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Short communication

Use of total reflection X-ray fluorescence (TXRF) for the evaluation of heavy metal poisoning due to the improper use of a traditional ayurvedic drug

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ABSTRACT

An Indian patient referred to Clinica del Lavoro 'L.Devoto' of Milano showed clinical signs of heavy metal poisoning, possibly related to a sustained 6-month use of approx. 3 g/day of a traditional preparation (a whitish powder with a 'mineral' appearance) to treat urological problems. To confirm the causal relationship between the disease and the use of such product, metal testing was performed on the patient's hair and the ayurvedic remedy samples by total reflection X-ray fluorescence (TXRF). For TXRF analysis 1-cm cut of the patient's hair was directly deposited onto the quartz glass sample carrier, then 10 μl of nitric acid 65% were added and dried in air. TXRF showed high versatility, rapid and simultaneous element detection, and short analysis time, thus supporting a wider use in emergency medicine and in forensic analyses.

1. Introduction

The monitoring of heavy metals during processes to obtain intermediates and final drug substances is an important task for pharmaceutical industries. Heavy metals, such as cadmium, lead, and mercury, if present in trace amounts in pharmaceuticals, are a source of strong health concern because of their toxicity. For this reason most Pharmacopoeias report a test for heavy metal presence in bulk pharmaceutical active principles as well as in formulated drug preparations. The test is usually based on visual determination of insoluble heavy metal sulphide precipitation in weakly acidic water solution. This classical reaction, however, shows serious limitations, due to the low sensitivity, poor specificity with respect to the element, and lack of quantitative information. Moreover, since several drugs are practically insoluble in water, weak acids, and alcohols or they have metal-complexing properties, the sample must be mineralized, usually by thermal ashing, before testing, thus lengthening the analytical procedure and possibly causing errors.

Another field where quantification of heavy metals in drugs is of increasing importance is that of traditional drugs, such as ayurveda preparations of the Indian traditional medicine. This practice, which is not recognized as conventional pharmaceutics by 'Western' Pharmacopoeias, is widely used throughout the Indian subcontinent and among South Asian ethnic worldwide communities. Moreover, ayurveda remedies are nowadays also available outside their traditional areas in all the world from ethnic markets, health and food stores and Internet. Due to the intrinsic presence of toxic metals such as mercury and lead in their composition, the use of ayurveda preparations has been worldwide associated with heavy metal intoxication cases, most often due to lead poisoning [1–4].

Heavy metal intoxication symptoms are often elusive and it is difficult for the clinicians to access rapid and effective screening procedures for toxic metal detection, delaying the heavy metal poisoning diagnosis, thus calling for unnecessary and invasive procedures to investigate generic symptoms, such as anaemia caused by lead poisoning, in patients suspected of intoxication.

In this paper we report a case of study of Pb intoxication, occurred in a patient who used ayurvedic medicine. He showed clinical signs of heavy metal poisoning [2], possibly related to a traditional Indian ayurvedic preparation to treat urological diseases. Total reflection X-ray fluorescence (TXRF) was employed to verify the drug composition and to perform a rapid and non-invasive biomonitoring of the patient, performing a direct analysis of his hairs. TXRF was already employed for chemical analysis of hair samples [5,6]. However, at the best of our knowledge, for the first time a direct quantitative analysis was proposed for hair investigation.

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2. Material and methods

2.1. Instrumentation

TXRF measurements were performed by a Bruker S2 Picofox TXRF (Bruker GmBH, Germany) portable system. The spectrometer was equipped with well-focussing polycapillary lens and the X-ray beam was about 8 mm × 0.1 mm. The excitation settings were 50 kV and 750 mA. Six-hundred second measurements were performed.

X-ray powder diffraction (XRD) measurements were performed by a PANalytical's X'Pert PRO system (PANalytical, Almelo, The Netherlands). Anode material was copper; generator settings: 40 mA, 40 kV.

2.2. Samples

Drug. The drug sample yielded (from the clinicians to the laboratory for the analysis) was an agglomerate of solid powders having different colours (white, red and brown). Thus, it was grinded in an agate mortar to obtain a powder with crystallite size less than 10 μm with a uniform colour.

Hair. A couple of clean hair of the patient was supplied.

Standard reference material. Method setup and analytical quality control was accomplished with the use of the standard reference material (SRM) IAEA-085 human hair sample, which was purchased from the International Atomic Energy Agency (IAEA) as an homogeneous powder [7].

