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**Determining preservatives in meat products by flow injection analysis (FIA):
A review**

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3 **Running head:** Preservatives in meat products by FIA
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9 **Determining preservatives in meat products by flow injection analysis**
10 **(FIA): A review**
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41 **Abstract**
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44 Various preservatives are added to meat products to extend their shelf-life stability and
45 enhance their safety. Determining preservatives is essential for purposes of legislation
46 and consumer safety. The analytical methodologies based on flow injection analysis
47 (FIA) can be highlighted among those available. FIA methodologies offer attractive
48 advantages compared with other procedures, including versatility, precision, low cost,
49 speed and ease of automation. This review considers the present status of published FIA
50 methodology available for the determination of preservatives in meat products. The
51 techniques are described with their application to different preservatives (nitrates and
52 nitrites, sulfites, sorbates, benzoates and p-hydroxybenzoate esters), emphasizing aspects
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3 related to extraction, separation, detection and quantification procedures in meat
4 matrices.
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10 **Key words:** Meat products, additives, preservatives, nitrates and nitrites, sulfites, sorbates,
11 benzoates and p-hydroxybenzoate esters, flow injection analysis.
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17 **Introduction**

18 Meat and meat products are highly perishable because they contain significant amounts
19 of the nutrients needed for micro-organism growth. The development of bacteria, yeast
20 and molds is one of the main agents responsible for the deterioration of these food
21 products, limiting their stability with important effects on the product quality and safety
22 levels, and relevant economic implications. Various preservation procedures are used to
23 protect meat from microbial spoilage, including those based on additives known as
24 preservatives. These are chemical compounds added to foodstuffs which prolong their
25 shelf-life by protecting them (by prevention or inhibition) from deterioration caused by
26 microorganisms and thereby enhance food safety.
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37 The control and regulation of the use of preservatives in meat products are very
38 important because they are effective and ensure consumer safety. The acceptable daily
39 intake (ADI) for preservatives has been calculated, just as for other food additives, since
40 if they are consumed in inappropriate quantities they may have adverse effects on
41 human health. This is why legislation on the use of additives is needed to establish
42 specific conditions for their use. Food additive legislation adopted by the European
43 Union is included in several European Parliament and Council Directives (Directives
44 95/2/EC and 2006/52/EC). The authorized preservatives for meat products are shown in
45 Table I. To be able to evaluate whether they are being used in the specified quantities
46 and/or foods, it is essential to have appropriate analytical methods available.
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56 Numerous methodologies for determining preservatives in foodstuffs are cited in the
57 relevant literature, including specific references to meat products. They are based on
58 separation, identification and quantification processes using a wide variety of
59 spectrophotometric, chromatographic and electrophoretic techniques etc. (Fazio and
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3 Warner 1990; Karovicová and Simko 2000; Wood et al. 2004; Ruiter and Bergwerff
4 2005; Jiménez Colmenero and Blazquez 2007). Among the methodologies, those based
5 on flow injection analysis (FIA) are of particular interest and have developed
6 significantly in recent years because of the many advantages they offer. FIA is a very
7 versatile, flexible, economical technique, suitable for the fast, routine analysis of a large
8 number of samples with high precision. Affordably priced and easy to use, it requires
9 minimum intervention of the operator and a lower consumption of samples and
10 reactants. This is especially important when working with toxic or expensive reactants.
11 It can be installed on-line and is compatible with a large number of detectors
12 (potentiometric, amperometric, spectrophotometric, fluorimetric, etc.). This
13 methodology responds to growing demands for the mechanization and automation of
14 analysis made by many laboratories faced with the increasing number of routine
15 analyses. This, along with its advantageous cost/efficiency relationship, favors the use
16 of this method over the alternative procedure for determining additives (Valcarcel and
17 Luque de Castro 1987; Sullivan et al. 1990; Ferreira et al. 1996; Pinho et al. 1998;
18 Kazemzadeh and Ensafi 2001; Andrade et al. 2003; Frenzel et al. 2004, Penteadó et al.
19 2005).

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Early studies of FIA focused on the determination of contaminants in water (a matrix generally easy to work with and with little interference except in waste water), but by the mid-80s the applications of FIA had been extended to other fields (food, biomedicine, control of industrial processes etc.), in more complex matrices (Valcarcel and Luque de Castro 1987). Although FIA has been trialed to determine some preservatives in meat products no in-depth critical analysis has been made of the use of this methodology and the possibilities it offers, as far as the authors are aware. This review considers the current status of the available published flow injection analysis methodology for the determination of authorized preservatives in meat products. To do this, different aspects are analyzed, related to extraction, separation, detection and quantification procedures in complex muscle matrices.

Brief description of FIA

The original concept of FIA was established by Ruzicka and Hansen in 1975, and has been continuously modified since then. Flow injection analysis is based on the injection of a liquid sample into a moving, non-segmented continuous carrier stream of a suitable

