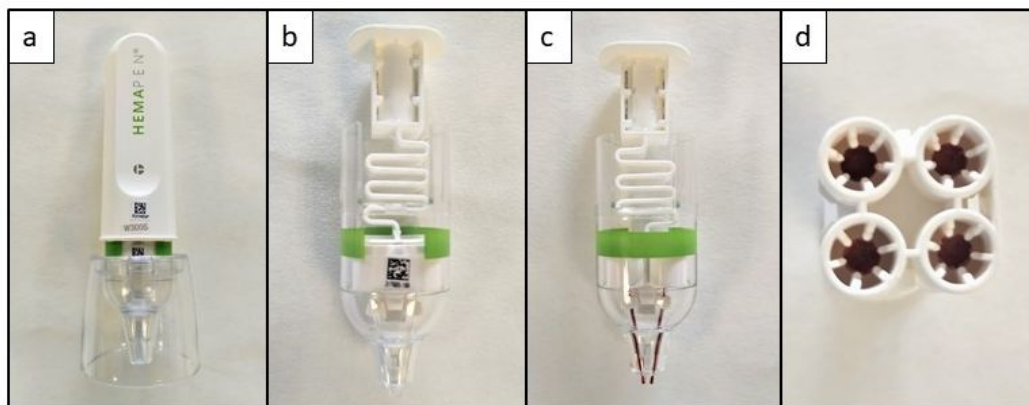


Figure 4. HemaPEN device: (a) fully commercial HemaPEN; (b) empty QCPEN (inner part of commercial HemaPEN); (c) QCPEN with microcapillaries filled with blood (approximately 2.74 μL each); (d) after deposition of blood onto the filter paper for DBS production.

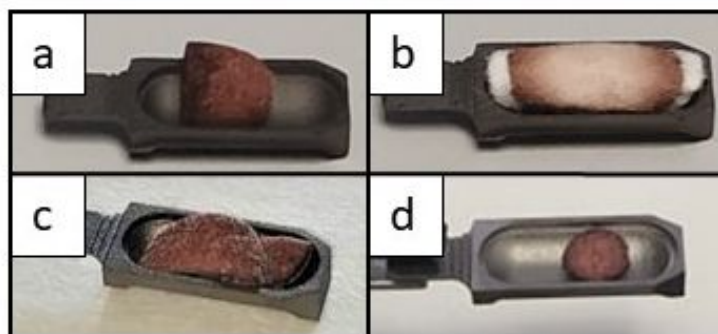


Figure 5. Deposition of DBSs onto the graphite platform before its introduction into the graphite furnace for subsequent HR CS GFAAS analysis. a) Mitra, b) HemaXis DB10, c) Capitainer qDBS and d) HemaPEN.

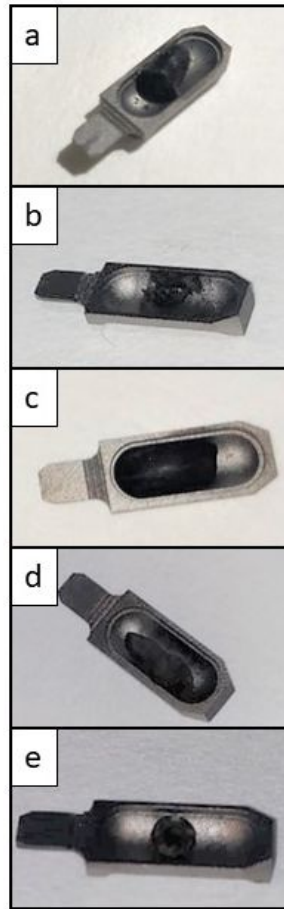


Figure 6. Devices after GFAAS temperature programme (See **Table 1**). a) Mitra using Pd; b) Mitra using Pt; c) HemaXis DB10 using Pt; d) Capitainer qDBS using Pt and e) HemaPEN using Pt.

