

Energy dispersive X-ray fluorescence spectrometry for the direct multi-element analysis of dried blood spots

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Abstract

Home-based collection protocols for clinical specimens are actively pursued as a means of improving life quality of patients. In this sense, dried blood spots (DBS) are proposed as a non-invasive and even self-administered alternative to sampling whole venous blood. This contribution explores the potential of energy dispersive X-ray fluorescence spectrometry for the simultaneous and direct determination of some major (S, Cl, K, Na), minor (P, Fe) and trace (Ca, Cu, Zn) elements in blood, after its deposition onto clinical filter papers, thus giving rise to DBS.

For quantification purposes the best strategy was to use matrix-matched blood samples of known analyte concentrations. The accuracy and precision of the method were evaluated by analysis of a blood reference material (SeronormTM trace elements whole blood L3). Quantitative results were obtained for the determination of P, S, Cl, K

and Fe, and limits of detection for these elements were adequate, taking into account their typical concentrations in real blood samples.

Determination of Na, Ca, Cu and Zn was hampered by the occurrence of high sample support (Na, Ca) and instrumental blanks (Cu, Zn). Therefore, the quantitative determination of these elements at the levels expected in blood samples was not feasible.

The methodology developed was applied to the analysis of several blood samples and the results obtained were compared with those reported by standard techniques.

Overall, the performance of the method developed is promising and it could be used to determine the aforementioned elements in blood samples in a simple, fast and economic way. Furthermore, its non-destructive nature enables further analyses by means of complementary techniques to be carried out.

Keywords: DBS, blood, clinical paper, EDXRF, elements

1. Introduction

Dried blood spots (DBS) are spots of capillary blood from a finger or heel prick dripped onto a clinical filter paper, dried and then used for laboratory testing [1]. From a medical point of view, DBS sample collection is simple, quick, relatively painless, less invasive than venepuncture, and entails minimal field storage requirements [2]. The first DBS application for glucose monitoring was developed by Ivar Bang in 1913 [3], but it was the work of Guthrie and Suzi [4] the one that demonstrated the advantages of this approach for the development of newborn screening programs worldwide [5]. Over the last decade, use of DBS is booming, as they have been widely used for the monitoring of a large number of biomarkers such as therapeutic drugs, drugs of abuse, environmental contaminants, toxins and (trace) elements, among others [6-7].

However, the direct analysis of the DBS is challenging due to the fact that most instrumental techniques are designed to analyze liquid samples. In the field of element monitoring, for instance, most of the published contributions are focussed on a previous extraction of the elements present in the DBS by incubation with a mixture of diluted nitric acid with a commercial surfactant (Triton X-100) followed by graphite furnace atomic spectrometry (GFAAS) or inductively coupled plasma mass spectrometry (ICP-MS) (see Table 1). In this sense, the major analytical problems reported are the development of efficient extraction protocols for the largest possible number of analytes, with adequate recovery values, and also the minimization of the contribution of background element contamination to the measurement from the filter papers [5].

To cope with some of these problems, a desirable and conceptually simpler alternative is the use of solid state techniques such as solid sampling GFAAS (SS-GFAAS) or laser ablation ICP-MS (LA-ICP-MS). As reported in Table 1, in this latter case, several contributions dealing with this topic have been recently published involving the use of different types of mass spectrometers including quadrupole, multi-collector and time-of-flight systems. Nevertheless, the direct analysis of the DBS by SS-GFAAS and LA-ICP-MS is also challenging mostly due to the fact that it is complicated to analyse a typical whole blood spot (25-50 μL , $\sim 15\text{ mm } \varnothing$) at once, and thus different results would be obtained depending on the sampling area selected [14]. In principle, the ideal situation would be that the amount of the element retained per cm^2 of filter paper will only be lineally dependant on the elemental concentration of the blood sample spotted onto it, and independent of other parameters such as total volume deposited, level of hematocrit or exact location from which the discs are punched/ablated. However, there is some controversy in the literature about the degree to which this requirement is fulfilled [9]. Other analytical challenges reported include the quantification if no suitable reference materials with similar matrix compositions are available for calibration [17] and the difficulties to use an adequate internal standard under real conditions [11]. Therefore, in spite of the advances performed in the field of the direct analysis of DBS samples, there is still an increasing need to explore new simple and efficient analytical methodologies to overcome the aforementioned limitations. There is also a lack of information regarding the use of solid sampling for the monitoring of minor elements (not only traces and ultratraces) in DBS. As it is shown in Table 1, most of the published contributions are focussed on the quantification of one or several

trace and ultratrace elements, but the determination of other elements present at higher concentrations (mg L^{-1} levels) in blood samples is still fairly unexplored.