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3 liquid. The injected sample forms a zone which is then transported towards a detector
4 that continuously records physical parameter such as the absorbance, electrode potential,
5 or other physical parameter, changing as the sample material passes through the flow
6 cell. The FIA response curve (FIAGram) is the result of two kinetic processes: the
7 physical process of dispersion of the sample zone within the carrier stream and the
8 chemical process of formation of a chemical species. These two processes occur
9 simultaneously and together with the dynamic characteristics of the detector, they yield
10 the FIA response curve.
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14 The simplest FIA system (see Figure 1) consists of a tube through which the carrier
15 stream moves towards the flow-through detector. Briefly, it consists of the following
16 basic elements:
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- 18 - **Propulsion system.** This is generally a peristaltic pump with a variable number
19 of channels, depending on the moving or reactive (carrier) phases which
20 intervene in the reaction. It provides a constant flow of a carrier solution.
21
- 22 - **Injection system.** This is normally a low pressure Rheodyne valve which allows
23 a small volume of the sample, usually measured in micro liters, to be inserted
24 into the flow of the carrier solution. The use of valves with various ports allows
25 the simultaneous determination of various analytes.
26
- 27 - **Reaction coils and complementary components.** The most frequently used
28 reactors are tubes made from various materials such as polytetrafluorethylene or
29 plastic tubing of different sizes and diameters (e.g. ID 0.8 mm), which can be
30 coiled, knitted or knotted. The purpose of this geometric deformation is to
31 decrease the zone dispersion. These coils can be thermostated. Sometimes the
32 system can also have other integrated elements in series or in parallel (Figures 2
33 and 3) such as columns (e.g. to cause chemical reactions – as with cadmium for
34 the transformation of nitrates to nitrites or to immobilize reactants), or gas
35 diffusion cells (e.g. for separating SO₂ or NO₂).
36
- 37 - **Detection systems.** A wide variety can be used, including potentiometric,
38 amperometric, spectrophotometric, fluorometric, etc.
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- 40 - **Data collector system.** This is normally carried out from a PC linked to the
41 system or to the detector, with signal integration software.
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Nitrites and nitrates

Nitrites and nitrates are used in processed meat because of their effect on its organoleptic characteristics (color stabilization, flavor development), oxidative stability of lipids and inhibition of pathogenous micro-organisms such as *Clostridium botulinum* or *Listeria monocytogenes* (Cassens et al. 1979; Cassens 1997; EFSA 2003). Although the positive effect of nitrite on human health has been indicated (protective role of nitric oxide), the interest in nitrite and its reaction is mainly centered on the potential formation of nitrosamines (on reaction with secondary amines), compounds with teratogenic, mutagenic and carcinogenic effects, highly dangerous for human health (Cassens 1997). For this reason and for some time now, the possibility has been put forward of reducing or even avoiding the use of these additives in the food industry.

In the EU, potassium and sodium nitrite and nitrate are authorized for use in different meat products, and maximum amounts (150 mg kg⁻¹ for nitrite and 300 mg kg⁻¹ for nitrate) are established for all products as well as maximum residual levels for some of them (Directives 95/2/EC and 2006/52/EC). Current regulations on the use of nitrite and nitrate in the US vary depending on the curing method used and the product to be cured. For comminuted products, the maximum ingoing nitrite and nitrate limits are 156 mg kg⁻¹ and 1718 mg kg⁻¹, respectively. For immersion-cured and massaged or pumped products those limits are 200 mg kg⁻¹ and 700 mg kg⁻¹, respectively. In dry cured products nitrite is limited to 625 mg kg⁻¹ and nitrate to 2187 mg kg⁻¹ (USDA 2007). The ADI for nitrites, as nitrite ion, is 0.07 mg kg⁻¹ body weight, and 3.7 mg kg⁻¹ for nitrates, as nitrate ion (FAO/WHO 2007a, b).

Different methodologies have been used to determine nitrates and nitrites, including ion chromatography, capillary electrophoresis, differential pulse voltammetry, enzymatic reduction and electrochemistry (biosensors with amperometric monitoring among others). For these, different detection systems based on UV, mass spectroscopy, fluorescence, chemiluminescence or electrochemical detection have been used (Usher and Telling 1975; Wood et al. 2004; Ruiter and Bergwerff 2005; Jiménez Colmenero and Blazquez 2007). Among these methodologies a prominent position is occupied by those based on flow analysis injection with their unquestionable advantages, which have been applied in numerous meat products (see Table II).

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3 Generally speaking, the determination of nitrates and nitrites by FIA can be divided into
4 two phases, as is also the case with other preservatives. The first phase is the extraction
5 process of these compounds from the meat matrix and the other is its evaluation *per se*
6 using FIA methodology. Both these phases are analyzed below.
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10 11 *Nitrate and nitrite extraction*

12 The extraction of nitrate and nitrite from meat products is the most critical in terms of
13 obtaining adequate recoveries for the nitrates and nitrites and to reduce possible
14 interferences. When nitrite is added to a biological material such as meat products, it
15 reacts with various components present naturally in the matrix, which means that the
16 levels of nitrite detectable analytically (known as residual) necessarily vary. This means
17 that the levels of residual nitrite fall during the different stages of processing, storage,
18 preparation and consumption depending on various factors related to the type of product
19 and the conditions of each of the phases mentioned above. (Cassens et al. 1979; Cassens
20 1997; EFSA 2003). Different procedures have been used in the extraction of nitrate and
21 nitrite from foods including processed meats.
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32 Just as with other analytical methods, most of the nitrite and nitrate extraction processes
33 by FIA determination use hot water and different systems for blending or homogenizing
34 the sample and filtration (see Table II). An extraction system for cured meat based on
35 microwave cooking of the samples following cold extraction (water and NH_4Cl at
36 different pH) in a nitrogen atmosphere has been reported by van Staden and Makhafola
37 (1996).
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46 Some compounds present in meat products (ascorbic acid, fat, protein, etc.) which can
47 interfere in the determination of nitrates and nitrites, may be present in the extract. This
48 is why it has been emphasized that purification or clarification steps need to be
49 followed. Several clearing techniques have been used for the analysis of nitrite and
50 nitrate in food especially in meat products. These may be based on de-proteinisation and
51 clarifying agents using reagents such as potassium aluminum sulfate, borax (sodium
52 tetraborate anhidra), mercuric chloride, zinc sulfate, or potassium hexacyanoferrate
53 (Carrez I) plus zinc acetate (Carrez II), the most commonly used clarifying agents for
54 aqueous extracts of meat samples (see Table II). Other clearing techniques may also be
55 used such as active carbon or gel filtration on Sephadex G25 - this last only used for
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3 beverages (Torro et al. 1998). Some of these reagents have been used to precipitate
4 proteins from meat extract and also to fix the pH between 6.0 and 6.5, to avoid nitrate
5 and nitrite loss. One of the major criticisms leveled at the use of Carrez is that the pH of
6 the final solution (approximately pH 5) tends to promote nitrite loss. However, this de-
7 proteinization procedure of samples was also complicated because it caused cloudy
8 filtrates and as a result serious experimental errors (Usher and Telling 1975).
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15 *Measurements of nitrite*