2.3. Sample preparation

Minimal sample preparation was necessary for the analysis of the ayurveda preparation and the patient's hair.

A weighted amount of the powder was transferred into a test tube containing a water solution of Triton X 100 (Acros Organics, Geel Belgium) and homogenized. After thorough homogenization, 10 μl of the suspension were deposited on quartz sample carrier, dried in air and then measured. A fast qualitative compositional analysis was done. TXRF quantitative analysis of the ayurvedic drug samples was performed by the internal standard procedure. For this purpose a suspension of the sample was prepared in the same way as for the previous qualitative analysis, then the right amount of gallium standard solution (as the nitrate; ICP standard solution, Fluka, Milano, Italy), was added in order to obtain a Ga concentration of 20 mg/l. After homogenization 10 μl of the slurry were deposited onto the quartz glass sample carrier, dried in air and then inserted into the TXRF machine to be measured. The concentration of the detected elements was estimated with respect to the known quantity of Ga.

To improve the accuracy of the TXRF analysis of the drug, three different suspensions were prepared and for each one of them three samples (drop deposited onto sample carrier) were measured.

One centimeter of patient's hair was placed in the centre of a quartz glass sample carrier, 10 μl of 65% nitric acid (Fluka Analytical, Milano, Italy) were added and allowed to dry in air at room temperature. The prepared sample is analysed by TXRF.

2.4. Calculations

The instrumental relative error for each one of the nine measures, was calculated according to theoretical approximation proposed by Fernandez-Ruiz [8], adding the contributes of sample weight uncertainty; then the value of the absolute error of the average was calculated considering the contribution of both the instrumental and empirical uncertainty according to the uncertainty propagation analysis [9].

3. Results and discussion

3.1. Characterization of the ayurveda preparation

Quantitative TXRF analysis of the drug is reported in Table 1. These values are the average concentrations of the nine different prepared specimens. TXRF analysis of the ayurveda preparation shows a Pb content of about 16.8 mg/g (16.8×10^3 ppm), in agreement with some literature results [10].

Two doses of the preparation were daily ingested by the patient during 6 months, before he was admitted to the hospital. His dose of the ayurvedic drug was approx. 5.3 g/day, corresponding to an ingested lead mass of approx. 90 mg/day or 16–20 g of Pb during the whole 6 months. From a clinical point of view, the ingestion of such large amount of lead is not compatible with life: an intake of 60 μg of Pb/day during a period of 1 month is sufficient to cause chronic poisoning, with life threatening manifestations such as kidney dysfunction, osteomalacia and obstructive lung disease [11]. Since the patient came into our attention in very poor, but not yet critical condition, we assumed that only a small part of the lead contained in the ayurveda preparation was assimilated by his body.

By means of X-ray diffraction (XRD) it was possible to identify the presence of sucrose, zinc oxide, calcium carbonate, tin sulphide, calcium hydroxide and lead oxide phases in the ayurvedic drug. In particular, the only detected lead containing crystalline compound was lead monoxide (PbO), an insoluble compound from which lead can be hardly leached in a toxic form in the biological environment of the gastro-intestinal tract.

Despite the fair number of papers dealing with the presence of heavy metals in ayurveda preparations and with their effects on the human health, only scanty data is available on the crystalline phases of this product. This is relevant since metal bioavailability is mostly determined by solubility of the specific phase in which the metal is present and their pharmacological or toxic effects in the human being are determined by the fraction of metal leached from the solid phase. It is possibly lack of this information that hampers a deeper understanding of the real *in vivo* behaviour of ayurveda preparations and can be the underlying cause of the publication of reports which emphasize the usefulness of these traditional medicinals.

3.2. Lead measurement in patient's hair

The final evidence of lead intoxication of the patient came from the measurement of lead in his biological fluids and specimens. In particular, hair is a convenient specimen for the evaluation of inorganic and organic chemicals of toxicological interest, from heavy metals to persistent environmental pollutants and drugs. Since the

Table 1
Quantification of the main elements in the ayurveda preparation by TXRF.

