

7 Analysis of iodine in milk by Inductively Coupled Plasma – Mass Spectrometry

7.1 Chemical property of iodine

Iodine (atomic number and weight: 53 and 126.9) is a halogen of 17th group and in the elemental state (I₂) is a slight-bright black solid which sublimes at atmospheric pressure as a nostrils-irritant violet gas. The solubility in water is 0.33 g/L at 25°C. In the environment iodine occurs principally in the marine areas as iodide (I⁻), sodium (NaIO₃) and calcium (Ca(IO₃)₂) iodates. Within the halogens and non-metallic elements, it is the least reactive and has some semi-metallic characteristic. The elemental form is soluble in organic solvents like chloroform or hexane. In amylase form of starch, iodine forms the well-known blue complex used as indicator in redox reactions. There are 30 isotopes of iodine, however, only ¹²⁷I is stable (Cotton and Wilkinson, 1999).

7.2 The over-estimation given by the acid extraction of iodine

The bioavailable form of iodine in foodstuffs need analytical methods capable of determining concentration of µg/g and ng/g levels. The most efficient analytical techniques were developed in spectrophotometry (Moxon & Dixon, 1980), X-ray fluorescence (Crecelius, 1975), neutron activation analysis (Rao & Chatt, 1991) and inductively coupled plasma - mass spectrometry, ICP-MS (Vanhoe *et al.*, 1993). The sensitivity of ICP-MS depends by the first ionization (IE) energy of elements; the iodine IE (10.45 eV) offers low ionization in the argon ICP, and it has been estimated at 29% (Jarvis *et al.*, 1992). Nevertheless, since iodine is practically monoisotopic (¹²⁷I), ICP-MS is capable of rapid and sensitive determination of iodine and since LOD is 0.5 ng/g (UNI EN 15111:2007).

The question in iodine determination by ICP-MS is the extraction procedure. According to Vanhoe *et al.* (1993), an extraction procedure by nitric acid in samples containing iodide can increase the iodine signal and establish severe memory effects. When digesting samples with nitric acid, iodine could be selective evaporated in HI of I₂ from the largest droplets in the spray chamber, and in advance carried with the nebulizer gas stream to plasma. Consequently, after uptaking 100 ng/g NaI solution (dissolved in 0.14 mol/L nitric acid), blanks were affected by memory effects for about 23 minutes. The previous effect is reduced, but not eliminated, transforming matrix iodide to iodate by oxidants like potassium bicromate (K₂Cr₂O₇), hydrogen peroxide (H₂O₂) and sodium hypochlorite (NaOCl). The extraction of iodide using of 0.5% v/v ammonia solution, instead, resolved completely the over-estimation. The prevention of selective evaporation is probable due to the formation of NH₄I in ammonia solution. Julshmann *et al.* (2001), instead proposed a twice dilution of the nitric digest with 3% ammonia solution v/v in a ratio HNO₃/NH₄OH 1:1.

Therefore, alkaline extractions are suitable for the determination of iodine by ICP-MS.

7.3 The alkaline extractions

Within the various methods for iodine analysis in food matrixes provided by literature only three have the status of an official method. The method 992.22 and 992.24 (AOAC, 2007) describe the determination of iodide in pasteurized milk and skim milk powder by liquid chromatography, and in infant formula by ion-selective electrode (ISE), respectively. The official method for the determination of total iodine in foodstuffs after alkaline extraction is the EN15111:2007 (CEN, 2007). The ammonia solution, instead, was experimentally used with some variations by different authors (Baumann, 1990; Vahnoe *et al.*, 1993; Fernandez-Sanchez & Szpunar, 1999).

The EN 15111:2007 procedure is conducted using the “25% in water TMAH” with an iodine content less than 1 µg/L. The procedure consists of a closed digestion at 90°C lasting 3 hours (sample weight: 0.1-0.5g), followed by two filtrations (from 5 to 0.45 µm) or ultracentrifugation step (10 000 g) in order to eliminate any suspended particulate which could cloud the inline filter of the ICP-MS. The internal standard is Tellurium (Te). The interlaboratory studies (n = 13) in ready-to-serve milk pudding (0.68 mg I/kg) gave a repeatability (s_r) and reproducibility (s_R) standard deviations of 0.05 and 0.09 mg/kg, respectively (EN 15111:2007). Digesting the certified reference material (CRM) BCR 150, Fecher *et al.* (1998) measure by ICP-MS a iodine content of 1.30 ± 0.05 µg/g, compared to the certified value of 1.29 ± 0.09 µg/g.