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17 In the FIA analytical methods the extracted samples are generally injected through the
18 injection system valve into the corresponding carrier streams (see Figure 2; see Table
19 II); this is generally a reagent composed of ammonium chloride and EDTA (van Staden
20 and Makhafola 1996; Oliveira et al. 2004; Ruiz-Capillas et al. 2006) or ammonium
21 chloride, sodium tetraborate and EDTA (Pinho et al. 1996; Ferreira et al. 1996) or a
22 buffer 7.5 (Ruiz-Capillas et al. 2007). These masking and buffering reagents draw the
23 injected extracted solution into the coil of different lengths; then it is mixed directly
24 with the color forming reagent (in most cases, since most of the methods used for nitrite
25 determination by FIA in meat products are colorimetric) in a second reaction coil (see
26 Figure 2; see Table II). The color forming reagents are mainly those used in the
27 traditional diazo-coupling reaction (the Griess reaction), generally composed of
28 sulphanilamide, (N-(1-naphthyl)ethylenediamine dihydrochloride - NED) and
29 phosphoric acid (Ferreira et al. 1996; Pinho et al. 1996; van Staden and Makhafola
30 1996; Ruiz-Capillas et al. 2006, 2007) or other acids such as HCl (Oliveira et al. 2004;
31 Frensel et al. 2004). These procedures are based on the well-known ability of nitrite to
32 convert aromatic amines (sulphanilamide) into diazonium ions, which are coupled to
33 another aromatic compound (NED) in order to produce an azo-dyamine which is
34 detected spectrophotometrically at 520-540 nm. This FIA system based on the Griess
35 reaction has been also used for the determination of protein-bound nitrite in frankfurter
36 and dry sausages (Ruiz-Capillas et al. 2007).
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54 Although the Griess reaction presents important advantages, including excellent
55 sensitivity, it also has certain limitations in relation to the control requirements of the
56 reaction conditions, stability of the reactants, interferences or the use of some reactants
57 which present carcinogenic effects. Many of these problems were also reported when
58 the FIA version of the Griess reaction was applied to meat matrices. E.g. van Staden
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3 and Makhafola (1996) reported that for certain samples, mainly from an acidic origin
4 (cured meat products), the experimental nitrite value obtained was lower than the
5 expected value. To solve these problems it has been recommended that the nitrite
6 samples should always be kept under nitrogen atmosphere during preparation and they
7 must always be analyzed immediately after preparation or stored under nitrogen
8 atmosphere. The nitrite solution was also standardized against a permanganate solution
9 treated with a few drops of chloroform and kept in the refrigerator (van Staden and
10 Makhafola 1996). SO₂ can also interfere with nitrite determination (Ahmed et al. 1996).
11 Haghghi and Tavassoli (2002) showed the importance of the dye instability, so that the
12 color reaction must be time controlled as it is affected by numerous oxidation reactions.
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23 Over recent years, and as the result of these drawbacks, a number of other amines have
24 been proposed as potential reactants for nitrite and potential coupling agents applied to
25 the analysis of food extracts by FIA spectrophotometric determination (see Table II).
26 For example, 3-nitroaniline, with NED, has been reported as a spectrophotometric
27 reagent (color-forming reagent) for nitrite and nitrate determination in beef and pork
28 sausages (Ahmed et al. 1996). Safranin O (3,7-diamino-2,8-dimethyl-5-
29 phenylphenazinium chloride) has also been reported to form diazonium salts in an
30 acidic solution which causes the reddish-orange dye color of the solution to change to
31 blue in acidic media and which absorbed at 520 nm has been used for flow injection
32 spectrophotometric determination of nitrite in beef and commercial sausages (see Table
33 II). The reaction of safranin with the nitrite decreases the color of the dye instead of its
34 appearance as in the case of diazodye (Kazemzadeh and Ensafi 2001; Penteadó et al.
35 2005).
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48 Andrade et al. (2003) also reported the development and application of FIA based on
49 spectrophotometric methods for the determination of nitrite in foodstuffs (vegetables
50 and meat products). This is based on the formation of FeSCNNO⁺ in the flow injection
51 system, with transient absorbance signal at 460 nm (see Table II). The method is based
52 on the reduction of the nitrite to NO in a sulfuric acid medium and the reaction of nitric
53 oxide with ferrous and thiocyanate ions forming FeSCNNO⁺.
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Oxidation/reduction reaction has been also used in the determination of nitrites. Ensafi
and Kazemzadeh (1999) proposed a flow injection method with spectrophotometric

