**Direct, rapid determination of tin in beverages using energy dispersive X-ray fluorescence**

ABSTRACT

In the work presented, a new method for determination of tin in beverages using EDXRF was developed, focusing on measurement simplicity and minimizing measurement time. Absorption coefficients for aqueous calibration samples were calculated and shown to be nearly identical to absorption coefficients of beverage samples. It was therefore possible to use aqueous standard solutions for external calibration. Beverage samples could then be measured directly, using the external calibration. The LOD and LOQ concentrations for this method, with a 4 minutes measurement time, were found to be 4 mg L-1and 15 mg L-1, and the precision was 3.89%. A variety of canned beverages (cold coffee, several fruit juices) were measured, and the results compared to concentrations obtained using ICP-OES(after digestion). The two methods showed good compatibility, establishing thenewly developed method as a rapid, simple, and accurate method for tin determination in beverages.

**1. Introduction**

Tin is a grey-white metal, obtained mainly from the mineral cassiterite, with many industrial applications. The primary uses of tin are for solder alloys (34% of global production) and for protective coatings, mainly in food containers (25-30% of global production)[1]. The main source of tin intake by humans is through diet. Today, most tin-lined food cans are lacquered to prevent tin reaction with food[2,3]. However, unlacquered tin-lined cans are still in use, mainly for light colored fruits juices, as tin helps maintain the color of the juice by preventing oxidization. Tin concentration in food was found to increase if the coating of the can is damaged, with increasing food acidity, with exposure to oxygen or heat, and with aging [3–5]. Tin is not considered highly toxic since the absorption of inorganic tin from the gastrointestinal tract is low for humans. However, consuming inorganic tin can cause liver and kidney problems, stomachaches, and anemia. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that acute manifestations of gastric irritation may arise from consumption of 150 mg L-1 of tin in canned beverages[1,2]. EU regulation sets the maximum allowed tin content in canned beverages, including fruit and vegetablejuices, at 100 mgkg-1[6].
There are a variety of methods for tin determination in beverages, includingICP-OES [7,8], FAAS [9], UV-Visible spectrophotometry [10], fluorimetry [11], and potentiometry [12]. All these methods provide very high sensitivity, with limits of detection usually in the ppb to sub-ppb levels. However, such sensitivity is not a requirement for tin determination in beverages, due to the relatively high permitted value. Additionally, these methods require sample preparation, increasing the complexity of the method and prolonging the measurement time required.
Energy dispersive X-ray fluorescence (EDXRF) is a multielement, nondestructive technique capable of measuring powder, solid and liquid samples. When a sample is exposed to X-ray radiation, electrons are ejected from the atoms in the sample (photoelectric effect). Higher energy electrons transfer to fill the vacancies formed, emitting either X-ray photons (with specific energy for each element) or Auger electrons. By measuring the energy of the X-ray fluorescence spectra EDXRF can quantitively identifythe elements in the sample [13].Modern EDXRF instruments have two main modes of measurement, direct and indirect(secondary target)[14]. Direct measurement uses the X-ray beam emitted from the source of the instrument, often placing a filter between the source and the sample. A filter adjusts the energy of the beam by absorbing much of the unused primary spectrum. Such filtration decreases background noise, reduces deadtime and increases the count throughput. Secondary target XRF uses a secondary target to excite the sample. First, the source beam is used to irradiate a secondary target crystal. The sample is excited by the emission beam of the target crystal (nearly monochromatic radiation) leading to a large decrease in the background noise and matrix effects.
EDXRF systems are implemented in element analysis in fields such as archeology [15,16], water quality[17,18], vegetation [19–21], nuclear industry [22,23] and foodstuff [20,21,24].The majority of EDXRF applications use solid samples (pressed pellets, fused beads or thin materials such as membranes) for optimal measurement sensitivity and accuracy [25]. The use of EDXRF in Liquid is not as common, due to higher background noise and possible bubble formation, therefore analytes in liquid samples are often separated and concentrated onto solids [26–28].However, the relatively high concentration of tin permitted in beverages makes direct liquid measurements by EDXRF viable. Sample preparation can there fore be practically eliminated, greatly increasing analysis speed and simplicity.
In this work, we show that due to the negligible differences in matrix absorption, aqueous calibration can be used for tin determination in beverages. The method is optimized by examining different measuring conditions. Characteristics such as limit of detection, accuracy, precision,and interferences are determined. The method is then applied to a variety of canned beverages, and the results are validated by comparison to results obtained using ICP-OES.