For instance, chloride is an electrolyte that helps in keeping a proper fluid and acid-base balance in human body, and its determination in blood samples is often a part of a comprehensive metabolic panel to diagnose certain health conditions. Phosphorous and potassium are also key elements to be determined in blood samples. Abnormal levels of the first one are related to kidney, liver and certain bone diseases, and the potassium levels are related to the hematocrit content in blood samples [18]. Iron has also an important role in blood as an indicator of a great number of anomalies including anaemia or thalassemia [19].

In the present contribution, for the first time, the capabilities and drawbacks of energy dispersive X-ray fluorescence spectrometry (EDXRF) have been evaluated for the quantification of P, S, Cl, K, Ca, Fe, Cu and Zn in DBS samples. The possibility to analyse directly solid samples and the inherent multi-elemental capabilities are the most important features among the many that have made EDXRF a very mature analytical tool in many fields, including biological tissue samples [20 to 23].

Most popular biomedical applications of EDXRF include the *in vivo* monitoring of toxicological metals in different biological tissues such as bone, kidney and skin [24]. For instance, recently, *in vivo* EDXRF in cancer and rheumatology patients has helped to understand how platinum and gold are retained in the human body [25]. In the field of clinical analysis, EDXRF technique has also been employed for multi-elemental determination of body fluids such as whole blood [26]. In this case, usually, EDXRF analysis is performed directly using a small volume of the venepuncture blood sample

deposited in a special polyethylene cup [27], or by means of a pressed powder pellet after blood sample lyophilisation [28]. Taking into account that EDXRF operates best on solid samples and gives optimal sensitivity and accuracy for thin homogeneous targets [29], blood samples have also been preconcentrated and analyzed after deposition of a sample aliquot ($>100\ \mu\text{L}$) on a laboratory filter paper (Whatman, no. 41) [19, 30].

In the current work, we want to explore the advantages of EDXRF for real DBS sample analysis, entailing the deposition of the advised amount of blood sample ($\sim 50\ \mu\text{L}$) on a standardized clinical filter paper (Whatman 903[®]), as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [28]. For that, blood sample preparation onto the clinical filter paper and EDXRF measurement conditions have been carefully evaluated so as to ensure the best sensitivity for analytes. Accuracy and precision of the results were tested by analyzing the human blood reference material Seronorm[™] Trace elements human whole blood L-3. To further prove applicability of the method for real blood samples, several real venous-blood samples from healthy volunteers were taken using standard phlebotomy technique and were analysed by DBS-EDXRF method and by ICP-OES or potentiometry (for Cl determination).

2. Experimental

2.1. Standards and reagents

Ca, Cu, Zn, Fe, Zn and P standard solutions were prepared from commercially available $1000\ \text{mg L}^{-1}$ single-standards in HNO_3 0.5 M (Romil Pure Chemistry, Cambridge, UK) by appropriate dilution. Cl and K standard solutions were prepared from commercially available $1000\ \text{mg L}^{-1}$ single-standards (Certipur, Merck, Darmstadt, Germany) in purified water. Na and Br standard solutions were prepared by dissolving a suitable

amount of analytical grade potassium bromide and sodium chloride salts (Sigma-Aldrich, Spain). In all cases, purified water from a Milli-Q system (Millipore Corp., Bedford, USA) was employed.

2.2. Blood samples

Three blood reference samples with reference values for the target analytes were considered for validation purposes. Seronorm™ Trace elements human whole blood L-1 (Lot. 1103128) and L-2 (Lot. 1103129) (Billingstad, Norway) were used as calibration standards to test the influence of matrix effects. The reference sample Seronorm™ Trace elements human whole blood L-3 (Lot. 1112691) (Billingstad, Norway) was used to evaluate the best measuring EDXRF conditions and the accuracy.

Real venous-blood samples from healthy volunteers from the Hospital Universitario Miguel Servet (Zaragoza, Spain) were taken using standard phlebotomy technique. These samples were both analyzed by the developed methodology (see next section for details) and by ICP-OES (Fe, K and P) or potentiometry (for Cl determination).

