FUEL ETHANOL QUALITY:

METHODS OF ANALYSIS AND REFERENCE MATERIALS

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INTRODUCTION

Biofuels constitute a viable alternative in relation to fuel derived from petrol and can be considered as important source of energy in the future. The participation of biofuel in the energetic matrix depends of a complex array of factors involving many production aspects (raw material and technology) and from the sustainability (social, economic and environmental), of this energy source. Since the start of Proalcool program the quality of ethanol was always connected with performance of engine working with hydrated alcohol and playing an important role in its sustainability economic.

Afterwards, ethanol quality was considered a priority factor for environmental sustainability, mainly when it compared with other vehicles working with other fuels. More recently, ethanol quality plays a decisive role on the possibility of becoming a commodity in the international market.

The validation of biofuels is markedly dependent of three basic factors: normalized specifications; official methods of analysis, and certified reference materials. The normalized specifications are a set of characteristics required to secure that the biofuel has the final destination as previously defined. The official methods are the tools necessary to construct a correct evaluation of the normalized specification of the fuel. The credited reference materials are tools utilized in the validation and official method control of fuel analysis.

Taking into consideration that the ethanol specifications are normalized by national (ANP e ABNT) and international (ASTM e CEN) agencies,

the present work has the aim to contribute with a critical analysis of the state-of-the-art (research and development) using the main analytical methods and to compile norms about reference materials certified for ethanol analysis.

METHODS OF ANALYSIS

The methods of analysis utilized for evaluation of fuel ethanol quality can be classified in two main groups: officials and alternatives methods. In both cases, there are a preponderancy of instrumental methods in relation to classic methods based on gravimetric and volumetric analysis. From the instrumental methods of ethanol analysis there is predominance of three classes: chromatographic, spectroscopic and electrochemical methods.

Official methods

The official methods destined to ethanol analysis and adopted by ANP have presented small changes since the first regulatory rules established to certify biofuel quality. The technical regulations emitted for CNP (n. 17/1985), DNC (23/1991), ANP (1/2001) and ANP (36/2005) shows that there is methodological evolution only for determined species.

This evolution was mainly based on the transference process occurring from classical to instrumental methods. As instance, the sulfate determination in ethanol was initially based on gravimetric method, afterwards was proposed a volumetric method and more recently the main analytical

method for sulfate determination is ionic chromatography. Also, the methodological evolution for chloride determination in fuel ethanol was from the volumetric methods, potentiometric analysis and finally by using ionic chromatography method.

On the other hand, some times the evolution occurred using own instrumental methods. For example, initial determination of copper and iron emigrated from UV-Vis spectrophotometric methods to atomic absorption spectroscopic methods. Paradoxically, for example, determination of hydrocarbons, esters and superior alcohols that were usually carried out by gas chromatography are not required in the actual specifications. In addition, there is introduction of regulations for new species, too. For example, it was introduced the necessity to determine some species that in the past was not required, such as hydrocarbons determination in fuel ethanol destined to importation, distribution and commercialization, by using gas chromatography.

Other official methods of analysis are proposed to assess the quality of fuel ethanol by international organisms, such as, American Society for Testing and Materials (ASTM) e Comité European de Normalisation (CEN). In general, the analytical methods utilized by these organisms present some differences with that adopted by the Agencia Nacional de Petróleo, Gás Natural and Biocombustíveis in Brazil (ANP).

In most cases these differences are centralized by the utilization of more specific method of analysis. Sometimes this difference involves the change of classical methods to instrumental methods or the specific type of instrumental method adopted. For example, the determination of water content in ethanol is carried out utilizing the karl Fisher method, but can be done by both volumetric (NBR 15531) and coulometric (EN 15489) methodology.

In summary, the official methods for fuel ethanol analysis have been the subject of several evolution over the years , which involves classical methods and simple instrumental methods but sometimes still demands modern and sophisticated methods, too. Thus, it is observed the disappearance of some analytical methods that are obsolete and the raising of others to attempt the

new specificities dealing with fuel ethanol. This dynamic involving the utilization of methods of analysis is due mainly the concern with environmental and economic factors. The environmental concerns determine the source for new species of importance in the environmental equilibrium; meanwhile the economic aspects are oriented to attempt the requirement of cost, speed and accuracy of the analysis.

Alternative methods of analysis

The main characteristics of the alternative methods for ethanol analysis are that they are essentially instrumental analysis. This occurs because most of them present high detectability and high selective of interest species.

Chromatographic methods

The chromatographic methods are predominant in organic species determination present in fuel ethanol, such as alcohols, aldehyde and acetones (Table 1). Among all these methods, predominates gas phase chromatography (GC) with fire ionization detection (FID). For determination of organic species are also utilized high performance liquid chomatography (HPLC) with spectroscopic (UV/Vis) and electrochemical detectors (DC).

From several chromatographic methods found in the literature [1] for fuel ethanol analysis we would like show some examples, such as the work carried out by Vilar *et al*. [7] that used gas chromatography with mass spectroscopy (GC-MS) for determining several organic compounds in fuel ethanol. The sample was submitted to solid phase extraction using XAD-4 resin and fractioned subsequently in a preparative liquid chromatographic column containing activated silica gel. It was detected linear saturated hydrocarbons and aromatic hydrocarbons in the first fraction and oxygenated compounds, such aldehydes, acetones and alcohol in the second fraction.

Pereira *et al*. [3] describes a useful analytical method for determining organic and inorganic contaminants in fuel ethanol sample utilizing capilar eletrophoresis technique. Chloride and

TABLE 1   Chomatographic methods proposed for ethanol fuel analysis.

Methods	Species		Useful work interval		Reference
			Minimum	Maximum	
GC	Acetaldehyde, Methanol, Propanaldehyde, Ethyl Formiate, Ethyl Propionate, Methyl acetate, Isopropyl Acetate, Dimethylcetone, Dimethyl Ether, Diethyl Ether, Superior Alcohols, Formaldehyde		1 ppm	N/S	[2]
CE	Acetaldehyde, Dimethylcetone		1.3 ppm	25 ppm	[3]
	Ammonium		0.15 ppm	N/S	[4]
	Calcium, Magnesium		0.17 ppm	N/S	[4]
	Potassium		0.22 ppm	N/S	[4]
	Sodium		0.16 ppm	N/S	[4]
	Chloride		0.065 ppm	0.65 ppm	[8]
			0.02 ppm	0.1 ppm	[20]
			0.08 ppm	N/S	[4]
	Nitrate		0.1 ppm	N/S	[4]
	Sulfate		0.25 ppm	4 ppm	[8]
			0.2 ppm	4 ppm	[3]
HPLC-ED	Acetaldehyde		3.8 ppm	379 ppm	[5]
	2-Furfuraldehyde, 5-Hidroximethylfurfural, Buthyraldehyde, Dimethylcetone, Methyl-ethylcetone		6 ppb	500 ppb	[6]

sulfate anions were analized using indirect detection at UV irradiation of 210 nm. The analysis of aldehyde was based on detection of adducts 3-metil-2-benzotiazole hydrated at 216 nm. Both methodologies were successful applied in real samples of fuel ethanol indicating inorganic ion concentration level from 0.15 to 6.64 mg L^{-1} and aldehydes from 32.0 to 91.3 mg L^{-1}.

