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Different analytical methodologies for the preconcentration and determination of trace chromium by XRF in medicinal herbs with effects on metabolism†

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The essentiality of chromium and the disturbances that its deficiency produces in humans are of interest, have the detection and determination of chromium in drugs of vegetable origin used in phytotherapy are of great importance. With the purpose of improving the sensitivity by increasing the peak-to-background ratios in trace element determinations by x-ray fluorescence (XRF) analysis in biological samples, the high concentration of lower atomic number elements (C, H, O, N) must be removed. These elements can produce non-specific enhancement and absorption effects on the characteristic radiation of the elements of interest. The analytical methodologies for eliminating interferences were realized in different ways in solid and liquid samples. In the first case, the assay was done on dried and calcined herbs and the variables related to the method such as drying time, grinding time, calcination time, calcination temperature, quantity of ash for pellet preparation, pressure for pellet preparation, etc, were studied. In the second case, two methods were evaluated: (a) lixiviation with different acids or acids mixtures and (b) infusion or brewing of medicinal herbs. In both cases, it was necessary to develop a preconcentration method for the determination of Cr by XRF. The optimum parameters for preconcentration and thin-film preparation were also studied. The methodologies were applied with very good results to the determination of trace Cr in the medicinal herb *Taraxacum officinale* and in synthetic samples. Copyright © 2002 John Wiley & Sons, Ltd.

INTRODUCTION

There is increasing evidence for the participation of chromium in lipids and glucids metabolism, in the activation of some enzymatic systems in thyroid metabolism and in the stabilization of proteins and nucleic acids. However, the main effect of chromium seems to be its participation in enhancing the potency of insulin in the form of tolerance to glucose (FTG).[1–3]

The return to the use of natural products in therapeutics is supported[4] by (1) the discovery of serious secondary effects with synthetic medication, (2) better chemical–pharmacological and clinical knowledge of vegetable drugs and their derived products[5] and (3) the development of analytical methods which guarantees better quality control.

Many methods have been developed to determine the concentrations of inorganic compounds in plants through instrumental methods such as atomic absorption spectrometry,[6–8] instrumental neutron activation analysis,[9,10] polarography,[11] by inductively coupled plasma atomic emission spectrometry (ICP-AES),[12–14] total reflection x-ray fluorescence[15] and x-ray fluorescence (XRF).[16,17] However, XRF methods are not useful for measuring concentrations of trace elements in biological specimens owing to the large error produced without matrix separation and preconcentration of the analyte.

Considering the importance of chromium and the disturbance that its deficiency produces in humans, its detection and determination of it in drugs of vegetable origin used in phytotherapy is of great importance.[18–21]

In some cases, the determination of the concentration of these elements can be realized in the natural matrix, whereas, for many elements present in complex matrices direct measurement is not possible. The reason is that the matrix components interfere in the measurement.

Wavelength-dispersive XRF is a simple multi-elemental detection method, which permits the rapid qualitative analysis of many elements in a specimen, generally without specimen preparation. However, in quantitative analysis, the use of proper standards, specimen preparation and/or the application of a correction method to eliminate matrix effects and spectral interferences[22] are essential.

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In the analysis of biological samples, a means of improving detection limits is to remove low atomic number elements (C, H, O, N) which are present in very high concentrations in these samples and are responsible for enhancement and absorption effects on the characteristic radiation (fluorescent emission) of the analyte. In these matrices, known as light matrices, the mass absorption coefficient (μ/ρ') is smaller than the analyte mass absorption coefficient, both for the analyte line and for primary radiation. This is the reason why the primary radiation suffers less attenuation when it goes through this matrix to reach the analyte, and secondary or fluorescent radiation is less attenuated when it emerges from this matrix (compared with pure analyte). Therefore, the analyte line intensity increases in a non-linear relation to the analyte concentration and the calibration curve shows a positive deviation.[23]

The purpose of this work was to develop different simple methodologies for the elimination of matrix effects produced by organic matter present in medicinal herbs for the determination of trace chromium by XRF and to improve the sensitivity by increasing the analyte line signal with respect to the background.

