

Rapid Determination of Mercury in Food Colorants Using Electrothermal Atomic Absorption Spectrometry with Slurry Sample Introduction

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The determination of mercury in different types of color food additives using electrothermal atomic absorption spectrometry is proposed. The procedure involves direct introduction of the additive samples as suspensions, and fast-program methodology is used to avoid mercury volatilization losses. Suspensions are prepared in a medium containing 0.1% w/v Triton X-100, 1% v/v concentrated nitric acid, 2% w/v potassium permanganate, and 3% w/v silver nitrate before being directly introduced into the furnace. Calibration uses aqueous standards, and compensation of the background is carried out using the Zeeman device. The characteristic mass is 61 pg and the detection limit is 59 pg. The reliability of the procedure is checked by comparing the results obtained with other results based on microwave-oven sample digestion, and by analyzing a certified reference material.

KEYWORDS: Electrothermal atomic absorption spectrometry; slurry sample introduction; mercury; color additives

INTRODUCTION

Mercury compounds are highly toxic because they are fat-soluble and easily absorbed and accumulated in erythrocytes and the central nervous system (1). Despite the widespread ban of these products in the European Community, they are still used in several applications, such as fungicides.

Color additives are added to foods and drinks to improve the color or modify the flavor (2), although such effects are not usually regarded as beneficial by consumers, who frequently suspect that they produce toxic effects. The authorization of a food additive requires that its safety be demonstrated, and many have been authorized by the European Community (3). However, any harmful effect could be increased by the presence of toxic metals proceeding from prior manufacturing stages. The presence of mercury is due to the addition of several chemical species that are used to modify the physical characteristics of the colorants. Thus, aluminum lakes are prepared by reaction of the colorant with aluminum hydroxide in aqueous conditions, and titanium dioxide additives consist of the purified product covered by small amounts of aluminum oxide or silica to improve the technical properties of the product. The specific criteria regarding the purity of color additives (3) nominate a limit for mercury of 1 µg/g, so that analytical methods with low limits of detection are needed.

The usual method for the determination of total mercury is atomic absorption spectrometry using the cold vapor method (CVAAS) (4). In this method, a digestion step at high temperature is necessary to destroy all organomercury compounds because only inorganic Hg(II) is quantitatively detected by CVAAS. This oxidation step is generally performed with potassium permanganate or potassium dichromate in a sulfuric acid medium, a step that runs the risk of contamination by the reagents used and which must be carried out in sealed vessels because of the high volatility of mercury. However, when the additives are aluminum lakes or titanium dioxide based products, the sample cannot be totally dissolved even with mixtures of concentrated acids or using digestion in closed pressurized systems. The ETAAS technique offers the advantage that solid samples can be directly introduced in the atomizer as suspensions (5), which is of special interest given the inherent difficulty in dissolving colorant samples. Several studies using the slurry-based approach have been proposed to determine mercury in different types of samples (6–11).

Procedures for the determination of metals in food color additives using atomic absorption spectrometry (AAS) are very scarce. Indeed, only three procedures have been found which use ETAAS to determine As, Cd, Cu, and Pb (12), or As, Pb, and Cd (13), or which use hydride generation (HGAAS) for measuring As (14). However, no studies have been found concerning the determination of mercury in these additives by using AAS.

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This paper represents the first study on the determination of mercury in food color additives using the ETAAS technique, fast-program methodology, and direct introduction of the samples into the atomizer in the form of slurries. The suspending solution contains Triton X-100, nitric acid, and potassium permanganate and silver nitrate as matrix modifiers. Zeeman-effect background correction is necessary to obtain consistent results. Contamination of the samples and losses due to volatilization of the mercury are avoided, and the sample preparation step is considerably simplified. The samples analyzed are colorants, aluminum lakes, and additives based on titanium dioxide. The procedure was checked by analyzing a standard reference material and by dissolving the samples using a closed system.

EXPERIMENTAL PROCEDURES

Instrumentation. An ATI-Unicam (Unicam Atomic Absorption, Cambridge, UK) 939QZ atomic absorption spectrometer equipped with a GF90 electrothermal atomizer were used. Pyrolytic platforms (ref 9423 393 95191) were obtained from ATI-Unicam. This instrument is equipped with both a deuterium-arc based corrector and a Zeeman correction device, which facilitate comparison between both correction modes. Measurements using a bandwidth of 0.5 nm were performed at 253.7 nm using a hollow cathode lamp operated at 8 mA. Argon was used as the inert gas, and the flow rate was 300 mL/min in all the stages, except atomization, during when the flow was stopped. Background-corrected integrated absorbance was used in all cases as the analytical signal. A Branson ultrasonic bath of 14-W constant power was used for homogenization of slurries.