Within laboratories, the most used methods are the EN15111:2007 and AOAC 992.24, which were recently compared in infant formula and certified materials by Hammer and Andrey (2008). The repeatability of methods, expressed as relative standard deviation (RSD), was similar ranging from 1.1 to 2.7. Also the recovery in BCR 063 (skim milk powder) and BCR 150 (milk powder) were both close to 100%.

Although efficient in precision and trueness, the procedure of TMAH extractions has a significant memory effect given by the use of not disposable vessels. The temperature control and the high temperature require hard and gas-tight materials, such as poly-tetrafluoro-ethylene (PTFE). Generally, nitric acid is used to digest many samples for the determination of various others minerals, unfortunately, the volatile compounds of iodine formed by the nitric oxidation can be absorbed by the walls of the PTFE vessels, providing a long-term contamination of iodine (Fecher *et al.*, 1998). The use of PTFE offers better results in new or fairly clean vessels, and therefore, the iodine extraction with TMAH requires much attention in washing procedure between sample charges.

The ammonia extraction can be a simple alternative to avoid the memory effect of not disposable materials. The procedure proposed by Fernandez-Sanchez & Szpunar (1999) for the determination of total iodine in milk is as follows: 2 ml of milk sample was added 5 ml of 0.5% v/v ammonia solution, then, sample was digested in a focussed microwave at 45 W for 2.5 min. Dilution (according the need) was followed with 0.5% v/v ammonia solution, and finally iodine content was determined by ICP-MS. Rhodium (Rh) was used as internal standard and the registered stability was probably because of the formation of Rh complexes in ammonia solution. The authors validated the method by analysing the certified reference material BCR 151: they obtained a total iodine concentration of 5.43 ± 0.06 µg/g, compared to the certified value of 5.35 ± 0.14 µg/g.

Since the ammonia extraction is performed in open system, it can be realized using disposable vessels (e.g. *DIGITUBE* by SCPScience) which permit to avoid source of error arising from the memory effect. In addition, the procedure is more rapid than the official method EN 15111:2007.

7.4 Speciation of iodine

The absorption and bioavailability of trace element is dependent by its chemical form, so it has been necessary to develop analytical techniques capable of determining the different form of organic and inorganic trace elements in foodstuffs. The speciation¹ methods capable of detecting iodine species in milk are the size-exclusion chromatography (SEC) and the ion chromatography (IC), both performed with coupled on line HPLC and ICP-MS.

Within HPLC techniques, SEC has the advantage of a high tolerance to the matrix and the compatibility of the mobile phase with ICP-MS. Sanchez & Szpunar (1999) investigated the iodine species in the whey and whole milk of different commercial cow, goat, human milk and infant formula by SEC-ICP-MS. The whey, casein and fat fractions were obtained by centrifugation at 50 000 rpm at 4°C for 15 min. The whey was filtered from the medium phase of centrifuged sample by 0.45 µm syringe filter prior to be injected in the SEC column. In commercial milk samples iodide was the mostly present chemical specie (from 70 to 86%), whereas, in infant formula the iodide recovery respect to total iodine was only 50%. Ultracentrifugation of samples with sodium dodecyl sulfonate (SDS) increased about 10-20% the iodide recovery in commercial milk, whereas for infant formula the increase was almost 40% (figure 18). Since infant formula are produced on the basis of hydrolysed cow's milk protein, the higher increase of iodine species recovery after SDS incubation was because of the disruption between iodine and protein bindings. In cow and goat milk, iodide appeared the major form of iodine, whereas the speciation in infant formula and human milk resulted in more complex profile (figure 19), particularly more than 50% of iodine in infant formula is bound in macromolecular compounds. The precision of peak area in SEC-ICP-MS was about 5% and the limit of specie-selective determination of iodide was about 1 µg/L.