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3 detection for determination of nitrite in beef samples based on the catalytic effect of
4 nitrite on the oxidation of galloxyanine by bromate in acidic media and the decrease in
5 absorbance of the system at 530 nm (see Table II). Sen et al. (1994) also developed a
6 FIA method based on nitrite reduction with potassium iodine followed by
7 chemiluminescence detection of the liberated NO using a thermal energy analyzer for
8 the determination of nitrites in food (cured meat) and other biological materials.
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16 Torró et al. (1998) developed a FIA method (tested on vienna sausages) that involved
17 the photochemical reduction of nitrate in a photo reactor consisting of a low pressure
18 mercury lamp. The nitrite produced is determined indirectly with the I_3^-/I^- system using
19 a flow-cell with two platinum (Pt) electrodes polarized at 100 mV (see Table II).
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25 Haghghi and Tavassoli (2002) reported a flow injection method on the basis of gas
26 phase molecular absorption for the determination of nitrite in meat sausages and
27 mortadella and aqueous solutions (see Table II). The sample solution is injected into a
28 stream of water which is then reacted with a stream of hydrochloric acid. The gaseous
29 products (NO, NO₂, HNO₂ and NOCl) are separated from the liquid stream by the
30 home-made gas liquid separator and they are swept by the carrier O₂ gas into a home-
31 made flow-through cell which has been positioned in the space of the cell compartment
32 of a UV/VIS spectrophotometer. The transient absorbance of the gaseous phase is
33 measured at 205 nm. The interference of anions in the methods proposed by Haghghi
34 and Tavassoli (2002) are overcome adding silver and sodium hydroxide solutions as the
35 precipitating agent. This method is less sensitive than the Griess methods but there are
36 also fewer limitations than in the Griess method.
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48 Frenzel et al. (2004) use a system of gas diffusion membrane-based optical flow
49 through optical sensor for the determination of nitrite in waste waters and meat extracts
50 (see Table II), using the modified Griess reaction where sulfuric acid was used as a
51 releasing agent to set HNO₂ and NO₂ free from the nitrite solutions. The color reagent
52 was prepared by a dissolution of sulfanilamide, *N*-(1-naphthyl)-ethylenediamine
53 dihydrochloride and concentrated hydrochloric acid in water. The main reason for
54 developing the proposed sensor was the inherent selectivity enhancement due to analyte
55 separation by the gas-diffusion process. Only a few analytes are sufficiently volatile to
56 pass through the membrane and they are effectively separated from the sample matrix.
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3 Species known to interfere in the Griess reaction are therefore almost entirely excluded.
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5 In particular, colored and turbid samples can be analyzed without problems and
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7 interferences as extreme pH values or the presence of high salt content in the samples
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9 are eliminated so that the proposed sensor is almost nitrite specific. However, the
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11 relatively low sensitivity of the developed sensor (compared to the common
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13 spectrophotometric method (ISO 1996) limits its applicability (Frenzel et al. 2004).
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15 16 *Measurements of nitrates and nitrites*

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18 Although initially FIA was used only for nitrite determination, nitrate determination
19
20 was also developed later. Since for nitrate analysis the previous reduction of nitrate to
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22 nitrite is required, FIA methodology included a reduction system. This means that in its
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24 application in meat products the nitrate is reduced on-line to nitrite in a cadmium
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26 column which is then treated with azo dye reagent and the absorbance due to the sum of
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28 nitrite and nitrate is measured: nitrate is determined from the difference in absorbance
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30 values (see Figure 2) (Ruiz-Capillas et al. 2006, 2007).
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33 The simultaneous determination of nitrates and nitrites has been trialed since this
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35 substantially reduces analysis times. Ahmed et al. (1996) determined nitrites and
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37 nitrates (beef and pork sausages) simultaneously using a selector valve to split the
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39 sample into two streams after it was injected into the carrier stream. The quantification
40
41 was based on the 3-nitroaniline+NED color reaction (see Table II). For different meat
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43 products Ferreira et al. (1996) and Pinho et al. (1998) also determined nitrate and nitrite
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45 simultaneously. The test was based on the splitting of the flow after injection and
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47 subsequent confluence of the flow before it reached the detector, allowing the reduction
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49 of nitrate to nitrite in part of the sample plug in an on-line copper cadmium reductor
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51 column. Since each channel has a different residence time two peaks were obtained for
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53 nitrite and for nitrite plus nitrate. The spectrophotometric determination was made after
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55 a diazotization coupling reaction. A similar system for simultaneous determination of
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57 nitrite and nitrate in various beef samples used flow injection with spectrophotometric
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59 detection based on a oxidation system where the nitrate is reduced to nitrite by using
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two sequential columns: copper and copper-coated cadmium (Ensafi and Kazemzadeh
1999). Kazemzadeh and Ensafi (2001) reported similar procedure for the simultaneous
determination of nitrate and nitrite but based on the reaction of nitrite with safranin O
(see Table II). On the other hand, Oliveira et al. (2004) reported a simultaneous

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3 determination of nitrate and nitrite in cured meat by sequential injection analysis, based
4 on the Griess reaction.
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7 8 **Sulfites** 9

