**Rapid direct determination of tin in beverages using energy dispersive X-ray fluorescence**

ABSTRACT

This article presents a new method for the simpler and faster determination of tin in beverages using EDXRF. Absorption coefficients for aqueous calibration samples were calculated and shown to be nearly identical to those of the beverage samples, thus permitting the use of aqueous standard solutions for external calibration. Beverage samples could then be measured directly using the external calibration. Determination of tin using this method takes 4 minutes. The LOD and LOQ were 4 mg L-1 and 15 mg L-1 respectively, and the precision was 3.89%. Different canned beverages (cold coffee, various fruit juices) were measured and the results compared to the concentrations obtained using ICP-OES after digestion. The two methods showed good compatibility, thus establishing the newly developed method as a rapid, simple, and accurate method for the determination of tin in beverages.

**1. Introduction**

Tin is a grey-white metal obtained mainly from the mineral cassiterite, and has many industrial applications. It is primarily used in solder alloys (34% of global production) and in 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 from reacting with food[2,3]. However, unlacquered tin-lined cans are still in use, mainly for light colored fruit juices, as tin helps maintain the color of the juice by preventing oxidization. Tin concentrations in food have been found to increase if the can coating is damaged, with increasing food acidity, with exposure to oxygen or heat, and with aging [3–5]. Tin is not considered highly toxic to humans, as the absorption of inorganic tin by the gastrointestinal tract is low. However, consuming inorganic tin can cause liver and kidney problems, stomach ache, 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 regulations set the maximum allowed tin content in canned beverages, including fruit and vegetable juices, at 100 mg kg-1[6].
There are a variety of methods for determination of tin in beverages, including ICP-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 required for tin determination in beverages, given the relatively high permitted value. Additionally, these methods require sample preparation prior to the determination, increasing their complexity and prolonging the measurement time.
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 identify the 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. This filtration decreases background noise, reduces dead time and increases the count throughput. With secondary target XRF, the source beam is used to irradiate a secondary target crystal. The sample is then excited by the beam emitted from the target crystal. The near-monochromatic radiation of this secondary beam means that background noise and matrix effects are greatly reduced.
EDXRF systems are implemented in element analysis in fields such as archeology [15,16], water quality [17,18], vegetation [19–21], the nuclear industry [22,23] and foodstuffs [20,21,24]. In most applications solid samples (pressed pellets, fused beads or thin materials such as membranes) are used, for optimal measurement sensitivity and accuracy [25]. The use of EDXRF in liquids is less common, due to higher background noise and possible bubble formation. For this reason, analytes in liquid samples are often separated and concentrated onto solids [26–28]. However, the relatively high permitted concentration of tin in beverages makes direct liquid measurements by EDXRF viable. Sample preparation can therefore be practically eliminated, greatly increasing the speed and simplicity of the analysis.
In this paper, we show that due to the negligible differences in matrix absorption, aqueous calibration can be used for the determination of tin in beverages. The method was optimized by examining different measurement conditions. Characteristics such as limit of detection, accuracy, precision, and interferences were determined. The method was then applied to a variety of canned beverages, and the results were validated by comparison with the results obtained using ICP-OES.

**2. Experimental**

*2.1. Materials*

Certified standard solutions (1 g L-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 HNO3 was purchased from J.T. Baker, and 30% H2O2 was purchased from Merck. Canned beverages were obtained from local supermarkets. High-purity water (18.2 MΩ·cm, Millipore 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, 400 W, with eight filters and eight targets) and emission intensity was obtained using nEXT (version 4.014.3) software. Instrument dead time was between 30% and 45%. Samples were contained in 10 mL plastic sample cups with a 6 µm polypropylene film support at the bottom of the cup. Each sample was measured three times under air. Absorption coefficients for different sample compositions were calculated using XCOM software (NIST).

Microwave digestion prior to ICP-OES 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 L min-1, auxiliary flow 0.8 L min-1, nebulizer flow 0.8 L min-1) using two tin emission lines (189.991 and 175.790 nm). Liquid transfers were performed using suitable micropipettes.

*2.3. Spiked beverage solution preparation*

A 2.5 mL mixture of a 1 g L-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 brought to 25 mL using the beverage sample, shaken thoroughly and refrigerated until measurement.

*2.4. Procedure for tin determination using EDXRF*

Standard tin calibration solutions with concentrations of 0, 40, 80, 120, 160, and 200 mg L-1 were prepared by dilution of a 1 g L-1 standard with purified water. For each standard, 5 mL were transferred to a plastic sample cup with a mylar support film. The Sn(Kα) intensity of each sample was measured and a calibration 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 sample’s tin concentration could then be derived from the calibration curve using its Sn(Kα) intensity.

*2.5.* *Sample digestion and tin determination by ICP-OES*

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

**3. Results and discussion**

*3.1. Method principle*

Under EU regulations, beverages may contain up to 100 mg L-1 of tin. This means that efforts can be shifted from increasing the sensitivity of the analysis to simplifying the determination. The goal of the method for tin determination in beverages presented herein was to minimize sample preparation and measurement time while ensuring sufficient sensitivity and accuracy. This was achieved by using EDXRF, which requires 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, the matrix effects are enhancement and absorption [13,14]. Enhancement effects are generally caused when the energy of the X-ray emissions of matrix elements is sufficiently high to be absorbed by the analyte. This effect is negligible in the case of tin 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 the atoms present in the sample (matrix and analyte) absorb photons from the X-ray beam 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[cm2 g-1]) of the samples can be calculated. Samples with similar matrix absorption coefficients can be considered matrix-matched, while samples with different coefficients require matrix correction.

