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Internal standardization combined with dilute-and-shoot preparation of distilled alcoholic beverages for Cu determination by high-resolution continuum source flame atomic absorption spectrometry

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ABSTRACT

Internal standardization (IS) and dilute-and-shoot preparation of distilled alcoholic beverages were evaluated for the direct determination of Cu by high-resolution continuum source flame atomic absorption spectrometry. Among Bi, In, Sn and Te evaluated as internal standards, Bi and Te furnished best results. Calibration curves were built up by plotting A^{Cu}/A^{Bi} and A^{Cu}/A^{Te} *versus* Cu concentration (0.05–4.0 mg L^{-1}) and linear correlation coefficients were 0.9994 and 0.9990, respectively. Contents of Cu in 22 commercial distilled beverages analyzed by the proposed method varied in the 0.029–3.608 mg L^{-1} interval. These results were in agreement (paired *t*-test) at 95% confidence level with those obtained by line source FAAS using standard addition calibration. Recoveries improved from 77–83% (without IS) to 99–101% (with IS) intervals. The relative standard deviation ($n = 12$) was 0.8–3.7% (IS-Bi), 0.4–4.4% (IS-Te), and 0.5–9.1% (without IS) and the limit of detection was *ca.* 5 μg L^{-1} using Bi or Te.

1. Introduction

Distilled alcoholic *beverages are* produced by distillation of fermented grains or fruits and are appreciated by world-wide people. The chemical composition, color, aroma and flavor of some *spirits* depend on the fermentation and distillation processes, and change appreciably by aging the distillate in wood barrels [1]. Most homemade spirit mills employ the traditional process based on copper alembics. The Cu and its compounds may be considered toxic to humans, so that accurate determination of this element in food samples is relevant [2].

Among main analytical spectrometric techniques, the flame atomic absorption spectrometry (FAAS) is suitable for the determination of Cu in distilled beverages providing that interferences caused by variations in chemical composition of samples are minimized. Standard addition [3], matrix-matching [4] and internal standardization [5] calibration methods are some strategies to reduce interference by transport effects. However, the time consuming (a full calibration for one sample), the relatively large sample volumes and extra mathematical treatments to obtain final results are among main disadvantages of the standard addition method. In matrix-matched calibration, the selection of the nature and amount of reagents to be used as a 'representative matrix' to calibrate a wide range of workable samples is not easy. Also, these reagents may influence the value of the analytical blank that often determines the *detection limit* capabilities. The internal standard calibration (IS) combines the simplicity of external calibration (a full calibration for all samples) with the effectiveness of standard addition, without the use of matrix-matching.

Since first proposal of IS in AAS in 1965 [6], homemade multichannel spectrometers were employed up to 1981 [7,8]. The applicability of IS has been further extended after 90s decade with the commercial availability of electrothermal atomization AAS instruments capable to monitor multiple lines simultaneously [9–12]. In theory, IS conceives a time-dependent correction as analyte and internal standard should be similarly influenced at a time by changes in experimental conditions. Most published papers about IS AAS employed simultaneous spectrometers with graphite furnace atomization because commercial flame instrument is not available yet. However, some studies on IS using flame AAS based on fast sequential mode have shown its potentiality to circumvent transport effects [13–16].

The Cu was determined in fruit juice [13] and sugar-cane spirits [14] and Fe in fuel ethanol [15] using respectively In, Ag, and Ni as internal standards and line source flame AAS instrument that uses a motor driven mirror to enable lamp selection. This type

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of equipment operates with a restricted number of fixed lamp sockets to set hollow cathode lamps. Furthermore, little combinations of *elements* are available for multi-element lamps, limiting then the wavelength election for IS [16]. Alternatively, a high-resolution double-Echelle monochromator and a charge-coupled device detector with a xenon short arc lamp continuum source makes feasible fast sequential detection in the 190–850 nm wavelength range [17]. A motor driven Echelle grade is used to vary the wavelength over CCD chip, so that the pixels are illuminated and read out simultaneously and act as independent detectors. This instrumental arrangement allows a quasi-simultaneous detection and seems suitable for IS AAS [18]. Transport effects in phosphoric acid and lubricant oil analysis for Pb [19] and Si [20] determination by high-resolution continuum-source flame AAS (HR-CS FAAS) were eliminated by using Co and W as internal standards, respectively.