10 Sulfites are used as preservatives in food and beverages to prevent oxidation and
11 bacterial growth. In meat, sulfites also help to give a bright color. Appearance (colour) is
12 one of the prime factors motivating consumer choice. In the EU the maximum permitted
13 amount of added sulfite agents in different meat products is 450 mg kg⁻¹, expressed as
14 SO₂ (Directives 95/2/EC and 2006/52/EC). These compounds are not permitted for use
15 in meat in the USA. Sulfites have been associated with allergic reactions and food
16 intolerance symptoms. They are also known to degrade thiamine (vitamin B1), of which
17 meat is a good source. The ADI for sulfite (expressed as SO₂) is 0.7 mg kg⁻¹ body
18 weight (FAO/WHO 2007c).
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28 There are many methods for analyzing sulfites in beverages and foods. These
29 procedures involve titrimetry, electrochemistry, fluorimetry, chemiluminescence
30 spectrometry, colorimetry, gas-liquid chromatography, high preformed liquid
31 chromatography, etc. (Fazio and Warner 1990; Karovicová and Simko 2000; Wood et
32 al. 2004; Ruitter and Bergwerff 2005; Jiménez Colmenero and Blazquez 2007). The
33 most commonly used techniques are based on the classic Monier-Williams method.
34 Although this method demonstrates sufficient sensitivity, it also presents certain
35 limitations because of the long analysis time (with important repercussions on the
36 analytical capacity of the laboratory) and the extreme control needed in some of the
37 analytical phases (e.g. N₂ stream adjustment).
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48 FIA procedures have been extensively researched for determining sulfites in beverages
49 and foods, in an attempt to overcome these limitations and reduce the time and effort
50 needed for those procedures. Most FIA procedures have been developed for liquid
51 foods, with far fewer for solid foods and especially for muscle foods (see Table III). It
52 has been reported that the FIA procedure is a more rapid, accurate and precise method
53 for the determination of total sulfite in food than the Monier-Williams procedure
54 (Sullivan et al. 1986, 1990).
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3 Sulfite determination by FIA generally consists of two phases: one related to the
4 extraction process of the sulfiting agent from the food and the other related to evaluating
5 it.
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10 *Sulfite extraction*

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12 When sulfiting agents are added to foods such as fish or meat products they can
13 combine with some of their components (aldehydes, proteins etc.), so that they may be
14 present as free, reversibly bound (probably as hydrosulphonate adducts) and irreversibly
15 bound (Wedzicha and Mountfort 1991; Pizzoferrato et al. 1998). The relative presence
16 of each of these depends on factors linked to composition, processing and conservation.
17 This is why the determination of sulfites in complex food matrices such as meat
18 products requires the application of some procedure for removing and recovering the
19 sulfites (free and reversibly bound both together called total sulfites). Since substantial
20 loss of sulfites can occur during extraction, different alternative extraction conditions
21 (distillation, acidic, alkaline extractant, etc.) have been tried, to obtain free and bound
22 sulfite fractions. For analysis using FIA various extraction media have been trialed
23 (including solutions containing acetone, formaldehyde and ethanol), but the most
24 efficient extraction procedure in muscle foods for FIA analysis includes pH extraction
25 with a sodium tetrachloromercurate (TCM) solution (Sullivan et al. 1986, 1990; AOAC
26 2005) (see Table III). TCM helps to release the sulfite from solid foods and stabilize it
27 forming a stable sulfite-mercuric complex, providing excellent sulfite recovery
28 (Sullivan et al. 1986; Ruiter and Bergwerff 2005).
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44 *Measurements of sulfites: separation/detection systems.*

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46 Afterwards, the extracting solution obtained from muscle food matrices (meat and fish)
47 containing the sulfiting agents is injected into the FIA system to proceed to the
48 separation of the SO₂ followed by the corresponding detection process (see Figure 3).
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53 The separation phase in these matrices is based on the use of a gas diffusion process
54 (Sullivan et al. 1986; AOAC 2005). For determination of total sulfites previous to this
55 separation in the gas diffusion system a hydrolysis treatment is applied with NaOH
56 aimed at the release of most of the bound sulfites from the extract solution which is
57 complete above pH 8 (Kim and Kim 1986; Sullivan et al. 1990; AOAC 2005) (see
58 Figure 3).
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5 The separation of SO₂ itself is carried out in the gas diffusion cell (see Figure 3). Two
6 liquid streams converge in this cell: the first is a donor stream which transports a strong
7 acidic donor solution intended to provoke the release of the SO₂ present in the test
8 solution. Although other donor reagents may be used in gas diffusion systems, sulfuric
9 acid is what is used in muscle based food (Sullivan et al. 1986, 1990; AOAC 2005).
10 This choice is closely linked to the detection system. The second is an acceptor stream
11 (indicator which collects the released SO₂), a solution containing an acid-base indicator
12 separated by a permeable membrane (generally made of teflon). The SO₂ released from
13 the donor diffuses through the membrane and is dissolved into the acceptor solution.
14 The membrane is not only necessary to separate the strong acidic donor solution from
15 the acceptor solution but also forms a barrier for potential interferences in the sample.
16 Different colorimetric reagents (iodine, bromocresol green etc.) can be found among
17 the acceptor solutions used, although in the determination of SO₂ by FIA in solid
18 matrices (muscle based food) a malachite green solution is used (Sullivan et al. 1986,
19 1990; AOAC 2005) (see Table III). The degree of discoloration of malachite green is
20 proportional to the amount of sulfite in the test solution and it is measured
21 spectrophotometrically at 615 nm. Spectrophotometric detection seems more
22 appropriate as it is more robust and it is usually available in routine control laboratories.
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39 The possible interferences in the determination of SO₂ by FIA with a gas diffusion unit
40 are related to the presence of compounds able to pass through the membrane, including
41 nitrite, sulfide and cyanide (Frenzel and Hillman 1995). Although these interferences
42 have been established in beverages, their incidence is plausible under any circumstances
43 in solid matrices such as meat products.
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49 **Sorbates, benzoates and p-hydroxybenzoate esters.**