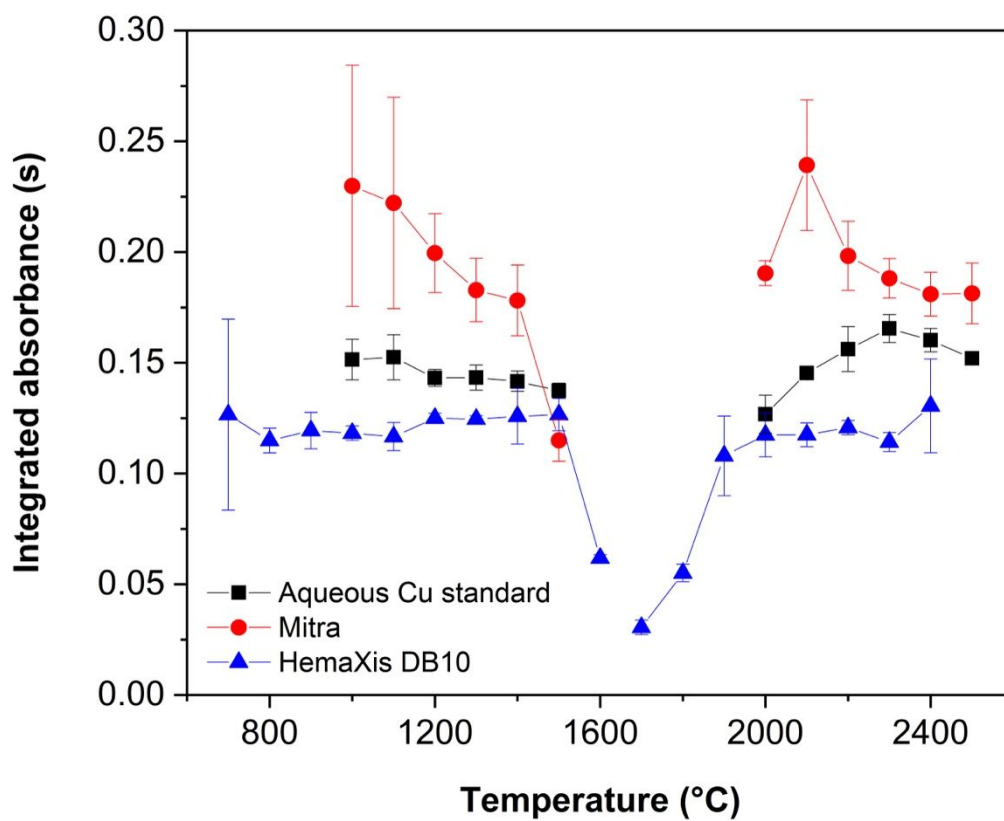


Figure 7. Atomization and pyrolysis temperature optimization. The error bars represent the standard deviation (n=5).

