Potential of total-reflection X-ray spectrometry for multielement analysis of biological samples using dilution or suspension sample preparation techniques

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In most clinical and nutritional studies, it is of significance to know information about the multielemental composition of biological samples. Conventional analysis of biological samples relies upon sample digestion followed by atomic spectrometry detection. This approach is essential for the quantification of ultratrace elements in biological samples. While in other applications it could be of interest to have simpler analytical methods with multielemental capability but involving a minimum sample treatment, reduce the amount of sample and a more cost-effective analysis. In the present contribution, the possibilities and drawbacks of simple sample treatments (i.e., dilution and suspension) in combination with total reflection X-ray fluorescence spectrometry (TXRF) for the analysis of different types of biological samples have been critically evaluated. For that, a set of reference materials or well-characterized biological human fluids (blood, serum, plasma and seminal plasma) and animal/vegetal tissues have been used to estimate the analytical capabilities in terms of limits of detection, trueness and precision of the proposed TXRF methods. The results are based on the authors’ experience in analysing biological samples using TXRF, and it is expected that they can be useful for new TXRF users in this field and they can provide a good basis for further application of this technique in clinical studies and other applications dealing with the analysis of biological samples in the future.

1 | INTRODUCTION

Elemental monitoring in human body fluids (i.e., blood, serum, plasma, seminal plasma…) is of special interest in view of the importance of body metal imbalances in (patho)physiological processes and diagnosis of various disorders.1,2 Likewise, knowing the elemental composition of vegetal and animal tissues can be useful for safety and nutritional purposes.3 In view of these premises, it is obvious the significance of multielemental analysis of this type of biological samples.

Some analytical challenges are related with the quantification of multiple elements in biological samples, including the complexity of sample matrices, the limited sample amount available (above all for human body fluids analysis) and the wide range in element concentrations. Commonly, atomic spectroscopic techniques such as flame atomic absorption spectrometry (FAAS),
electrothermal atomic absorption spectrometry (ETAAS), inductively coupled plasma emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) are selected for element determination in biological samples.\[^{14-16}\] In the last years, the number of studies dealing with the use of ICP-MS has been increased due to its multielemental capability, high sample throughput and high sensitivity. However, these kinds of systems are designed for the analysis of low-salty liquid samples and therefore, biological samples have to be processed before analysis. In the case of human body fluids, a dilution step using a high dilution factor or the use of additional treatments for the destruction of the organic matrix is required.\[^{7}\] Likewise, solid biological samples have to be transformed to liquid state by means of a digestion procedure, which is in most cases time-consuming.\[^{8}\]

This approach is important for the quantification of ultratrace elements in biological samples. While in other applications it could be of interest to have alternative simpler and faster analytical methods with multielemental capability but involving a minimum sample treatment. In this regards, it is interesting to mention the potential role of total reflection X-ray fluorescence spectrometry (TXRF). TXRF offers some advantages with respect to other spectroscopic techniques, such as simultaneous multielemental capability, easy quantification by means of internal standardization and the low amount of sample required, which are of paramount importance for the analysis of human biological fluids.\[^{9}\]

Moreover, TXRF systems are cost-effective in comparison with ICP-based techniques since they do not require gas or cooling media for function. An additional advantage of TXRF is the possibility to analyse complex liquid or solid samples using simpler sample treatments (i.e., dilution or suspension), which are more in line with the Green Analytical Chemistry principles. Although these analytical approaches have been used for multielemental analysis of solid samples in the last few years,\[^{10-12}\] suspension preparation of solid samples (without digestion) accounts only for the 15% of the sample treatment procedures used in TXRF analysis, as it has been reported in a recent tutorial review published by De La Calle and co-workers.\[^{13}\]

The main aim of the present contribution is to bring out the analytical potential and constraints of using simple treatment methods in combination with TXRF for multielemental analysis of biological samples. For that, dilution and suspension preparation have been tested as treatment strategies for TXRF analysis of human fluids and vegetal/animal tissues, respectively. The results are based on the authors’ experience in analysing biological samples using TXRF, and we hope it can be useful for new TXRF users and to provide a good basis for further application of this technique in clinical trials and other applications dealing with the analysis of biological samples.