Also ion-chromatography is a sensitive technique for the speciation of iodine species, particularly it is efficient for a rapid separation of iodide and iodate (figure 20). The methods described in literature differed principally for the mobile phase in which to eluate samples: for instance the buffer Na₂CO₃/NaHCO₃ at 3.5/1.0 mM/L ratio (Leiterer *et al.*, 2001), and 0.03 mol/L ammonium carbonate solution at pH 9.4 (Yoshida *et al.*, 2007; Li Bing *et al.*, 2006).

¹ Speciation analysis is the analytical activity of identifying and/or measuring the quantities of one or more individual chemical species in a sample. The chemical species are specific forms of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure. The speciation of an element is the distribution of an element amongst defined chemical species in a system (IUPAC, 2000).

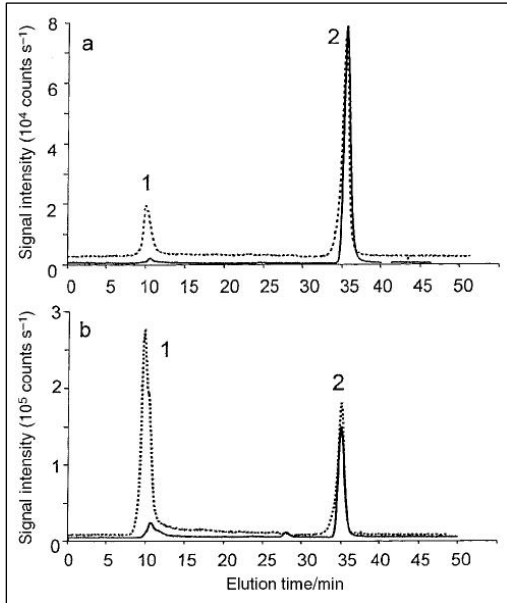


Figure 18. Effect of the SDS addition on the chromatographic profiles by SEC-ICP-MS of different milk samples. Sample without SDS (—) and sample incubated with SDS (···) according to Fernandez-Sanchez and Szpunar (1999).

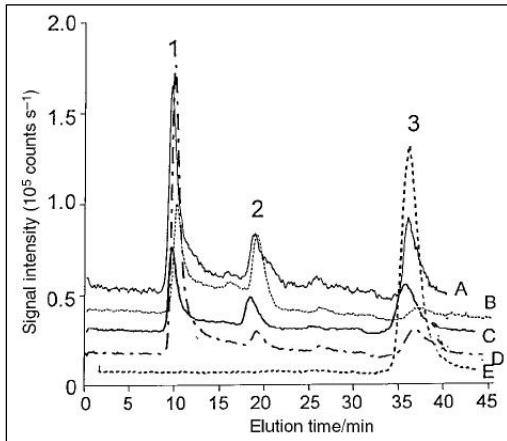


Figure 19. SEC-ICP-MS chromatographic profiles of infant formulas of different brands (A, B, C, D, E). Source: Fernandez-Sanchez and Szpunar (1999)

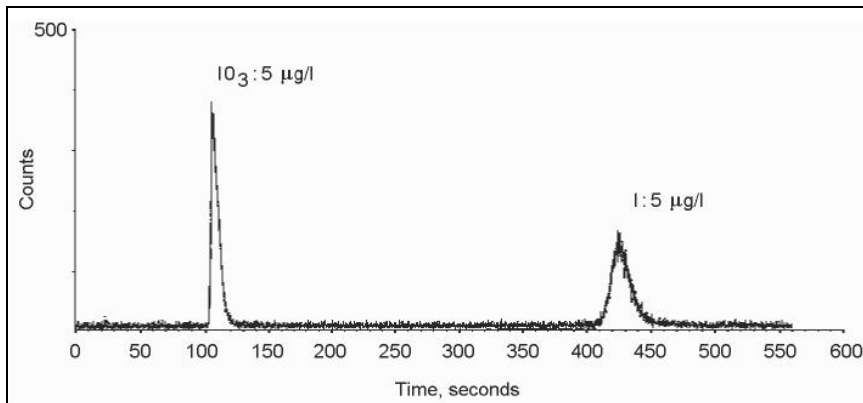


Figure 20. IC-ICP-MS chromatographic profiles for standard solution containing 5 µg/L of iodide and iodate. Source: Yoshida *et al.* (2007).