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Determination of iron and molybdenum in a dietetic preparation by flame AAS after dry ashing

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Abstract

Methods for the determination of iron and molybdenum in a dietetic pharmaceutical preparation by flame atomic absorption spectrometry (FAAS) after dry ashing at 600°C have been validated. Linearity, precision, accuracy, detection and quantification limits, specificity and robustness have been determined. Linearity of response was verified for concentrations ranging from 0.50 to 4.00 mg l^{-1} of iron and 1.00 to 6.00 mg l^{-1} of molybdenum. Precision of the methods, performed under conditions of repeatability and reproducibility, gave relative standard deviations of 0.4 and 1.1%, respectively, for the iron determination and of 1.0 and 6.5%, respectively, for the molybdenum determination. Mean recoveries determined after spiking dietetic preparation placebos ranged from 97.1 to 102.6% for iron and 95.2 to 102.9% for molybdenum. The limit of detection for iron was 126 μg g^{-1} and for molybdenum 129 μg l^{-1}. Quantification limits were 420 and 433μg l^{-1} for iron and molybdenum, respectively. No interference in the iron and molybdenum determination due to other components present in the dietetic capsules was found. Day-to-day and analyst-to-analyst variability was less than 1.1% for iron and 4.5% for molybdenum. Results show the suitability of the method for measurement of iron and molybdenum in a complex matrix sample such as a dietetic pharmaceutical preparation.

Keywords: Flame atomic absorption spectrometry; Iron; Molybdenum; Dietetic pharmaceutical preparation

1. Introduction

The determination of essential metals for human nutrition in different matrix samples is considered of great importance. This is due to the fact that a safe and adequate daily intake for these elements is necessary since important diseases can appear when they reach low levels in the human body.

Atomic absorption spectrometry (AAS) is now probably the most used technique for determination of metals in different samples [1]. AAS determinations are usually made by flame-AAS

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(FAAS) when the concentration of the analyte is high enough, or by graphite furnace (GFAAS) when the concentration is low. To get the sample into solution before analysis, most types of samples require a previous treatment. Dry ashing and wet digestion with mineral acid are the most commonly used. Generally, dry ashing consume more time than wet digestion, but due to the resulting ash can be dissolved in a small amount of diluent, it provides much better detection limits than wet digestion.

Analytical laboratories need validated methods of analysis for determining essential metals, such as iron and molybdenum, in a wide range of matrixes. Validation of an analytical method is a necessary step in controlling the quality of quantitative analysis, and can be defined as the process by which it is established that the analytical parameters of the method meet the requirements for the intended analytical applications.

Validated methods for determining iron and molybdenum using AAS, in general in presence of other elements, are available for some drinks and foods [2–8]. In pharmaceutical preparations, a method for the determination of molybdenum in Chinese herbal medicine by GFAAS after dry ashing of the sample is available [9]. There are also available methods for the determination of iron in Yunduowei capsules, Fuxuesu tablets and Tothema oral ampoules by FAAS [10–12]. However, to the best of the authors' knowledge there are no validated methods for the determination of these metals in more complex pharmaceutical samples such as dietetic preparations in the literature. In fact, these preparations contain, besides a wide range of vitamins and minerals, high amounts of carbohydrates, fats and proteins, so they need a more complicated sample treatment than other pharmaceutical preparations.

Because more methods for quality control of iron and molybdenum content in pharmaceutical preparations are necessary, the purpose of this work was to validate an easy analytical method for the determination of these metals using FAAS in a dietetic preparation after dry ashing.

2. Experimental

2.1. Instrumentation

A Perkin Elmer model 1100 B atomic absorption spectrophotometer equipped with an air/acetylene burner or a nitrous oxide/acetylene burner was used for the iron and molybdenum determination, respectively. Hollow cathode lamps operating at 20 mA were employed for iron and molybdenum that provided resonance lines of 248.3 and 313.2 nm, respectively. The instrumental settings of the spectrometer for the iron and molybdenum determination are summarised in Table 1.

2.2. Reagents

All reagents were analytical reagent grade. Water used in preparations of standard solutions and samples were obtained from a Millipore Milli-Q-System (Waters, Millipore, Medford, MA). Concentrated HNO_3 (60% w/v) and concentrated HCl (37% w/v) were obtained from Panreac. $AlCl_3$ used as a modifier in the molybdenum determination was purchased from Aldrich.

Iron standard solution (1 mg ml^{-1}) was obtained from Merck and molybdenum standard solution (1 mg ml^{-1}) was obtained from Fluka. These solutions were diluted as necessary to obtain stock solutions containing 10 and 20 mg l^{-1} of iron and molybdenum, respectively. Iron

Table 1
Instrumental conditions for measurement of iron and molybdenum by flame atomic absorption spectrometry

Parameter	Element	
	Fe	Mo
Wavelength (nm)	248.3	313.2
Bandwidth (nm)	0.2	0.7
Light source	Iron holow lamp	Molybdenum holow lamp
Power supply (mA)	20	20
Flame, flow setting (l min^{-1})	Air (8), acetylene (2.5)	Nitrous (7), acetylene (6)
Integration time (s)	3	3

working standard solutions for analysis, ranging from 0 (blank) to 3 mg l^{-1}, were prepared by diluting appropriate amounts of the iron stock solution to 100 ml with Milli-Q water after the addition of 0.5 ml of 6% HCl (v/v) and 0.5 ml of 2% HNO_3 (v/v). Molybdenum working standard solutions for analysis, ranging from 0 (blank) to 6 mg l^{-1}, were prepared by diluting appropriate amounts of the molybdenum stock solution to 100 ml with Milli-Q water after the addition of 6 ml of concentrated HCl, 2 ml of concentrated HNO_3 and 10 ml of 5% $AlCl_3$ (w/v).