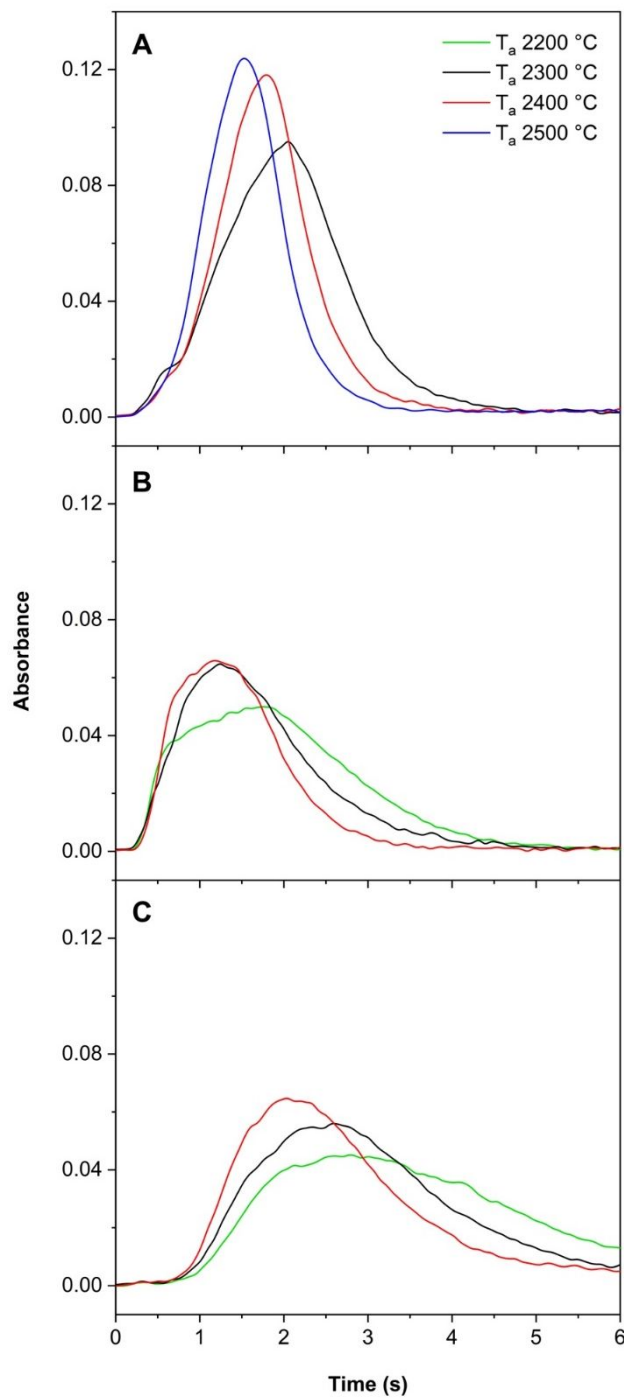


Figure 8. Time-resolved absorbance profile of the three central pixels, without blank correction, obtained from the optimization of the atomization temperature setting the pyrolysis temperature at 1300 °C for: a) Mitra, b) HemaXis DB10 and c) Cu standard solution.

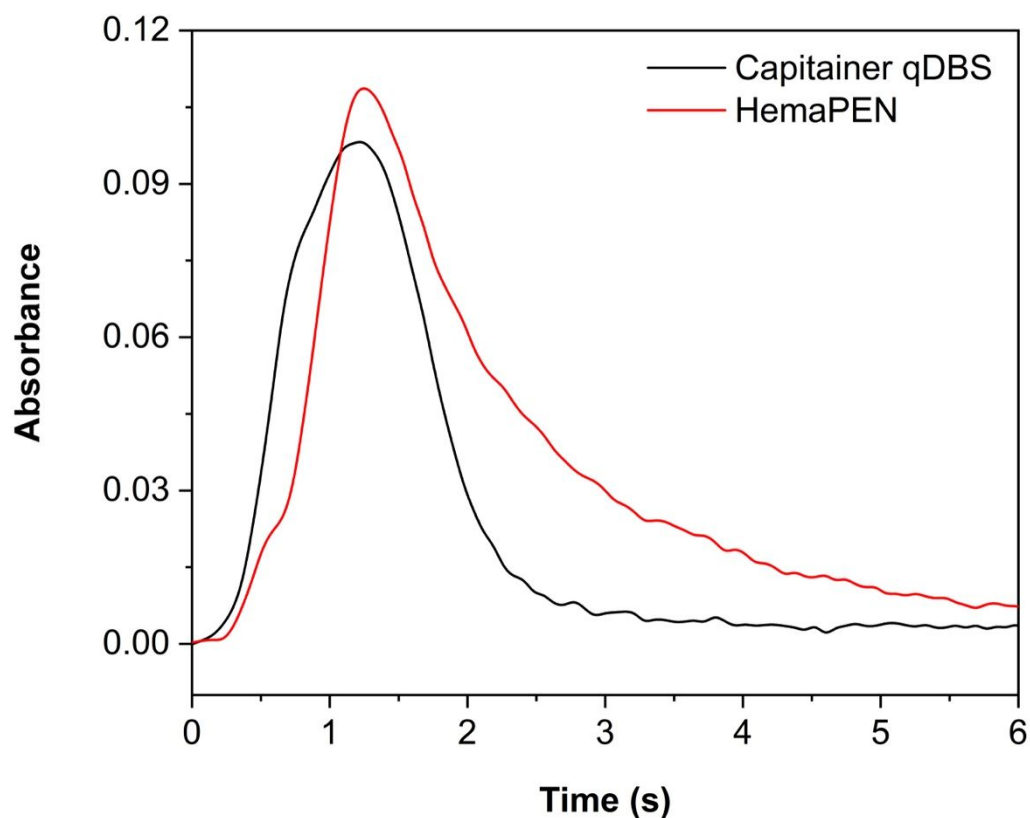


Figure 9. Time-resolved absorbance profile of the three central pixels, without blank correction, obtained using the temperature program shown in **Table 1** for DBSs produced *via* Capitainer qDBS ($\lambda=249.215$ nm) and for HemaPEN. ($\lambda=222.570$ nm).

Table 1. Graphite furnace temperature program for Cu determination in DBS and liquid blood samples using HR CS GFAAS. Argon was used as purge gas.

Step	Temperature (°C)	Ramp (°C s ⁻¹)	Time (s)	Gas flow (L min ⁻¹)
Drying 1	120	3	30	2.0
Drying 2	150	5	30	2.0
Pyrolysis	1300	50	35	2.0
Gas adaption	1300	0	5	0
Atomization	2400 ^a /2300 ^b	3000	6	0
Cleaning	2500	500	4	2.0

a: Atomization temperature used for Mitra devices

b: Atomization temperature used for liquid RMs, liquid real blood samples and for HemaXis DB10, Capitainer qDBS and HemaPEN devices.

