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Development of a new analytical method for the determination of sulfites in fresh meats and shrimps by ion-exchange chromatography with conductivity detection

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ABSTRACT

An accurate and reliable analytical method, based on ion chromatography and suppressed conductivity detection, has been developed and validated for the quantitative determination of sulfites in fresh meats and shrimps. The chromatographic separation was accomplished by using an anion-exchange column eluted with sodium carbonate and sodium hydroxide. The optimized step-change elution, followed by column re-equilibration at the initial mobile phase composition, guaranteed a good resolution even toward endogenous interfering peaks, and an excellent retention time repeatability (1.1%, $n = 6$). Good results in terms of sample extract stability, recovery efficiency were achieved with an extraction solvent mixture based on sodium hydroxide, fructose and EDTA. The method validation, performed by an in-house model according to Decision 657/2002/EC and Regulation 882/2004/EC, provided excellent results with respect to linearity (correlation coefficient up to 0.9998), limits of detection and quantification (2.7 and 8.2 mg kg^{-1}, respectively, expressed as SO_2), expanded measurement uncertainty (below 10%), recovery values (ranging from 85% to 92%) and repeatability (down to 8%), demonstrating the conformity of the proposed method with the European directives. Finally, by major changes ruggedness studies, the method applicability to the quantitative analysis of cow hamburger, pork and horse sausage, and shrimps was demonstrated.

1. Introduction

Sulfites have been widely used as preservatives and blanching agents for many years in a large variety of foodstuffs and beverages (fish, potatoes, wine, etc.) to prevent oxidation and bacterial growth [1–3]. Sulfites were also used because of their food technology effects in improving the appearance and flavour of food during preparation, storage and distribution, by stabilizing the product colour and inhibiting discoloration [4]. Before 1986 sulfites were considered incorrectly harmless for consumers and they received the "Generally Recognized As Safe" (GRAS) status. On the contrary, the ingestion of foods containing large amounts of sulfites was associated with asthmatic reactions and food intolerance symptoms, experienced by sensitive individuals [5,6], and in 1986 the U.S. Food and Drug Administration (FDA) revoked the GRAS status [7]. Owing to the potential toxicity, the occurrence of sulfite in foods and beverages can be considered a hazard to human health and should be strictly limited. The acceptable daily intake (ADI) of sulfite (expressed as SO_2) is 0.7 mg kg^{-1} body weight [8]. The US Food and Drug Administration required sulfite declaration on the label of any food containing 10 ppm amounts of sulfite [7]. The European Community has set a maximum sulfites content (expressed as SO_2) for the different foodstuffs and beverages in the Directives 2/1995/EC [9] and 52/2006/EC [10], which include food additives, colorants and sweeteners. In fresh meats the total absence of sulfites has been established, differently from fish products where maximum levels, expressed in mg/kg of edible part, vary dependently on the product-size. Hence, sulfites determination in food products is essential for the purposes of legislation, nutrition and public health, and the development of sensitive, selective, fast and low-cost methods represents an important topic for food assurance and quality control.

The determination of sulfite anion is not an easy task because of a low-level presence in complex matrices and its rapid oxidation to sulfate in aqueous solution. The Monier–Williams [11–13] method is the most common indirect procedure for quantifying sulfite in foods and beverages, traditionally adopted as the official method. This method is based on distillation under acid condition of SO_2 that is adsorbed into a hydrogen peroxide solution. The volumetric titration with NaOH of the sulfuric acid produced by SO_2 oxidation

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allows the indirect quantification of sulfites. The Monier–Williams method is a rather time consuming and quite labor-intensive procedure, requiring a high degree of the operators' expertise, and a complete control of the analytical steps.

Very recently, flow injection analysis procedures in conjunction with detection techniques based on spectrophotometry, pH-change, chemiluminescence, conductimetry and amperometry or enzymatic reactions for the determination of sulfites in foods and beverages have been reviewed [4]. Methods including capillary electrophoresis [14,15] and HPLC with UV [16,17] or amperometric detection [18,19] were developed in the past decades. HPLC is the most frequently used technique, but satisfactory results have not been completely achieved. Although ion chromatography (IC) has become very popular for the determination of anion compounds, there is a very limited number of papers dealing with the sulfite determination by IC coupled to electrochemical detection [20–23]. Among them, only two works are referred to suppressed conductivity detection of SO_2 in air [21] and sulfite ions in spring waters [22]. In the latter case a stoichiometric conversion of sulfite to sulfate prior to the chromatographic run is required. In addition, all these methods have been applied to simple and fat-free matrices, including drinks, without performing comprehensive validation studies, as currently required for the official methods.

In the present study, the development and validation of a novel and reliable analytical method based on ion-exchange chromatography and suppressed conductivity for the determination of the sulfite anion in fresh meats and shrimps is described. Separation experimental conditions and sample extraction protocol were carefully evaluated in order to prevent sulfites oxidation and to remove endogenous interfering peaks. The proposed method has been submitted to an in-house validation procedure, in agreement with the recent European regulations, to assess accuracy, sensitivity, reproducibility and ruggedness.