## MATERIALS AND METHODS

### 2.1 Reagents and materials

Monoelemental stock solutions of 1,000 mg/L (ROMIL PrimAg@ Monocomponent reference solutions) were used to prepare internal standard solutions and spiked biological liquid samples. Ultrapure de-ionized water (Millipore Corp., Bedford, MA) and 1% solution of Triton™ X-100 were tested as diluting or disperser agents to prepare liquid and solid biological samples before TXRF analysis. Silicone solution in isopropanol (Serva GmbH & Co, Germany) was used to coat all the quartz glass disc reflectors (diameter: 30 mm, thickness: 3 mm) in order to obtain a hydrophobic film so as to facilitate sample deposition.

### 2.2 Biological samples

The samples were a set of different reference materials. In the case of the biological fluid samples, four different types of human body fluids were considered including serum (Seronorm™ Trace elements serum L-1, Ref. 201,405), plasma (Clinchek® Plasma control Level-II, Ref. 8,883–8,885), blood (Seronorm™ Trace elements whole blood L-1, Ref. 210,105) and seminal plasma (SP). In the latter case, due to the lack of reference material available, six real seminal plasma samples (S1-S6) previously analysed by ICP-OES were used. These samples were obtained from leftovers of semen samples collected for diagnostic purposes, as a part of the standard procedure of infertility diagnostics and treatment. Specific details of the sampling procedure can be found elsewhere.\[^{14}\]

Different types of biological solid reference materials were also considered, including plant and animal tissues: SRM 1515 (apple leaves), SRM 1547 (peach leaves), SRM 1570a (spinach leaves), SRM 1573a (tomato leaves) purchased from the National Institute of Standards & Technology, NIST; NCS ZC73012 (cabbage), NCS ZC73013 (spinach), NCS DC73349 (bush branches and leaves), NCS DC73350 (leaves of the poplar), GBW08571 (Mussel muscle tissue) from China National Analysis Centre for Iron and Steel, NACIS, and RM 003 (strawberries) provided in the frame of the PRO-METROFOOD Project from the Italian National Agency for New Technologies, Energy and Sustainable Economic Development, ENEA.
2.3 | Sample preparation strategies for biological samples analysis by TXRF

In Figure 1, a summary of the main steps involved in the sample preparation of biological liquid and solid samples for the subsequent TXRF analysis is displayed. It is interesting to remark that the main goal of this study was to investigate the potential and constraints of TXRF as a simple and sustainable method for multielemental analysis of biological samples. For that, only dilution (liquid samples) and suspension (solid samples) were considered as sample treatment strategies and more sophisticated sample treatments such as microwave digestion or cold plasma ashing, which have been used in biological sample analysis by TXRF, were not considered.[15,16] As it is shown in Figure 1, the general procedure involves the addition of a diluent agent to a small volume of biological liquid sample (<1 ml) or the suspension of several mg of sample in an adequate disperser agent, in the case of solid biological samples. In both cases, usually, quantification by TXRF is performed by means of internal standardization and therefore, a few μl of a suitable internal standard have to be added to the diluted or suspended sample. After, in order to increase the homogeneity of the prepared specimens, a vortex mixing process (10–20 s) is carried out before deposition of a small amount of sample (5–20 μl) into a suitable sample carrier (usually a quartz reflector). In the case of solid suspensions, an additional sonication step (5–10 min) is recommended prior to vortex mixing to improve particle suspension and homogeneity. Finally, once the sample is deposited on the reflector, a drying process is needed. Usually, a hot plate or an IR lamp is used for such a purpose. It is noteworthy that in both cases, the complete sample treatment procedure is simple, quite fast and took less than 10–15 min to obtain suitable specimens to be analysed by TXRF.

In Table 1, specific details about sample preparation of liquid and solid biological samples are displayed. Preliminary studies were performed to select the best experimental conditions to prepare each type of biological sample. More information about optimization studies can be found in the scientific publications referred in Table 1.

2.4 | Instrumentation

TXRF analysis of all samples was performed using a benchtop TXRF system (S2 PICOFOX, Bruker AXS Microanalysis GmbH, Berlin, Germany) equipped with a W X-ray tube anode. The detailed instrumental characteristics and measurement conditions are summarized in Table 2. TXRF spectra evaluation and the calculation of the analyte net peak areas were performed with the equipment’s software (Spectra Plus 5.3, Bruker AXS Microanalysis GmbH, Berlin, Germany). With this software, the spectral background is calculated using a mathematical algorithm and subtracted from the spectrum.