Plastic (polypropylene) vessels of the type commonly used to collect clinical samples were found suitable for preparing and storing the solutions or suspensions. They were nitric acid-washed and rinsed with ultrapure water. Pipet tips were made of polypropylene.

Mineralization of the samples for comparison purposes was carried out in closed Teflon cups using a MLS-1200 MEGA microwave oven (Milestone) and a MDR-1000/6 rotor (Radiometer).

Reagents. All the chemicals used were of the highest purity available. High-quality water from a Milli-Q purification system (Millipore) was used exclusively. A mercury standard solution (1000 µg/mL) from Panreac (Spain) was diluted as necessary to obtain working standards. High-quality concentrated (65% w/v) nitric acid (Merck), Triton X-100 (Merck), 30% w/v aqueous emulsion of antifoam A concentrate 100% active silicone polymer (Sigma), silver nitrate (Sigma), and potassium permanganate (Sigma) were also used.

Samples and Reference Materials. Three different kinds of samples, dyes or colorants, lakes, and titanium dioxide additives, were used. They were commercial products supplied by a sweet factory. The aluminum lakes were prepared by reaction of the colorant with aluminum hydroxide in aqueous conditions. Next, the product was filtered, washed with water, and dried. The final product contained some unreacted alumina. The titanium dioxide consisted of the purified product covered by small amounts of aluminum oxide or silica to improve the technical properties of the product. The method was validated using a standard reference material, fly ash SRM 1633a, supplied by the National Institute of Standards and Technology (NIST; Gaithersburg, MD).

Procedures. The suspensions of color additives were prepared by weighing the samples (0.25–1 g) in plastic vessels and adding 5 mL of a solution containing 0.1 w/v Triton X-100, 1% v/v nitric acid, and 0.1% w/v silicon antifoam. The slurries were homogenized, sonicated for a few minutes to ensure the absence of lumps, and continuously stirred with a magnetic stirrer. Into an eppendorf tube were added, in the order specified, a 200-µL aliquot of the suspension, 20 µL of 40% w/v silver nitrate, and 60 µL of 10% w/v potassium permanganate, and the mixture was manually stirred. Aliquots of 10 µL were immediately taken and injected into the furnace. The fast-heating program given in **Table 1** was run, and the background-corrected peak

Table 1. Furnace Heating Program

step	temperature (°C)	ramp (°C/s)	hold (s)
dry	150	20	30
atomization ^a	800	0	8
cleaning	2600	0	3

^a The flow of argon was stopped during the atomization step.

areas due to the analyte were obtained using Zeeman-effect background correction. Calibration was performed using aqueous standards. The certified reference sample was analyzed in the same way.

To confirm the reliability of the procedure, the samples were previously analyzed for comparison purposes. Fractions (0.5 g) of the samples were weighed into Teflon cups, and 4 mL of concentrated nitric acid and 0.5 mL of concentrated hydrogen peroxide were added. The program used in the microwave oven consisted of 1 min at 250 W, 2 min at 400 W, 4 min at 600 W, 2 min at 750 W, 1 min at 1000 W, 1 min at 500 W, and 1 min at 250 W. For mineralization of the additives based on titanium dioxide, it was necessary to add 1.5 mL of concentrated sulfuric acid in a second step, and to apply the same heating program. However, even with this second treatment, mineralization of the samples was not complete. After this treatment, the samples were maintained in the closed cups for 10 min before being diluted with deionized water in 10-mL volumetric flasks. Solutions were analyzed by ETAAS.

RESULTS AND DISCUSSION

The suspensions of color additives tend to form particle agglomerates, thus hindering reproducible sampling by micropipet, while the aliquots deposited inside the atomizer escape through the hole, resulting in analyte losses. Consequently, several surfactants were added to the suspensions, and aliquots of 10 µL were injected. The addition of surfactants such as glycerol, poly(vinyl alcohol), or detergents considerably improved the stability of the suspension with respect to those in water, and better results were obtained using Triton X-100. Foaming was prevented by adding a silicon antifoam agent. However, only partial stabilization was achieved, and suspension aliquots were taken while they were being continuously stirred using a magnetic stirrer.