50 Sorbic acid, benzoic acid and the methyl and ethyl esters of 4-hydroxybenzoic acid
51 (parabens) or their (Na, K, or Ca) salts are organic acids used as meat product
52 preservatives (see Table I). In the EU they are allowed in the surface treatment and jelly
53 coating of many processed meats, used singly or in combination (sorbates, benzoates
54 and parabens in concentrations ranging from 1000 mg kg⁻¹ to *quantum satis*) and in
55 pâtés (sorbates and parabens used singly or in combination), up to a maximum level of
56 1000 mg kg⁻¹. (Directives 95/2/EC and 2006/52/EC). The acceptable daily intake is 25
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3 mg kg⁻¹ for sorbic acid (FAO/WHO 2007d) and 5 mg kg⁻¹ body weight for benzoic
4 acid (FAO/WHO 2007e). The EFSA has established a full-group ADI of 10 mg kg⁻¹
5 body weight for the sum of methyl and ethyl p-hydroxybenzoic acid and esters and their
6 sodium salts (Directive 2006/52/EC).
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12 Given that these compounds present strong UV absorption, the method most commonly
13 used to determine organic preservatives in foods is spectrophotometry. Other methods
14 have also been reported including thin-layer chromatography, gas chromatography, high
15 performance liquid chromatography and capillary electrophoresis among others
16 (Karovicová and Simko 2000; Wood et al. 2004; Ruiter and Bergwerff 2005; Jiménez
17 Colmenero and Blazquez 2007).
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25 The application of FIA method for the determination of sorbic acid, benzoic acid and
26 parabens in meat products has not been found in the literature. A sorbic acid
27 determination method by FIA in wine has been proposed (Molina et al. 1999). The
28 advantages offered by the traditional spectrophotometric methods in terms of simplicity
29 and specificity may help to understand the lack of interest in the application of other
30 methodologies, including FIA, to the determination of these compounds in foods. There
31 is no methodological reason which limits the application of FIA in the determination of
32 these preservatives in meat products. The procedure requires, just as for other methods,
33 an extraction process (for the extraction of the compound and, where relevant, the
34 elimination of interferences related to the nature of the matrix) before it is introduced
35 into the equipment, where the color reaction is developed and later detection, both
36 visible and UV, takes place. In this case a basic advantage of the FIA system is the
37 possibility of automation, which would mean an increase in the volume of samples
38 analyzed and reduced handling.
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51 CONCLUSIONS

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53 The application of FIA to determine preservatives in meat is an important alternative to
54 more conventional methods and presents several advantages in routine laboratory
55 analysis for official bodies and industry. FIA is a method which allows a simple,
56 precise, fast determination of small amounts of sulfite with low reagent consumption,
57 small volumes of samples required and the use of different detection systems. However,
58 more studies are necessary in order to develop new FIA procedures for the
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3 determination of other preservatives present in meat and meat products (e.g. sorbates,
4 benzoates etc.). It is also necessary to improve the present procedure in order to reduce
5 the quantity of toxic reagent that is used in some of them. More studies of the extraction
6 and cleaning procedures will be necessary for the application of FIA in complex
7 matrices such as muscle based foods. Therefore preservative recovery should be
8 improved and the reduction of possible interference associated with both natural matrix
9 components and other food additives should be studied. Studies regarding the
10 automatization of all the procedures must also be carried out, as well as reviewing the
11 stabilising reagent for the extraction solution and its conservation to avoid substantial
12 losses of preservatives. In depth studies will also be necessary to apply FIA procedures
13 to new products (including meat based functional foods), which have been developed
14 using new non-meat ingredients (e.g. from plant and marine sources).
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Figure 1.- Diagram of a basic Flow Injection Analyser

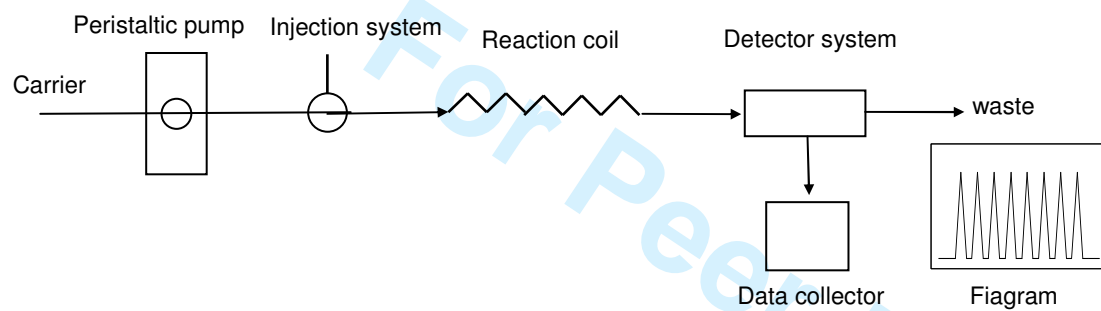
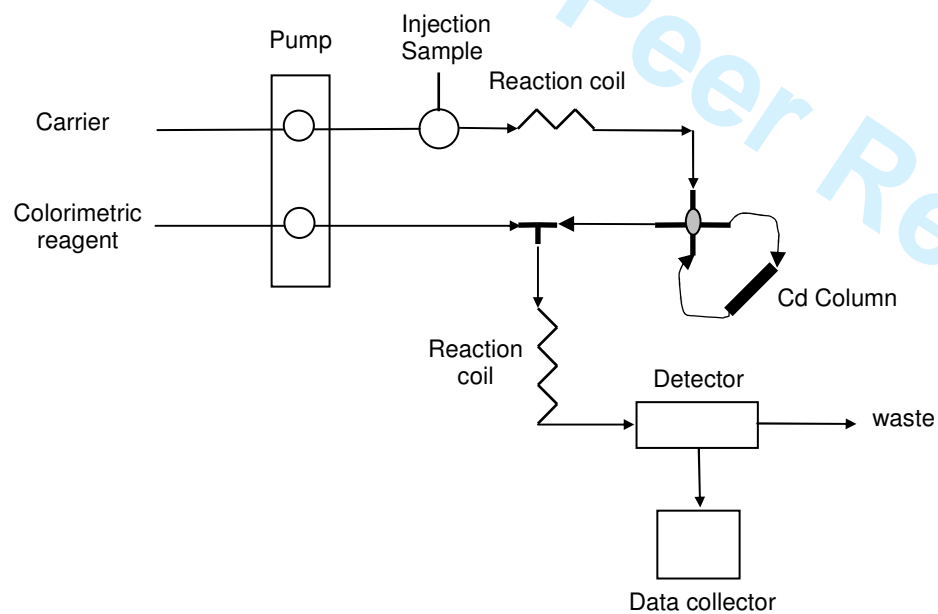