Zinc content in seminal plasma samples was also determined by ICP-OES (Agilent 5,100, Agilent Technologies, Spain) using the experimental conditions reported in Table 2. In order to study the spatial distribution of Zn in the dried seminal plasma residue on the reflector, mappings were performed by μ-EDXRF.
using a benchtop small-spot EDXRF spectrometer (XDV-SDD model, Helmut Fischer GmbH, Sindelfingen, Germany). In previous studies, we showed the potential of such a system for biological sample mappings.\textsuperscript{[18,19]} Detailed features and analytical conditions are displayed in Table 2. Spectral data from EDXRF analysis were evaluated using the WinFTH software, version 6.35 linked to the instrument. A stereoscopic optical microscope (NIKON SMZ-1000) was used for homogeneity studies of sample suspensions deposited on quartz reflectors.

### 2.5 Estimation of detection limits and quantification approaches

To evaluate the capabilities of the proposed TXRF methods for trace analysis, limits of detection were calculated by using the following expression:

\[
\text{LOD} = \frac{3C_i \sqrt{\frac{N_{\text{bkg}}}{N_i}}}{N_i},
\]

where $C_i$ is the concentration of a given analyte; $N_{\text{bkg}}$ is the background area and $N_i$ is the analyte net peak area. This equation is analogous to the $3\sigma$ definition of the LOD.\textsuperscript{[9]}

Quantitative analyses were carried out by internal standardization\textsuperscript{[9]}:

\[
C_i = \left( \frac{N_i C_{\text{IS}} S_i}{N_{\text{IS}} S_i} \right),
\]

where $C_i$: analyte concentration, $N_i$: analyte net peak area, $C_{\text{IS}}$: IS concentration, $S_i$: instrumental sensitivity for the IS, $N_{\text{IS}}$: IS net peak area, and $S_i$: instrumental sensitivity for the analyte.

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### Table 1 Specific experimental details about sample preparation of liquid and solid biological samples for TXRF analysis

| Sample biological samples | Diluent | Amount (g) | Type | Amount (g) | IS (mg/kg) | Vortex (s) | V (μl) | Drying mode | Ref. | Type | Amount (g) |
|---------------------------|---------|------------|------|------------|------------|------------|--------|-------------|------|------|------------|
| Seminal plasma            | 0.2     | 1% triton X-100\textsuperscript{a} | 0.2  | 5 (Y)      | 10         | 5          | IR lamp | [14]        |      |      |            |
| Blood                     | 0.1     | 1% triton X-100\textsuperscript{a} | 0.4  | 5 (Ga)     | 10         | 10         | IR lamp | [17]        |      |      |            |
| Serum                     | 0.2     | None       | None | 5 (Y)      | 10         | 12.5       | IR lamp | \textsuperscript{b} |      |      |            |
| Plasma                    | 0.2     | None       | None | 5 (Y)      | 10         | 20         | IR lamp | \textsuperscript{b} |      |      |            |

\textsuperscript{a}Units: weight/volume (w/v). \textsuperscript{b}Unpublished data.

### Table 2 Instrumental characteristics and measurement conditions

#### S2 PICOFOX TXRF benchtop spectrometer (Bruker Nano)

- X-ray tube anode: W
- Power: 50 W
- Optics: Multilayer monochromator (35.0 keV)
- Detector: SDD, 10 mm\textsuperscript{2}, <150 eV resolution at Mn-K\textsubscript{a}
- Working environment: Air
- Measurement time: 2000 s

#### XDV-SDD μ-XRF spectrometer (Helmut Fischer GmbH)

- X-ray tube anode: W
- Voltage: 50 kV
- Current: 0.1–1 mA (max. Power 50 W)
- Primary filter: Al 1,000 μm
- Detector: SDD, 145 eV resolution at Mn-K\textsubscript{a}

#### 5,100 ICP-OES spectrometer (Agilent)

- Nebuliser: Pneumatic concentric
- RF power: 1,200 W
- Plasma flow: 12 L min\textsuperscript{-1}
- Torch configuration: Radial
- Detector: Multichannel charge transfer
- Wavelength: Zn: 202.548 nm
3 | RESULTS AND DISCUSSION

3.1 | Analysis of biological liquid samples by TXRF

In some medical studies, it is of relevance to have information about the concentration levels of trace elements (i.e., Fe, Zn, Cu and Se) in biological human fluids such as whole blood, serum, plasma or seminal plasma among others. As mentioned in the introduction section, one of the most commonly used techniques for multielemental analysis of these types of samples is ICP-MS. Nevertheless, usually, a previous sample treatment such as digestion or dilution (using a mixture of different components: surfactants, complexing agents and bases) of the sample needs to be carried out before ICP-MS to reduce matrix interferences and minimise the risk of clogging the nebuliser, torch injector or sampling interface. [20] Moreover, external calibration using matrix-matched standards or standard addition needs to be employed as a quantification approach to obtain reliable results. In this sense, it is interesting to note the benefits that the use of TXRF can contribute in the field of biological human fluids analysis.