Selection of the Background Corrector System. The determination of mercury was carried out using the two most commonly used systems for background correction. Thus, a comparison study was performed using a 10% w/v suspension of a color additive and injecting consecutive aliquots into the atomizer using both correction systems. The best results as regards correction capability and analytical accuracy were obtained using the Zeeman correction system, which was therefore used in this study.

Optimization of the Furnace Heating Program. The use of fast-program methodology (15), in which both the drying and calcination steps were replaced by a modified drying step, seemed useful because of the high volatility of mercury, which makes it necessary to use low temperatures for the calcination step to avoid volatilization losses (4). Optimization of the furnace program was carried out using platform atomization and in the presence of matrix modifiers for both a 100 ng/mL Hg-(II) aqueous solution and a 15% w/v suspension of a colorant based on titanium dioxide (E-110) spiked with 100 ng/mL Hg-(II).

For the modified drying step, a temperature of 150 °C with a slow ramp of 30 s was selected to avoid sample sputtering and to minimize low-temperature losses of mercury. The influence of the holding time was also studied between 10 and

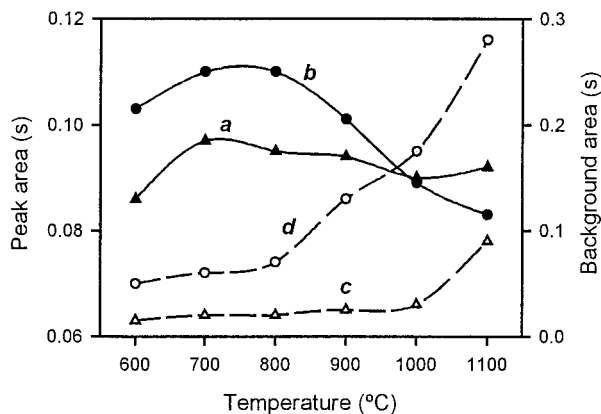


Figure 1. Effect of the atomization temperature on the signals for an aqueous solution containing 100 ng/mL Hg(II) (curves a and c) and for a 15% w/v E-171 additive suspension spiked with 100 ng/mL Hg(II) (curves b and d). Solid lines indicate the analytical signals and dotted lines indicate the backgrounds.

45 s to ensure that no losses were produced at long times. It was found that the signal was only reproducible between 30 and 45 s. Thus, a holding time of 30 s, which was sufficient for the total drying of the sample, was selected. No mercury losses were observed during this time because the analytical signal remained constant. Thus, it was proved that volatile inorganic mercurials were not lost because the temperature was not very high.

The influence of the atomization temperature on both the atomic signal and the background was studied between 600 and 1100 °C (Figure 1). The atomic signal increased up to 700–800 °C and decreased at higher temperatures for both the aqueous solution and the color additive suspension. A temperature of 800 °C, which provided the maximum signal for mercury and a low background signal for the suspension, was selected. The atomization time at this temperature was 8 s, whereas longer times were required for lower atomization temperatures. This low temperature permitted the mercury to be atomized before the matrix element (as can be seen in Figure 1, curve d), thus decreasing nonspecific absorption. However, since a powerful background correction system is employed, the presence of background was unimportant. To avoid the accumulation of carbonaceous residues inside the atomizer, it was also necessary to include a cleaning step of 2600 °C, the maximum temperature permitted by the atomizer. Table 1 shows the program as finally recommended.

Optimization of the Chemical Agent Concentrations. The addition of Triton X-100 was necessary to prevent the sample escaping through the tube. Several concentrations in the 0.01–0.5% w/v range were tried, and good dispersion of the additive suspensions and best reproducibility were achieved using 0.1% w/v. Silicon antifoam (0.1% w/v) was also added to avoid foaming.

Next, the concentration of the matrix modifiers was optimized. The loss of mercury by volatilization might be due to the reduction of Hg(II) and the vaporization of elemental Hg(0), adsorption of Hg, or the formation of stable compounds or amalgams (4). Thus, to determine the total mercury content, the addition of an oxidant agent such as permanganate (10, 16) or dichromate (16, 17) is recommended.