Figure 2.- Diagrams of Flow Injection Analyser for nitrite and nitrate determination



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6 **Figure 3.-** Diagrams of Gas diffusion-Flow Injection Analyser for sulphite determination
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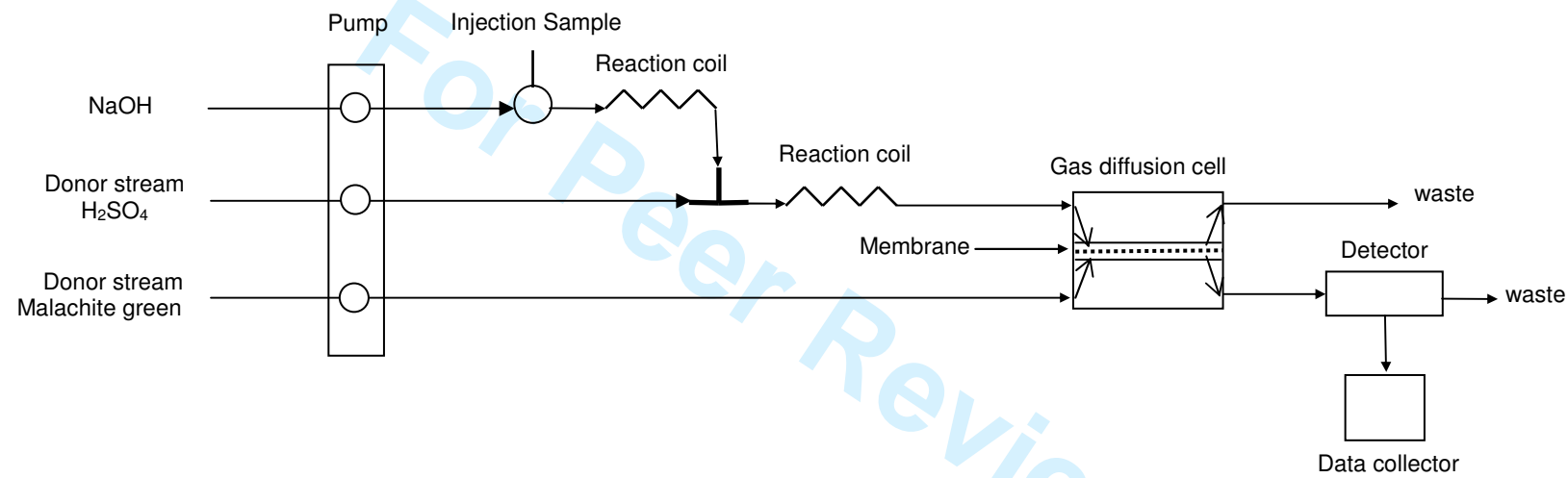


Table I.- Food Additives approved by the European Union (1) for use in meat products (E-Number list)

Groups	E numbers	Names
Sorbates (Sa)	E-200	Sorbic acid
	E-202	Potassium sorbate
	E-203	Calcium sorbate
Benzoates (Ba)	E-210	Benzoic acid
	E-211	Sodium benzoate
	E-212	Potassium benzoate
	E-213	Calcium benzoate
Paraben (PHB)	E-214	Ethyl-p-hydroxybenzoate
	E-215	Sodium ethyl-p-hydroxybenzoate
	E-216	Propyl p-hydroxybenzoate ¹
	E-217	Sodium propyl p-hydroxybenzoate ¹
	E-218	Methyl-p-hydroxybenzoate
	E-219	Sodium methyl-p-hydroxybenzoate
Sulphites (SO ₂)	E-220	Sulphur dioxide
	E-221	Sodium sulphite
	E-222	Sodium hydrogen sulphite
	E-223	Sodium metabisulphite
	E-224	Potassium metabisulphite
	E-226	Calcium sulphite
	E-227	Calcium hydrogen sulphite
	E-228	Potassium hydrogen sulphite
	Nitrates (NO ₃) & Nitrites (NO ₂)	E-249
E-250		Sodium nitrite
E-251		Sodium nitrate
E-252		Potassium nitrate

¹ The European Food Safety Authority (EFSA) has recommended their withdrawal of approval.

An SO₂ content of not more than 10 mg/kg is not considered to be present

Food additive legislation: European Parliament and Council Directives (Directives 95/2/EC and 2006/52/EC)

Table II.- Nitrate and nitrite determination in meat and meat products by flow injection analysis