On the one hand, TXRF analysis can be performed without any sample treatment or by means of a simple dilution step (using ultrapure water or dilution with surfactant solution) depending on the complexity and protein content of the biological matrix (see Table 1 for details). In the case of light elements, the dilution of the sample is mandatory in most cases to obtain quantitative results. This fact was discussed in detail in a previous study published by Zarkadas et al. [17] who found that the direct TXRF analysis of biological fluids was only viable for elements with atomic number higher than 23.

As an example, in Figure 2, the comparison of TXRF spectra obtained in the analysis of seminal plasma, serum, plasma and blood using different dilution ratios is displayed. As it is shown in the resulting spectra, in all cases, in addition to the light elements (S, Cl, K and Ca), trace elements relevant for medical diagnostics such as Fe, Zn and Cu can be also detected in a simple and fast way, among others. An interesting aspect to be highlighted is also the possibility to easily determine Br, which is difficult to be measured by other spectrometry techniques and it could be of interest in some clinical studies. [21]

In the case of blood and seminal plasma, the direct TXRF analysis was not possible since the residue on the reflector was too thick and it was detached from the surface of the quartz reflector. For that, a dilution step using a solution of 1% w/v Triton X-100 was required. As reported in previous studies, better deposition is obtained using a solution of 1% w/v Triton X-100 compared to ultrapure water due to the non-ionic nature of

![Comparison of TXRF spectra obtained in the analysis of seminal plasma (dilution 1:1), serum (without dilution), plasma (without dilution) and whole blood (dilution 1:5). Analytical conditions are reported in Table 1](image)
this surfactant and the attainment of a more homogeneous diluted sample \[21\]. As it is shown in Figure 2, the dilution factor has a high impact in the light elements region (2–6 keV). Although the analytical signals in this region are decreased due to the dilution factor, the background is also decreased and the signal-to-noise ratio is not so different for diluted and non-diluted biological fluids. Nevertheless, the dilution factor greatly affects the determination of trace and ultratrace elements as can be seen from the calculated detection limits for trace elements in human fluids (see Figure 3). As it is shown, the limits of detection for blood sample analysis are significantly higher compared to other studied fluids mainly due to the higher dilution factor needed to perform the TXRF analysis. Nevertheless, in all cases, the limits of detections for some biologically relevant trace elements (i.e., Fe, Cu and Zn) are adequate. It is also interesting to remark that the limits of detection can be significantly reduced when using TXRF systems equipped with Mo X-ray tubes instead of W X-ray tubes \[22\]. Using Mo-based TXRF systems, other trace elements present at lower concentrations such as Se, which can play a critical role as antioxidant defence system enzymes (selenoproteins), have also been estimated, for instance, in seminal plasma samples \[23\].

In order to evaluate the detection limits for potentially toxic metals, the biological fluids studied were spiked with Cd and Pb at the level of 0.9 mg/kg. The calculated limits of detection were in the range of 0.05–0.3 mg/kg (Cd) and 0.07–0.2 mg/kg (Pb), respectively. These values are not adequate to monitor these potentially toxic metals in biological fluids and are significantly higher compared to the values obtained in similar sample matrices using ICP-MS \[24\]. Therefore, despite the fact that dilution + TXRF analysis can be a simple, fast and cost-effective approach to monitor minor and trace elements in biological fluids, more sophisticated sample treatments (i.e., digestion followed by a pre-concentration step) or more sensitive techniques such as ICP-MS are required for the determination of other potentially toxic metals that are present at lower concentrations \[25,26\].