**2. Experimental**

*2.1. Materials*

Certified standard solutions (1 gL-1) of Sn, Ag, Al, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Ni, P, Pb, Sb, Zn, and Zr were purchased from CPA chem. Superpure HNO3was purchased from J. T. Baker, and 30% H2O2 waspurchased from Merck. Canned beverages were obtained from local supermarkets. High-purity water (18.2 MΩ·cm, Millpore Co., USA) was used for all measurements.

*2.2**. Instruments and apparatus*

EDXRF measurements were performed on a Xenemetrix X-7600 EDXRF (Rh tube, SDD detector, 60 kV, 400W, with eight filters and eight targets) and emission intensity obtained using nEXT(version 4.014.3) software. Instrument dead time was maintained between 30% to 45%. Samples were contained in plastic sample cups (10 mL volume)with a 6 µm polypropylene film support at the bottom of cup.Each sample was measured three times, under air. Absorption coefficients for different sample compositions were calculated using XCOM software (NIST). Microwave digestion was performed using a milestone ETHOS EASY microwave,using preloaded digestion methods.
ICP-OES measurements were made on a Spectro Arcos ICP-OES (plasma power 1200 W, coolant flow 13 Lmin-1, auxiliary flow 0.8 Lmin-1, nebulizer flow 0.8 Lmin-1), using two Tin emission lines (189.991&175.790 nm).Transfer of liquids was performed using suitable micropipettes.

*2.3. Spiked beverage solution preparation*

A 2.5 mL mixture of a 1 gL-1 standard tin solution and purified water was transferred to a 25 mL volumetric flask. The purified water:tin volume ratio was determined by the final tin concentration required in the sample (a spike of 0, 50 or 100 mg L-1). The solution volume was then filled up to 25 mL using the beverage sample, shaken thoroughly and kept in a refrigerator until measurement.

*2.4. Procedure for tin determination using EDXRF*

Standard tin calibration solutions with concentrations of 0, 40, 80, 120, 160, 200 mg L-1 were prepared by dilution of a 1 gL-1 standard with purified water. For each standard, 5mL were transferred to a plastic sample cup with a mylar support film. The Sn(Kα) intensity of each sample was measured and acalibration curve was produced by plotting the Sn(Kα) intensities against the tin concentrations. Samples were prepared by adding 2 mL of purified water to 3 mL of the beverage sample, in a sample cup. The tin concentration of the sample could then be derived from the calibration curve using the sample's Sn(Kα) intensity.

*2.5.* *Sample digestion and tindetermination by ICP-OES*

A sample volume of 2 mL was transferred to a modified PTFE microwave vessel. Next, 9 mL of concentrated nitric acid and 1mL of 30% hydrogen peroxide were slowly added to the vessel sequentially. The vessel was capped, and the sample was digested in a microwave (180o C, 15 minutes heat up, 10 minutes heating). Once digestion was complete, the sample was transferred to a 50 mL volumetric flask and diluted using purified water. Finally, 4 mL of the sample was added to 6 mL of purified water. The tin concentration of the sample was then determined by ICP-OES. ICP calibration curve solutions (0, 0.5, 1, 1.5, 2, 2.5 mg L-1) were diluted from the 1 g L-1 tin standard.

**3. Results and discussion**

*3.1. Method principle*

According to EU regulations, the permitted concentration of tin in beverages is 100 mg L-1. This allows shifting the effort from an increase ofthe sensitivity of the analysis to ease of operation. The goal of the method for tin determination in beverages presented in this work was to minimize sample preparation and shorten measurement time while ensuring sufficient sensitivity and accuracy. This was achieved by using EDXRF, which required minimal sample preparation, and provides adequate sensitivity within a short acquisition time.