EXPERIMENTAL

Reagents and apparatus

A chromium standards solution (200 mg L^{-1}) was prepared by dissolving 113 mg of $K_2Cr_2O_7$ in water and diluting to 200 ml with distilled water. Dilute working standard solutions were prepared by adding water to the concentrated solutions.

A 2% solution of sodium diethyldithiocarbamate (NaDDTC) was prepared by dissolving 2 g of the reagent in water and diluting to 100 ml in a volumetric flask.

A 50 mg l^{-1} solution of $CuSO_4$ was prepared by dissolving 125 mg of $CuSO_4$ in and diluting to 1000 ml with water.

A buffer solution of pH 9.17 was prepared by dissolving $Na_2B_2O_7 \cdot 10H_2O$ in water.

Membrane filter-papers of 0.45 μm pore size were obtained from Millipore.

All other chemicals were of analytical grade and doubly distilled water was used throughout.

Instrumentation

A Philips PW1400 x-ray fluorescence spectrometer was used to measure the Cr Kα line. The operating parameters were Rh tube at 50 mA and 60 kV, goniometer at $2\theta = 63.56°$, counting time for peak and background 50 s, 75–25 V window width, LiF(200) crystal and gas flow proportional–scintillation detector in tandem arrangement.

ICP-AES measurements were performed with a sequential inductively coupled plasma spectrometer (Baird ICP 2070).

The pH values of solutions were measured with an Orion 701-A pH meter with an Ag/AgCl electrode.

Filtrations were performed in a special filtration apparatus with a vacuum pump. A Thermovac vacuum freeze-dryer was used for lyophilizing liquid samples, operated at 500 mTorr and a temperature of −70 °C.

Study of the analytical methodology for the determination of Cr

With the purpose of determining the quantity of chromium in the medicinal herb *Taraxacum officinale* and the pharmaceutical liquid forms prepared from it, different analytical methodologies were studied and numerous experiments were carried out on the preparation and quantization and for the presentation of the sample to the XRF spectrometer in solid and liquid forms.

Solid samples

The variables which affect the preparation and presentation of the sample were studied, namely calcinations time, quantity of ashs for preparing pellets, pellet preparation pressure, temperature of drying, calcination time, etc. The procedure for studying variables related to pellet preparation was the following:

(1) the sample, properly conditioned (washed, etc.), obtained from a herbalist shop (good quality), was placed in an oven for 3 h at 105 °C;
(2) the dried sample was pulverized as finely as possible in an agate mortar (to avoid any contamination with Cr);
(3) 30 g of ground sample were taken, dried on a burner and calcined in a muffle furnace for 3 h at 500 °C;
(4) a support of boric acid with a circular central depression of 25 mm diameter and 0.45 mm depth was prepared, which was achieved by pressing 4 g of boric acid at 3–4 atm in a mold which contained a ledge with the same measurements; this support permits a thin film of the ash obtained to be deposited;
(5) on these supports were added different quantities of ash, at different pressures, and measured by XRF.

Study of calcination temperature

The procedure was similar to that described above but in step 3 the solid sample was calcined at 450, 500, 600, 700, 800 and 900 °C. Standards of chromium were prepared by adding increasing and known quantities of $K_2Cr_2O_7$ and a calibration curve was constructed for each temperature (standard addition method).

Validation of the method

The method was validated through its application to samples of *Taraxacum officinale* spiked with known quantities of Cr.

Liquid samples

Lixiviation of herb ash with different acids or mixtures of acids

Different acids, mixtures of acids and procedures were studied, and the most suitable procedure for dissolving the sample was the following:

(1) 10 g of dried plant were ground and ashed at 500 °C in a muffle furnace;
(2) 500 mg of the ash were weighed and 20 ml of 6 mol l^{-1} HCl were added;
(3) the mixture was heated to boiling;
(4) the solution was cooled, filtered and washed with HCl–H_2O (1 : 1);

(5) the obtained liquid was diluted to volume in a 25 ml volumetric flask with 6 mol l^{-1} HCl.