For ICP-OES analysis, samples were diluted in water by a factor of 241, and were subsequently analyzed. Seronorm L-2 reference material was analyzed in the same manner and used for calibration, in order to correct for possible matrix effects, even though deviations from the reference values using aqueous standards for calibration were relatively low (lower than 15%).

Prior to Cl determination, samples were also diluted a factor of 3.

2.3. Sample preparation for EDXRF analysis

Adequate volumes (50 or 200 μ L) of aqueous element standards, blood reference samples and real venous-blood samples were carefully deposited onto the surface of the clinical filter paper Whatman 903®, following the recommendations of the

National Committee for Clinical Laboratory Standards (NCCLS) [31]. Then, samples and standards were left to dry at room temperature for at least 4 h and, once dried, were kept in sealed plastic bags at room temperature. For EDXRF analysis, loaded filters were placed between two 4.0 μm -thick Prolene[®] X-ray foils (supplied by Chemplex Industries, Inc., Palm City, FL, USA) mounted in special sample holders, which incorporates snap-on ring at the end of the cell for attachments of thin-film supports. Afterward, samples were sealed in the sample holder of the equipment for EDXRF analysis.

2.4. EDXRF instrumentation

A commercially available benchtop EDXRF spectrometer (S2 Ranger, Bruker AXS, GmbH, Karlsruhe, Germany) was used in the present study. This instrument is equipped with a Pd target X-ray tube (max. power 50 W) and a XFLASH[™] LE Silicon Drift Detector (SDD), ultra-thin beryllium window (0.3 μm thickness) with a resolution lower than 129 eV at Mn-K α line for a count rate of 100000 counts-per-second. The distance tube to sample is 62.1 mm and the distance sample to detector is 33.8 mm. In this LE configuration of SDD detectors the intensities for Na K-alpha and Mg K-alpha are, respectively close to 8 and 4 times higher than the intensity recorded by conventional SDD detectors. The instrument is also equipped with nine primary filters that can be used in front of the tube before X-ray beam impinges the sample surface to improve measuring conditions for the elements of interest and it can operate under vacuum conditions. More detailed information about the equipment can be found elsewhere [32]. The software used to control the equipment, to build the calibrations and to perform the data treatment was SPECTRA EDX (Bruker AXS, GmbH, Germany). This software can perform the full line profile fitting, deconvolution when lines are

overlapped, intensity correction for inter-element effects and full-quantitative routines.

3. Results and Discussion

3.1 Evaluation of sample preparation and EDXRF measurement conditions

3.1.1. Measuring conditions

To obtain the most efficient excitation for analyte determinations, an evaluation of the best EDXRF measuring conditions was carried out. In Table 2 a summary of the main instrumental conditions used is displayed. Tests were performed by analysing a 200 µL DBS of the blood reference material Seronorm™ Trace elements human whole blood L-3. In all cases, the intensity was adjusted automatically to obtain a maximum count rate of 100000 cps and the measuring time (300s) was selected as a trade-off between an acceptable repeatability of measurements and total analysis time. In this study, the choice of the primary beam filters and the tube operating conditions was based on the best signal-to-noise ratio attainable for each element. It was found that for elements with emission lines in the range of 1.040-2.622 keV (Na, P, S, Cl) the best choice was the use of a voltage and current of 20kV/1.22mA without the use of any primary filter. Otherwise, a combination of a primary filter made of Al (500 µm thickness) and a ratio between the X-ray tube voltage and current of 40 kV/1.25 mA was the better option for the determination of elements with higher energy emission lines (K, Ca, Fe, Cu, Zn). As it is also shown in Table 2, variable alphas model was used for weak absorption corrections and secondary fluorescence effects, together with different line overlap corrections, depending on the element to be calibrated. The proper selection of the correction models was based on the best linear calibration curve for each of the target elements.

3.1.2. Blank contributions

Some recent contributions have highlighted the limitation of DBS method for fully quantitative analysis at trace levels due to the high background signals for some elements arising from the clinical filter papers [6]. For instance, Resano and co-workers demonstrated the significant presence of Co, Cu, Sn and Ni in the ink of the printed areas of the clinical papers used for patient sample positioning [33]. Similar problems for several elements have been reported when using volumetric absorptive microsampling devices instead of filter paper [34-35].

