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Development of an ultrasonic slurry sampling method for the determination of Cu and Mn in antibiotic tablets by electrothermal atomic absorption spectrometry

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ABSTRACT

A new method is described for simple, efficient and rapid determination of Cu and Mn in tablets of antibiotics (ciprofloxacin and cephalexin) by electrothermal atomic absorption spectrometry (ETAAS) using slurry sampling. In order to optimize the procedure, several variables that could affect the performance of the method were investigated. In the best conditions, the tablets could be analyzed by introducing into the graphite tube 20 μl of a slurry prepared with approximately 90–100 mg of the sample and 2 ml of a solution containing 5% m/v of Triton X-114 and 2.8 M of HNO_3. Before the introduction, the slurries were sonicated for 15 min at 40% of amplitude (130 W maximum power) with an ultrasonic probe. The developed method was applied in the determination of Cu and Mn in four samples, and the results were compared with those obtained by focused microwave acid digestion with aqua regia (1:3 mixture of HNO_3:HCl). There was no statistical difference between the obtained values at 95% confidence level when a paired Student *t*-test was applied.

1. Introduction

One of the most important steps in the production of pharmaceuticals is the quality control, which is the step that ensures their safe use [1]. According to the Brazilian Pharmacopeia [2], three assays must be carried out to attest the quality of a medicine: (i) identification, (ii) dosing of the active principle and (iii) purity. The tests to verify the presence of inorganic contaminants such as chlorides, sulfates and metals are included in these assays and make part of the common analysis of the raw materials and final products.

Manganese and copper are considered essential elements for living organisms [3]. However, their ingestion (or exposure) in high amounts can cause serious problems to the human health. Manganese is associated with neurological dysfunctions and pulmonary problems [4,5], while copper can damage the liver and kidneys [6].

Brazilian Pharmacopeia [2] indicates that the determination of metals in medicines can be performed by two different approaches. The first approach is a non-selective and semi-quantitative assay that is based on the precipitation of metallic cations with sulfide ion. In this assay, a visual comparison between the standard (precipitate of PbS) and the sample is carried out to decide if the limit (20 $\mu g\,g^{-1}$, total metal content) is achieved or not. Obviously, several problems can be pointed out for this kind of procedure such as the lack of selectivity and the great influence that the experience of the analyst has on the results [7–9]. Another possibility is to determine the metals in the samples using atomic spectrometric techniques. In this case, the methods are selective for each element of interest and have convenient sensitivity, making possible the identification and quantification of individual species in the samples. Recently, the Brazilian Pharmacopeia has established a limit for the content of Cu and Mn, among other metals, in oral medicines and in parenteral solutions that are 250 $\mu g\,g^{-1}$ and 25 $\mu g\,g^{-1}$, respectively, for both elements.

In general, the employment of atomic spectrometric techniques for the determination of metals in solids (as some tablets of antibiotics) requires a previous step of treatment of the samples that should be applied to convert them into a convenient form before introduction into the instrument. This step is, in most cases, laborious and time-consuming. Besides, it is subjected to some problems such as the contamination of the samples during their handling and losses [10]. Several strategies have been used to decompose (or dissolve) solid samples of pharmaceuticals for the analysis by atomic spectrometric techniques. Classical procedures commonly used for this task are the acid digestion with conventional or microwave assisted heating, and the dry ashing at high temperatures [11–13].

The use of the slurry sampling approach can be considered a good alternative to the decomposition of the samples because it does not require the destruction of the matrix, which simplifies

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Table 1
Operational conditions employed in the determination of Cu and Mn in the slurries by ETAAS.

Parameter	Copper	Manganese
Wavelength (nm)	324.8	403.1
Lamp current (mA)	4	5
Slit width (nm)	0.5	0.2
Purge gas	Argon	Argon

the whole procedure. The main advantages of the slurry sampling strategy are the simplification of the sample treatment, the minimization of contamination risks and analytes losses, the use of less aggressive reagents and the possibility of application to organic and inorganic samples [14].

Nowadays, there are a number of papers regarding the application of the slurry sampling approach to the direct analysis of different kinds of solid samples, such as geological materials [15–17], glasses [18], foods [19–21], biological materials [22–24] and others [25–28], by ETAAS. However, to the best of our knowledge, there is no application of such strategy in the determination of trace metals in solid medicine samples as the tablets of antibiotics analyzed in this present work. So, the goal of this work was to optimize a method for the determination of trace concentrations of Cu and Mn, as contaminants, in tablets of some antibiotics (cephalexin and ciprofloxacin) using the slurry sampling approach and ETAAS. Also, a study was performed to evaluate the influence of the sonication (time and power) of the slurry on the performance of the method.