2.3. Samples

Alcalá Farma S.L. (Spain) furnished a dietetic multivitamin-multimineral preparation. This preparation contained among others a wide range of vitamins and minerals (Ca, Mg, Cu, Mn, Zn, Fe, Mo), soybean oil and soybean lecithin. Iron was present in the preparation as $FeSO_4$ and molybdenum as $MoO_4Na_2 \cdot 2H_2O$. Different dietetic preparation placebos (all the components except the analytes to be determined) were also supplied by the pharmaceutical company.

2.4. Procedures

In order to optimise the sample treatment preliminary experiments were carried out. These experiments consisted basically in the optimisation of the temperature and time for the complete sample calcination in the muffle. In order, to obtain an optimum dissolution of the resulting ash, different acidic treatment conditions were also assayed.

Approximately 12.9 g of product were weighted in an analytical balance, dried in a sand-bath for 2 h and then calcined in a muffle at 600°C for 2 h. After ashes were cool, they were treated with 20 ml of Milli-Q water, 15 ml of concentrated HCl and 5 ml of concentrated HNO_3 and maintained in heavy boiling for 15 min. This solution was put into a 250 ml volumetric flask and diluted with water (solution A). An aliquot of solution A was filtered through a 0.45-μm pore size cellulose membrane filter (Millipore).

In order to determine the iron content of the sample, 5 ml of the filtered solution A were transferred into a 100 ml volumetric flask and volume was filled with Milli-Q water. Finally, 10 ml of the later solution were diluted to 100 ml with water (solution B).

To determine the molybdenum content of the samples, 9 ml of the filtered solution A were transferred into a 10 ml volumetric flask, and volume was filled with 1 ml of 5% $AlCl_3$ (w/v) to obtain solution C.

Blanks, working standard solutions for iron and molybdenum and sample solutions B and C were measured in the spectrophotometer under the instrumental conditions described in Table 1 for iron and molybdenum.

3. Results and discussion

To assess the validity of the proposed methods, analytical performance characteristics for determination of iron and molybdenum in the dietetic preparation were estimated. Linearity of response was studied by using iron standard solutions containing 0.00, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00 and 4.00 mg l^{-1}, and molybdenum standard solutions containing 0.00, 1.00, 2.00, 3.00, 4.00, 5.00 and 6.00 mg l^{-1}. By plotting absorbance for each solution versus its metal concentration, a linear relationship was obtained at 4 mg l^{-1} for iron and 6.00 mg l^{-1} for molybdenum. Thus, in order to obtain the linear calibration curves, standard solutions containing 0.00, 1.00, 1.50, 2.00 and 3.00 mg l^{-1} of iron and 0.00, 2.00, 3.00, 4.00 and 6.00 mg l^{-1} of molybdenum were analysed. Slope, intercept and correlation coefficient (r) of three curves obtained in different days over a period of 30 days are shown in Table 2.

To evaluate the precision of the methods, measurements were performed under conditions of repeatability and reproducibility. Repeatability was checked in order to show if the instrument response for a standard solution of each metal was always the same. This parameter considers only the error attributable to the operating system and not the error attributable to sample handling and preparation. The instrumental precision was

Table 2
Slope, intercept and correlation coefficient (r) for three curves obtained using different standard solutions of Fe and Mo

	Fe			Mo		
	Slope	Intercept	r	Slope	Intercept	r
Curve 1	0.053	1.50×10^{-3}	0.999	8.65×10^{-3}	2.5×10^{-4}	0.999
Curve 2	0.055	1.65×10^{-3}	0.999	7.80×10^{-3}	2.1×10^{-4}	0.999
Curve 3	0.055	1.50×10^{-3}	0.999	7.85×10^{-3}	2.5×10^{-4}	0.999
Mean	0.054			8.10×10^{-3}		
RSD, %	2.7			5.9		

calculated from ten consecutive measurements of a 2.00 mg l^{-1} iron standard solution and from ten consecutive measurements of a 4.00 mg l^{-1} molybdenum standard solution. A good repeatability, expressed as relative standard deviation (RSD, %), was obtained since RSD was equal to 0.40 and 1.03% for iron and molybdenum determination, respectively.