Munoz et al. [4] developed a method for determination of inorganic ions in fuel ethanol using capilar eletrophorese technique. The method was used to Na, K,Ca, Mg e NH$_4$ cation and sulfate, chloride and nitrate anions. The limit detections for these ions are in a concentration interval from 0.06 to 0.18 mg L^{-1}. Inferior detection limit can be reached by increasing of injection time and/or injection pressure.

Okumura et al. [5] describes an analytical method for acetaldehyde determination in fuel ethanol by using high performance liquid chromatography (HPLC) with UV-vis detection. The acetaldehyde was dissolved with 2.4-dinitrophenil-hydrazine. The calibration graphs are linear from concentrations of 3 to 300 mg L^{-1} by injection of 20 μL sample. The limit detection was estimated to 2.03 μg L^{-1}. The method was successful applied in analysis of acetaldehyde in fuel ethanol sample.

Bruning et al. [2] developed a method for identification and quantification of organic contaminants presents in fuel ethanol utilizing the gas phase chromatographic technique (GC) couple to fire ionization detector (FID). The analysis carried out in ethanol presented relative standard deviation around 10%. The main organic contaminants

found were ethyl acetate, n-propanol, isobutyl acetate and isoamyl acetate.

SACKZ *et al.* [6] describes the determination of aldehydes and several acetones in fuel ethanol by high performance liquid chromatography (HPLC) with electrochemical detection (ED). The compounds 5-hydroximethylfurfural, 2-furfuraldehyde, butyraldehyde, acetone and methyl-ethyl-acetone, were derizatized with 2.4 – dinitrophenilhydrazine (DNPH). The analytical curves present linear relationship for concentration from 5.0 to 400.0 ng L^{-1}, and detection limit from 1.7 to 2.0 ng L^{-1} (20 µL). The proposed methodology is simple, rapid (15 min/analysis) and present high recovery (> 95%).

Spectroscopic methods

The spectroscopic methods are utilized in fuel ethanol analysis for determining inorganic species, mainly metallic ions, such as, copper, iron and sodium (Table 2). In the set there is predominance of methods involving atomic absorption spectroscopy with fire (Faas) and graphite oven (GFaas). In the determination of metallic ions species are also utilized plasma methods induced by mass detectors (ICP-MS).

From the spectroscopic methods for ethanol analysis found in literature [1] a relevant contribution was shown by SAINT'PIERRE *et al.* [10]. The authors describe the development of a method for metal determination in fuel ethanol utilizing ICP-MS. A solution of 10% v/v ethanol was introduced directly in the ultrasonic nebulizer formed with carbon deposit in the cones. The detection limits for each analyzed metal were: Ag: 0.08; Cd: 0.12; Co: 0.04; Cu: 0.4; Fe: 27; Mn: 0.7; Ni: 0.4 e Pb: 0.2 µg L^{-1}. The results illustrated that the proposed method present rapidity, accuracy and enough sensibility when compared with that obtained by ETV-ICP-MS.

Oliveira *et al.* [11] developed a simultaneous determination method of Al, As, Cu, Fe, Mn e Ni in fuel ethanol utilizing atomic absorption spectrometry with electro thermal source (ETAAS) using permanent modifier of W-Rh and conventional modifier of $Pd(NO_3)_2$ + $Mg(NO_3)_2$. The detection limit was 1.9 µg L^{-1} Al; 2.9 µg L^{-1} As; 0.57 µg L^{-1} Cu; 1.3 µg L^{-1} Fe; 0.40 µg L^{-1} Mn e 1.3 µg L^{-1} Ni. The relative standard deviation between the measurements was 4%; 4%; 3%; 1.5%; 1.2% e 2.2% for Al, As, Cu, Fe, Mn e Ni respectively. The proposed method was applied for metal determination and ten samples of fuel ethanol showing a good accuracy.

Roldan *et al.* [12] describes an analytical method for determining Cu, Ni e Zn in fuel ethanol utilizing the atomic absorption spectroscopy with fire (Faas) subsequently to a pre-concentration step containing silica gel modified with 2-aminotiazole (Siat). The results shown recovery around 100% for all metallic ions adsorbed in the column containing 500 mg of Siat. The adsorption and desorption studies of studied metallic ions contributed to the development of a sensible method of these metals at trace levels in fuel ethanol using FAAS for its quantification.

Teixeira *et al.* [13] developed a spectrophotometric method for simultaneous determination of copper and iron in fuel ethanol using ferroine as derivatizing reagent. 1.10-fenantroline can be used to detect both compounds after controlled reaction up to limit detection of 7 and 8 µg L^{-1} and variation coefficient between measurements of 1.8 and 2.3% for iron and copper, respectively. The 2.2-bipyrine reagent can also be utilized for simultaneous determination of iron and copper and the method reaches limit detection of 11 and 32 µg L^{-1} and variation coefficients of 1.9 and 2.5 for iron and copper, respectively.

Santos *et al.* [14] also developed a spectrophometric method for sulfate determination in fuel ethanol using previous reaction with dimethylsulphonazo reagent. The analytical method was based on absorbance of the product monitored at 665 nm. The method was used for determination of sulfate up to 10 mg L^{-1} with a relative standard deviation less than 2.5% between measurements and limit detection of 0.27 mg L^{-1}. The proposed method was successful used in determination of sulfate in real samples of fuel ethanol.

Teixeira *et al.* [15] describes a simple method for determination of formadehyde in fuel ethanol utilizing the spectrophotometric technique.

TABLE 2   Spectroscopic methods proposed for fuel ethanol analysis.