Consequently, potassium permanganate was selected and its effect was double, because it maintained mercury as Hg(II), avoiding its reduction to Hg(0), and also destroyed the organic matter of the color additive. Figure 2 shows the variation of

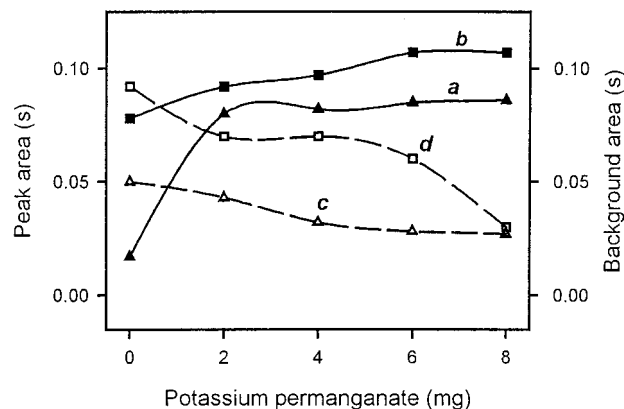


Figure 2. Effect of potassium permanganate on the analytical signals obtained for (a) a 100 ng/mL Hg(II) solution and (b) a 15% w/v E-171 color additive suspension fortified with 100 ng/mL Hg(II); effect on the background values (c) and (d) for the same samples, respectively.

the atomic signals and the backgrounds when the concentration of permanganate was varied between 0 and 8 mg for both an aqueous solution of 100 ng/mL Hg(II) and a 15% w/v E-171 additive suspension fortified with 100 ng/mL Hg(II). The atomic signals increased when the oxidant concentration was increased to 2 mg in the aqueous solution (curve a), and to 6 mg potassium permanganate in the suspension (curve b), after which it remained constant. Conversely, the background values decreased when the permanganate concentration increased (curves c and d). Thus, a 6 mg potassium permanganate (equivalent to 2% w/v) concentration, which led to the maximum atomic signal and minimum background values, was selected.

One of the chemical agents added to the colorants for modifying its texture is sodium chloride, which strongly interferes with mercury determination (18). The presence of chloride in the samples caused the appearance of double peaks and, depending on the conditions, losses through volatilization and interference in the gaseous phase. This effect can be noted in both the peak shape and the atomic signal. It has been demonstrated that the presence of silver substantially decreases chloride interference (10, 11) due to the formation of solid silver chloride, which acts as a collector, thus avoiding volatilization losses, or in a similar way to palladium, by forming an amalgam with mercury, which is retained.

Consequently, the effect of the presence of silver on the atomic signal of mercury and the background was studied for an aqueous solution of 100 ng/mL Hg(II) and a 15% w/v E-171 additive suspension spiked with 100 ng/mL Hg(II). Figure 3 shows that, for the Hg(II) aqueous solution, the atomic signal slightly decreased in the presence of silver, whereas for the suspension, the atomic signal increased with the silver concentration up to 8 mg. Consequently, this concentration (equivalent to approximately 3% w/v silver nitrate) was selected. The order of addition of the matrix modifiers (potassium permanganate and silver nitrate) must be kept as indicated to avoid interference by chloride (10).

The influence of the nitric acid concentration was studied in the 0–5% v/v range for a 15% w/v suspension of the E-110 lake and values practically constant for the atomic signal were observed throughout the range studied (Figure 4A). Background values only decreased when using a high (5% v/v) acid percentage which could damage the pyrolytic platform. Next, the influence of this variable on the fraction of mercury extracted into the supernatant was assayed by preparing different suspensions of the E-110 lake with varying nitric acid concentrations

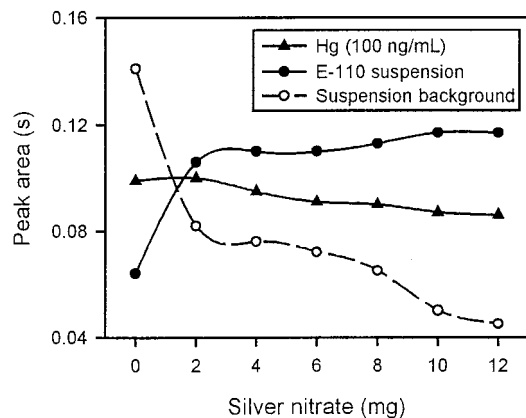


Figure 3. Effect of the silver nitrate concentration on the analytical signals for a 100 ng/mL Hg(II) aqueous solution and a 15% w/v E-171 additive suspension fortified with 100 ng/mL Hg(II). The dotted line represents the suspension background.

