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

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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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19 The simplest FIA system (see Figure 1) consists of a tube through which the carrier  
20 stream moves towards the flow-through detector. Briefly, it consists of the following  
21 basic elements:  
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- 24 - **Propulsion system.** This is generally a peristaltic pump with a variable number  
25 of channels, depending on the moving or reactive (carrier) phases which  
26 intervene in the reaction. It provides a constant flow of a carrier solution.  
27
- 28 - **Injection system.** This is normally a low pressure Rheodyne valve which allows  
29 a small volume of the sample, usually measured in micro liters, to be inserted  
30 into the flow of the carrier solution. The use of valves with various ports allows  
31 the simultaneous determination of various analytes.  
32
- 33 - **Reaction coils and complementary components.** The most frequently used  
34 reactors are tubes made from various materials such as polytetrafluorethylene or  
35 plastic tubing of different sizes and diameters (e.g. ID 0.8 mm), which can be  
36 coiled, knitted or knotted. The purpose of this geometric deformation is to  
37 decrease the zone dispersion. These coils can be thermostated. Sometimes the  
38 system can also have other integrated elements in series or in parallel (Figures 2  
39 and 3) such as columns (e.g. to cause chemical reactions – as with cadmium for  
40 the transformation of nitrates to nitrites or to immobilize reactants), or gas  
41 diffusion cells (e.g. for separating SO<sub>2</sub> or NO<sub>2</sub>).  
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- 44 - **Detection systems.** A wide variety can be used, including potentiometric,  
45 amperometric, spectrophotometric, fluorometric, etc.  
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- 48 - **Data collector system.** This is normally carried out from a PC linked to the  
49 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<sup>-1</sup> for nitrite and 300 mg kg<sup>-1</sup> 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<sup>-1</sup> and 1718 mg kg<sup>-1</sup>, respectively. For immersion-cured and massaged or pumped products those limits are 200 mg kg<sup>-1</sup> and 700 mg kg<sup>-1</sup>, respectively. In dry cured products nitrite is limited to 625 mg kg<sup>-1</sup> and nitrate to 2187 mg kg<sup>-1</sup> (USDA 2007). The ADI for nitrites, as nitrite ion, is 0.07 mg kg<sup>-1</sup> body weight, and 3.7 mg kg<sup>-1</sup> 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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33 Just as with other analytical methods, most of the nitrite and nitrate extraction processes  
34 by FIA determination use hot water and different systems for blending or homogenizing  
35 the sample and filtration (see Table II). An extraction system for cured meat based on  
36 microwave cooking of the samples following cold extraction (water and  $\text{NH}_4\text{Cl}$  at  
37 different pH) in a nitrogen atmosphere has been reported by van Staden and Makhafola  
38 (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). SO<sub>2</sub> 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<sup>+</sup> 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<sup>+</sup>.  
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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, NO<sub>2</sub>, HNO<sub>2</sub> and NOCl) are separated from the liquid stream by the  
30 home-made gas liquid separator and they are swept by the carrier O<sub>2</sub> 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 HNO<sub>2</sub> and NO<sub>2</sub> 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*