An additional advantage of TXRF compared to other atomic techniques such as ICP-MS is the possibility of performing quantitative analysis without the need for external calibration. This fact is of significance in the analysis of biological fluids since external calibration using a set of matrix-matched standards is usually required to obtain quantitative results. On the contrary, in TXRF analysis, quantification can be performed by the addition of a suitable internal standard to the sample (see Section 2.5). The internal standard should not be present in the original sample, not interfere with the target elements and have an adequate analytical response. It is important to mention that the TXRF system used in this study was equipped with a W anode to generate X-rays (see Section 2.4). This fact allowed the use of mid-Z elements (i.e., Ga) as internal standards but also the use of higher Z elements such as Y, which is generally not recommended when employing TXRF systems equipped with Mo X-ray tubes. This fact of significance in view of the overlapping of Ga-lines with Zn-lines is observed in some biological fluid samples (see Figure 2). It is also important to ensure a homogeneous distribution of the internal standard within the sample. As an example, in Figure 4, spatial distribution of Zn and Y (internal standard) in a seminal plasma sample deposited on a quartz reflector is displayed. As it can be seen, both elements are accumulated in the borders of the spot deposited (coffee-ring effect) and therefore the distribution of the elements within the residue is not homogeneous. This fact shows the importance of analysing the whole residue on the reflector to obtain representative element concentrations.

**FIGURE 3** Limits of detection estimated for seminal plasma, serum, plasma and blood sample analysis by TXRF. Analytical conditions are reported in Table 1
In order to check the quality of the results obtained using the best analytical conditions reported in Table 1 and using internal standardization as a quantitative approach, the TXRF results obtained from the analysis of several reference materials were compared with the reference values for Fe, Cu and Zn. As it is shown in Table 3, good agreement was obtained between both data sets for all biological matrices studied (plasma, serum and blood). It is also interesting to mention that the relative standard deviations (RSD) estimated for the triplicate analysis were below 10% in most cases, except for the elements present at concentration levels close to the detection limit (i.e., Cu in blood, RSD ≈ 30%). In view of the lack of suitable seminal plasma reference materials, a set of seminal plasma samples containing different amounts of zinc were analysed by TXRF and the results obtained were compared with those obtained by ICP-OES analysis. Zn is an essential element for normal spermatogenesis of mammals and it is associated with sperm quality and inflammation.[27] As it can be seen in Figure 5, no significant statistical differences at 95% confidence level were obtained between both techniques in the studied concentration range. This fact reinforces the potential of TXRF as a simple, rapid and cost-effective technique in some clinical applications. In this sense, it is important to remark that to carry out ICP-OES measurements, SP samples had to be diluted (1:20 ratio) with a water solution containing 0.0003% EDTA, 0.004% NH₃ and 0.07% Triton X-100 and matrix-matched calibration standards, using the same solution, were used for quantification purposes.

### 3.2 Analysis of solid biological samples by TXRF

In some biological, clinical, nutritional or toxicological studies, it is also important to get information about multielemental composition of vegetal and animal tissues. Commonly, the techniques used for multielemental analysis of solid biological samples involved a previous digestion step of the sample matrix, which is time-consuming and entails the use of harmful reagents. An interesting analytical feature of TXRF in this field is the possibility to analyse solid samples by suspending several milligrams of material in an adequate disperser agent without the need to digest the sample (see Figure 1). In Figure 6, the limits of detection for trace elements in the range of Z = 25–38 in some plant tissues using this analytical approach are displayed. As it is shown, the values are in the low mg/kg range with values lower than 1 mg/kg for heavier elements. The limit of detection of potentially toxic elements such as Pb was also estimated and it was in the range of 0.7–3.5 mg/kg, depending on the plant tissue. These values are not adequate in view of the very low concentration levels found in these types of samples. In such cases, the use of more sensitive analytical techniques such as ICP-MS or the use of more sophisticated sample treatments is mandatory. Finally, it is interesting to note that the limits of detection for light elements such as K and Ca were also calculated and they were in the range of 130–170 mg/kg for K and 81–84 mg/kg for Ca, respectively, for all plant tissues studied.

| Element | Plasma | Reference | Serum | Reference | Blood | Reference |
|---------|--------|-----------|-------|-----------|-------|-----------|
| Fe      | 1.39 ± 0.09 | 1.2 (1.05–1.42) | 1.36 ± 0.17 | 1.47 (1.17–1.77) | 320 ± 20 | 334* |
| Cu      | 1.27 ± 0.08 | 1.26 (1.08–1.45) | 1.02 ± 0.07 | 1.066 (0.852–1.281) | 0.6 ± 0.2 | 0.64 ± 0.13 |
| Zn      | 1.94 ± 0.07 | 2.13 (1.81–2.45) | 1.0 ± 0.1 | 1.057 (0.844–1.269) | 4.4 ± 0.4 | 4.3 ± 0.9 |

**Note:** Results are expressed as mean concentration values of three replicates with the associated standard deviation (in mg/L).