The main purpose of this work is enlarge the achievability of the IS HR-CS FAAS for direct determination of Cu in a sort of distilled alcoholic beverages (cognac, whisky, vodka, rum, tequila, grappa, gin, and cachaça) presenting large variety of matrices. The elements Bi, In, Sn and Te were evaluated as internal standards as alternative for Ag employed for sugar-cane spirits [14] due to its weakness to precipitate as silver chloride and photo reduction for some special sample lots.

2. Experimental

2.1. Instrumentation

An Analytik Jena ContrAA 300 high-resolution flame atomic absorption spectrometer equipped with a xenon short-arc lamp (XBO 301, 300 W, GLE, Berlin, Germany) as a continuum radiation source, a compact high-resolution double - Echelle grating monochromator correspondent to a spectral band width < 2 pm per pixel in the far ultraviolet range and a charge-coupled device (CCD) array detector were used throughout the work. The optimization of the method was evaluated using the major atomic lines of Cu (324.754 nm), Bi (223.061 nm), In (325.609 nm), Sn (224.605 nm), and Te (214.281 nm). All measurements were carried out in five replicates using an injection module (SFS 6) enabling the computer-controlled aspiration of the solutions. The absorbance of the blank, analytical solutions and samples were obtained under optimum equipment conditions using a wavelength-integrated absorbance (WIA) equivalent to 3 pixels (CP ± 1, CP central pixel associated to measurement of central absorbance line) and aspiration rate fixed at 5.0 mL min^{-1}. An oxidizing air-acetylene flame was used to atomize Cu, Bi, In, Sn and Te. High-purity acetylene (99.7% Air Liquide, Brazil) was used as fuel gas. A Perkin Elmer AAnalyst 100 spectrometer (Shelton, CT, USA) with a deuterium lamp background corrector was used as comparative technique. The determination of Cu was carried out employing standard addition technique using an air-acetylene flame with Perkin-Elmer Lumina™ single-element hollow cathode lamps.

2.2. Reagents, analytical solutions and samples

High-purity de-ionized water obtained using a Millipore Rios 5® reverse osmosis and a Millipore Milli-Q Academic® deionizer system (resistivity 18.2 MΩ cm^{-1}, Millipore, Bedford, MA, USA), and nitric acid (70%, Spectrum Chemical®) were used throughout to prepare all solutions. All solutions and samples were stored in high-density polypropylene bottles (Nalgene®, Rochester, USA). Plastic bottles and glassware materials were cleaned by soaking in 10% (v/v) HNO_3 at least 24 h and rinsed abundantly in de-ionized water before use.

The elements Bi, In, Sn and Te were evaluated as possible internal standards, and were chosen due to their atomization characteristics and low probability to be found in distilled beverage samples.

Analytical working solutions containing 0.05, 0.10, 0.20, 0.50, 1.00, 1.50, 2.00, 3.00 and 4.00 mg L^{-1} Cu plus 10.0 mg L^{-1} IS (Bi, In, Sn and Te) were prepared daily by appropriate dilution of the 1000 mg L^{-1} stock standards solution (Tritisol®, Merck, Germany) and acidified to 1.0% (v/v) HNO_3 solution. The blank solution was 1.0% (v/v) HNO_3 + 10.0 mg L^{-1} IS.

Working solutions containing 1.00 mg L^{-1} Cu were prepared in ethanol–water medium at different ethanol (Merck, Germany, 99.8% purity) concentrations [(0.0, 1.0, 2.5, 5.0, 7.5, 10, 20, 30, 40, 50, 60, 70, 80 and 90% (v/v)]. These solutions were directly aspirated by the burner/nebulizer system of the spectrometer in order to evaluate the influence of transport effects on Cu absorbance.

The effectiveness of IS HR-CS FAAS was evaluated by means of direct analysis of 22 distilled alcoholic beverages (cognac, whisky, vodka, rum, tequila, grappa, gin, and cachaça) purchased at the local market in Araraquara city, São Paulo State, Brazil. A minimum sample preparation was adopted: 10-mL sample volume was spiked with 100 μL of 1000 mg L^{-1} Bi or Te plus 100 μL of concentrated nitric acid, and properly homogenized.

2.3. Measurement procedure

The measurement procedure is relatively fast and simple: absorbance for blank, analytical solutions and samples were measured successively at the optimized operating conditions. The analytical curve for Cu determination employing IS was built up by plotting the ratio of absorbance of Cu and Bi (A^{Cu}/A^{Bi}) or Cu and Te (A^{Cu}/A^{Te}) *versus* concentration of Cu in analytical working solutions. Contents of Cu in samples were obtained by interpolation of corresponding A^{Cu}/A^{Bi} or A^{Cu}/A^{Te} ratios on that plot.