	Sample	Extraction/Clarification	Carrier	Reduction NO ₃ to NO ₂	Colorimetric reagent	Detection system	Ref
Nitrite	Cured meat	Homogenised with hot water and NaOH solution, transferred flask to a bath (50-60°C) and occasional shaking, then addition of zinc acetate and filter.	-----	-----	Iodine	Chemiluminescence	Sen et al. 1994
Nitrate and Nitrite (simultaneous)	Ham, sausages, smoked pork sausages and smoked ham	Homogenised with hot water and followed by purification and filtration by addition of disodium tetraborate and Carrez reagent (potassium hexacyanoferrate and zinc acetate)	Ammonium chloride + sodium tetraborate + EDTA	Copper - cadmium reductor column	Sulphanilamide NED Phosphoric acid	Spectrophotometric 530 nm	Ferreira et al. 1996
Nitrite	Cured meat samples	Cooking of the samples in a microawave oven and then cold extraction under N ₂ atmosphere with water and + NH ₄ Cl	Ammonium chloride + EDTA	-----	Sulphanilamide NED Phosphoric acid	Spectrophotometric 540 nm	van Staden and Makhafola 1996
Nitrate and Nitrite (simultaneous)	Beef and pork sausages	Sample was mixed with sand and homogenised in a mortar and digested in a bath (100°C) during 2h and occasional shaking.	Ammonium chloride	Copper column + Copperised cadmium column	3-nitroaniline + NED	Spectrophotometric 535 nm	Ahmed et al. 1996
Nitrate and Nitrite (simultaneous)	Liver pâtés	Homogenised with hot water and followed by purification and filtration by addition of disodium tetraborate and Carrez reagent (potassium hexacyanoferrate and zinc acetate)	Ammonium chloride + sodium tetraborate + EDTA	Cadmium reductor column	Sulphanilamide NED Phosphoric acid	Spectrophotometric 530 nm	Pinho et al. 1998
Nitrite and nitrate	Vienna sausages	Chopped and placed with hot water in a flask that was boiled for 2h and cooled, then 2g of active carbon were added to the clean.		Photochemical reduction in a photoreactor (a low-pressure mercury lamp)	H ₂ SO ₄ /iodine	I ³ /I ⁻ System potentiostat	Torró et al. 1998
Nitrate and Nitrite (simultaneous)	Beef	Sample was mixed with sand and homogenised in a mortar and digested in a bath (100°C) during 2h and shaking occasionally.	Ammonium chloride	Copperised cadmium reductor column	Potassium permanganate at 23°C	Spectrophotometric 530 nm	Ensafi and Kazemzadeh 1999
Nitrate and Nitrite (simultaneous)	Beef	Sample was mixed with sand and homogenised in a mortar and digested in a bath (100°C) during 2h and occasional shaking.	HCl +KCl	Two sequential column Copper and copper-coated cadmium	Safranin O	Spectrophotometric 520 nm	Kazemzadeh and Ensafi 2001
Nitrite	Meat products mortadella	Homogenised with water containing 4 drops of NaOH.	Detilled water	-----	HCl nitrite zone O ₂ gas carrier	Spectrophotometric 205 nm	Haghighi and Tavassoli 2002
Nitrate and Nitrite	Pork sausages and hot-dogs	Homogenised with hot water and followed by clarification of the extract by addition of disodium tetraborate and Carrez reagent	Ammonium chloride + EDTA	Cadmium cooper reductor column	Ferrous and thioacyanate solution	Spectrophotometric 460 nm	Andrade et al. 2003

		(potassium hexacyanoferrate and zinc acetate)					
Nitrate and Nitrite (simultaneous)	Cured meat	Homogenised with hot water and followed by purification and filtration by addition of disodium tetraborate and Carrez reagent (potassium hexacyanoferrate and zinc acetate)	Ammonium chloride + EDTA	Copperized cadmium mini column	Sulphanilamide NED HCl	Spectrophotometric 538 nm	Oliveira et al. 2004
Nitrite	Homogenised meat samples	Homogenised with hot water and transferred flask to a bath and left for 2h, occasional shaking.	H ₂ SO ₄	Gas diffusion membrane-based optical flow-trough sensor	Sulphanilamide and NED and Hydrochloric acid	Optical flow-trough sensor (absorbance or reflectance)	Frenzel et al. 2004
Nitrite	Meat sausage	Homogenised with hot water and transferred flask to a bath (80°C) and left for 2h, occasionally shaking.	Detilled water	-----	Safranine O	Spectrophotometric 520 nm	Penteado et al. 2005
Nitrate and Nitrite	Frankfurter sausages	Homogenised with hot water and transferred flask to a bath (100°C) and left for 2h, occasional shaking.	Ammonium chloride+ EDTA	Cadmium reductor column	Sulphanilamide NED, Phosphoric acid	Spectrophotometric 540 nm	Ruiz-Capillas et al., 2006
Nitrite and Protein bound nitrite	Frankfurter sausages and Spanish dry sausages	Homogenised with hot water and transferred flask to a bath (100°C) and left for 2h, occasional shaking.	Buffer 7. and 7.5	Cadmium reductor column	Sulphanilamide NED, Phosphoric acid	Spectrophotometric 540 nm	Ruiz-Capillas et al. 2007

NED: N-(1-naphthyl)ethylenediamine dihydrochloride)

Safranine O (3,7-diamino-2,8-dimethyl-5-phenylphenazinium chloride)

Table III.- Sulphite determination by flow injection analysis in muscle based foods.

	Sample	Extracion	Hydrolysis	Donor Reagent	Separation	Acceptor Reagent (colorimetric reagent)	Detection system	Ref
Sulphites	Gulf shrimp, shrimp and other food and beverages (wine, juice, guacamole, apricots, potatoes, onions, apples lettuce, cabbage)	Sodium Tetrachloromercurate (TCM)	-----	Sulfuric acid	Gas diffusion cell	Malachite green	Spectrophotometric 615 nm	Sullivan et al. 1986
Total sulphites	Shrimp and other food and beverages (patatoes, dried pineapple, white wine)	TCM	NaOH	Sulfuric acid	Gas diffusion cell	Malachite green	Spectrophotometric 615 nm	Sullivan et al. 1990
Total Sulphite	Food and beverages	TCM	NaOH	Sulfuric acid	Gas diffusion cell	Malachite green	Spectrophotometric 615 nm	AOAC 2005 (n° 990.29)