Table 2. Figures of merit for the Cu determination using the current methods *via* HR CS GFAAS. For the method limits: 10 blank replicates were measured in each case, except for Mitra for which 15 measurements were carried out.

Wavelength (nm)	Device	Sensitivity (s ng ⁻¹)*	Characteristic mass (ng)*	Sample volume (μL)	Instrumental Limits	Method Limits
					LOD (ng / mg L ⁻¹)	LOD (mg L ⁻¹)
249.215	Mitra	0.0092 ^a	0.48	10	0.20 / 0.02	0.78 ^a
	HemaXis DB10				0.22 / 0.02	0.06 ^b
	Capitainer qDBS					0.48 ^b
222.570	HemaPEN (Perkin Elmer 226)	0.050 ^b	0.089	2.74	0.07 / 0.02	0.08 ^b
	HemaPEN (Whatman 903)					0.23 ^b

* Calculated as the median of: a) Sensitivity values from 8 different days; b) Sensitivity values from 4 different days.

Table 3. Cu concentrations obtained for the analysis of the blank devices tested using the method described in section 2.4. Results are expressed as $\bar{x} \pm U$, where $U = (t_s)/\sqrt{n}$; for a 95% confidence interval. The concentration was calculated considering the volume absorbed by each type of DBS device.

	DBS Device				
	Mitra ^a	HemaXis DB10 (Whatman 903) ^b	Capitainer qDBS (Ahlstrom 222) ^b	HemaPEN (Perkin Elmer 226) ^b	HemaPEN (Whatman 903) ^b
Cu Concentration (mg L⁻¹)	0.40 ± 0.14	0.07 ± 0.01	0.33 ± 0.12	0.20 ± 0.02	0.19 ± 0.05
RSD (%)	65	25	52	13	39
Median of Cu Concentration (mg L⁻¹)	0.27	0.08	0.25	0.20	0.18

a) n=15; b) n=10.

Table 4. Direct Cu determination in whole blood reference materials *via* HR CS GFAAS using different DBS devices. Results are expressed as $\bar{x} \pm U$, where $U = (t s)/\sqrt{n}$ for a 95% confidence interval. The concentration was calculated taking into account the volume absorbed by each type of DBS device.

Sample	Reference value (Cu, mg L ⁻¹)	Direct (Cu, mg L ⁻¹) (n=3)	Mitra (Cu, mg L ⁻¹) (n=5)	HemaXis DB10 (Cu, mg L ⁻¹) (n=5)	Capitainer qDBS (Cu, mg L ⁻¹) (n=5)	HemaPEN (Cu, mg L ⁻¹) (n=5)
RM L-1	0.64 ± 0.13	-	0.73 ± 0.19 (RSD = 21%)	0.60 ± 0.05 (RSD = 7%)	0.64 ± 0.10 (RSD = 13%)	0.59 ± 0.08 (RSD = 10%)
RM L-2	1.34 ± 0.27	1.30 ± 0.16	1.51 ± 0.27 (RSD = 14%)	1.42 ± 0.10 (RSD = 7%)	1.59 ± 0.18 (RSD = 9%)	1.40 ± 0.08 (RSD = 4%)
RM L-3	2.08 ± 0.42	-	2.42 ± 0.16 (RSD = 5%)	2.30 ± 0.08 (RSD = 3%)	2.48 ± 0.13 (RSD = 4%)	2.36 ± 0.15 (RSD = 5%)

Table 5. Results obtained for analysis of real blood samples both in liquid form and as DBS prepared with the different devices tested.

Results are expressed as $\bar{x} \pm U$, where $U = (t_s)/\sqrt{n}$ for a 95% confidence interval.

Volunteers	Liquid blood (Cu, mg L⁻¹)	Mitra (Cu, mg L⁻¹)	HemaXis DB10 (Cu, mg L⁻¹)	Capitainer qDBS (mg L⁻¹)	HemaPEN (Cu, mg L⁻¹)
V1	0.85 ± 0.06^a	1.12 ± 0.26^b (RSD = 15%)	0.97 ± 0.24^c (RSD = 10%)	0.97 ± 0.16^c (RSD = 7%)	0.84 ± 0.15^c (RSD = 7%)
V2	0.83 ± 0.04^a	1.02 ± 0.44^b (RSD = 27%)	0.91 ± 0.05^c (RSD = 2%)	0.92 ± 0.09^a (RSD = 8%)	0.80 ± 0.09^a (RSD = 9%)
V3	0.98 ± 0.08^a	1.24^e	0.94 ± 0.12^c (RSD = 5%)	0.98 ± 0.46^d (RSD = 5%)	1.19 ± 0.14^c (RSD = 5%)
V4	0.99 ± 0.06^a	1.32^e	1.01 ± 0.16^c (RSD = 6%)	0.91 ± 0.29^d (RSD = 4%)	1.00 ± 0.51^d (RSD = 6%)

^a(n=5), ^b(n=4), ^c(n=3), ^d(n=2) and ^e(n=1).