To evaluate the effect of the blank contribution for the determination of target analytes, a blank clinical filter paper and a 200 μ L DBS of the blood reference material Seronorm™ Trace elements human whole blood L-3 were analyzed by EDXRF using the aforementioned measurement conditions. As it is shown from the EDXRF spectra displayed in Figure 1, significant Na, Ca, Cu and Zn peaks were identified in the blank filter paper. In fact, the signal for these elements in the blank was more or less the same than that obtained in the analysis of the blood reference material. Therefore, it is obvious that the determination of these elements at real blood level concentrations is not possible. In a former contribution using similar instrumentation we detected traces of Cu and Zn arising to the background signals received from the equipment design [36]. Therefore, Na and Ca are probably arising from the clinical paper used to prepare the DBS, but the small traces of Cu and Zn are probably due to instrumental blank. In view of these results, additional studies are needed to fully evaluate the impact that the use of cleaner filter papers would have on the final results.

3.1.3. Sample holder

In XRF instruments, the sample is usually placed into a metal holder cup. Hence, radiation coming from these materials can interfere to the spectrum shape and the results of the determination. This fact is particularly relevant when thin layers or light matrices are analyzed. For this reason, different sample holder types were evaluated for clinical filter paper analysis (see Figure 2): (1) a commercial sample holder designed to filter analysis (Bruker AXS), (2) a teflon cup which incorporates snap-on ring at the end of the cell for attachments of thin-film supports and (3) a teflon cup with a teflon backstopper at the bottom (in-house). Teflon has got a low transmittance at 20 kV that makes it a proper mask material for our determination. It was found that when using a double-Teflon piece configuration (sample holder 3) the signal obtained was slightly higher than that obtained when using the commercial carrier (sample holder 1) or the teflon cup (sample holder 2), but the signal-to-noise ratio for element peaks was almost three times lower compared to sample holder 2 and similar to the one obtained when using the sample holder 1. Taking into account these results, a teflon cup (2) was used as sample holder to carry out the analysis of the clinical filter papers.

3.1.4. Blood spot size

According to the literature (see Table 1), for real applications, the total amount of blood sample deposited onto the clinical filter paper varied between 50 and 80 μL . In fact, some studies demonstrated that 100 μL is already a high volume, clearly exceeding the limits of the circle used for patient sample positioning [9]. Usually, a deposition of 50 μL onto the clinical paper gives a blood spot around 12-15 mm \varnothing . For many solid state techniques (LA-ICP-MS or GFAAS) this spot is too large for complete analysis in reasonable time, and only one or various portions of the DBS are sampled.

This fact may lead to inaccuracies of the results because different analytical responses could be obtained depending on the sampling area selected [14]. These problems can be avoided when using EDXRF analysis since the whole blood spot can be fully irradiated and analysed. Taking into account that the EDXRF system used is equipped with a 30mm mask, even blood spots with higher areas ($>15\text{mm } \varnothing$) can be analysed at once. For this reason, two aliquots of 50 μL and 200 μL of the blood reference material SeronormTM Trace elements human whole blood L-3 were deposited on a clinical filter paper and after drying they were analyzed by EDXRF. In Table 3, element analytical responses obtained for both samples are displayed. As it is shown, for most elements, similar results were obtained independently of the blood volume deposited onto the filter. This fact is surprising taking into account that when using 200 μL , the amount of blood analysed is four times higher. A possible explanation for that are the potential absorption issues when depositing larger sample volumes that weaken the analytical responses, besides the already commented presence of significant Na, Cu and Zn peaks in the blank filter paper.

All in all, the fact that the volume of blood deposited onto the filter is not a critical factor is of special relevance when applying the method to analysis of real samples since the exact volume of blood sampled onto the filter paper is unknown in most cases. In view of the obtained results and for practical reasons, aliquots of 50 μL of blood were used in further experiments.

3.2. Calibration approach and analytical performance

Empirical calibration was used to predict concentrations and it was based on a regression plot of corrected intensity (considering deconvolution overlaps and correction for inter-element effects, see Table 2) versus concentration.