*3.2. Calibration*

Analysis of edible samples usually requires a relatively long sample preparation step in order to eliminate the effects of the sample matrix. In X-ray fluorescence, matrix effects arecomposed of enhancement and absorption [13,14]. Enhancement effects are generally caused when the X-ray emission of matrix elements is at close energetic proximity to be absorbed by the analyte. This effect is negligible in the case of tin determination in beverages, as there are no elements in the sample matrix which emit X-rays energetic enough to be absorbed by tin. Absorption effects occur because all atoms present in the sample (matrix and analyte) absorb photons from the X-raybeam emitted by the instrument. As more photons are absorbed by the matrix, fewer photons are left to excite the analyte.
The absorption of each element is determined by its mass absorption coefficient, and if the atomic composition of a sample is known, the absorption coefficient (µ[cm2g-1]) of the samples can be calculated. Samples with similar matrix absorption coefficients can beconsidered matrix matched, while samples with different coefficients require matrix correction. Absorption coefficients were calculated for the beverage samples and calibration standards to determine whetherthey are matrix matched. The calibration standards matrix is composed of purified water, with small amounts of nitric acid and hydrofluoric acid (originating from the tin standard). Beverage samples are composed of water, carbohydrates,and potassium (examination by EDXRF of several beverages found no other elements above trace concentration). Calculation results show the absorption coefficient at 25.27 keV(the Sn(Kα) X-ray energy) to be nearly identical: 0.498 cm2g-1 for the calibration standard, and 0.492to 0.499 cm2g-1for the beverages (dependent on carbohydrates concentration, see SI, fig. S1). It is therefore possible to utilize an external calibration curve produced using aqueous standard solutions to analyze the tin content of various beverages directly, with no matrix correction.

*3.3. Evaluation of measurement conditions*

*3.3.1. Emission lines of tin*

Three tin emission energies were examined: Sn(Kα), Sn(Kβ) and Sn(Lα) (at energies of 25.27, 28.45, 3.44 keV, respectively). For Sn(Kα) and Sn(Kβ) measurements were performed using a tungsten filter, while Sn(Lα) intensity was measured using a titanium target. Since the Sn(Kβ) intensity was significantly lower than that of Sn(Kα) and gave less reproducible results the Sn(Kβ) emission line was rejected. The Sn(Lα) measurements gave good results (SI, fig. S2). While L lines intensity is lower than K lines, indirect measurement resulted in greatly reduced background noise. However, the potassium Kα line (3.31 keV) produced significant interference for Sn(Lα). Since beverages often contain potassium, it was decided the method would be based on Sn(Kα) intensity measurements. It should be noted that due to tin soldering in the instrument, a permanent Sn(Kα) signal (calculated to be 65 mg L-1, by standard addition) is observed at the measuring conditions used. However, since the signal is stable and consistent in all measurements, it can be discarded by referencing.

*3.3.2. Voltage and current*

Emission line intensity is dependent on the source voltage and current, therefore it is essential to find optimized values for the measurement. Optimized values were determined by keeping one variable constant and varying the other while examining Sn(Kα) intensity (fig.1). Increasing the voltage shifts the intensity distribution of the continuum radiation towards higher energies. Optimized results are obtained when beam energy is slightly higher than the Sn(Kα) absorption edge. Optimized voltage is usually equal to about 3 times the binding energy on the analyte line. An aqueous sample containing 50 mg L-1 of tin was measured using voltages of 45-60 kV (current was kept at 5.5 mA). As expected, increasing the voltage led to a great increase in the intensity of the Sn(Kα) line, as well as improving the instrument dead time. Next, the sample was measured at different currents (4-5.5 mA), at 60 kV. The measured intensity was proportional to the current, increasing from 187.41 to 283.43 cps. Therefore, a voltage of 60 kV and a current of 5.5 mA were selected for this method.

*3.3.3. Sample volume*

Samples with "infinite thickness" are always preferred for XRF measurements[14]. Therefore, the effect of different sample volumes on Sn(Kα) intensity was examined. Samples of 120 mg L-1 tin with volumes of 2, 3, 4, 5, 6 mL were measured (fig.2). The Sn(Kα) intensity increased with the volume, nearly reaching a constant value ("infinite thickness") at the higher volumes. However, in order to avoid spillage from the sample cup, a volume of 5 mL was used. Since the samples do not have "infinite thickness", it is important to maintain precisely the same sample volume for all samples.