Reproducibility of the methods was estimated from results of analyses of four sample solutions. Quantification of the metals in the dietetic formulation was carried out according to the following equations:

$$C_{Fe} = (50X_{sol\,B})P^{-1}$$

$$C_{Mo} = (2.5X_{sol\,C})(9P)^{-1}$$

where C_{Fe} is the iron concentration of the sample (mg g^{-1}), C_{Mo} is the molybdenum concentration of the sample (mg g^{-1}), P is the sample weight (g), $X_{sol\,B}$ is the iron concentration in the solution B (mg l^{-1}) and $X_{sol\,C}$ is the molybdenum concentration in the solution C, being $X = Y - b/a$ (Y = instrument response, a = slope of the calibration curve, b = intercept). As it can be seen in Table 3, a good reproducibility was achieved for both metals since RSD was less than 1.1% for iron determination and 6.5% for molybdenum determination.

Due to the fact that a reference material containing a well-known amount of the metals to be determinated was not available, the accuracy of the methods was expressed as the percent of analyte recovered from samples spiked with different amounts of each metal. Thus, accuracy of the methods was calculated by preparing samples containing the same quantity of placebo (all the components except the analyte to be determined) as the real sample, and increasing amounts of $FeSO_4$ (80, 100 and 120% of the theoretical iron sample content) or of MoO_4Na_2. $2H_2O$ (80, 100 and 120% of the theoretical molybdenum sample content). Results expressed as the percent of analyte recovered (%R) from the spiked samples are shown in Table 4. As it can be seen, the accuracy of the studied methods was very good, and recoveries determined for iron ranged from 97.1 to 102.6% and for molybdenum from 95.2% to 102.9.

Table 3
Reproducibility in the determination of iron and molybdenum in four samples of a dietetic formulation prepared by two analysts

Sample	Element[a]	
	Fe	Mo
Analyst A		
1	7.86	0.086
2	7.94	0.083
3	8.05	0.081
4	8.02	0.082
Mean	7.97	0.083
RSD, %	1.07	2.60
Analyst B		
1	8.06	0.075
2	8.10	0.086
3	7.99	0.086
4	8.14	0.085
Mean	8.07	0.083
RSD, %	0.79	6.45

[a] Results are given in mg of Fe or Mo per gram of sample.

Table 4
Analytical recovery of iron and molybdenum added to placebo samples of the dietetic preparation

Addition of element (%)[a]	Recovery (%)	
	Fe	Mo
80	101.7	99.6
80	99.8	97.3
100	102.6	99.4
100	97.1	95.2
100	98.8	101.3
100	101.8	102.9
100	101.3	97.8
100	100.1	95.3
120	98.5	97.0
120	98.8	97.9
Mean	100.05	98.37
RSD, %	1.77	2.47

[a] Percentage added of the theoretical content of element in the sample.

The limit of detection was calculated as 3 S.D. a^{-1}, where S.D. is the standard deviation of the calibration straight line and a is the slope of the regression lines obtained for iron and molybdenum standard solutions [13]. Results obtained were 126 and 129 μg g^{-1} for iron and molybdenum, respectively. Quantification limits, calculated as 10 S.D. a^{-1}, were 420 and 433 μg l^{-1} for iron and molybdenum, respectively.

Specificity of the methods was evaluated to check the absence of matrix effects for the two metals by measuring in the spectrophotometer two placebos containing the same quantity of all components as the real sample, except the corresponding amount of $FeSO_4$ or $MoO_4Na_2 \cdot 2H_2O$. Due to absorbance obtained for placebos were similar to that obtained for the blank, it can be concluded that there was no interference in the FAAS determination of iron or molybdenum due to the other components present in the dietetic formulation.

Robustness was evaluated in order to know how much affect the change of some variables in the final results obtained with the method. The RSD obtained for the results of the dietetic formulation analysis performed by two different analysts in different days (analyst-to-analyst and day-to-day fluctuation) was 1.1 and 4.5% for iron and molybdenum, respectively.

To the best of the authors' knowledge there are no validated methods for the determination of Fe and Mo in dietetic pharmaceutical preparations in the literature. These preparations need a sample treatment more complicated than other pharmaceutical preparations because they contain, besides a wide range of vitamins and minerals, high amounts of carbohydrates, fats and proteins. Analytical performance characteristics obtained in the present work for the determination of iron in the dietetic preparation by FAAS are in agreement with those obtained by other authors in other pharmaceutical preparations. Thus, Wang [10] reported an iron recovery of 102.2% (RSD 0.57%) after FAAS analysis of Yunduowei capsules diluted in HCl 1%. In the determination of iron in Fuxuesu tablets by FAAS, after dilution of the sample using polytetrafluoro ethylene in a high pressure digest bomb, Ye and Li [11] obtained a recovery between 98 and 102% (RSD $\leq$ 1%). FAAS determination of iron in Tothema oral ampoules diluted with water showed an iron recovery of 102% [12]. Reproducibility of the method expressed as RSD was equal to 1.7%.

4. Conclusions

On the basis of linear range of calibration, precision, accuracy, detection and quantification limits, specificity and robustness obtained for the proposed methods, it can be concluded that these methods are suitable for determining iron and molybdenum in the dietetic preparation under study. The procedure is very practical and useful for routine laboratory analyses and could be applied to dietetic preparations which are similar to a less complex composition.

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