Element	Direct – TXRF Concentration ($\times 10^3$ ppm)
S	6.6 ± 1.3
Cl	0.78 ± 0.09
K	1.4 ± 0.3
Ca	88 ± 3
Ti	0.10 ± 0.04
Cr	0.02 ± 0.02
Fe	0.9 ± 0.2
Ni	0.03 ± 0.04
Cu	0.3 ± 0.1
Zn	29.4 ± 1.6
Ga	20.0 ± 1.2
Sr	0.5 ± 0.2
Sn	23.3 ± 4.4
Pb	16.8 ± 1.4

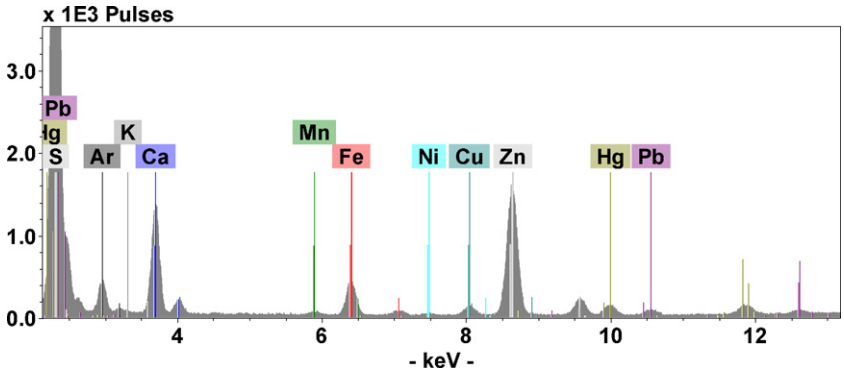

Fig. 1. TXRF spectrum of the standard reference material IAEA-085 trace elements in hair.

Table 2
TXRF quantitative analysis of (SRM) IAEA-085 human hair sample. The comparison between the certified concentration values [7] and the ones obtained in our laboratory is also reported.

Element	Certified values		Obtained concentration
	Confidence interval (ppm)	Concentration (ppm)	Direct – TXRF (ppm)
S	nd	nd	17 480 ± 2843
Cl	nd	nd	105 ± 18
Ca	847–1010	929	922 ± 150
Mn	8.4–9.2	8.8	6.9 ± 1.2
Fe	71–87.8	79.3	74 ± 12
Ni	nd	nd	1.6 ± 0.3
Cu	15.7–17.8	16.8	12.4 ± 2
Zn	156–170	163	163 ± 27
Se	0.96–1.17	1.07	nd
Hg	22.4–24.0	23.2	24.6 ± 4
Pb	nd	nd	6.1 ± 1.0
Sc	0.0084–0.0100	0.0092	nd

growth of hairs is fairly constant over the time and similar in all human beings, at a rate of approx. 1 cm/month, it is also possible to estimate the time of exposure or contact with the chemical agent. Since exogenous components were incorporated in the keratinous matrix at the time of hair growth, the period of the exposure can be estimated by measuring the distance from the hair root. In this particular case, the patient had short hair, thus lead concentration in the hair could have been evaluated only for the month before his hospital admission.

In view of the low mass quantity of the sample and the complexity of the digestion procedure, a different quantitative procedure was used.

TXRF analysis was first made on hair certified standard reference material IAEA-085. The spectrum is shown in Fig. 1 and the concentrations of the elements are reported in Table 2, together with the certified values. Zn was chosen as reference [12,13], for comparison with the SRM, and the amount of all the other detected elements was calculated on the basis of its concentration reported in the reference sheet.

Fig. 2 shows the TXRF pattern collected on the patient hair sample. In that sample, an intense signal highlighted an extraordinary presence of lead. The Zn content was considered equal to that of SRM since it is known that its concentration is almost a constant value in human beings independently from the head sampling point. Indeed, the Zn content in hair could vary from people and food habits but the reported values are in the range around 200 ± 40 ppm [5,14,15]. This is because the source of zinc embedded in the keratine matrix of hair is blood and its zinc concentration is tightly controlled, except in conditions of severe malnutrition, and does not significantly increase. The TXRF quantitative analysis of the main elements present in the patient's individual hair is reported in Table 3. Taking into account the Zn relative error