*3.4. Analytical parameters*

*3.4.1 Limits of detection and quantification*

The limits of detection (LOD) and quantification(LOQ) were determined using the IUPAC definition [29], presented in the equation:

$$x\_{L}=\overbar{x}\_{bl}+k∙sd\_{bl}$$

Where XL is the lowest intensity which can be measured with reasonable certainty of detection or quantification, $\overbar{x}\_{bl}$is the average intensity of 10 blank measurements, *k*is a factor (3 for LOD, 10 for LOQ) and *sdbl*is the standard deviation of the 10 blank measurements. While the LODusing EDXRF improves with analysis time, one of the goals of this method was short measurement time. Long exposure of samples to the X-ray beam may also heat up the sample and increases the odds of interference from bubbles formation. It was therefore decided to use a measurement time of four minutes for the method.The intensity was converted to concentration using a calibration curve, and the LOD value of 4 mg L-1, and LOQ value of 15 mg L-1 were derived. While many beverages contain tin concentrations below these values, they are very suitable to examine whether tin content is within the allowed limits.

*3.4.2. Precision*

The precision and accuracy of the method were examined for two tin concentrations. Each sample, containing50 or 150 mg L-1 of tin, was prepared separately from the calibration standards and measured 10 times. The precision of the method was determined using the coefficient of variation of the 10 measurements, giving a value of 3.89% and 1.49% for the 50 and 150 mg L-1 samples, respectively.

*3.4.3. Interference*

As mentioned previously, elements in the sample matrix can affect tin measurement. Solutions containing 50 mg L-1 of tin, along with 100 mg L-1 of various potentially interfering ions, were measured using our method and results are presented in Table 1.The 16 interfering ions examined include elements with emission energy similar totin (Sb, Cd, Ag), higher than tin (Pb, Ba) and elements likely to found in beverages (Al, Ca, K, Mg). Out of all the interfering ions examined, a noticeable effect was only observed in a solution containing 100 mg L-1 of silver. An overlap between the Ag(Kβ) and Sn(Kα) emissions (24.94 and 25.27 keV, respectively) leads to an increase in intensity at the Sn(Kα) emission energy. The overlap had only a slight effect when 50 mg L-1 Ag was present. However, since the presence of silver is not expected at all in beverages, this interference presents no real limitation on the method.

*3.5. Application of method*

Our method was applied for tin determination in eightcanned beverages. Samples included canned coffee drinks and several types of fruit juices, some in tin cans and some in aluminum cans. The Sn(Kα) spectra and intensity for the calibration standards along with a mango juice sample are presented in fig.3. For each beverage three measurements were performed, with spikes of 0, 50 and 100 mg L-1tin. Tn concentration in the beverages was then determined using both EDXRF and ICP-OES. Results are presented in Table 2. Neither can composition nor beverage type exhibited an effect on tin concentration. For all beverages, with no tin spike, only trace amounts of tin were observed. Such concentrations are lower than the LOD of the EDXRF method and could only be determined using ICP-OES. Examining the results of spiked beverages, the results obtained by the two instruments showed good compatibility. Comparing the measurement time using both instruments, calibration time was similar for both methods (ca. 4 minutes per standard). However, ICP-OES measurement, which requires sample digestion and dilution, was over an hour (for each sample). Meanwhile, the direct measurement using the EDXRF was only about 5 minutes per sample, highlighting the large advantage that comes from using EDXRF.

**4. Conclusions**

We have developed a method for tin determination in beverages using EDXRF. The goal of the method was to simplify the measurement and minimize measurement time. Absorption coefficients for aqueous calibration sample were calculated at Sn(Kα) and shown to be nearly identical to the absorption coefficients of beverage samples. It was therefore possible to use aqueous standard solutions for calibration and then measure beverage samples directly, without any digestion of the beverages required. The methods LOD and LOQ were found to be 4 and 15 mg L-1, respectively. The precision of the method was determined to be 3.89%. A variety of canned beverage samples (cold coffee, several fruit juices) were then measured, and the results compared to results obtained using ICP-OES. The two methods showed good compatibility, establishing the developed method as a rapid, accurate method for tin determination in beverages.

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