As pointed in several works, when analysing thin samples (i.e. loaded filters) by XRF spectrometry matrix effects are usually considered negligible [29]. However, taking into account the complexity of the blood matrix, in addition to aqueous element standards deposited onto the clinical papers, also calibration curves using blood samples were considered for quantification purposes. In this latter case, two reference materials (SeronormTM Trace elements human whole blood L-1 and L-2) and three real venous-blood samples (previously analyzed to obtain reference values, as discussed in section 2.2.) were used. It is important to remark that it was not possible to obtain a linear response between Na, Ca, Cu and Zn concentration and analytical responses in any case. As discussed in section 3.1.2, significant peaks of these elements were identified in a blank clinical filter paper this fact real hamper their quantification at real blood level concentrations. Regarding the other elements, in general, similar calibration curves were obtained when using aqueous element standards and real blood samples. Nevertheless, better correlation coefficients were assessed when using aqueous element standards since a better spread of calibration data points over the suitable concentration range of each element can be assessed in comparison with real blood samples (Figure 3). However, for some elements such as Fe, significant differences (18%) were obtained between the slopes of both calibration curves (aqueous standards/real blood samples). This fact highlights the presence of matrix effects and the need to use blood samples as calibration standards. In order to study the quality of the results obtained using the DBS-EDXRF method, the blood reference material SeronormTM Trace elements human whole blood L-3 was analyzed and obtained results are summarized in Table 4. As it is shown, no significant statistical differences at 95% confidence level were found between experimental results and

reference values, demonstrating the usefulness of the developed methodology for the quantification of P, S, Cl, K and Fe in blood samples. Limits of detection (LODs) were also estimated from the EDXRF spectrum of the same sample using the 3σ criteria [37]: P (20 mg L^{-1}), S (50 mg L^{-1}), Cl (50 mg L^{-1}), K (70 mg L^{-1}) and Fe (15 mg L^{-1}). Despite the fact that these values are significantly higher in comparison with other published methodologies (see Table 1), they are suitable taking into account concentration levels of the target elements in real blood samples.

Global precision of the DBS-EDXRF method was also tested by analysing six independent replicates of the same reference material mentioned above. Besides, one of the replicates was measured six times and the relative standard deviation (RSD) associated was also calculated. This uncertainty is related to the instrument stability and counting statistics. Therefore, by means of error propagation, the uncertainty due to sample preparation (deposition of the blood aliquot onto the filter paper) can also be estimated. Results obtained are displayed in Figure 4. As it is shown, global precision is acceptable for all the studied elements with RSD values between 2.9 to 9.3%. From Figure 4 it can also be deduced that uncertainties in sample preparation have a significant contribution (>50%) to the global precision of the obtained results above all for light elements such as P and S.

3.3. Application to real blood samples

To further prove applicability of the method for real blood samples, four real venous-blood samples from healthy volunteers were taken using standard phlebotomy technique and were analysed by DBS-EDXRF method (using a blood sample volume of $50 \mu\text{L}$), and by ICP-OES, after a proper dilution of the blood sample, or potentiometry (for Cl determination). The results are displayed in Figure 5. As it can be seen, similar

results were obtained using both analytical approaches highlighting the suitability of the developed methodology for the quantification of P, Cl, K and Fe in real blood samples.

It can be stressed that one of the problems associated with direct analysis of DBS by means of microsampling techniques (e.g., LA-ICP-MS) when only a portion of the sample is analyzed instead of the whole filter [38], is the influence of the hematocrit level, which may have a significant effect on the spreading of the sample [12]. However, it has also been published that there is a direct correlation between such level and the content of K, such that knowing the K level it is possible to accurately predict the hematocrit level of a DBS [18].

Therefore, the development of a simple and fast, non-destructive XRF method seems promising to complement ultratrace elemental analysis of DBS using more sensitive but destructive solid sampling techniques (e.g., LA-ICP-MS), as not only elements present at higher levels can be directly determined, but the XRF method can also provide valuable information to gain accuracy in the subsequent analysis (e.g., correction of hematocrit bias, use of the Fe signal as internal standard), overall enabling more and better information to be obtained without the need for any sample treatment.

4. Conclusions

For the first time, a methodology for the direct analysis of DBS samples by EDXRF is provided. While the analysis of DBS using EDXRF was found to be unsuitable for several major elements of clinical interest (Na, Ca, Cu and Zn), reasonable data were obtained for P, S, Cl, K and Fe. Further work would be needed to explore the usefulness of this approach for electrolyte elements (Cl and K) since they are often measured in either

plasma or serum. The determination of Fe in DBS might be useful, bearing in mind that from a clinical perspective, Fe status is normally assessed as either serum ferritin or by means of the total iron binding capacity (TIBC) test.