Accuracy of the proposed IS HR-CS FAAS method was assessed using addition/recovery tests with and without IS for spiked beverage samples and anhydrous ethanol by adding appropriate aliquots of 1000 mg L^{-1} Cu stock standard in order to obtain solutions containing 0.50 and 1.00 mg L^{-1} Cu. For comparison propose, samples were also analyzed by LS FAAS using standard addition calibration method.

The limit of detection (LOD) and limit of quantification (LOQ) for Cu were calculated according to the IUPAC recommendation [21].

3. Results and discussion

3.1. Evaluation of the Bi, In, Sn and Te as internal standards

The elements Bi, In, Sn and Te were evaluated as internal standard alternatively to Ag employed for sugar-cane spirits [14] due to weakness of Ag ion to precipitate as silver chloride and photo reduction for some special sample lots. The concentration of a given element candidate as internal standard should be within the limit of quantification and the upper limit of linear response, and the optimum concentration of this element depends on its usual concentration in workable samples and the required precision. Absorbance–concentration plots were built up in the 1.0–50.0 mg L^{-1} Bi, In, Sn and Te, and corresponding plots were linear up to 15, 20, 20, and 10 mg L^{-1}, respectively. The suitable concentration interval to evaluate all elements together in mixed solutions was 1.0–10 mg L^{-1}. Besides Bi, In, Sn and Te are foreign elements in most distilled beverages, the selected concentration of Bi, In, Sn and Te for further studies was 10 mg L^{-1}, that is high enough to 'mask' absorbance fluctuations due to eventual presence of them at trace levels in samples. Thereafter, the efficacy of Bi, In,

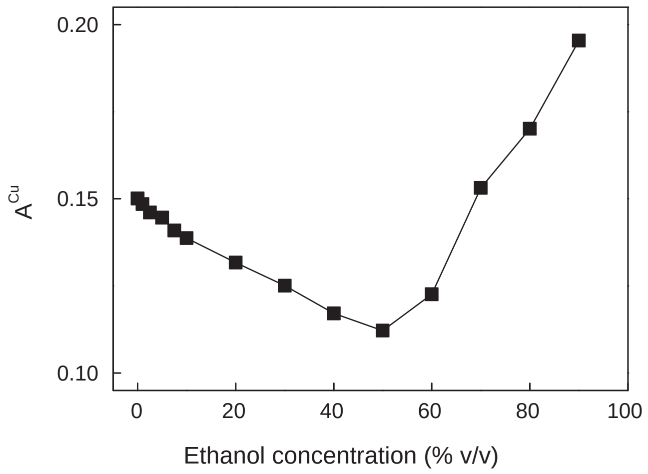

Fig. 1. Influence of variation of ethanol concentration on Cu absorbance. Measured absorbance corresponds to 1.0 mg L^{-1} Cu solution using WIA equivalent to 3 pixels.

Sn and Te as internal standards was checked by evaluating the influence of variation of ethanol concentration and flame composition on Cu absorbance.

The influence of ethanol concentration on absorbance of 1.0 mg L^{-1} Cu working standard was evaluated for different water–ethanol media (Fig. 1). When the ethanol concentration varied from 0 to 50% (v/v), absorbance decreased due to reduction of the aspiration rate of nebulizer from 5.0 to 2.0 mL min^{-1}. For hydro-ethanol solutions varying from 60 to 90% (v/v) ethanol, the aspiration rate increased, but only up to 3.6 mL min^{-1}. For sample introduction systems based on nebulization chambers, the efficiency of sample transport is high dependent on viscosity and surface tension. According to Rocha and Nobrega [22] the maximum viscosity of hydro-ethanol solution is reached for 50% (v/v) ethanol, which explains the effect observed in Fig. 1. It should be pointed out that high viscosities reduced the aspiration rate, and so the absorbance. However, the influence of hydro-ethanol solutions > 60% (v/v) ethanol may be caused by the surface tension, another physical property of these particular solutions. Solutions presenting lower surface tension are easily introduced into the nebulization chamber, facilitating the formation of a larger population of droplets. These transport effect in flame AAS can be circumvented by calibration methods based on matrix matching or standard addition, which require prior knowledge of alcoholic content of sample. Depending on the large scale analysis of routine, these calibration methods make the hole analytical procedure laborious and time consuming.