2. Experimental

2.1. Apparatus

All measurements were carried out with a Varian (Mulgrave, Australia), model AA240Z, electrothermal atomic absorption spectrometer equipped with a Varian GTA 120 atomizer unit and a Varian PSD 120 autosampler. The background correction was performed with a Zeeman effect-based background corrector with a constant magnetic field strength of 0.8 T.

The atomization was carried out directly from walls of the graphite tubes using a tubes made of electrolytic graphite covered with pyrolytic graphite also supplied by Varian. Monoelementar hollow cathode lamps of copper and manganese were used as light sources and Argon (99.99% grade), supplied by Linde Gases (Macaé, Brazil), was employed as protective gas. The operational conditions of the instrument for the measurements are summarized in Table 1.

The slurries were prepared with the aid of an ultrasound processor, furnished by Sonics (Newtown, CT, USA), model VCX 130, equipped with a titanium probe with 6 mm diameter.

The acid dissolution of the samples was carried out with a focused microwave oven supplied by CEM (Matthews, NC, USA), model Star 2, using quartz flasks. It is important to remark that the microwave oven was coupled to a scrubber unit in order to avoid the emission of acid gases.

An analytical balance with 0.1 mg precision, model AY-220, from Shimadzu (Tokyo, Japan) was employed.

2.2. Reagents and solutions

All solutions and slurries were prepared with purified water (18.2 MΩ cm) obtained with a DirectQ-3 system, supplied by Millipore (Bedford, MA, USA).

Analytical solutions of Cu and Mn were prepared from adequate dilution of the individual standard stock solutions with 1000 mg l^{-1} concentration of each metal, supplied by Tedia (Fairfield, OH, USA).

Table 2
Heating program employed in the acid dissolution of the samples.

Stage	Temperature (°C)	Ramp (min)	Hold (min)
1	90	3	10
2	110	1	15

Diluted nitric acid solutions were prepared by convenient dilution of concentrated HNO_3 (P.A. grade), furnished by Tedia, using purified water.

The aqueous solution used in the preparation of the slurries (5% m/v Triton X-114 in 2.8 M HNO_3) was prepared by dissolving 5 g of Triton X-114 (Acros Organics, St. Louis, USA) in approximately 50 ml of 2.8 M HNO_3 solution. After total dissolution of the surfactant and foam decrease, the obtained solution was transferred to a 100 ml volumetric flask and the volume was completed to the mark with the 2.8 M HNO_3 solution.

The solution used in the acid dissolution of the samples was prepared by gentle mixing of 90 ml of concentrated HCl (P.A. grade, Tedia) with 30 ml of concentrated HNO_3. The mixture was continuously cooled in an ice bath during the preparation of this solution.

2.3. Sample processing

The samples analyzed in this present work were purchased in the local market and ground with an agate mortar in order to obtain samples with a convenient homogeneity in terms of particle size. Each sample was a set of 20 tablets.

2.4. Acid dissolution of the samples

The acid dissolution of the samples was performed employing a mixture of concentrated nitric and hydrochloric acids (1:3 ratio). For this task, approximately 400 mg of the samples were weighed directly into the microwave oven flask and 5 ml of the oxidant mixture was added. The condenser was adapted to the flask, which was adjusted to the cavity of the oven, and the heating program (Table 2) was run. After running the heating program, the quartz flask was taken out of the microwave oven cavity and kept on the bench (inside the fume hood) for cooling to room temperature. Then, it was opened, the content was quantitatively transferred to a 25 ml volumetric flask and the volume was completed to the mark with purified water. The final solution obtained was employed in the determination of Cu and Mn by ETAAS, using the same temperature program employed for the analysis of the slurries (Table 3). When necessary, this solution was filtered, in order to eliminate residual amounts of amorphous silica, and diluted with purified water. All determinations were made in triplicate by the standard addition method and the content of Cu and Mn in the reference sample was 8.1 ± 0.9 μg g^{-1} and 2.1 ± 0.5 μg g^{-1}, respectively.

2.5. Slurry preparation and measurement

The slurries used in the determination of Cu and Mn in the antibiotic tablets were prepared by dispersing approximately

Table 3
Program of temperatures employed for the determination of Cu and Mn by ETAAS.

Step	Temperature (°C)	Ramp (s)	Hold (s)	Ar flow rate (ml min^{-1})
Drying	85	5	0	300
	95	40	0	300
	120	10	0	300
Pyrolysis	1100 (Cu); 1000 (Mn)	2	6	300
Atomization	2300 (Cu); 2400 (Mn)	1	4	0
Cleaning	2400 (Cu); 2500 (Mn)	2	0	300