17 Although initially FIA was used only for nitrite determination, nitrate determination  
18  
19 was also developed later. Since for nitrate analysis the previous reduction of nitrate to  
20  
21 nitrite is required, FIA methodology included a reduction system. This means that in its  
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23 application in meat products the nitrate is reduced on-line to nitrite in a cadmium  
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25 column which is then treated with azo dye reagent and the absorbance due to the sum of  
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27 nitrite and nitrate is measured: nitrate is determined from the difference in absorbance  
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29 values (see Figure 2) (Ruiz-Capillas et al. 2006, 2007).  
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31 The simultaneous determination of nitrates and nitrites has been trialed since this  
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33 substantially reduces analysis times. Ahmed et al. (1996) determined nitrites and  
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35 nitrates (beef and pork sausages) simultaneously using a selector valve to split the  
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37 sample into two streams after it was injected into the carrier stream. The quantification  
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39 was based on the 3-nitroaniline+NED color reaction (see Table II). For different meat  
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41 products Ferreira et al. (1996) and Pinho et al. (1998) also determined nitrate and nitrite  
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43 simultaneously. The test was based on the splitting of the flow after injection and  
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45 subsequent confluence of the flow before it reached the detector, allowing the reduction  
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47 of nitrate to nitrite in part of the sample plug in an on-line copper cadmium reductor  
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49 column. Since each channel has a different residence time two peaks were obtained for  
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51 nitrite and for nitrite plus nitrate. The spectrophotometric determination was made after  
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53 a diazotization coupling reaction. A similar system for simultaneous determination of  
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55 nitrite and nitrate in various beef samples used flow injection with spectrophotometric  
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57 detection based on a oxidation system where the nitrate is reduced to nitrite by using  
58  
59 two sequential columns: copper and copper-coated cadmium (Ensafi and Kazemzadeh  
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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<sup>-1</sup>, expressed as  
14 SO<sub>2</sub> (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 SO<sub>2</sub>) is 0.7 mg kg<sup>-1</sup> 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. N<sub>2</sub> 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 SO<sub>2</sub> 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 SO<sub>2</sub> 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 SO<sub>2</sub> 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 SO<sub>2</sub>), a solution containing an acid-base indicator  
12 separated by a permeable membrane (generally made of teflon). The SO<sub>2</sub> 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 SO<sub>2</sub> 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 SO<sub>2</sub> 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<sup>-1</sup> 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<sup>-1</sup>. (Directives 95/2/EC and 2006/52/EC). The acceptable daily intake is 25  
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3 mg kg<sup>-1</sup> for sorbic acid (FAO/WHO 2007d) and 5 mg kg<sup>-1</sup> body weight for benzoic  
4 acid (FAO/WHO 2007e). The EFSA has established a full-group ADI of 10 mg kg<sup>-1</sup>  
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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determination of other preservatives present in meat and meat products (e.g. sorbates, benzoates etc.). It is also necessary to improve the present procedure in order to reduce the quantity of toxic reagent that is used in some of them. More studies of the extraction and cleaning procedures will be necessary for the application of FIA in complex matrices such as muscle based foods. Therefore preservative recovery should be improved and the reduction of possible interference associated with both natural matrix components and other food additives should be studied. Studies regarding the automatization of all the procedures must also be carried out, as well as reviewing the stabilising reagent for the extraction solution and its conservation to avoid substantial losses of preservatives. In depth studies will also be necessary to apply FIA procedures to new products (including meat based functional foods), which have been developed using new non-meat ingredients (e.g. from plant and marine sources).

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### **References**

- Ahmed MJ, Stalikas CD, Tzouwara-Karayanni SM, Karayannis MI. 1996. Simultaneous spectrophotometric determination of nitrite nitrate by flow-injection analysis. *Talanta* 43:1009-1018.
- Andrade R, Viana CO, Guadagnin SG, Reyes FGR, Rath S. 2003. A flow-injection spectrophotometric method for nitrate and nitrite determination through nitric oxide generation. *Food Chemistry* 80:597-602.
- Official Methods of Analysis of AOAC International; 2005; 17th Ed., AOAC International; Gaithersburg, MD, USA.
- Cassens RG. 1997. Residual nitrite in cured meats. *Food Technology* 51:53-55.
- Cassens RG, Greaser ML, Ito T, Lee M. 1979. Reaction of nitrite in meat. *Food Technology* 33:46-57.