Methods	Species	Useful work interval		Reference
		Minimum	Maximum	
FIA-UV/Vis	Formaldehyde	0.063 ppm	1.9 ppm	[15]
	Sulfate	12 ppm	N/S	[14]
Colorimetry	Cyanide	100 ppb	N/S	[2]
FTIR	Methanol	N/S	N/S	[11]
GFAAS	Arsenic, Aluminum, Iron, Manganese, Nickel	3 ppb	63 ppb	[16]
		0.004 ppb	N/S	[16]
	Aluminum	3 ppb	N/S	[16]
	Copper	2.5 ppb	63 ppb	[11]
		0.72 ppb	N/S	[16]
	Iron	1.6 ppb	N/S	[16]
	Manganese, Nickel	0.5 ppb	N/S	[16]
FAAS	Nickel	1.6 ppb	N/S	[16]
	Cadmium	35 ppm	141 ppm	[17]
		28 ppm	359 ppm	[18]
		0.3 ppb	N/S	[19]
	Chromium, Copper, Iron, Nickel, Potassium, Sodium	1.3 ppb	N/S	[2]
	Chromium	16.4 ppm	65.8 ppm	[17]
FAAS	Cobalt	14.8 ppm	185 ppm	[18]
		1.8 ppb	N/S	[19]
	Copper	20 ppm	80 ppm	[17]
		16 ppm	200 ppm	[18]
		19 ppm	252 ppm	[12]
		1.8 ppb	N/S	[19]
		2.9 ppb	N/S	[20]
		11 ppb	N/S	[21]
		8.9 ppb	N/S	[22]
	Iron	18 ppm	70 ppm	[17]
		14 ppm	140 ppm	[18]
		6.3 ppb	N/S	[20]
		11 ppb	N/S	[21]
		5 ppb	N/S	[22]

Methods	Species	Useful work interval		Reference
		Minimum	Maximum	
FAAS	Lead	14 ppm	176 ppm	[18]
	Manganese	2.5 ppb	N/S	[22]
	Nickel	18 ppm	74 ppm	[17]
		15 ppm	182 ppm	[18]
		19 ppb	253 ppb	[12]
		10 ppb	N/S	[20]
		4 ppb	N/S	[21]
		5 ppb	N/S	[22]
	Sodium	N/S	0.375 ppm	[23]
	Zinc	20 ppm	205 ppm	[17]
		16 ppm	2.5×10^{-3} mol L^{-1}	[18]
		13 ppb	126 ppb	[12]
		1.3 ppb	N/S	[19]
		0.1 ppb	N/S	[20]
		2.5 ppb	N/S	[21]
		2.5 ppb	N/S	[22]
ET-AAS	Arsenic	3 ppb	N/S	[24]
	Cadmium	0.06 ppb	N/S	[24]
	Lead	1.4 ppb	N/S	[24]
ETV-ICP-MS	Cadmium	0.1 ppb	N/S	[25]
	Copper	0.12 ppb	N/S	[25]
	Lead	0.06 ppb	N/S	[25]
	Silver	0.03 ppb	N/S	[25]
	Thallium	0.001 ppb	N/S	[25]
	Cadmium	0.15 ppb	N/S	[10]
	Copper, Nickel	0.5 ppb	N/S	[10]
	Cobalt	0.05 ppb	N/S	[10]
	Iron	34 ppb	N/S	[10]
	Lead	0.25 ppb	N/S	[10]
	Manganese	0.9 ppb	N/S	[10]
	Silver	0.10 ppb	N/S	[10]
UV/Vis	Copper	40 ppb	N/S	[13]
	Iron	14 ppb	N/S	[13]

The product assigned as 3.5-diacetyl-1,4-dihy-drolutidine generated after reaction between formaldehyde and fluoral P was kept in the solid phase cartridge (C18) and the spectrophotometric detection carried out at 412 nm. The proposed method presents linear response for concentration interval from 0.05 to 1.5 mg L^{-1}. The detection limit obtained was 30 µg L^{-1} and the relative standard deviation was 2.2% (625 µL). The method was successful applied in determination of formaldehyde in fuel ethanol samples.

Electrochemical methods

The electrochemical methods utilized for determination of inorganic species in fuel ethanol, are preponderantly metallic ions, such as copper, lead and zinc (Table 3). From these methods are predominance of them based anodic stripping voltammetric techniques (ASV) coupled to differential pulse (DP) and square wave (SW) as scan mode. The potentiometric and conductometric methods are also utilized for species no metallic determination as, chloride and sulfate.

From the several electrochemical methods describes in the literature [1] for fuel ethanol analysis, there is relevant contribution from work developed for Godinho et al. [27]. The authors developed a methodology for sulfur determination in fuel ethanol using Raney nickel base don cathodic stripping voltammetric coupled to differential pulse scan. The calibration curve present linear relationship for concentration of sulfur from 1-40 ppb and the method was successful applied in ethanol samples containing 15 ng g^{-1} to 20 µg g^{-1}. The values of precision and accuracy obtained with

TABLE 3 Electrochemical methods proposed for ethanol fuel analysis.

Methods	Species	Useful work interval		Reference
		Minimum	Maximum	
Voltammetry	Acetaldehyde	0.05 ppm	5 ppm	[31]
		0.05 ppm	0.5 ppm	[33]
	2-Furfuraldehyde	115 ppm	704 ppm	[34]
	Solvent Orange 7	1.4 mg L^{-1}	6.3 mg L^{-1}	[32]
ASV	Cadmium	0.14 ppb	70 ppb	[30]
DPV	Nickel	5.0×10^{-9} mol L^{-1}	5.0×10^{-7} mol L^{-1}	[35]
		7.5×10^{-9} mol L^{-1}	1.0×10^{-6} mol L^{-1}	[36]
DP-ASV	Copper, Zinc	0.08 ppb	40 ppb	[30]
	Copper	6 ppb	200 ppb	[37]
	Lead	2.5 ppb	312 ppb	[28]
		0.26 ppb	130 ppb	[30]
	Zinc	40 ppb	411 ppb	[38]
SW-ASV	Copper	0.15 ppb	N/S	[39]
	Iron	0.42 ppm	1.4 ppm	[40]
	Lead	0.297 ppb	N/S	[39]
DP-CSV	Sulfur	1 ppb	40 ppb	[27]
Potentiometry	Copper	12 ppb	N/S	[41]
	Chloride, Sulfate	1 ppm	N/S	[2]
Conductometry	Chloride	1.1 ppm	N/S	[29]

the proposed method present values comparable with that previously described in the literature.

Takeuchi *et al.* [28] described a method for determining copper in fuel ethanol utilizing paraffin containing impregnated graphite powder with the 2-aminotiazole as modifier. The technique for detection was anodic stripping voltammetry with differential pulse mode. The analytical curve presents linearity from 7.5×10^{-8} to 2.5×10^{-6} mol L^{-1} copper concentration and detection limit of 3.1×10^{-8} mol l^{-1}. The method was successful applied in the determination of copper in fuel ethanol sample.