Owing to the interference of Fe, an extraction step was carried out with small volumes of diethyl ether.

Preconcentration method

This method was carried out using Cu as a carrier element and sodium diethyldithiocarbamate (NaDDTC). The optimum pH of coprecipitation was studied by adjusting the solutions to different pH values with 0.1 mol l^{-1} NaOH or 0.1 mol l^{-1} HCl. The coprecipitate was filtered through a Millipore membrane using special filtration equipment. The membranes were placed in the XRF spectrometer. With the optimum conditions of preconcentration established, standards of different Cr concentration were prepared.

Infusion and brewing of medicinal herbs

The quantity of Cr consumed through infusion or brewing of the medicinal herb *Taraxacum officinale* is also of interest. The form of their preparation was as follows:

(1) for infusion, 5% according to the National Argentine Pharmacopoeia,[24] adding boiling water to the dried plant;
(2) for brewing, 5% according to the National Argentine Pharmacopoeia,[24] brewing the dried plant for 20 min.

Cr could not be detected in either solution by XRF when presenting the liquid sample directly to the spectrometer chamber. It was necessary to use a preconcentration method. The method selected here was lyophilization.

Preconcentration procedure through lyophilization

This method consists of rapid freezing of the sample followed by sublimation. Once the liquids from infusion and brewing of *Taraxacum officinale* had been preconcentrated, the lyophilized samples were calcined and the ash was used to prepare pellets. The pellets were produced using the same parameters as for solid samples. Standards were prepared by adding increasing and known quantities of Cr (standard addition method).

RESULTS AND DISCUSSION

The numerous experiments used to study the variables for pellet preparation gave optimum values of 9–10 atm pressure and 400 mg of ash. These values allowed us to prepare uniform and plane surface pellets, without loose powder, and, at the same time, with a detectable and reproducible Cr signal. These last considerations are of great importance taking into account the low concentration of Cr in these samples.

Table 1 contains the Cr concentrations at each calcination temperature. Although these values are different at each temperature, the experiments showed that the concentration values obtained at 500 °C were more reproducible (each experiment was repeated six times). At higher temperatures greater variation in Cr concentration was found, possibly due to the existence of other volatile compounds (e.g. alkali metal halides) which, at the same time, could cause the loss of Cr. At lower temperatures (400 °C) we found that the organic matter was not totally eliminated. Therefore, in the following experiments, 500 °C was taken as the optimum temperature for calcining these samples.

Table 1. Determination of Cr concentration in *Taraxacum officinale* at different calcination temperatures ($n = 6$)

Temperature (°C)	Cr conc. (μg g^{-1})
450	4.17 ± 0.07
500	4.55 ± 0.03
600	4.80 ± 0.03
700	4.84 ± 0.02
800	4.11 ± 0.05
900	4.11 ± 0.05

Table 2. Validation of the method for the determination of Cr in samples of *Taraxacum officinale* ($n = 6$)

Cr determined (μg g^{-1})	Cr spiked (μg g^{-1})	Cr found (μg g^{-1})
4.55 ± 0.03	4.00	8.33 ± 0.03

The results were confirmed by measuring specimens of the medicinal herb *Taraxacum officinale* spiked with known quantities of Cr. The results, shown in Table 2, agree with the sum of Cr found in samples plus the amount of Cr added.

The other method proposed for the determination of total Cr in *Taraxacum officinale* was developed by placing the sample in solution. The procedure was realized through lixiviation of the herb with different acids or acid mixtures [HCl, HNO_3, HCl–HNO_3 (3 : 1)] and applying different procedures. The experiments demonstrated the efficiency of HCl–HNO_3 (3 : 1) in dissolving the plant. In spite of this, the cellulose contained in the herb remained as an insoluble residue, even after filtration. This treatment was unsatisfactory and the lixiviation was carried out on ash, which eliminated the organic matter of the plant.