Absorption coefficients were calculated for the beverage samples and calibration standards to determine whether they were matrix-matched. The calibration standard matrix consisted of purified water with small amounts of nitric acid and hydrofluoric acid (originating from the tin standard). The beverage samples consisted of water, carbohydrates, and potassium (examination by EDXRF of several beverages found no other elements above trace concentrations). The absorption coefficients at 25.27 keV (the Sn(Kα) X-ray energy) were found to be nearly identical: 0.498 cm2 g-1 for the calibration standard, and 0.492 to 0.499 cm2g-1 for the beverages (depending on carbohydrate concentration: see SI, fig. S1). An external calibration curve produced using aqueous standard solutions can therefore be used 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 25.27, 28.45, and 3.44 keV respectively. Measurements for Sn(Kα) and Sn(Kβ) 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 line intensity was lower than K line intensity, this indirect measurement resulted in greatly reduced background noise. However, there was significant interference from the potassium Kα line at 3.31 keV. Since beverages often contain potassium, it was decided to base the method 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 under the measurement 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 the optimal values for the measurement. These are determined by measuring Sn(Kα) intensity while keeping one variable constant and varying the other (fig. 1). Increasing the voltage shifts the intensity distribution of the continuum radiation towards higher energies. Optimal results are obtained when the beam energy is slightly higher than the Sn(Kα) absorption edge. The optimal voltage is usually approximately 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 at a current of 5.5 mA. As expected, increasing the voltage led to a great increase in the intensity of the Sn(Kα) line, as well as reducing the instrument’s dead time. The sample was then measured at different currents (4-5.5 mA) at a constant voltage of 60 kV. The measured intensity was proportional to the current, increasing from 187.41 to 283.43 cps over the range tested. A voltage of 60 kV and a current of 5.5 mA were therefore selected for this method.

*3.3.3. Sample volume*

Samples with "infinite thickness" are always preferred for XRF measurements [14]. The effect of different sample volumes on Sn(Kα) intensity was therefore investigated. Samples containing 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, approaching a constant value ("infinite thickness") at the higher volumes. However, it was decided to use a volume of 5 mL to avoid spillage from the sample cup. Since the samples do not have "infinite thickness", it is important to maintain precisely the same 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 definitions [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 LOD with EDXRF improved with a longer measurement time, one of the goals for this method was a short measurement time. Long exposure of samples to the X-ray beam may also heat up the sample and increase the risk of interference from bubble formation. It was therefore decided to use a measurement time of four minutes.

The intensity was converted to concentration using a calibration curve, and an LOD of 4 mg L-1 and an LOQ of 15 mg L-1 were derived. Although many beverages contain tin concentrations below these values, the permitted limits are much higher, hence the method’s LOD and LOQ are highly suitable for determining whether tin content is within said limits.

*3.4.2. Precision*

The precision and accuracy of the method were examined for two tin concentrations. Each sample, containing 50 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 values 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 and 100 mg L-1 of various potentially interfering ions were measured using our method: the results are presented in Table 1. The 16 interfering ions included elements with emission energies similar to tin (Sb, Cd, Ag) and higher than tin (Pb, Ba) as well as elements likely to be found in beverages (Al, Ca, K, Mg). Of all the interfering ions examined, a noticeable effect was only observed in a solution containing 100 mg L-1 of silver, in which an overlap between the Ag(Kβ) and Sn(Kα) emissions (24.94 and 25.27 keV respectively) led to an increased Sn(Kα) emission energy intensity. This overlap had only a slight effect with 50 mg L-1 Ag. In any case, as silver is very unlikely to be present in beverages, this interference is not a real limitation.

*3.5. Application of the method*

The method was used to determine the tin concentration in eight canned 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 and for a mango juice sample are presented in fig. 3. Three measurements were performed for each beverage after spiking with 0, 50 or 100 mg L-1 tin. The tin concentration was then determined using both EDXRF and ICP-OES. The results are presented in Table 2.

Neither the can composition nor the beverage type affected tin concentration. Only trace amounts of tin were observed in all unspiked beverages. Their concentrations were below the LOD of the EDXRF method and could only be determined using ICP-OES. Examining the results for spiked beverages, the two instruments showed good compatibility. Although the calibration time was similar with both methods (approximately 4 minutes per standard), the sample digestion and dilution required by the ICP-OES method prior to the determination lead to a test time of over an hour per sample. In contrast, direct measurement by EDXRF only took about 5 minutes per sample, thus highlighting the great advantage of this method.

**4. Conclusions**

We have developed a method for the determination of tin in beverages using EDXRF, with the aim of simplifying the measurement and minimizing measurement time. Absorption coefficients for aqueous calibration sample were calculated at Sn(Kα) and shown to be nearly identical to those of the beverage samples, thus permitting the use of aqueous standard solutions for calibration and direct measurement of beverage samples without the need for digestion. The method's LOD and LOQ were 4 and 15 mg L-1 respectively, and its precision was 3.89%. Different canned beverage samples (cold coffee, various fruit juices) were then measured, and the results compared to the concentrations obtained using ICP-OES after digestion. The two methods showed good compatibility, thus establishing the new method as a rapid, accurate method for the determination of tin in beverages.

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