Avelar *et al.* [29] developed a determination method for chloride and total acidity in fuel ethanol sample using conductometric titulation method. The proposed methodology is simple and rapid and can be applied with good repeatability and detectability of total acidity and also chloride content when compared with the official methods of analysis.

Oliveira *et al.* [30] describes the development of a method for simultaneous determination of zinc, copper, lead and cadmium in fuel ethanol using a mercury film on vitreous carbon electrode by stripping voltammetric technique (ASV). The measurements were carried out using linear scan (LSV), differential pulse (DPV) and square wave (SWV) modes. The obtained detection limit for all metals was in the interval from 10^{-9} to 10^{-8} mol L^{-1}. The proposed method was tested for quantification of zinc, copper, lead and in commercial samples of fuel ethanol.

Rodgher *et al.* [31] proposed a method for acetaldehyde determination in fuel ethanol utilizing previous mixed reaction with hydrazine by using square wave voltammetry. The analytical curves were linear for concentrations changing from 1.0×10^{-6} to 10×10^{-6} mol L^{-1}, with a detection limit of 2.38×10^{-7} mol L^{-1}. The method was satisfactory applied in determination of total aldehydes in ethanol samples without any pretreatment step.

Romanini *et al.* [32] describes the development of a method for determination of solvent orange 7 dye used as dye marker in fuel ethanol using linear scan voltammetry (LSV) and square wave voltammetry (SWV. The SWV technique presents lower detection limit and linear calibration curve from 4.0×10^{-6} to 18×10^{-6} mol L^{-1}. The method was successful applied in direct quantification of this marker in fuel ethanol sample without any pretreatment step.

THE IMPORTANCE OF THE REFERENCE MATERIAL ON QUALITY CONTROL OF ETHANOL

The current usage of biofuels is global concern with environmental sustainability. Therefore the quality of Brazilian bioethanol due to its origin from renewable sugarcane becomes even more important [42-44]. Since well-characterized reference values are essential to the development and validation of measurement methods, the usage of Certified Reference Material (CRM) will contribute to the quality of the measurement around the world, giving support to international trade.

In 2007, experts and government representatives from the United States of America (USA), European Union (EU) and Brazil agreed that there are different standards for biofuels which were known to be an obstacle to the free circulation of biofuels among the three regions. On a White Paper [45] issued by the tripartite task force, they identified areas where greater compatibility could be achieved in the short to long term. Bioethanol was classified into category B. It has specifications with significant differences between parameters and methods.

Because of the importance of bioethanol to the Brazilian economy [46], and considering that the international trade in biofuels will increase significantly [47, 48], Inmetro, the Brazilian Metrology Institute, has focused its attention on the need for accurate and reliable measurements in a variety of parameters, through the work for developing and producing CRM of bioethanol [49] in order to establish quality and comparable measurements in bioethanol matrix. However, the use of a CRM of bioethanol will also contribute to Brazilian producers, to avoid possible technical barriers imposed by bioethanol importers in the near future.

Reference Material (RM) and Certified Reference Material (CRM)

Reference material is a material sufficiently homogeneous and stable with reference to speci-

fied properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties [50-54]. On the other hand, a CRM is a reference material accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceability, using valid procedures. To summarize, the RMs and CRMs, as defined above, are widely used for the calibration of measuring apparatus, evaluation or validation of methods of analysis or test and for long-term quality assurance of measurements. All kinds of RMs and CRMs are playing an increasingly important role in national and international standardizations activities, for the trade, in proficiency testing and in the accreditation of laboratories.

In the chemical area, to establish the traceability to the measurement, the use of the so considered primary measurement methods or certified reference materials is needed. The Consultative Committee for Amount of Substance — Metrology in Chemistry (CCQM) has identified several methods with the potential of being a primary method of measurement, which in the field of chemical measurement, such a method is defined by CCQM as "a method having the highest metrological properties, whose operation can be completely described and understood, for which a complete uncertainty statement can be written down in terms of the units of SI (International System of Units)". However, they are as yet very few [55, 56], thus, the CRMs are strongly used to guarantee the quality of chemistry measurements.

Although the importance of the use of CRMs is known, however, the great variety of chemical substances and diverse matrices in which the analytes can be encountered limit the number of the CRMs available. Thus, there are not sufficient CRMs to all measurements. To this difficulty can be added the high cost for acquisition and importation of this materials, to the bureaucracy and the long time spent in the transport and liberation of the imported materials in the Brazilian customs [57].

Hence, Inmetro has been developing CRMs which can be used by the laboratories in the country from different areas such as industrial, research & development and environment. The high metrological level of the measurements performed by the laboratories of Inmetro is mainly due to its participation in key comparisons with the supervision of the *International Committee for Weights and Measures* (CIPM)/CCQM. On the other hand, the CRMs prepared by Inmetro follow the requisites established in the ISO Guides 30 [58], 31, 32 [59], 33 [60], 34 e 35 [54]. The ISO guides from 30 to 34 have already been translated to Portuguese by ABNT, the Brazilian forum for standardization.

Some countries have already had an accreditation system for producers of reference materials. The accreditation is based on the ISO Guides 30, 31, 34, 35 and on the standard ISO/IEC 17025 [61], as well as the standards or guides which are related to the area to be accredited. Thus, Inmetro is structuring a similar system in Brazil. This system of accreditation will contribute to increase the production of CRMs as well as the quality of the chemical measurements in the country.

Development of a certified reference material

In the development of a CRM, the producer must take into account the requisites and the recommendations stated in the ISO Guides above mentioned.

The ISO Guide 35 can be seen as an application of the *Guide to the Expression of Uncertainty in Measurement (GUM)* [62] specifically for the production of CRMs. ISO Guide 35 complements the GUM because it provides additional guidance with respect to the inclusion of the uncertainties due to the batch in homogeneity and instability of the CRM in the uncertainty of the property values, and the determination of these uncertainty contributions.

Homogeneity and stability are two important characteristics of any CRM. Hence care must be taken during preparation to produce materials as homogeneous and stable as possible. However, careful preparation by itself is not enough. Demonstration of homogeneity and stability is required by taking into account the uncertainty calculation [63]. According to the GUM, the uncertainty of a CRM should consider all the uncertainty sources relevant to the user. This not only includes the un-

certainty of the batch characterization (u_{char}), but also all uncertainties relating the possible between-bottle variation (u_{bb}), instability upon long-term storage (u_{lts}) and instability during transport to the customer (u_{sts}). The combined standard uncertainty for a CRM can thus be expressed as in Equation 1 [64].

$$u_{CRM} = \left(u_{char}^2 + u_{bb}^2 + u_{lts}^2 + u_{sts}^2 + \right)^{1/2} \quad (1)$$

It is important to highlight the difference between characterization and certification. Characterization of a reference material is a process of determining the property values of a reference material, as part of the certification process. The characterization process provides the values for the properties to be quantified. In batch certification, the characterization refers to the property values of the batch. Certification is the whole process of obtaining the property values and their uncertainty, which includes homogeneity testing, stability testing, and characterization [65].

