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Indirect determination of cysteine in pharmaceutical formulations by high-resolution continuum source graphite furnace molecular absorption spectrometry

Jorge S. Almeida[a,b,*], Lucilia A. Meira[a], Leonardo S.G. Teixeira[a,b]

[a] Universidade Federal da Bahia, Instituto de Química, Departamento de Química Analítica, Campus Universitário de Ondina, 40170-115 Salvador, Bahia, Brazil
[b] INCT de Energia e Ambiente - Universidade Federal da Bahia, Instituto de Química, Campus Universitário de Ondina, 40170-115 Salvador, Bahia, Brazil

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ABSTRACT

An indirect method for the determination of cysteine in pharmaceutical formulations involving the monitoring of CS (carbon monosulfide) molecular absorption bands using high-resolution continuum source graphite furnace molecular absorption spectrometry (HR-CS GF MAS) is reported. The best conditions for the determination were based on the sum of the absorbance signals of the molecular transition lines for CS at 258.034 and 258.056 nm with a pyrolysis temperature of 1000 °C and a volatilization temperature of 2400 °C using Pd/Mg as a chemical modifier. The linear range for cysteine was 1.4 to 100 mg kg^{-1} with a limit of detection and limit of quantification of 0.4 and 1.3 mg kg^{-1}, respectively. The precision, evaluated as relative standard deviation (RSD), was 3.7% (n = 10) for a sample containing 25.0 mg kg^{-1} of cysteine. The accuracy of sulfur determination was evaluated using a standard reference material (NIST-SRM-1573a tomato leaves) and concordant values were observed within the 95% confidence interval. The method was successfully applied to the analysis of pharmaceutical formulation samples containing cysteine, lysine, and alanine. The concentrations of cysteine found in the samples were between 9.8 and 20.1 mg kg^{-1}.

1. Introduction

Cysteine is an amino acid present in a wide variety of proteins and acts as a natural antioxidant in several biological processes, including protein synthesis, metabolism, and detoxification. Due to its thiol group, cysteine can easily be oxidized to cystine, a dimeric amino acid (CySS). This reaction is reversible and allows the control of a wide range of biological activities and protein structures, and, therefore, the determination of cysteine in biological matrices and pharmaceutical preparation is highly important [1–5].

Since its introduction, high-resolution continuous source atomic absorption spectrometry (HR-CS AAS) has opened new possibilities for chemical analysis [6–10]. The use of a high intensity continuous source, combined with a high-resolution monochromator and a coupled charge detector (CCD), made possible the selection of optimum wavelengths for the determination of desired chemical species with the exclusion of most spectral interferences [7, 11]. One of the main advantages of this technique is the ability to monitor different chemical species simultaneously [12–16] and, more recently, the ability to determine non-metals using molecular absorption bands based on the electronic excitation spectrum of the fine structure of molecular rotation [7, 8, 17].

Sulfur is not easy to determine directly using atomic absorption spectrometry (AAS), since its main resonance line at 180.671 nm, and two other lines at 181.974 and 182.565 nm, are present in vacuum-UV, and therefore are not accessible with conventional instrumentation. Indirect methods for sulfur determination, based on the molecular absorption of sulfur species (CS, SH, SO_2, etc.) using high-resolution continuum source atomic absorption spectrometers, were extensively reviewed [7, 8, 18]. Using a flame or graphite furnace, molecules containing sulfur evolve in the gas phase, and the characteristic molecular absorption of sulfur can be measured.

Two analytical conditions must be considered in order to achieve good results in the determination of sulfur using high-resolution continuum source graphite furnace molecular absorption spectrometry (HR-CS GF MAS). First, the sulfur must be stabilized using an appropriate chemical modifier at a high pyrolysis temperature to remove the matrix and destroy the original molecules containing sulfur, without loss of the analyte. The second condition is to ensure the formation of a diatomic molecule, in this case CS, in order to obtain maximum sensitivity [6–8].

* Corresponding author.
E-mail address: jorgealmeidas@hotmail.com (J.S. Almeida).

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Using molecular absorption, sulfur was previously determined by high-resolution continuum source molecular absorption spectrometry (HR-CS MAS) in food samples [19–21]; biological samples [22]; wine [23, 24]; plants [25, 26]; cast iron, spinach, and peach leaves [27, 28]; coal [29–31]; agricultural samples [32]; natural, rivers, lakes, and mineral waters [33]; polyethylene, biological, steel, and petroleum coke samples [34]; petroleum products and diesel [35–37]; airborne particulates [38]; and in coconut water samples [39]. However, to the best of our knowledge, the quantification of sulfur by the indirect determination of cysteine using HR-CS GF MAS, has yet to be described. Consequently, the aim of this study was to develop an indirect method for the determination of cysteine in pharmaceutical formulations based on the molecular absorption of CS using HR-CS GF MAS, considering the presence of the thiol group in cysteine.

2. Experimental

2.1. Instrumentation

A high-resolution continuum source atomic absorption spectrometer model contrAA 700 (Analytik Jena AG, Jena, Germany) was employed for the determination of cysteine content in a pharmaceutical formulation. The spectrometer was equipped with a xenon short-arc lamp (GLE, Berlin, Germany) as a continuum radiation source, a charge-coupled device (CCD) array detector, and a double-echelle monochromator. A 300 W xenon short-arc lamp operating in hot-spot mode was used as the continuous radiation source with a wavelength ranging from 190 to 900 nm. To increase the analytical sensitivity, the analytical signals were measured by summing the absorbance of the molecular transition lines for CS at 258.034 nm and 258.056 nm using the center pixel (CP) and the two adjacent pixels at either side (CP ± 1). Interactive background correction (IBC) was used for all experiments. All measurements were carried out in triplicate.