- 1  
2  
3 Directive No 95/2/EC of the European Parliament and of the Council of 20 February  
4  
5 1995 on food additives other than colours and sweeteners.  
6  
7 Directive 2006/52/EC of the European Parliament and of the Council of 5 July amending  
8  
9 Directive 95/2/EC on food additives other than colours and sweeteners and  
10  
11 Directive 94/35/EC on sweeteners for use in foodstuffs.  
12  
13 EFSA. 2003. The effects of Nitrites/Nitrates on the microbiological safety of meat  
14  
15 products. The EFSA Journal 14:1-31.  
16  
17 Ensafi AA, Kazemzadeh A. 1999. Simultaneous determination of nitrite and nitrate in  
18  
19 various samples using flow injection with spectrophotometric detection,  
20  
21 Analytica Chimica Acta, 382:15-21.  
22  
23 Joint FAO/WHO. 2007a. Expert Committee of Food Additives Potassium nitrite  
24  
25 Sodium nitrite. [www.inchem.org/documents/jecfa/jecval/jec\\_1702.htm](http://www.inchem.org/documents/jecfa/jecval/jec_1702.htm)  
(accessed 06/27, 2007).  
26  
27 Joint FAO/WHO. 2007b. Expert Committee of Food Additives Potassium nitrate  
28  
29 Sodium nitrate. [www.inchem.org/documents/jecfa/jecval/jec\\_1701.htm](http://www.inchem.org/documents/jecfa/jecval/jec_1701.htm)  
30  
31 (accessed 06/27, 2007).  
32  
33 Joint FAO/WHO. 2007c. Expert Committee of Food Additives Sulfur dioxide.  
34  
35 [www.inchem.org/documents/jecfa/jecval/jec\\_2215.htm](http://www.inchem.org/documents/jecfa/jecval/jec_2215.htm) (accessed 06/27, 2007).  
36  
37 Joint FAO/WHO. 2007d. Expert Committee of Food Additives Sorbic acid.  
38  
39 [www.inchem.org/documents/jecfa/jecval/jec\\_2181.htm](http://www.inchem.org/documents/jecfa/jecval/jec_2181.htm) (accessed 06/27, 2007).  
40  
41 Joint FAO/WHO. 2007e. Expert Committee of Food Additives Benzoic acid.  
42  
43 [www.inchem.org/documents/jecfa/jecval/jec\\_184.htm](http://www.inchem.org/documents/jecfa/jecval/jec_184.htm) (accessed 06/27, 2007).  
44  
45 Fazio T, Warner CR. 1990. A Review of sulphites in foods: analytical methodology and  
46  
47 reported finding. Food Additives and Contaminants 7:433-454.  
48  
49 Ferreira IMPLVO, Lima JLFC, Montenegro MCBSM, Olmos RP, Rios A. 1996.  
50  
51 Simultaneous assay of nitrite, nitrate and chloride in meat products by flow  
52  
53 injection. Analyst 121:1393-1396.  
54  
55 Frenzel W, Schulz-Brussel J, Zinvirt B. 2004. Characterisation of a gas-diffusion  
56  
57 membrane-based optical flow-through sensor exemplified by the determination  
58  
59 of nitrite. Talanta 64:278-282.  
60  
61 Frenzel W, Hillmann B. 1995. Gas-diffusion flow-injection analysis to the  
62  
63 determination of sulfite and sulfur-dioxide in environmental-samples. Chemia  
64  
65 Analityczna 40:619-630.