*Approximate value.
Nevertheless, this is not a critical point taking into account that K and Ca are usually found as major elements, present at higher concentrations in solid biological samples.

When using suspension preparation as a sample treatment approach for the analysis of solid biological samples by TXRF, it is of paramount importance to select the appropriate amount of sample and type of disperser agent. These parameters have a significant impact in the homogeneity of the resulting suspension and the shape of the residue deposited on the reflector. In Figure 7, optical microscope images in transmitted light for 10 μl sample spots in quartz reflectors prepared by suspending different types of solid biological samples are displayed. As it is shown, the size of the drop on the reflector is highly influenced by the type of disperser agent. Usually, ultrapure water or diluted solutions of a non-ionic surfactant are used to suspend the solid material. An important aspect to be considered is that the diameter of the sample spot on the sample carrier has to be within the beam size for complete exposure of the drop to the X-ray beam. For that, only a few microliters (usually 5–10 μl) of the suspension should be deposited on the reflector. As it is shown in Figure 7, when using a 1% w/v solution of Triton X-100 as disperser agent, the diameter of the sample spot on the reflector is bigger than that obtained when using ultrapure water. This trend is also valid for liquid biological samples such as whole blood as it is shown also in Figure 7.

In the case of suspension preparation, another important aspect to get homogeneous mixtures is the particle size of the powdered material. Usually, the particle size of biological reference materials is lower than 75 μm and at this particle size level the homogeneity of the sample suspension is acceptable. However, when dealing with the analysis of real solid biological samples, it is important to ensure a reduction of the particle size to these values (i.e., using a cryogenic grinder, a ball mixer mill) to obtain a good homogeneity of the resulting suspensions.

As stated above, one of the advantages of TXRF compared to other multielemental analytical techniques is the possibility to use internal standardization as a quantification approach. However, light elements are more prone to be affected by absorption matrix effects as it has been pointed by Mal'tsev et al. in a recent publication dealing with sample preparation techniques for TXRF analysis of tea leaves. Therefore, for some biological matrices, the results for light elements using internal standardization are not as good as for mid Z or high Z elements. In such cases, an improvement of the TXRF results can be assessed by external calibration using a set of reference materials with a similar matrix to the real samples. As it can be seen in Figure 8, the obtained results for the determination of K (light element) in vegetal samples using the external calibration (Mean recovery: 98%) are much better than using internal
standardization (Mean recovery: 75%). For other mid-Z elements (such as Zn), similar results are obtained using both quantification approaches, and thus usually internal standardization is preferred due to the simplicity of the procedure and the lower consumption of reagents and materials.

Finally, in Table 4, the results obtained for multielemental analysis of some vegetal and animal tissue
reference materials by using suspension preparation and TXRF analyses are displayed. It is interesting to mention that, working with the best analytical conditions (see Table 1), in most cases, acceptable results can be obtained compared to reference values with recovery values in the range of 90–110% in a simple and fast way. The precision of the results, estimated by triplicate analysis, is around 10% in most cases. Only, higher discrepancies are found for elements present at very low concentration levels (i.e., Cd and Pb).

4 | CONCLUSIONS

This study gives insight into the possibilities and drawbacks of analytical methods based on the use of TXRF instrumentation for biological samples analysis. In addition to the inherent advantages of TXRF systems (simultaneous multielemental information, microanalytical capability and low operating costs), the technique also allows the possibility to carry out biological sample analysis using simpler sample treatments compared to other atomic spectroscopic techniques. For instance, biological human fluids can be prepared by means of a simple dilution step using a small volume of an innocuous solvent (i.e., ultrapure water or a diluted solution of a surfactant) and animal or vegetal tissues can be analysed by suspending a few milligrams of the powdered material in an adequate disperser agent without the need for a time-consuming digestion step. Results obtained showed that using these analytical approaches in combination with TXRF analysis, acceptable results can be obtained for the determination of minor and trace elements relevant in the field of medical diagnostics. However, for the determination of elements present at ultratrace levels, more sophisticated sample treatment strategies (i.e., digestion) and/or more sensitive analytical techniques such as ICP-MS are required. It is also interesting to mention that using the proposed TXRF methods, in most cases, quantification can be carried out by adding a suitable internal standard to the target sample (internal standardization) and thus external calibration is not needed. This fact is of special relevance in the analysis of biological samples since usually matrix-matched standards are required to obtain reliable results when using other atomic spectroscopic techniques.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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