Ash of the sample could be satisfactorily dissolved in concentrated HCl. The low Cr concentration in these solutions could not possible be determined by XRF. In addition, the high background produced by the matrix reduced the peak to background ratio and, therefore, increased the low detection limit.

The preconcentration method proposed involved coprecipitation[25] using Cu as carrier element, NaDDTC as precipitant and the optimum pH range determined by our experiments, 9–9.5, and subsequent experiments were performed at pH 9.17 with a buffer solution.

The first problem that appears when working at this pH value was the presence of Fe, which begins to precipitate at pH 4 and, owing to its high concentration in these plants, it produces a bulky precipitate at the working pH. The principal inconvenience is related to the thickness of the film that must be prepared with the coprecipitate. For this reason, Fe was eliminated by extraction with diethyl ether.

For the preparation of thin films, it was first necessary to determine the critical thickness of the films, which means the thickness above which the intensity of the emerging

fluorescent x-rays is not proportional to concentration. Thus, for very thin films attenuation of the incident primary and emergent secondary spectral line radiation is almost negligible and the plot of intensity vs thickness is linear. For films of intermediate thickness, attenuation of both primary and secondary x-rays increases with depth. The emerging secondary x-rays continue to increase in intensity with increase in thickness, but at a decreasing rate. At the critical thickness, secondary x-rays are excited at depths from which they cannot reach the surface. A further increase in thickness results in no further increase in intensity.

In our case, the thickness of the films depended on Cr concentration: higher Cr concentrations lead to greater thickness of the films. The critical thickness determined by us was that prepared from a solution containing $\sim$50 μg ml^{-1} of Cr. Hence the coprecipitate obtained below this concentration value and filtered on membranes constituted a thin film. At higher Cr concentrations the coprecipitate obtained became a thick film. In thin films practically all matrix effects are eliminated since each atom in the film emits in an independent form. In addition, working with these optimum experimental parameters, the enrichment factor obtained was 500, considering a 10 ml initial volume of sample, a final volume in the thin film of 20 mm^3 and practically 100% of retention determined in the filtrate by ICP-AES. The detection limit, using the described methodology, is 0.027 μg ml^{-1}. The calibration curve for the determination of Cr by this method is shown in Fig. 1. The concentration of Cr obtained was 4.78 ± 0.02 μg g^{-1}, which is in good agreement with the results obtained previously by the analysis of solid samples.

The quantity of Cr in liquids prepared by infusion or brewing of the medicinal herb was also of interest. The importance of the concentration of Cr on the therapeutic effects of this plant is still under study. Hence it is important to determine the quantity of Cr in these preparations, in order to ensure that the patient is not consuming Cr above the toxic level when he or she is under treatment with this herb.

The determination of Cr in *Taraxacum officinale* infusion or brew requires a previous preconcentration step. This was carried out by lyophilizing the liquids, calcining the lyophilized material and preparing pellets. The results are shown in Table 3. These values led to important conclusions

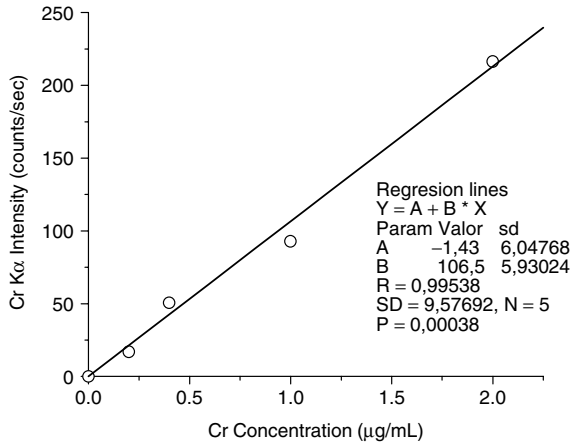

Figure 1. Calibration curve of Chromium determined through lixiviation of ashes and preconcentration by coprecipitation.