- 1  
2  
3 Haghghi B, Tavassoli A. 2002. Flow injection analysis of nitrite by gas phase  
4 molecular absorption UV spectrophotometry. *Talanta* 56:137-144.  
5  
6  
7 ISO. 1996. ISO 13395, Water Quality—Determination of Nitrite Nitrogen and Nitrate  
8 Nitrogen and the Sum of Both by Flow Analysis (CFA and FIA) and  
9 Spectrometric Detection, Beuth, Berlin.  
10  
11 Jiménez Colmenero F, and Blazquez J. 2007. Additives: Preservatives. In: Handbook of  
12 Processed Meats and Poultry Analysis. Ed. Nollet, L.; Toldrá, F. Taylor and  
13 Francis, LLC, Boca Raton, USA. (in press).  
14  
15  
16 Karovicová J, Simko P. 2000. Determination of synthetic phenolic antioxidants in food  
17 by high-performance liquid chromatography. *Journal of Chromatography A*  
18 882:271-281.  
19  
20  
21  
22 Kazemzadeh A, Ensafi AA. 2001. Sequential flow injection spectrophotometric  
23 determination of nitrite and nitrate in various samples. *Analytica Chimica Acta*  
24 442:319-326.  
25  
26  
27  
28 Kim HJ, Kim YK. 1986. Analysis of free and total sulfites in food by ion  
29 chromatography with electrochemical detection. *Journal of Food Science*  
30 51:1360-1986.  
31  
32  
33  
34 Molina AR, Alonso EV, Cordero MTS, de Torres AG, Pavon JMC. 1999.  
35 Spectrophotometric flow-injection method for determination of sorbic acid in  
36 wines. *Laboratory Robotics And Automation* 11 (5): 299-303.  
37  
38  
39 Oliveira SM, Lopes TMS, Rangel AOSS. 2004. Spectrophotometric determination of  
40 nitrite and nitrate in cured meat by sequential injection analysis. *Journal of Food*  
41 *Science* 69:C690-C695.  
42  
43  
44 Penteadó JC, Angnes L, Masini JC, Oliveira PCC. 2005. FIA-spectrophotometric  
45 method for determination of nitrite in meat products: An experiment exploring  
46 color reduction of an azo-compound. *Journal of Chemical Education* 82:1074-  
47 1078.  
48  
49  
50  
51 Pinho O, Ferreira IMPLVO, Oliveira MBPP, Ferreira MA. 1998. FIA evaluation of  
52 nitrite and nitrate contents of liver pates. *Food Chemistry* 62:359-362.  
53  
54  
55 Pizzoferrato L., Di Lullo G, Quattrucci E. 1998. Determination of free, bound and total  
56 sulphites in foods by indirect photometry-HPLC. *Food Chemistry* 63:275-279.  
57  
58  
59 Rüter A. Bergwerff AA. 2005. Analysis of chemical preservatives in foods, in methods  
60 of analysis of food components and additives, Ötles, S., Ed., Taylor & Francis,  
Boca Raton, FL, chap.14.

- 1  
2  
3 Ruiz-Capillas C, Aller-Guiote P, Carballo J, Jiménez-Colmenero F. 2006. Biogenic  
4 amine formation and nitrite reactions in meat batter as affected by high-pressure  
5 processing and chilled storage. *Journal of Agricultural and Food Chemistry*  
6 54:9959-9965.  
7  
8  
9  
10 Ruiz-Capillas C, Aller-Guiote P, Jiménez-Colmenero F. 2007. Application of flow  
11 injection analysis to determine protein-bound nitrite in meat products. *Food*  
12 *Chemistry* 101:812-816.  
13  
14  
15 Ruzicka J, Hansen EH. 1975. Flow Injection Analyses .1. New Concept of Fast  
16 Continuous-Flow Analysis. *Analytica Chimica Acta* 78:145-157.  
17  
18  
19 Sen NP, Baddoo PA, Seaman SW. 1994. Rapid and sensitive determination of nitrite in  
20 foods and biological-materials by flow-injection or high-performance liquid-  
21 chromatography with chemiluminescence detection. *Journal Of Chromatography*  
22 *A* 673:77-84.  
23  
24  
25  
26 Sullivan JJ, Hollingworth TA, Wekell MM, Meo VA, Etemadmoghadam A, Phillips  
27 JG, Gump BH. 1990. Determination of total sulfite in shrimp, potatoes, dried  
28 pineapple, and white wine by flow-injection analysis - collaborative study.  
29 *Journal of the Asociation of Official Analytical Chemists* 73:35-42.  
30  
31  
32  
33 Sullivan JJ, Hollingworth TA, Wekell MM, Newton RT, Larose JE. 1986.  
34 Determination of sulfite in food by flow-injection analysis. *Journal of the*  
35 *Asociation of Official Analytical Chemists* 69:542-546.  
36  
37  
38  
39 Torro IG, Mateo JVG, Calatayud JM. 1998. Flow-injection biamperometric  
40 determination of nitrate (by photoreduction) and nitrite with the  $\text{NO}_2^-/\text{I}^-$  reaction.  
41 *Analytica Chimica Acta* 366:241-249.  
42  
43  
44  
45 USDA. 2007. Processing Inspector's Calculations Handbook.  
46 <http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7620-3.pdf> (accessed  
47 26.6.07).  
48  
49  
50 Usher CD, Telling GM. 1975. Analysis of nitrate and nitrite in foodstuffs. *Critical*  
51 *Review Journal of The Science of Food and Agriculture* 26:1793-1805.  
52  
53  
54 Valcarcel M, Luque de Castro MD. 1987. Flow injection Análisis. Principles and  
55 Applications. Ellis Horwood, Chichester, Englasnd .  
56  
57  
58 van Staden JF, Makhafola MA. 1996. Spectrophotometric determination of nitrite in  
59 foodstuffs by flow injection analysis. *Fresenius Journal of Analytical Chemistry*  
60 356:70-74.