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Table 1

Overview of analytical procedures published in the last decade for element determination in dried blood spots

Technique	Sample treatment	Amount of blood (µL)	Elements	Detection limits (µg L ⁻¹)	Ref.
GFAAS	Extraction: 5% HNO ₃ . Mixing, centrifugation	≈50	Pb	<40	8
SS-GFAAS	None (analysis of a 3.2 mm Ø discs)	≈50	Pb	2.5	9
DRC-Q-ICPMS	Extraction: 5% HNO ₃ +0.01%Triton X-100. Incubation, sonication, centrifugation	60	As, Pb, Hg, Cd	<1	10
CCT-ED-Q-ICPMS	Mineralization: HNO ₃ +H ₂ O ₂ , 65°C, 4h	n.a.	As, Cd, Cu, Pb, Mo, Se, Zn	0.15-15	11 ^a
CCT-ED-Q-ICPMS	Extraction: 5% HNO ₃ +0.01%Triton X-100 (1h, 2 cycles). Centrifugation	≈75	Na, Mg, K, Ca, Mn, Fe, Cu, Zn, As, Se, Cd, Pb	0.5-200 Na, Mg, K, Ca, Fe (12-580 mg L ⁻¹)	1
Q-ICPMS	Extraction: 5% HNO ₃ +0.01%Triton X-100. Incubation, sonication, centrifugation	≈60	As, Cd, Pb, Hg	Low µg L ⁻¹ range	12
Q-ICPMS	Extraction: 2-mercaptoethanol+Triton X-100+L-cysteine+EDTA+hydrochloric acid. Agitation.	≈6	Hg	0.7	13
SF-ICPMS	Extraction: 5% HNO ₃ +0.01%Triton X-100. Incubation, sonication, filtration	≈80	Ca, Cs, Cu, Fe, K, Mg, Na, P, Rb, S, Zn	<0.1	5
LA-MC-ICPMS	None (5-6 mm Ø carters by ablating 250-285 concentric circumferences)	5-15	Cd, Co, Cu, Pb	0.04-0.5	14
LA-TOF-ICPMS	None (ablation of 4mm line scans)	n.a.	Pb (other potential elements: As,Cd,Cu,Sb,Se)	9	15
LA-Q-ICPMS	None	0.5	Be, Mn, Co, Ni, Tl, Bi, Sb, Pb, Cu, Zn, Ba, Mg, Cd	0.14-29	16 ^a
EDXRF	None	50	P, S, Cl, K, Fe	15-70 mg L ⁻¹	This work

^a PVDF membranes were used instead of the commonly used clinical filter paper 903[®]

GFAAS: Graphite furnace atomic absorption Spectrometry, SS: Solid sampling, ICP-MS: Inductively coupled plasma mass Spectrometry, DRC: Dynamic reaction cell, CCT-ED: Collision cell with kinetic energy discrimination, Q: Quadrupole, SF: Sector field, LA: Laser ablation, MC: Multi-collector, TOF: Time of Flight, EDXRF: Energy dispersive X-ray fluorescence spectrometry

Table 2

Instrumental characteristics and measuring parameters used for EDXRF measurements

EDXRF benchtop system (S2 Ranger, Bruker AXS)	
Anode X-ray tube	Pd
kV/mA	20/1.22 (Na, P, S, Cl) 40/1.25 (K, Ca, Fe, Cu, Zn)
Primary filter	None (Na, P, S, Cl) Al 500 μm (K, Ca, Fe, Cu, Zn)
Detector	XFLASH TM SDD, <129 eV at Mn-K $_{\alpha}$
Focal spot	30 mm
Measuring mode	Vacuum
Measuring time	300 s (per analytical condition)
Absorption effect corrections	Variable alphas, Intensity model
Line overlap corrections by intensity	Fe-K $_{\alpha 1}$ (Cu), Cu-K $_{\alpha 1}$ (Zn)
Analytical lines (keV)	Na- K $_{\alpha}$: 1.040, P- K $_{\alpha}$: 2.010, S- K $_{\alpha}$: 2.309, Cl- K $_{\alpha}$: 2.622, K- K $_{\alpha}$: 3.314, Ca- K $_{\alpha}$: 3.692, Cu- K $_{\alpha}$: 8.046, Zn- K $_{\alpha}$: 8.637

Table 3

Influence of paper size and blood sample volume on elemental EDXRF responses.



Spot	Drop size (Ø, mm)	Blood volume (µL)	EDXRF analytical response (Cps/mA)								
			Na	P	S	Cl	K	Ca	Fe	Cu	Zn
	23	200	28.9	225.5	1446.5	3826.5	43.2	114.9	154.7	15.0	16.4
	12	50	29.0	179.7	1235.0	3333.0	26.8	101.0	98.3	16.9	17.1

Table 4

Results obtained for the determination of P, S, Cl, K and Fe in the commercial blood reference material (Seronorm™ Trace elements whole blood L-3) by means of the developed DBS-EDXRF method. Results are expressed as mean concentration values of three replicates with the associated standard deviation.