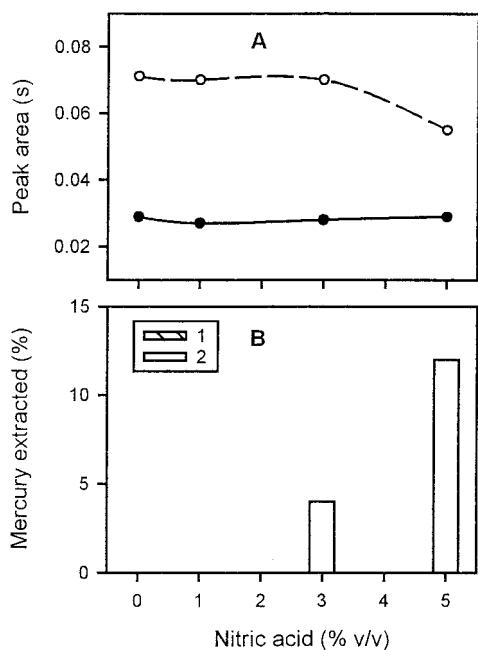


Figure 4. (A) Influence of the nitric acid concentration on the analytical signal (solid line) and the background (dotted line) for a 15% w/v E-110 lake suspension. (B) Effect of nitric acid on the extraction percentage of mercury to the liquid phase from the 15% w/v E-110 lake suspension, using procedures 1 and 2 indicated in the text.

in the 0–5% v/v range. The experiments were carried out using two procedures. First, the suspension containing the sample and nitric acid was centrifuged, and the supernatant was separated. Then, an aliquot was taken and the modifiers (permanganate and silver) were added before the sample was injected into the atomizer. In the second procedure, the suspension of the sample was directly prepared in the presence of nitric acid, permanganate, and silver. After centrifugation, an aliquot was injected into the furnace. **Figure 4B** shows the results obtained. As can be seen, in the absence of, or with low percentages of, nitric acid, no mercury was extracted into the supernatant by either procedure. This percentage slightly increased for the maximum concentration of the acid, particularly when the second procedure (with the matrix modifiers present during the extraction process) was used. In both cases the extraction percentage was very low, and a 1% v/v nitric acid concentration was selected.

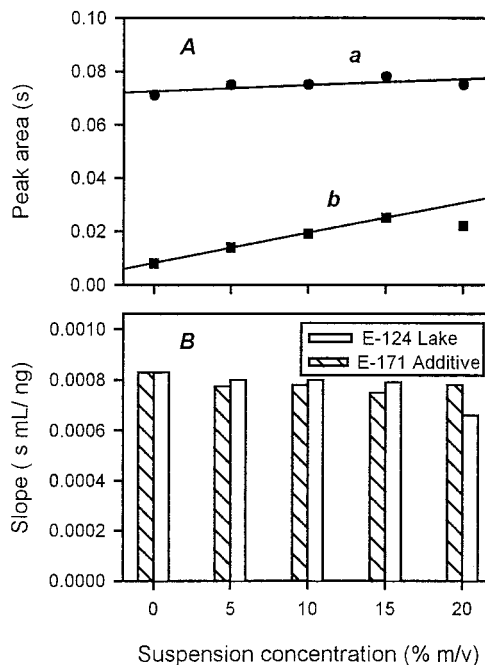


Figure 5. Influence of the suspension concentration on the analytical signals for mercury atomization from (A) an E-171 additive suspension spiked with 75 ng/mL Hg(II) and (B) an E-124 lake suspension. The bar graphs indicate the slopes of aqueous calibration and standard additions calibration graphs for the color additives at different concentrations.

Influence of the Suspension Concentration and Study of Matrix Effect. The influence of the suspension concentration was first studied for two samples of different natures such as the E-124 lake (with aluminum hydroxide) and the E-171 additive (with titanium dioxide) spiked with 75 ng/mL Hg(II). **Figure 5A** shows the variation of the atomic signals obtained for mercury when varying the suspension concentration in the 0–20% w/v range. As can be seen in curve b, which represents the variation of the signal for the lake containing mercury, linearity was observed up to 15% w/v, but at higher concentrations the signal decreased due to the appearance of a matrix effect. However, this limit must be considered as a guideline because it depends on the type of additive and the level of mercury present. For the additive which contained only added mercury, the signal was also constant up to 15–20% w/v.

The slopes of the aqueous calibration and standard additions calibration graphs for the E-124 lake and the E-171 additive suspensions at different concentrations were compared. The bar graphs included in **Figure 5B** show the results obtained. Each graph was constructed from four points, and each point was measured three times. As can be seen, the slopes for mercury standards and standard additions for different concentrations of the color additive suspensions were quite similar up to a 15% w/v concentration, but for higher suspension concentrations the slopes were lower due to interference by the matrix.