All experiments were performed using pyrolytically-coated transversely heated graphite furnaces and platforms (Analytik Jena Part No. 407-A81.025). Samples were introduced using an MPE-60 furnace autosampler (Analytik Jena). Argon with a purity of 99.996% (White Martins, São Paulo, Brazil) was used as a purge and protective gas. The temperature program for the graphite furnace used in the determination of the CS molecules is shown in Table 1.

2.2. Reagent, solution, and sample preparation

All reagents used were of at least analytical grade. Water with a resistivity of 18.2 MΩ cm was obtained from a Milli-Q system (Millipore, Bedford, MA, USA) and used in all solution preparations. Nitric acid was purified by double sub-boiling distillation in a quartz still (Kürner Analysentechnik, Rosenheim, Germany). All flasks were cleaned by soaking in a 1.4 mol L^{-1} HNO_3 solution for at least 24 h, and rinsed thoroughly with high-purity deionized water before use.

A 1000 mg kg^{-1} L-cysteine stock solution was prepared daily in a dark brown bottle by dissolving the reactant (Sigma-Aldrich, Japan) in a 0.01 mol L^{-1} HNO_3 solution. Working standard solutions were prepared for the construction of calibration curves by diluting the stock solutions with the 0.01 mol L^{-1} HNO_3 solution to obtain cysteine concentrations ranging from 1.4 to 100 mg kg^{-1}. A 100 mg kg^{-1} lysine solution was used as an analytical blank, due to the absence of sulfur in the carbon chain of this amino acid.

The following reagents were investigated as chemical modifiers: Pd^{2+} 0.1% (w v^{-1}) (Fluka, Switzerland), Mg^{2+} 0.04% (w v^{-1}) (Fluka, Switzerland) and Ca^{2+} 0.04% (w v^{-1}), all three in their nitrate form. Hydrogen peroxide 0.6% (v v^{-1}) was prepared by gradually diluting a 30% (w w^{-1}) concentrated solution (Merck, Germany). An organic standard (diesel, SDF-30x-4, New Haven, USA), an inorganic sulfur standard (Sigma-Aldrich, SP, Brazil), and a standard reference material (SRM-1573a tomato leaves), supplied by the National Institute of Standards and Technology (Gaithersburg, Maryland, USA), were used to evaluate the accuracy of the analytical procedure.

Pharmaceutical formulations used as supplements for amino acid replacement were purchased in the region of Salvador, Bahia, Brazil. All analyzed samples were in capsule form and contained lysine, alanine, and cysteine; however, the formulations did not contain methionine. The samples were weighed and diluted with 0.01 mol L^{-1} nitric acid solution in a similar way to the standard solutions.

2.3. Procedure for the determination of CS

The pyrolysis and atomization temperature were important parameters for the successful generation of CS spectra in the graphite tube and the determination of cysteine. After establishing sufficient vaporization conditions, the absorption peaks were more reproducible. In this way, it was possible to select the combination of lines of interest for the determination.

After adjusting the number of peaks used for the determination, cysteine was monitored indirectly using the sum of the absorbance measurements obtained for the CS molecule at 258.034 nm and 258.056 nm by HR-CS GF MAS. These lines were chosen considering their proximity and sensitivity compared with other lines.

For the determination, 20 μL of the solution containing cysteine, 10 μL of H_2O_2 0.6% (v v^{-1}), and 10 μL of the Pd/Mg 0.1/0.04% (w v^{-1}) modifier solution were collected using the auto-sampler for liquids, and injected into the vaporization system. After injecting these solutions, the heating program was initiated (Table 1). The results obtained for cysteine content were compared with the values published by the manufacturers of the drug.

2.4. Analyses of reference materials

After establishing the best conditions for the indirect determination of cysteine using CS band absorption, the method was applied to the determination of sulfur in the standard reference materials (SRM). The sulfur content was also determined in the organic and inorganic standards.

The SRM-1573a tomato leaves were digested prior to analysis in a closed microwave-assisted system [20]. For the digestion, 0.5 g of the sample was placed into a Teflon vessel, and 6 mL of concentrated HNO_3 and 1 mL of H_2O_2 (30%, m m^{-1}) were added. The heating program was as follows (power/temperature/standby time): (I) 250 W/110 °C/2 min; (II) 0 W/110 °C/2 min; (III) 250 W/185 °C/6 min; (IV) 400 W/185 °C/5 min; and (V) 600 W/185 °C/5 min. The obtained solutions were transferred to glass vessels, evaporated to near dryness, and then diluted to 10 mL with a 0.01 mol L^{-1} nitric acid solution. After digestion of the tomato leaf samples, the total sulfur content was determined using the sum of the CS absorption lines located at 258.034 and 258.056 nm.

For the analysis of the organic standard (diesel, SDF-30x-4, New Haven, USA), the sample was diluted with 50% ethanol solution (v v^{-1}) prepared in 0.01 mol L^{-1} HNO_3 [36]. After stirring the mixture, the sample was used to determine the total sulfur content by HR-CS GF AAS

Table 1
Temperature program for the determination of CS molecules by HR-CS GF MAS.

Stage	Temperature, °C	Ramp, °C s^{-1}	Hold time, min	Ar flow rate, L min^{-1}
Drying 1	100	3	60	2.0
Drying 2	130	5	10	2.0
Pyrolysis	1000	300	10	2.0
Adaptation	1000	0	5	Stop
Vaporization	2400	FP[a]	5	Stop
Clean	2450	500	4	2.0

[a] Full power.

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