Element	Reference value (mg L ⁻¹)	EDXRF method (mg L ⁻¹)	% Dif.
P	214 ± 6	200 ± 10	6.5
S	1268 ± 39	1352 ± 40	6.6
Cl	3315 ± 41 ^a	3400 ± 200	2.4
K	1074 ± 19	1070 ± 40	0.4
Fe	383 ± 9	380 ± 20	0.8

^a value determined by potentiometry (no reference value was available)

Figure 1

EDXRF spectra of the commercial blood reference material (Seronorm™ Trace elements whole blood L-3) and a clinical filter paper blank (Whatman 903®). Legend: Red (filter paper blank), black (blood reference material onto the filter paper blank).

(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

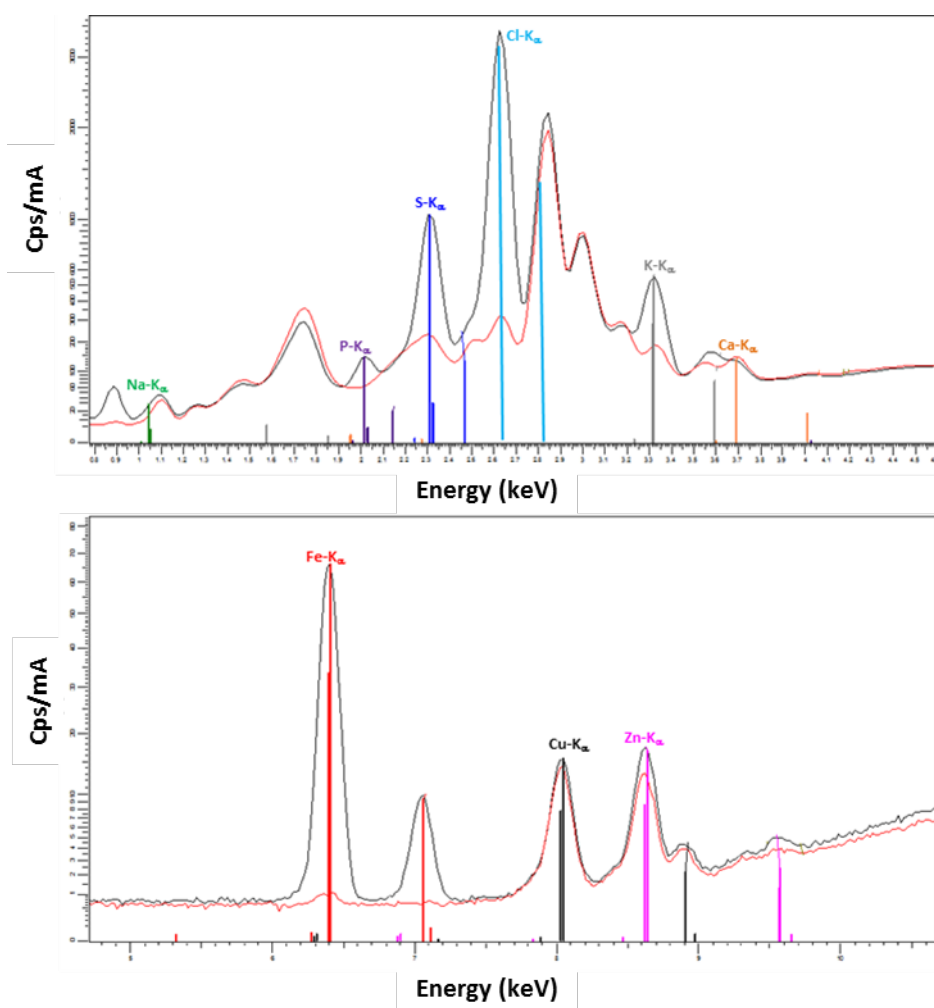


Figure 2

Sample holder types tested for clinical filter paper analysis: (1) Commercial sample holder for filter analysis (Bruker AXS), (2) Teflon cup, (3) Teflon cup + Teflon backstopper.