This study was extended to a wide number of additive samples of different bases, such as colorants, lakes of aluminum hydroxide, and titanium dioxide-based additives, to test whether matrix effects existed for a suspension concentration up to 15% w/v. The results depicted in **Table 2** show that the slopes of the best-fit regression lines for standard additions to the different suspensions varied by 1.6% (RSD) ($n = 13$). Consequently, for concentrations below 15% w/v, no matrix effect was evident and calibration could be carried out using aqueous standards.

Table 2. Slopes of Standard Additions Calibration Graphs to Different Additives

sample	slope (s mL/ng) ^a
aqueous standards	0.00078 ± 0.00005
E-171 (batch 1)	0.00077 ± 0.00008
E-171 (batch 2)	0.00080 ± 0.00012
E-171 (batch 3)	0.00078 ± 0.00007
E-171 (batch 4)	0.00079 ± 0.00009
E-171 (batch 5)	0.00080 ± 0.00013
lake E-124	0.00079 ± 0.00009
lake E-110	0.00077 ± 0.00011
lake E-102	0.00079 ± 0.00013
lake E-104	0.00079 ± 0.00008
lake E-132	0.00077 ± 0.00009
lake E-133	0.00078 ± 0.00006
green lake	0.00076 ± 0.00010

^a Mean ± standard deviation ($n = 3$).**Table 3.** Mercury Content of Color Additives and a Certified Reference Material

sample	suspension ^a ($\mu\text{g/g}$)	mineralization ^a ($\mu\text{g/g}$)
lake E-124	0.18 ± 0.02	0.18 ± 0.11
lake E-133	0.19 ± 0.05	0.19 ± 0.03
lake E-102	0.12 ± 0.01	0.14 ± 0.07
lake E-110	0.19 ± 0.02	0.17 ± 0.04
lake E-132	0.14 ± 0.02	0.21 ± 0.06
lake E-104	0.15 ± 0.03	0.13 ± 0.06
green lake	0.13 ± 0.01	0.14 ± 0.06
fly ash (1633a)	0.15 ± 0.01	0.16 ± 0.01 ^b

^a Mean ± standard deviation ($n = 4$). ^b Certified value NIST SRM.

Calibration Graphs and Repeatability. The calibration graph for mercury was linear up to 600 ng/mL and the characteristic mass was 61 pg. The detection limit calculated for 10 successive injections of the blank and using the 3 σ criterium was 59 pg. When using the maximum recommended suspension concentration (15% w/v), the DL was 39 ng/g. The repeatability was calculated using the relative standard deviation for 10 successive injections and the value found was $\pm 7.2\%$.

Results and Accuracy. The results obtained for the different color additives analyzed using the proposed procedure, as well as a reference method based on mineralization using a closed system, are summarized in **Table 3**. Mercury was only found in the lakes based on aluminum hydroxide at levels between 0.1 and 0.2 $\mu\text{g/g}$ (values below the maximum permitted level of 1 $\mu\text{g/g}$); however, mercury was not detected in the colorants or the additives based on titanium dioxide. A comparison of the results obtained by both procedures was carried out using the paired t -test and the value of the statistical $t = -0.737$ ($P = 0.489$) indicated no significant differences between the results obtained by both methods (confidence level 0.05). Consequently, the results demonstrate that the addition of aluminum hydroxide to the colorants produces a significant increase in the mercury content.

The reliability of the method was further established by using a certified reference material, fly ash (SRM 1633a), with a physical aspect similar to that of the colorants because certified color additives could not be found. The result obtained for mercury is also shown in **Table 3**, together with the certified value.

CONCLUSION

Acid mineralization of color additives is very difficult and may lead to volatile mercury losses or to incomplete dissolution of the sample when closed systems are used. Thus, the direct introduction of the color additives into the atomizer in the form of suspensions has considerable advantages since it reduces total analysis time by avoiding the prior stage of sample dissolution. The use of a fast program is advantageous because mercury losses through volatilization during the calcination step are also avoided. Calibration can be carried out using aqueous standards in the presence of both potassium permanganate and silver nitrate, which act as matrix modifiers. The presence of permanganate also helps to reduce the deposition of carbonaceous residues inside the atomizer. The results obtained demonstrate that the addition of aluminum hydroxide to the colorants produces a significant increase in the mercury content.

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