IS can be considered an effective strategy to eliminate errors by transport provided that a multi-element spectrometer is available. Shown in Fig. 2 are effects of Bi and Te (Fig. 2a) and Sn and In (Fig. 2b) on absorbance of Cu prepared in different ethanol concentrations. As the smallest variation in the ratio A^{Cu}/A^{IS} was observed for Bi and Te, these elements were then selected as promising internal standards for further studies.

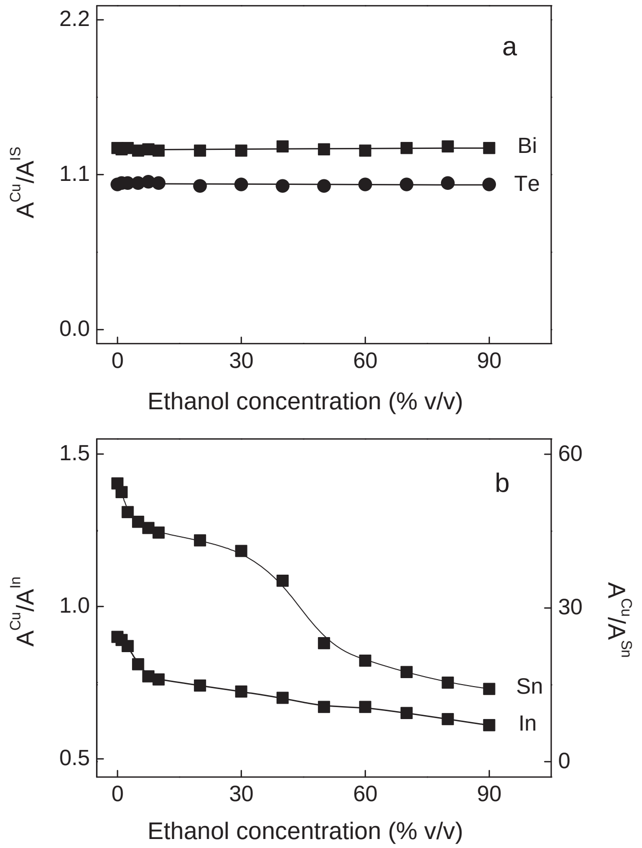

Fig. 2. Correlation graphics correspond to the absorbance ratios of 1.0 mg L^{-1} Cu to 10.0 mg L^{-1} Bi, In, Sn and Te at different water–ethanol media: (a) A^{Cu}/A^{Bi} and A^{Cu}/A^{Te}, and (b) A^{Cu}/A^{In} and A^{Cu}/A^{Sn}.

Variations in flame composition may change the flame temperature and atomization degree, which may vary analyte absorbance. However, if analyte and internal standard are similarly affected by alterations in flame conditions, the IS may minimize these absorbance variations and improve accuracy [5]. The influence of variation of flame composition on absorbance of 1.0 mg L^{-1} Cu was studied by varying the acetylene flow-rates (40–45–50–55–60–70 and 80 L h^{-1}) and keeping fixed the air flow-rate at 514 L h^{-1}. Shown in Fig. 3 is the behavior of Cu absorbance by variation of flame composition and Fig. 4 illustrates the compensation of this effect by IS. Analysis of this figure reveals that the behavior of Cu absorbance at different acetylene flow-rates is unstable, but the fast sequential measurements of Cu and Bi or Cu and Te by HR-CS FAAS allowed the use of IS to minimize effects caused by variation of flame composition. The Bi and Te furnished similar performances, so both elements were employed as internal standards for further experiments.

The poor performance of In and Sn as internal standards for Cu may be attributed to dissimilarity of physical properties to those elements and that for analyte. Analysis of fifteen chemical

Table 1
Some physical properties for elements under study and their oxides [23–27].

Property	Cu	Bi	In	Sn	Te
Enthalpy of fusion (J mol^{-1} K^{-1})	13.3	11.3	3.26	7.03	17.3
Molar heat capacity (J mol^{-1} K^{-1})	24.44	25.52	26.74	26.99	25.73
Dissociation energy of $MO_{(g)}$ (kJ mol^{-1})	269	333	320	529	263

	CuO	Bi_2O_3	In_2O_3	SnO	TeO_2
Melting point (°C)	1227	825	1912	1080	733

Table 2
Results (mg L^{-1}) expressed as mean ± standard deviation for Cu in distilled alcoholic beverages samples determined ($n = 5$) by HR-CS FAAS without IS, using Bi and Te as internal standards and by LS FAAS using standard addition calibration method (SAC).