2. Materials and methods

2.1. Chemicals and working standard solutions

Sodium sulfite (98%) and D-(−)-fructose (≥99%) were purchased from Sigma–Aldrich (Stenheim, Germany). Carbonate-free sodium hydroxide (50%, w/w), sodium carbonate anhydrous (>99.5%) and ethylenediamine tetraacetic acid (EDTA, >99%) was purchased from J.T. Baker (Deventer, Netherlands). All solutions were prepared with ultrapure water with a specific resistance of 18.2 MΩ cm, supplied by Milli-Q RG unit from Millipore (Bedford, MA, USA). Sodium hydroxide solution used as the eluent was prepared by dilution of a carbonate-free 50% (w/w) NaOH solution in water, filtration with a 0.45-μm membrane and degassing with nitrogen. Owing to the easy sulfite oxidation to sulfate, samples and standards should be analyzed in a short period of time and a particular care should be taken to reduce air exposure.

A stabilizing solution (StS) containing 50 mM NaOH, 10 mM fructose and 0.1 M EDTA has been used for both the preparation of standard solutions and the sample extraction [24]. This solution was stored in a refrigerator and used not longer than a week. From a stock solution of sodium sulfite at a concentration of 1000 mg L^{-1}, working standard solutions were prepared by dilution in StS to the required concentration, just before injection, to minimize the slow sulfites oxidation.

2.2. Sample preparation

A 4-g portion of the homogenized sample (fresh sausage and hamburger with a fat percentage around 26%, and shrimps) was mixed to 40 mL of the stabilizing solution in a horizontal shaker for 30 min. After centrifugation for 5 min at 1200 rpm at room temperature, the extract (~2 mL) was filtered on paper (Whatman No. 40), and then on Anotop 10 LC (0.2 μm, 10 mm, Whatman) prior to chromatographic analysis. No further cleanup step was required.

2.3. Apparatus and method

Chromatographic separations were performed on a Dionex system (Dionex Corporation, Sunnyvale, CA) composed of a GP50 quaternary gradient pump, an electrochemical detector (model ED40) set to conductivity mode, equipped with a temperature compensated conductivity cell, and a Rheodyne injection valve (model RH9125, Cotati, CA, USA) with a 25 μL injection loop. A Dionex anion self-regenerating suppressor (ASRS II, 4 mm) was used for the electrochemical suppression, at an operating current of 50 mA. All the separations were performed using an IonPac AS9-HC column (250 mm × 4 mm i.d., particle size: 9 μm) eluted in gradient mode at a flow rate of 1.0 mL min^{-1}. The mobile phase consisted of 8 mM Na_2CO_3 and 2.3 mM NaOH (A) and 24 mM Na_2CO_3 (B). The solvent gradient program started with an isocratic step at 100% A for 15 min, a gradient step to 50% A and 50% B in 1 min, and then 4 min at this eluent concentration. Finally, the system was re-equilibrated for 20 min at 100% A. The plastic reservoir bottles (DX500 2 L bottles, Dionex) were closed and pressurized with pure nitrogen to 0.8 MPa. The system was interfaced, via proprietary network chromatographic software (PeakNet™), to a personal computer for instrumentation control, data acquisition and processing (Dionex).

2.4. Validation procedure

Since there are no specific normatives aimed at the validation of protocols of sulfites determination in foods, the proposed method has been validated by an in-house validation model, in agreement with Decision 657/2002/EC [25] and Regulation 882/2004/EC [26], which describe the analytical parameters to be tested to assure the method reliability. These parameters are linearity, selectivity, recovery, precision, limits of detection (LOD) and quantification (LOQ), and ruggedness. The linearity test has been performed by three series of analyses on three different days, by injecting five standard solutions of sulfites at concentration of 1.0, 2.5, 5.0, 10.0 and 20.0 mg L^{-1}. To test selectivity, 20 independent blank samples of fresh meats (7 cow meats, 7 horse meats and 6 pork meats) found negative by a qualitative test [27], have been analyzed, in order to verify the absence of interfering peaks in the retention time-window of interest (±2.5% of SO_3^{2-} retention time). Precision and recovery have been determined by performing tests on three sets of blank cow hamburger samples (six replicates each) fortified with sulfites at concentrations of 10, 40, and 80 mg kg^{-1} (expressed as SO_2). The experiments have been performed in different days with the same instruments, but by different operators. The method ruggedness, under conditions of major changes, has been valued toward three different matrices (pork and horse sausages, and shrimps) by using the Youden factorial experimental design [28].

3. Results and discussion

3.1. Sample extraction and chromatographic separation

Sulfites are rather labile and can be degraded during extraction from the sample, and to prevent this problem different extraction conditions were reported for chromatographic [19,22,29,30] and FIA analysis [4,31,32], based on solutions containing acetone, formaldehyde [29,30,33,34], ethanol, mannitol [23], EDTA [35], glycerol and tetrachloromercurate. Although various compounds were proposed, it is difficult to find a stabilizer that is compatible