Homogeneity study

A homogeneity study is necessary in batch certification to demonstrate that the batch of bottles (units) is sufficiently homogeneous. It also finds application in the preparation and checking of proficiency testing material [66]. Even when a material is expected to be homogeneous, as in the case of solutions, an assessment of the between-bottle inhomogeneity is required. When dealing with solid-state reference materials, a within-bottle homogeneity study should be foreseen to determine the minimum sample intake.

The results of a between-bottle homogeneity test should be evaluated in accordance with the design of the study. In most cases, however, an approach based on a fully-nested analysis of variance, as described in ISO Guide 35 and in [66, 67] can be used for the purpose.

Stability testing

Stability testing aims to determine the remaining degree of instability of the reference material after preparation, or to confirm the stability of the material. A distinction is made between the stability under specified storage conditions (long-term stability) and transport conditions (short-term stability).

The long-term stability concerns the remaining instability of property values of the CRM. A reference temperature should be chosen so that it is practically certain that the material is stable at that temperature.

The short-term stability study is typically carried out at different temperatures, to study the effect of different temperatures on the properties of the material. A short-term stability study takes typically 1 to 2 months. On the other hand, a long-term stability studies last between 24 and 36 months [68, 69].

A flow diagram for evaluation of measurement uncertainty in a complete process for certifying a reference material [65] can be seen in Figure 1. The measurements of the homogeneity, stability and the characterization studies of the material should be combined in order to obtain a proper estimate for the property value and its standard uncertainty.

Choice of measurement methods

For the characterization of the reference material, especially in the case of matrix reference materials, it is often highly desirable to use multiple methods, and often also multiple laboratories. Both the methods and the performance of the laboratories should represent "state-of-art", and they should be able to make their measurements traceable to the references specified in the design of the project to develop a CRM. This approach is known as a collaborative study. This underpins the joint effort of the coordinator and participants to characterize the reference material.

The method used in a certification project should be properly validated, and it should be demonstrable that any result obtained with the measurement method meets the specifications established during the validation of the method.

The property values of a CRM need to be traceable to appropriate units and/or references due to its role as a measurement standard. Ensuring the
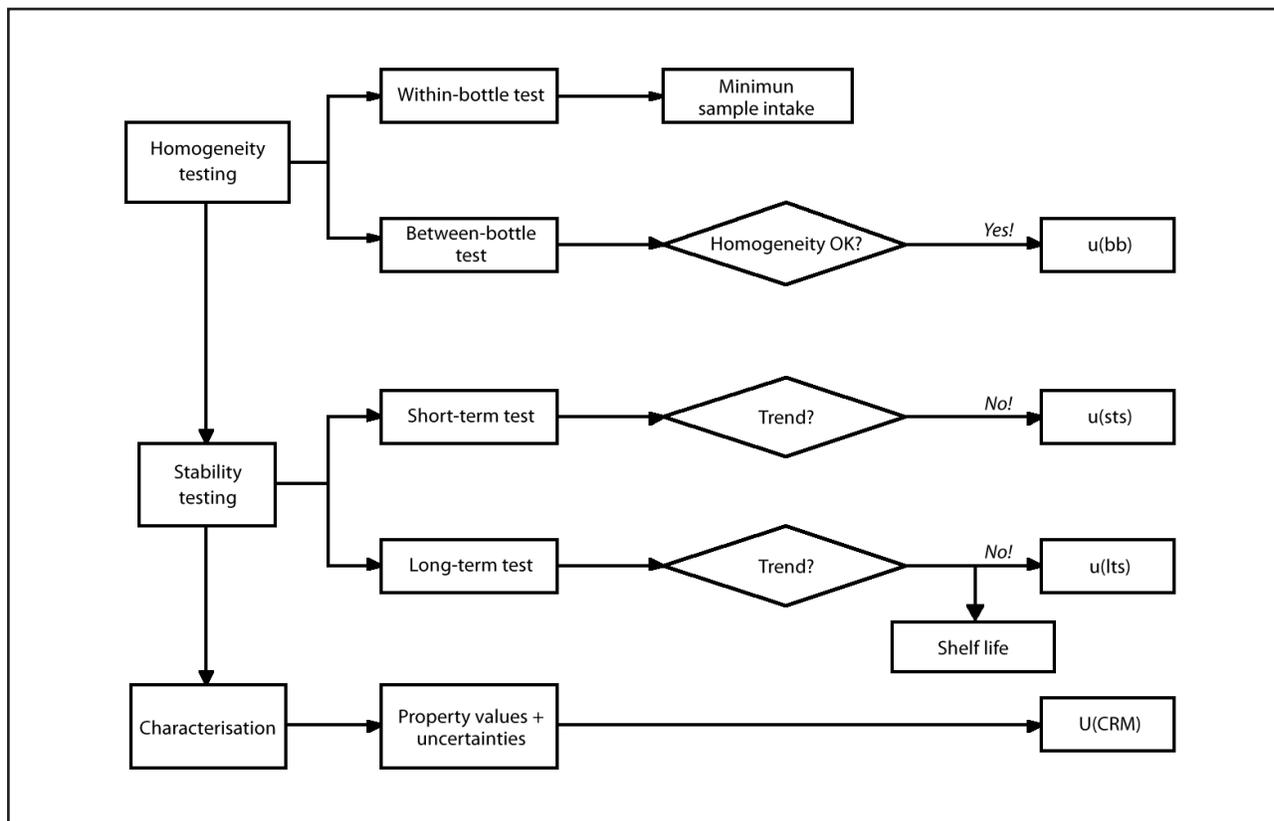
FIGURE 1   Flow diagram of a complete process for certification of a reference material.

traceability of all measurements in a certification of a reference material is an important requirement [53, 70, 71].

The CRM of bioethanol

The necessity of developing CRM of bioethanol has been claimed by the producers of this kind of product for a long time in order to guarantee its quality and to increase the exportation of their products, since the specific parameters for bioethanol could be measured with traceability and reliability and accepted by the international trade.

The development of reference materials and commercial barriers are two of the most sensitive areas needing proper Chemical Metrology support. Therefore, the first step, as stated above, would be to define the traceability chain for the measurement of these parameters. A side bonus derived from this effort would be the proposal of which parameters should be considered in the specifications of bioethanol as a fuel. This is something that would naturally come from the scientific background that will be associated with the proposed metrological work.