Sample	HR-CS FAAS			LS FAAS
	Without IS	IS-Bi	IS-Te	SAC
Tequila	0.391 ± 0.005	0.553 ± 0.028	0.534 ± 0.026	0.549 ± 0.007
Gin	0.661 ± 0.009	0.745 ± 0.021	0.753 ± 0.019	0.735 ± 0.013
Cachaça	2.832 ± 0.031	3.608 ± 0.092	3.763 ± 0.135	3.677 ± 0.031
Tequila	0.225 ± 0.003	0.245 ± 0.012	0.282 ± 0.014	0.256 ± 0.002
Cachaça	2.793 ± 0.022	3.157 ± 0.063	3.249 ± 0.086	3.285 ± 0.021
Cachaça	2.645 ± 0.021	3.306 ± 0.045	3.324 ± 0.057	3.303 ± 0.025
Cachaça	1.784 ± 0.022	2.149 ± 0.050	2.198 ± 0.070	2.211 ± 0.013
Cachaça	1.393 ± 0.014	1.874 ± 0.041	1.914 ± 0.062	1.862 ± 0.019
Grappa	<LOD	<LOD	<LOD	<LOD
Gin	0.701 ± 0.004	0.889 ± 0.022	0.871 ± 0.034	0.859 ± 0.007
Cachaça	0.716 ± 0.004	0.802 ± 0.023	0.797 ± 0.034	0.775 ± 0.006
Cachaça	1.619 ± 0.020	2.284 ± 0.048	2.115 ± 0.063	2.202 ± 0.016
Rum	0.021 ± 0.001	0.029 ± 0.002	0.032 ± 0.001	0.033 ± 0.003
Rum	0.029 ± 0.002	0.038 ± 0.001	0.039 ± 0.002	0.040 ± 0.002
Cognac	0.019 ± 0.001	0.030 ± 0.002	0.031 ± 0.002	0.033 ± 0.001
Cognac	<LOD	<LOD	<LOD	<LOD
Cognac	0.145 ± 0.009	0.185 ± 0.005	0.190 ± 0.009	0.192 ± 0.011
Vodka	<LOD	<LOD	<LOD	<LOD
Vodka	<LOD	<LOD	<LOD	<LOD
Whisky	<LOD	<LOD	<LOD	<LOD
Whisky	0.103 ± 0.004	0.122 ± 0.008	0.124 ± 0.006	0.128 ± 0.010
Cognac	<LOD	<LOD	<LOD	<LOD

and/or physical data for Cu, Bi, In, Sn and Te reveals that enthalpy of fusion, molar heat capacity, dissociation energy of $MO_{(g)}$, and melting point of oxides (Table 1) are properties more significant than others that could explain the poor performance of In and Sn. Although dissociation energies of InO and BiO are close, the high melting point of In_2O_3 may explain the weakness of In as internal standard. This element offers more tendency of formation of stable volatile compounds at lower atomization conditions caused by changes in aspirating rate of sample solution and flame temperature and/or composition. In spite of the fact that SnO presents melting point lower than that for CuO, the shortcoming of Sn as internal standard may be related to the high dissociation energy of its oxide. These assumptions make In and Sn elements presenting higher temperature dependence of atomization efficiency than Bi and Te.

3.2. Analysis of samples

After internal standard selection, calibration curves in the 0.05–4.00 mg L^{-1} Cu were built up by plotting A^{Cu}/A^{Bi} and A^{Cu}/A^{Te} *versus* Cu concentration and typical linear correlation coefficients were 0.9994 and 0.9990 for Bi and Te, respectively. Acceptable precision and linearity were obtained with Bi and Te, hence both elements were applied to the determination of Cu in 22 commercial