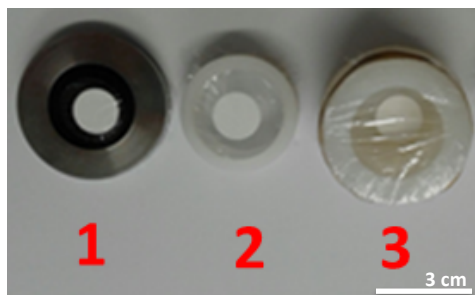


Figure 3

Comparison of the calibration curves obtained for aqueous standard solutions (in rhombus) and for blood reference materials (in squares): K (A), Fe (B). Error bars represent standard deviation of triplicate analysis.

(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

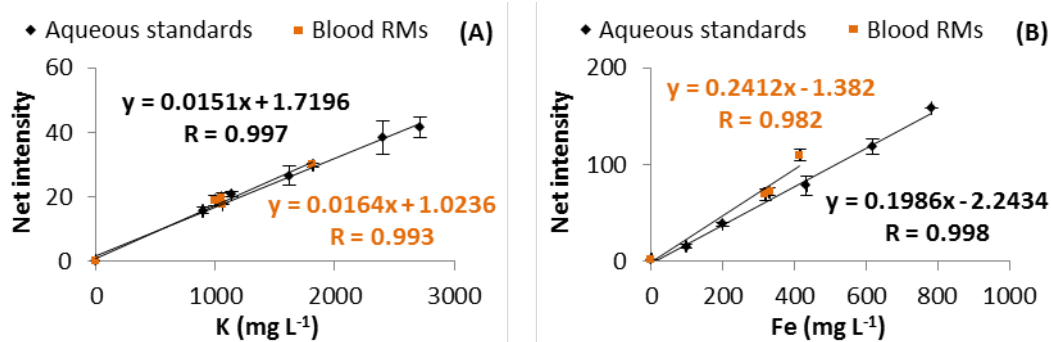


Figure 4

Contribution of instrument and sample preparation uncertainties to the global precision of the DBS-EDXRF results.

Legend: Dark grey: instrument uncertainty, light grey: sample preparation uncertainty

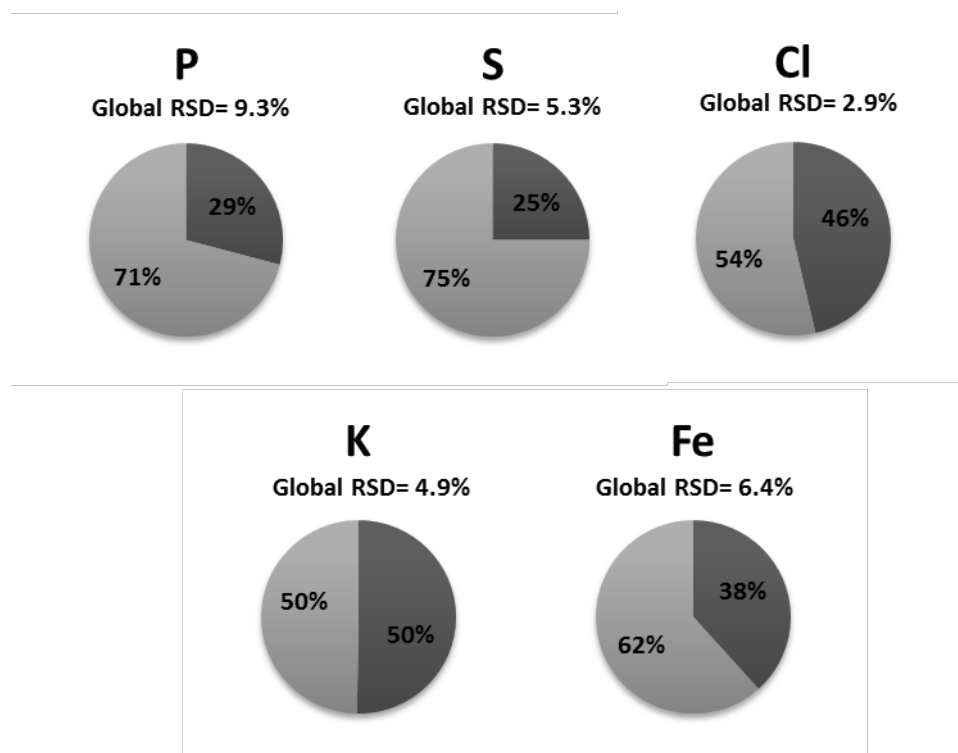


Figure 5

Results obtained after DBS-EDXRF analysis of four blood samples in comparison with the venous method results. Results are expressed as mean concentration values of three replicates with the associated standard deviation.