Since 2003, Inmetro has been studying different parameters which are established in the specifications of the National Agency of Petroleum, Natural Gas and Biofuels (ANP). This agency is responsible for the control of quality of biofuels in Brazil. It regulates the limits for several parameters in biofuels [72], according to the Brazilian Standards.

The parameters that are being studied for certification and the reasons of their relevance are given below. They are common for both anhydrous bioethanol and hydrated bioethanol.

Alcoholic content and density: for the stability and quality of the product, since the original alcoholic content as well as evaporation may affect calorific power and motor performance.

Acidity: for avoiding corrosion in the fuel chain, since it contributes to the corrosion of the metallic parts present in the production process, transport and motors.

Electrolytic conductivity: conductivity values above 500 μS×m^{-1} contribute to corrosion.

Water content: high water content may increase the conductivity and therefore corrosion, as well as decreases the calorific power.

pH: range from 6.0-8.0 for hydrated bioethanol to avoid corrosion in the fuel chain, as well as it contributes to the corrosion of metallic parts in the production process, transport and use (motors).

Copper and iron content: these metals in contact with gasoline may cause polymerization (probably gum formation) in automotive motors. ASTM Standard [73] recommends copper content values below 0.1 mg×kg^{-1}.

Chloride and sulphate: the presence of chlorides by external contamination and sulphates which cause deposits in the electronic injection systems can also contribute for increasing the corrosion.

Since 2008, the USA and Brazil have established a partnership attending to American and Brazilian government throughout of their National Metrology Institutes, National Institute of Standards and Technology (NIST) and Inmetro, respectively. In this partnership, the development of CRM for bioethanol was proposed because of the great visibility which the bioethanol has today, mainly in the environmental issues. Besides, both countries are the major biofuel producers in the world. Nowadays the studies of this CRM are in progress.

European Union has been focusing on working with Brazil also in the relevant task of comparing and searching for the harmonization of the analytical methods for the biofuel. The main objective of this partnership is to coordinate a proficiency testing (PT) in order to measure different parameters of bioethanol. In this PT the participation of various countries which has interest in providing the quality management in the trade of their products will be possible.

At Inmetro, the studies of these several parameters in anhydrous and hydrated bioethanol from Brazilian producers have become possible because this work has the support of different laboratories from Mechanical and Chemical Division from Inmetro in this challenge. Each of them

carries out the measurements which are needed in the homogeneity, characterization and short-term and long-term stability studies. All the studies relating to the CRM production and development have been done according to ISO Guide 35.

The studies of homogeneity of each parameter were carried out with random measurements. If the results present homogeneity, the characterization study is carried out with the best method available for each parameter.

The short-term and long-term stability studies were done in two different temperatures simulating transport and storage conditions. Both temperatures were at 50 ºC and 20 ºC, respectively.

The total time of the stability study for CRM for anhydrous and hydrated bioethanol was done during one year and all parameters showed stable, then the shelf-life (validity) of this CRM was established by one year.

It is very important to highlight that the period of validity of the CRM for bioethanol can be increased if the stability is maintained during the stability studies which will continue for all the certified parameters.

FUTURE PERSPECTIVES

Nowadays, despite the high evolution that can be seen in the used methods for analyzing the fuel ethanol, there is a constant need for new specifications that require analytical methods even more precise and accurate for determination of great numbers of species. Therefore, the development of new methods of analysis might find great challenges in order to guarantee the quality of this biofuel.

From this perspective, one can designate them as: i) development of analytical methods with very low detection limits for diverse species responsible for the quality of the fuel ethanol; ii) development of analytical methods with very great selectivity to guarantee the quality of the fuel ethanol from different matrices; iii) development of analytical methods for determination of new important species for the environmental sustainability; iv) development of analytical methods for on line monitoring of the quality of the biofuel; v)

development of low cost analytical methods joined with operational simplicity to guarantee the quality of the fuel ethanol.

From the perspective of the research and development of CRM for bioethanol, it has showed great importance to Brazil, since there was an extreme urgency to produce it. The Brazilian producers, in order to prove accuracy of the measured parameters for the trade, needed a reference material which could be used as a standard reference to chemical measurements. Therefore, this CRM of bioethanol, which is in development by means of a collaborative study from NIST and Inmetro, will guarantee the traceability and quality of the measurement results from quality parameters of the bioethanol in order to contribute for a reliable and fair trade; moreover, it will help to increase the trade for bioethanol from sugarcane.

REFERENCES

[1]   Firmino, MF, Saczk AA, Okumura LL, Stradiotto NR, Analytical methods employed at quality control of fuel ethanol. **Energy & Fuels** 2009; on line.

[2]   Bruning IMRA, Malm EB. Identificação e quantificação das impurezas presentes no etanol. **Bol Tec Petrobras** 1982; 25:217-28.

[3]   Pereira EA, Tavares MFF, Stevanato A and Cardoso AA. **Evaluation of inorganic and organic contaminants in alcohol fuel by capillary electrophoresis.** Quím Nova 2006; 29:66-71.

[4]   Munoz RAA, Richter EM, Jesus DP, Lago CL, Angnes L. **Determination of inorganic ions in ethanol fuel by capillary electrophoresis.** J Braz Chem Soc 2004; 15:523-6.

[5]   Okumura LL, Saczk AA, De Oliveira MF, Zanoni MVB and Stradiotto NR. **Rapid and sensitive method for the determination of acetaldehyde in fuel ethanol by high-performance liquid chromatography with UV–Vis detection.** Anal Bioanal Chem 2005; 381:1619–24.

[6]   Saczk AA, Okumura LL, De Oliveira MF, Zanoni MVB and Stradiotto NR. **Determination of aldehydes and ketones in fuel ethanol by high-performance liquid chromatography with electrochemical detection.** Chromatographia 2006; 63:45–51.

[7]   Vilar RBC, Da Silva R, Schossler P,Veses RC, Piatnicki CMS, Samios D *et al*. **Preliminary characterization of anhydrous ethanol used in Brazil as automotive fuel.** J. Chrom. A 2003; 985:367-73.

[8]   Pereira EA, Tavares MFF, Stevanato A and Cardoso AA. **Indirect determination of chloride and sulfate ions in alcohol fuel by capillary electrophoresis.** Anal Bioanal Chem 2004; 380:178-82.

[9]   Okumura LL, Saczk AA, De Oliveira MF, Zanoni MVB and Stradiotto NR. **Determination of acetaldehyde in fuel ethanol by high-performance liquid chromatography with electrochemical detection.** Anal Sci 2005; 21:441-4.