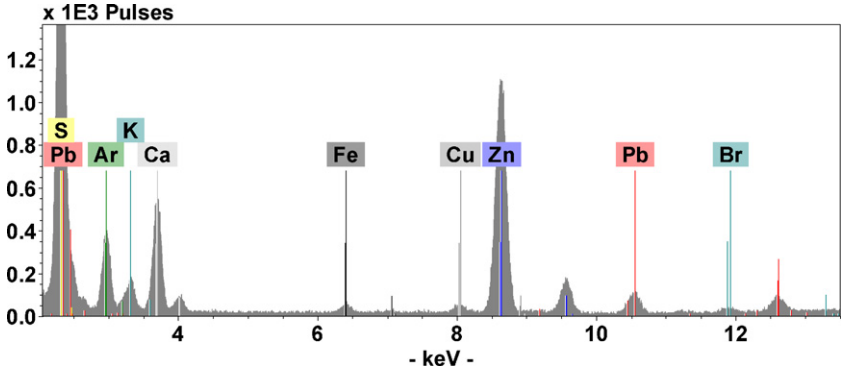

Fig. 2. TXRF spectrum of patient's individual hair.

Table 3
TXRF quantitative analysis of patient's individual hair.

Element	Direct – TXRF Concentration (ppm)
S	13 733 ± 1019
K	175 ± 14
Ca	453 ± 34
Fe	8.8 ± 0.7
Cu	4.7 ± 0.4
Zn	163 ± 12
Br	1.3 ± 0.1
Pb	17.2 ± 1.3

reported in the SRM reference sheet, the corresponding calculated lead range for the patient hair is 17.2 ± 1.3 ppm. It is evident that the lead content of the SRM sample, belonging to unhexposed donors, is much lower than that of the patient hair. In fact the Pb content of hair reported in these other studies [5,14,15] is considerably lower than the one obtained in this case.

In our recent studies about Mn biomonitoring in the Brescia province, performed in the frame of PHIME European project [16], dealing with the hair analysis of about 140 persons, we applied the previously described methodology. Pb presence was detected only in the hair of 22 persons with an average concentration of 1.6 ppm.

The abnormal quantity of Pb detected in the patient's hair of our paper strongly suggested his lead poisoning, that was also confirmed by blood and urine analises. Moreover the reported value is totally in agreement with previously reported studies [17]. The half-life of lead is about 1 month in blood, 3–5 years in trabecular bone such as patella, and 15–25 years in cortical bone such as tibia. Hence, blood lead is considered to reflect acute exposure, and bone lead, especially cortical bone lead, is representative of cumulative exposure [18]. However the analysis of bone is extremely invasive and should be avoided. Thus hair sample analysis opens a new opportunity of non-invasive and cheap biomonitoring analysis. Indeed, considering the growth rate of hairs, in principle it would be possible also the monitoring of chelating therapy results.

4. Conclusions

In this work we report how TXRF analysis was used to identify an acute lead poisoning due to use of a traditional Indian medicine. The availability of suitable analytical techniques in the clinical setting is mandatory to deal with such cases, with a high probability of correct and fast diagnosis. TXRF showed in this study a high versatility, demonstrated by the analysis of the different matrices (drug and hair matrix samples), with rapid and simultaneous element detection, short measuring time, high accuracy and high sensitivity, requesting very low samples quantity (mg).

In particular, we showed that TXRF is well suited for drug monitoring, thanks to the possibility of easy procedure for sample preparation. In addition, due to other advantages, such as the small amount of sample required for the measurements (≤g), low detection limits, wide dynamic range (at least four order of magnitude), simple quantification, negligible matrix effects, rapidity of the detection and the spectra analysis, and simultaneous detection of all contaminants, TXRF appears extremely interesting for a rapid screening of biological samples (for example hair), with the advantage of non-invasive patient monitoring analysis.

In the case of hair, the quantitative analysis can be performed considering Zn as the internal standard, considering its variability in the range 200 ± 40 ppm, with a good results accuracy for the other elements concentration, for example Pb. Thus procedures for Pb quantification, involving digestion, that can be expensive, time-consuming, and operator dependent, could be avoided.

In principle, considering that the hair growth rate is about 1 cm/month, it is possible to evaluate also the patient exposition for a long period of time, investigating a pollutant and/or a drug effect in a pollutant removing therapy, opening a wide range of interesting possibilities for pharmaceutical tests [19].

The proposed direct hair sample analysis proved to be very useful for clinical service laboratories especially for the routine tests and screening procedures, where a very large number of samples must be analyzed and so easy samples preparation and short experiments are mandatory [20,21].

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