1  
2  
3 Wedzicha BL, Mountfort KA. 1991. Reactivity of sulfur-dioxide in comminuted meat,  
4  
5 Food Chemistry 39:281-1991.

6  
7 Wood R, Foster L, Damant A, Key P. 2004. Analytical Methods for Food Additives,  
8  
9 CRC Press Woodhead Publishing Limited, Boca Raton, Cambridge, 253.

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**Figure 1.-** Diagram of a basic Flow Injection Analyser

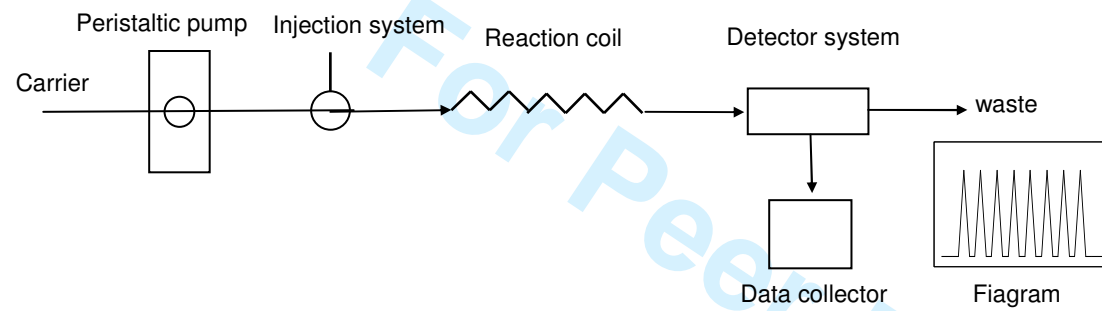
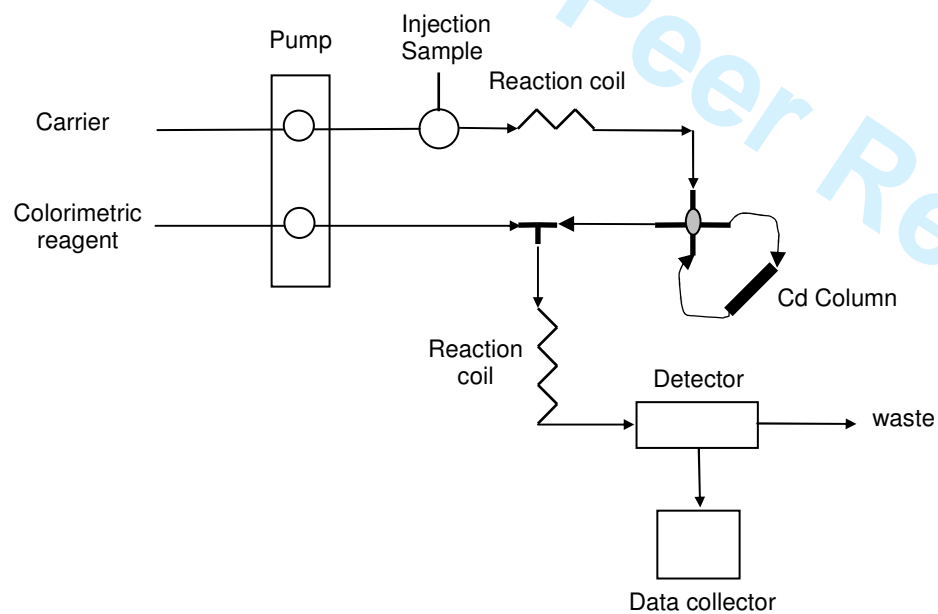
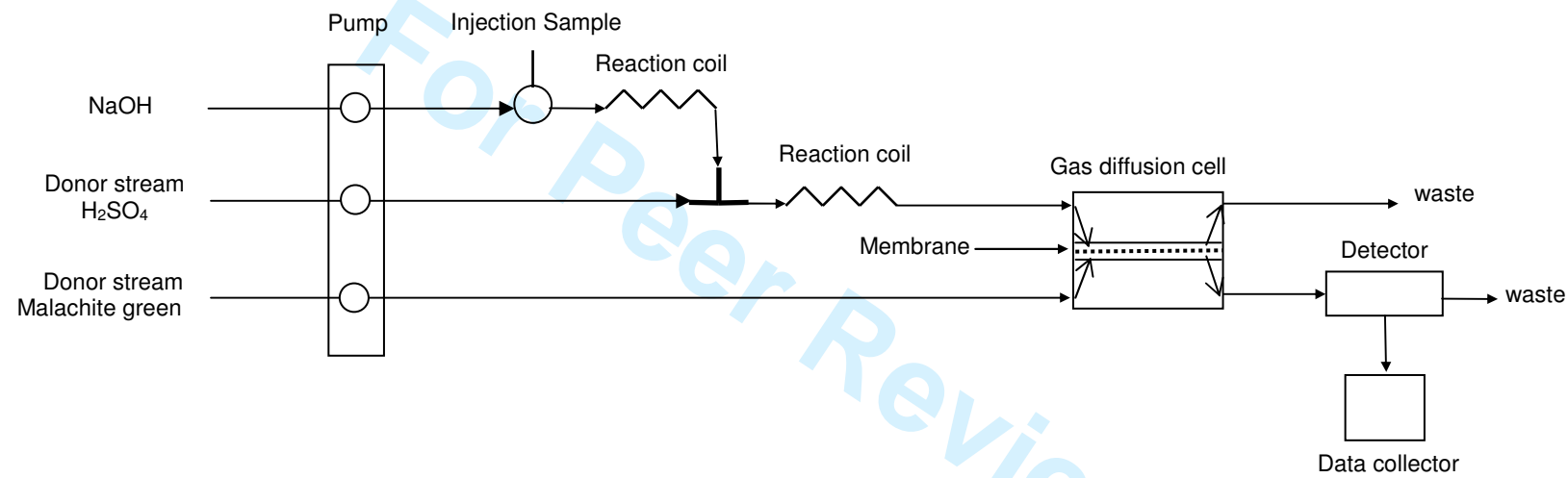


Figure 2.- Diagrams of Flow Injection Analyser for nitrite and nitrate determination



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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 <sup>1</sup>
	E-217	Sodium propyl p-hydroxybenzoate <sup>1</sup>
	E-218	Methyl-p-hydroxybenzoate
	E-219	Sodium methyl-p-hydroxybenzoate
Sulphites (SO <sub>2</sub> )	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 <sub>3</sub> ) & Nitrites (NO <sub>2</sub> )	E-249
E-250		Sodium nitrite
E-251		Sodium nitrate
E-252		Potassium nitrate

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

An SO<sub>2</sub> 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 <sub>3</sub> to NO <sub>2</sub>	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 <sub>2</sub> atmosphere with water and + NH <sub>4</sub> 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 <sub>2</sub> SO <sub>4</sub> /iodine	I <sup>3</sup> /I <sup>-</sup> 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 <sub>2</sub> 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 <sub>2</sub> SO <sub>4</sub>	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	-----	Safranin 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)

Safranin 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)