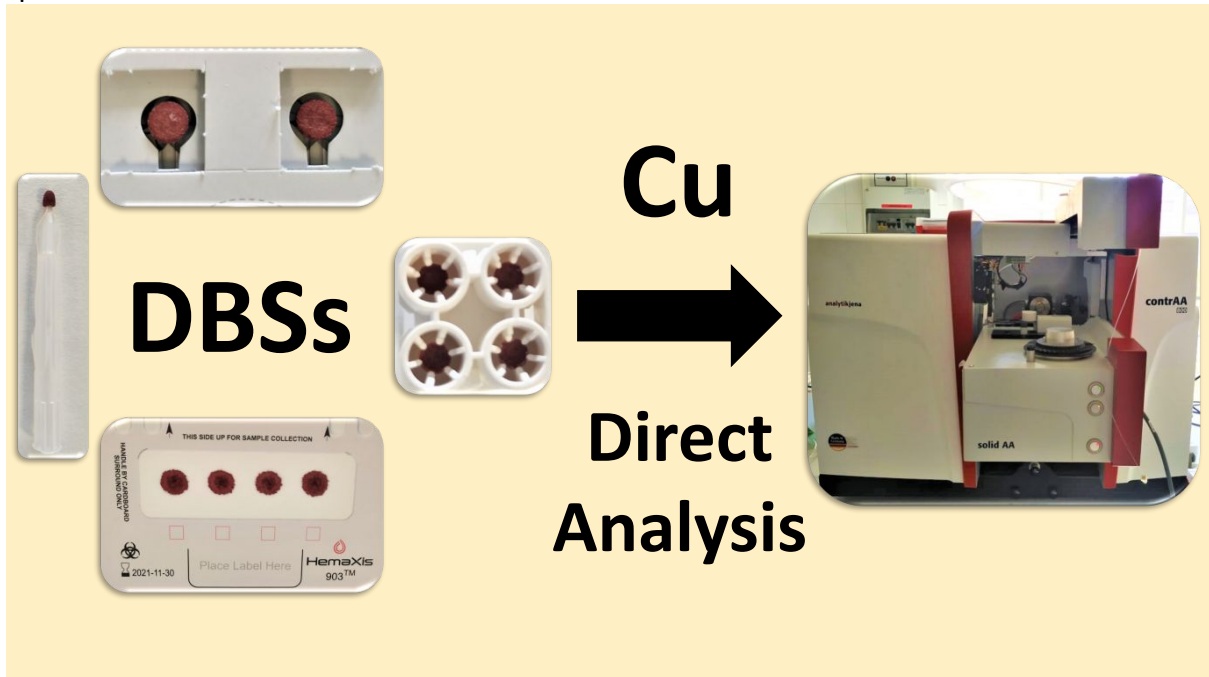
Perkin Elmer 226 and Whatman 903 filter papers test (HemaPEN)

For testing the two filter papers (Perkin Elmer 226 and Whatman 903) supplied by the HemaPEN manufacturer, DBSs were prepared by taking 2.74 μL of the whole blood RMs with a positive displacement micropipette and depositing that volume onto the filter papers. The analyses were carried out as explained in Section 2.4.

Table S1. Direct Cu determination in whole blood reference materials *via* HR CS GFAAS using different DBS filter papers. Results are expressed as $\bar{x} \pm U$, where $U = (t_s) / \sqrt{n}$ for a 95% confidence interval. The concentration was calculated taking into account the volume absorbed by the DBS device ($n=5$).

Sample	HemaPEN (Perkin Elmer 226 (mg L ⁻¹))	HemaPEN (Whatman 903 (mg L ⁻¹))
RM L-1	0.66 \pm 0.07 (RSD = 8%)	0.65 \pm 0.05 (RSD = 6%)
RM L-2	1.30 \pm 0.07 (RSD = 4%)	1.35 \pm 0.10 (RSD = 6%)
RM L-3	2.26 \pm 0.14 (RSD = 5%)	2.48 \pm 0.15 (RSD = 5%)

Fast and simple determination of Cu in blood is proposed via direct analysis of dried blood spots of known volume



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