Table 3. Determination of Cr by XRF in liquid samples prepared by infusion and brewing up of *Taraxacum officinale* ($n = 6$)

Sample	Cr found (μg g^{-1})
Infusion	1.88 ± 0.05
Brew	2.98 ± 0.04

with respect to toxicity, maximum doses of the liquids to be consumed in a day and, most important, the relation of chromium concentration in the herb to the therapeutic effect. Logically, the content of Cr is lower in the infusion than in the brew.

CONCLUSIONS

The development of these analytical methods in conjuction with XRF spectrometry makes an important contribution to the study of these medicinal plants, their quality control (their composition depends on their origin) and their medicinal uses. Simultaneously, it enables one to take advantage of the use of a unique instrumental technique to perform qualitative and quantitative analyses of the components of the plant, even at trace levels, thus overcoming the difficulties that this type of sample presents when employing XRF.

REFERENCES

1. Picard H. *Utilización Terapéuticas de los Oligoelementos*. Sirio: Málaga, 1989; 85.
2. Baran E. *Acta Farm. Bonaerense* 1989; **8**: 43.
3. *Remington. Farmacia*, (19th edn), vol. 2 Médica Panamericana: Buenos aires, Argentina, 1998; 1714.
4. *Fitoterapia–Vademecum de Prescripción*. Masson: Barcelona, 1998.
5. Houghton PJ. *J. Alt. Complem. Med.* 1995; **1**: 131.
6. Pluta J, Figura B, Lorenz K, Wendt L. *Pharmazie* 1984; **39**: 63.
7. Felcman J, Tristáo Bragança ML. *Biol. Trace Elem. Res.* 1988; **17**: 11.
8. Wong MK, Tan P, Wee YC. *Biol. Trace Elem. Res.* 1993; **36**: 135.
9. Rajurkar NS, Pardeshi BM. *Appl. Radiat. Isot.* 1997; **48**: 1059.
10. Singh V, Garg AN. *Appl. Radiat. Isot.* 1997; **48**: 97.
11. Sviceková M, Havránek E. *Pharmazie* 1995; **50**: 302.
12. Markert B. *Fresenius'Z. Anal. Chem.* 1989; **333**: 11.
13. Ødegård KE, Lund W. *J. Anal. At. Spectrom.* 1997; **12**: 403.
14. Awadallah R, Sherif M, Mohamed A, Grass F. *Int. J. Environ. Anal. Chem.* 1984; **19**: 41.
15. Günther K, von Bohlen A, Strompen C. *Anal. Chim. Acta* 1995; **309**: 327.
16. Mino Y, Usami H, Ota N, Takeda Y, Ichihara T, Fujita T. *Chem. Pharm. Bull.* 1990; **38**: 2204.
17. Nas S, Gokalp HY, Sahin Y. *Z. Lebensm.-Unters.-Forsch.* 1993; **196**: 32.
18. Tolonen M. *Vitaminas y Minerales en la Salud y la Nutrición*. Acribia: Zaragoza, 1995.
19. de Portella ML. *Vitaminas y Minerales en Nutrición*. López Librero Editores: Buenos Aires, 1993; 71, 103.
20. Seiler H, Sigel A, Sigel H. *Handbook on Metals in Clinical and Analytical Chemistry*. Marcel Dekker: New York, 1994.
21. Scholz H. *Los Minerales y su Salud*. Lidium: Buenos Aires, 1987; 121.
22. Van Grieken R, Markowicz A (eds). *Handbook of X-Ray Spectrometry*. Marcel Dekker: New York, 1993.
23. Bertin E. *Principles and Practice of X-Ray Spectrometric Analysis*. Plenum Press: New York, 1984.
24. *National Argentine Pharmacopoeia* (6th edn). Buenos aires, Argentina, 1978.
25. Van Grieken R. *Anal. Chim Acta* 1982; **143**: 3.