[10] SaintPierre TD, Tormen L, Frescura VLA, Curtius AJ. **The direct analysis of fuel ethanol by ICP-MS using a flow injection system coupled to an ultrasonic nebulizer for sample introduction.** J Anal Atom Spectrom 2006; 21:1340–4.

[11] De Oliveira AP, De Moraes M, Neto JAG, Lima EC. **Simultaneous determination of Al, As, Cu, Fe, Mn, and Ni in fuel ethanol by GFAAS.** Atom Spectrosc 2002; 23:39-43.

[12] Roldan PS, Alcantara IL, Castro GR, Rocha JC, Padilha CCF, Padilha PM. **Determination of Cu, Ni, and Zn in fuel ethanol by FAAS after enrichment in column packed with 2-aminothiazole-modified silica gel.** Anal Bioanal Chem 2003; 375:574-7.

[13] Teixeira LSG, Brasileiro JF, Borges JR MM, Cordeiro PWL. **Simultaneous spectrophotometric determination of iron and copper using ferroin reagents.** Quim Nova 2006; 29:741-5.

[14] De Oliveira FS, Korn M. **Spectrophotometric determination of sulphate in automotive fuel ethanol by sequential injection analysis using dimethylsulphonazo (III) reaction.** Talanta 2006; 68:992-9.

[15] Teixeira LSG, Leão ES, Dantas AF, Pinheiro HCL, Costa ACS, Andrade JB. **Determination of formaldehyde in Brazilian alcohol fuels by flow-injection solid phase spectrophotometry.** Talanta 2004; 64:711-15.

[16] De Oliveira AP, De Moraes M, Neto JAG, Lima EC. **Direct determination of Al, As, Cu, Fe, Mn, and Ni in fuel ethanol by simultaneous GFAAS using integrated platforms pretreated with W-Rh permanent modifier together with Pd plus Mg modifier.** Atom Spectrosc 2002; 23:190-5.

[17] Padilha PM, Padilha CCF, Rocha JC. **Flame AAS determination of metal ions in fuel ethanol after preconcentration on acid carboxymethylcellulose.** Quim Anal 1999; 18:299-303.

[18] Gomes LAM, Padilha PM, Moreira JC, Filho NLD, Gushikem Y. **Determination of metal ions in fuel ethanol after preconcentration on 5-amino-1,3,4-thiadiazole-2-thiol modified silica gel.** J Braz Chem Soc 1998; 9:494-8.

[19] Teixeira LSG, Bezerra MA, Lemos VA, Santos HC, Jesus DS, Costa ACS. **Determination of Copper, Iron,**

Nickel, and Zinc in Ethanol Fuel by Flame Atomic Absorption Spectrometry Using On-Line Preconcentration System. Sep Sci Tech 2005; 40: 2555-65.

[20] Teixeira LSG, Bezerra MA, Lemos VA, Santos HC, Jesus DS, Costa ACS. Determiantion of copper, iron, nickel and zinc in ethanol fuel by flame atomic absorption spectrometry using on-line preconcentration system. Sep. Sci. Tech. 2005; 40:2555-65.

[21] Moreira JC and Gushikem Y. Preconcentration of metal-ions on silica-gel modified with 3(1-imidazolyl) propyl groups. Anal Chim Acta 1985; 176:263-7.

[22] Filho NLD, Gushikem Y, Polito WL, Moreira JC and Ehirim EO. Sorption and preconcentration of metal ions in ethanol solution with a silica gel surface chemically modified with benzimidazole. Talanta 1995; 42:1625-30.

[23] De Oliveira AP, Okumura LL, Neto JAG, De Moraes M. Evaluation of analyte additions method for sodium determination in fuel ethanol by flame atomic emission spectrometry. Eclet Quim 2002. 27:285-91.

[24] Curtius AJ, Giacomelli MBO, Silva JBB, SaintPierre TD. Use of iridium plus rhodium as permanent modifier to determine As, Cd and Pb in acids and ethanol by electrothermal atomic absorption spectrometry. Microchem J 2004; 77:151-6.

[25] SaintPierre TD, Frescura VLA, Curtius AJ. The development of a method for the determination of trace elements in fuel alcohol by ETV-ICP-MS using isotope dilution calibration. Talanta 2006; 68:957–62.

[26] Oliveira FS, Leite BCO, Andrade MVAS, Korn M. Determination of total aldehydes in fuel ethanol by MBTH method –SIA. J Braz Chem Soc 2005; 16:87-92.

[27] Aleixo LM, Rath S, Godinho OES. Determination of total sulphur in ethanol by reduction using Raney nickel and detection by cathodic stripping voltammetry. Analyst 1988; 113:1427-9.

[28] Takeuchi RM, Santos AL, Padilha PM and Stradiotto NR. Copper determination in ethanol fuel by differential pulse anodic stripping voltammetry at a solid paraffin-based carbon paste electrode modified with 2-aminothiazole organofunctionalized silica. Talanta 2007; 71:771-7.

[29] Avelar HM, Barbeira PJS. Conductometric determination of total acidity and chloride content in automotive fuel ethanol. Fuel 2007; 86:299-302.

[30] De Oliveira MF, Saczk AA, Okumura LL, Fernandes AP, De Moraes M, Stradiotto NR. Simultaneous determination of zinc, copper, lead, and cadmium in fuel ethanol by anodic stripping voltammetry using a glassy carbon–mercury-film electrode. Anal Bioanal Chem 2004; 380:135-40.

[31] Rodgher VS, Okumura LL, Saczk AA, Stradiotto NR and Zanoni MVB. Electroanalysis and determination of acetaldehyde in fuel ethanol using the reaction with 2,4-dinitrophenylhydrazine. J Anal Chem 2006; 61:889-95.

[32] Romanini, DC, Trindade MAG, Zanoni MVB. A simple electroanalytical method for the analysis of the dye solvent orange 7 in fuel ethanol. Fuel 2009; 88:105–109.

[33] Rodgher VS, Stradiotto NR and Zanoni MVB. Electroanalysis of acetaldehyde derivatized with hydrazine and determination in fuel ethanol by square wave voltammetry. Quim Nova 2006; 29:662-5.

[34] Saczk AA, De Oliveira MF, Okumura LL and Stradiotto NR. Voltammetric behavior of 2-furfuraldehide reduction in ethanol using glassy carbon electrode. Eclet Quim 2002; 27:141-51.

[35] Tartarotti FO, De Oliveira MF, Balbo VR and Stradiotto NR. Determination of nickel in fuel ethanol using a carbon paste modified electrode containing dimethylglyoxime. Microchim Acta 2006; 155:397–401.