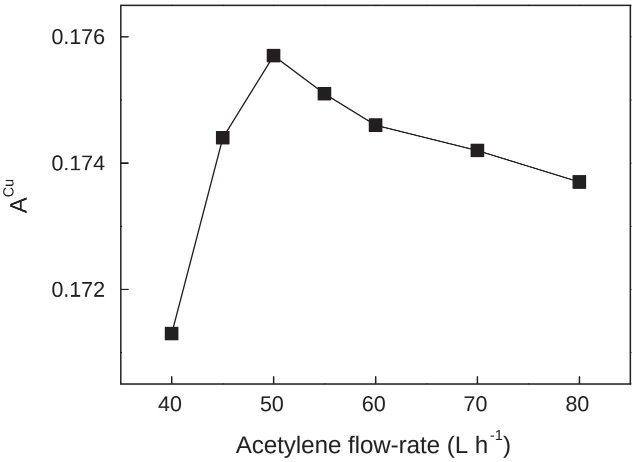

Fig. 3. Influence of variation of flame composition on Cu absorbance. Measured absorbance corresponds to 1.0 mg L^{-1} Cu using WIA equivalent to 3 pixels.

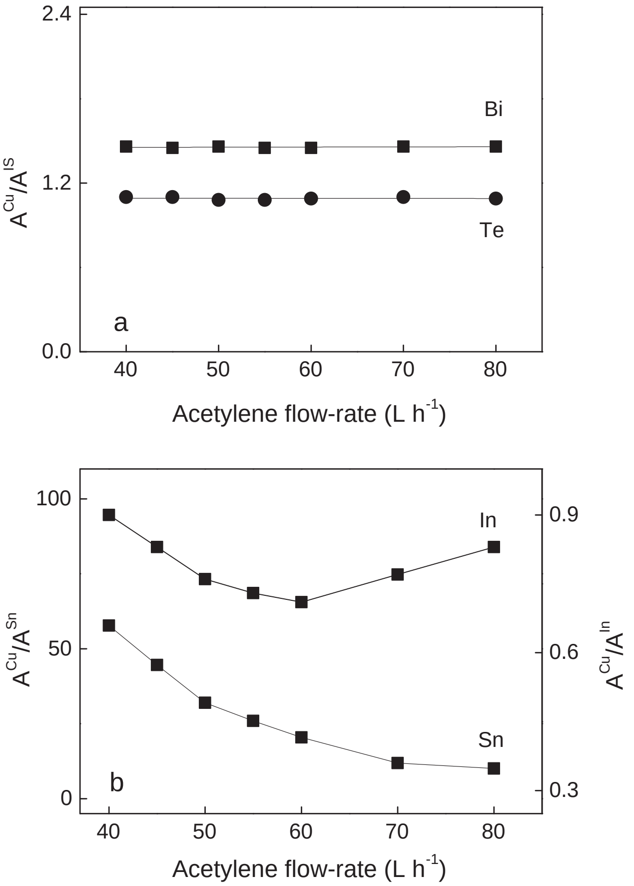

Fig. 4. Correlation graphics correspond to the absorbance ratios of 1.0 mg L^{-1} Cu to 10.0 mg L^{-1} Bi, In, Sn and Te at different flame compositions: (a) A^{Cu}/A^{Bi} and A^{Cu}/A^{Te}, and (b) A^{Cu}/A^{In} and A^{Cu}/A^{Sn}.

beverages such as cognac, whisky, vodka, rum, tequila, grappa, gin, and cachaça. Contents of Cu in samples obtained by the proposed IS HR-CS FAAS method varied in the 0.029–3.608 mg L^{-1} (Table 2). Analysis of this table reveals that both Bi and Te furnished agreeable results at 95% confidence level. Samples were also analyzed by LS FAAS employing analyte-addition calibration method (without IS), and results were in accordance (paired *t*-test) with those obtained by IS HR-CS FAAS. On the other hand, results obtained by HR-CS FAAS employing only external calibration (without correction method) did not agree with those obtained with IS or standard addition calibration methods. Relative standard deviations of 12 successive measurements were in the 0.8–3.7% (IS-Bi), 0.4–4.4% (IS-Te), 0.9–5.1% (standard addition method), and 0.5–9.1% (without IS) intervals. Limits of detection were 4.8 $\mu g L^{-1}$ Cu (IS-Bi) and 5.2 $\mu g L^{-1}$ Cu (IS-Te). In the addition/recovery tests, recoveries were in the 99–101% (IS), 77–83% (HR-CS FAAS without IS) and 98–102% (standard addition method) intervals.

4. Conclusion

The internal standardization was effective to the direct determination of Cu in a large spectrum of distilled alcoholic beverages by HR-CS FAAS using dilute-and-shoot for sample preparation. In general, the proposed method here is rugged and simple, and may be considered environmentally friendly since saves time and the consumption of reagents, and the minimum generation of wastes or residues.

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