[36] Bergamini MF, Santos AL, Stradiotto NR. Lead ions determination in ethanol fuel by differential pulse anodic stripping voltammetry using a carbon paste electrode modified with ion-exchange resin Amberlite IR120. Eclet Quím 2006; 31:45-52.

[37] Takeuchi RM, Santos AL, Padilha PM and Stradiotto NR. A solid paraffin-based carbon paste electrode modified with 2-aminothiazole organofunctionalized silica for differential pulse adsorptive stripping analysis of nickel in ethanol fuel. Anal Chim Acta 2007; 584:295-301.

[38] De Oliveira MF, Saczk AA, Okumura LL, Stradiotto NR. Determination of zinc in fuel alcohol by anodic stripping voltammetry. Eclet Quim 2002; 27:153-60.

[39] Munoz RAA, Angnes L. Simultaneous determination of copper and lead in ethanol fuel by anodic stripping voltammetry. Microchem J 2004. 77:157-62.

[40] Mattos CS, Carmo DR, De Oliveira MF and Stradiotto NR. Voltammetric determination of total iron in fuel ethanol using a 1,10 fenantroline/nafion carbon paste-modified electrode. Int J Electrochem Sci 2008; 3:338-45.

[41] Neves EFA, Neto GO and Serrano SHP. A new type of precipitating agent for copper membrane-electrode. Anal Lett 1987; 20:1363-77.

[42] Moreira JR, Goldemberg J, The alcohol program, Energy Policy, 1999; 27: 229-245.

[43] Lima LR, Marcondes AA, Álcool Carburante uma Estratégia Brasileira, Curitiba, UFPR, 2002.

[44] Futuro para o mercado do álcool e açúcar. Available at: <http://www.biodieselbr.com/energia/alcool/mercado-etanol.htm>, 30 September, 2009.

[45] White paper on internationally compatible biofuel standards. Tripartite task force: Brazil, European Union & United States of America, Dec. 31, 2007. Available at: www.inmetro.gov.br/painelsetorial/biocombustiveis/whitepaper.pdf.

[46] Bioetanol de caña de azúcar: energía para el desarrollo sostenible. Coordinación BNDES y CGEE, Rio de Janeiro: BNDES, 2008.

[47] Biofuels and other renewable energy in the transport sector (2009). Available at: <http://ec.europa.eu/energy/renewables/biofuels/biofuels_en.htm>, 30 August, 2009.

[48] Energy independency and Security Act of 2007. Available at: <http://www.govtrack.us/congress/billtext.xpd?bill=h110-6>, 30 August, 2009.

[49] Inmetro, **Material de Referência Certificado de Bioetanol**. Available at: <http://www.inmetro.gov.br/metcientifica/MRC/material_bioetanol.pdf>, 30 September, 2009.

[50] VIM, **International Vocabulary of Metrology – Basic and general concepts and associated terms**, 2008.

[51] Emons H, Fajgelj A, Van Der Veen, A.M.H., Watters, R., **New definitions on reference materials**, Accred. Qual. Assur., 10, pp. 576-578, 2006.

[52] ABNT ISO Guia 31:2004, **Materiais de Referência – Conteúdo de certificados e rótulos**. Segunda edição.

[53] ABNT ISO Guia 34:2004, **Requisitos gerais para a competência de produtores de materiais de referência**. Primeira edição.

[54] ISO Guide 35:2006, **Reference materials** – General and statistical principles for certification. Third edition.

[55] Richter W, **Primary methods of measurement in chemical analysis**, Accred Qual Assur 1997; 2: 354-359.

[56] Máriássy M, Vyskocil L, Mathiasová A, **Link to the SI via primary direct methods**, Accred Qual Assur 2000; 5:437-440.

[57] Fraga ICS, Rodrigues JM, Borges PP, Araújo TO, Cunha VS, **Os conceitos envolvendo materiais de referência**, Metrologia & Instrumentação, abril/maio, 2009; 40-43.

[58] ABNT ISO Guia 30:2000, **Termos e definições relacionados com materiais de referência**. Primeira edição.

[59] ABNT ISO Guia 32:2000, **Calibração em química analítca e uso de materiais de referência certificados**. Primeira edição.

[60] ABNT ISO Guia 33:2002, **Utilização de materiais de referência certificados**. Primeira edição.

[61] ABNT NBR ISO/IEC 17025:2005, **Requisitos gerais para a competência de laboratórios de ensaio e calibração**.

[62] **Guia para a Expressão da Incerteza de Medição**, 3 edição brasileira, ABNT / Inmetro, SERIFA comunicação: Rio de Janeiro, 2003.

[63] Linsinger TPJ, Pauwels J, Van der Veen AMH, Schimmel H, Lamberty A, **Homogeneity and stability of reference materials**, Accred Qual Assur 2001; 6: 20-25.

[64] Pauwels J, Van der Veen AMH, Lamberty A, Schimmel H, **Evaluation of uncertainty of reference materials**, Accred Qual Assur 2000; 5: 95-99.

[65] Van der Veen AMH, Linsinger TPJ, Schimmel H, Lamberty A, Pauwels J, **Uncertainty calculations in the certification of reference materials. 4. Characterisation and certification**, Accred Qual Assur 2001; 6: 290-294.

[66] Van der Veen AMH, Linsinger T, Pauwels J, **Uncertainty calculations in the certification of reference materials. 2. Homogeneity study**, Accred Qual Assur 2001; 6: 26-30.

[67] Van der Veen AMH, Pauwels J, **Uncertainty calculations in the certification of reference materials. 1. Principles of analysis of variance**, Accred Qual Assur 2000; 5: 464-469.

[68] Lamberty A, Schimmel H, Pauwels J, **The study of the stability of reference materials by isochronous measurements**, Fresenius J Anal Chemistry 1997; 360:359-361.

[69] Van der Veen AMH, Linsinger TPJ, Lamberty A, Pauwels J, **Uncertainty calculations in the certification of reference materials. 3. Stability study**, Accred Qual Assur 2001; 6: 257-263.

[70] International Laboratory Accreditation Cooperation, **ILAC G12 Guidelines for the Requirements for the Competence of Reference Materials Producer**, ILAC, 2000.

[71] **Guidelines for feasibility studies on certified reference materials**, European Commission, EUR 20574 EN, 2002.

[72] Resolução ANP n. 36, de 16 de dezembro de 2005.

[73] American Society for Testing and Material (ASTM) Standard, D4806:1998, **Denatured fuel ethanol for blending with gasoline, for use as automotive spark-ignition engine fuel**.