

Protocol nº 006/2016 (Dimci/GT-PEP) – Revision nº 02

Proficiency Testing in Cachaça – Organic Parameters

4th Round – Methanol, 1-butanol, 2-butanol, isobutanol, 1-propanol and ethyl carbamate

ORGANIZATION AND COORDINATION

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SPECIFIC OBJECTIVES

- To determine the performance of laboratories for the proposed test;
- To contribute for the confidence increase in laboratory measurement results;
- To contribute for the continuous improvement of the measurement techniques of each laboratory;
- To aggregate value to the Brazilian cachaça in order to increase the health protection of consumers and to conquer international markets.

PARTICIPATION

Any laboratory that performs organic contaminants analysis in distilled alcoholic beverages may take part in this proficiency testing, provided it meets the criteria outlined below:

1. Accredited laboratory by Inmetro on the basis of ABNT NBR ISO/IEC 17025 for the scope of this PT;
2. Laboratory that is undergoing Inmetro's accreditation process;
3. Cachaça Producer;
4. Other distilled alcoholic beverages producers;
5. South Korea KTR (Korea Testing & Research Institute) indicated laboratories.

The maximum number of participants in this round is 30 (thirty) laboratories, 20 participants that meet the criteria 1 to 4 and 10 participants that meet the criterion 5. If the number of registrations exceeds the maximum number of participants, it will be taken into consideration the criteria order established above.

For participation, laboratories that fit criteria 1 to 4 shall fill out the registration form through Inmetro's website (<http://www.inmetro.gov.br/metcientifica/profiCachaca.asp>). Laboratories that fit criterion 5 will be invited through KTR contact. KTR is responsible for sending the registration forms of these laboratories to Inmetro via e-mail pep-inmetro@inmetro.gov.br.

We emphasize that participation in this PT is free of charge and the deadline for registration expires on **28/October/2016**.

PROFICIENCY TEST ITEM

The test items are 10 mL glass bottles with rubber cover and metal seal containing 6 mL cachaça. Each bottle will be duly identified, containing the program name in its label, the round number and the sample number. For this round it was selected the following parameters described in the Normative Instruction (IN) nº 13 of 29/June/2005 from

the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA)¹, which establishes the maximum cachaça contaminant limits as follows:

1. **Methanol** – Due to its high toxicity, its presence in cachaça is consequence of degradation of pectin present in sugar cane. It's considered a cachaça organic contaminant, with maximum amount established by IN nº13/2005 of MAPA in 20,0 mg/100 mL.
2. **1-butanol** – Coming from the bacterial contamination in the fermentation process. It is considered an organic cachaça contaminant, with maximum amount established by IN nº13/2005 of MAPA in 3 mg/100 mL.
3. **2-butanol** – Coming from the bacterial contamination in the fermentation process. It is considered an organic cachaça contaminant, with maximum amount established in 10 mg/100 mL.
4. **Isobutanol** – One of the superior alcohols that enter the Congeners Coefficient calculation, with maximum amount established by IN nº13/2005 of MAPA in 360 mg/100 mL of superior alcohols (expressed by the sum of n-propyl alcohol, isobutyl alcohol and isoamyl alcohols). The congeners are chemical substances found in cachaça and formulated during fermentation, distillation and maturation, comprising properties that have direct relevance to its flavor and aroma.
5. **1-propanol** – One of the superior alcohols that enter the Congeners Coefficient calculation, with maximum amount established by IN nº13/2005 of MAPA in 360 mg/100 mL of superior alcohols (expressed by the sum of n-propyl alcohol, isobutyl alcohol and isoamyl alcohols). The congeners are chemical substances found in cachaça and formulated during fermentation, distillation and maturation, comprising properties that have direct relevance to its flavor and aroma.
6. **Ethyl carbamate** – Due to its carcinogenesis potential, it appears as a fermentation product of sugar cane. It is considered a cachaça organic contaminant, with maximum amount established by IN nº28/2014 of MAPA in 210 µg/L, which changed the maximum limit established by IN nº13/2005 of MAPA (which was 150 µg/L).

Each participant will receive 4 bottles, 2 of them for ethyl carbamate analysis and the other 2 for the other parameters.

In the registration form each laboratory shall indicate on which parameters will perform the measurements.

TEST ITEM DISPATCH

On the date set in the schedule it will be sent to each participating laboratory that fit the criteria 1 to 4 of the "Participation" item 1 (one) cardboard box (12,5 cm x 11,5 cm x 2,5 cm dimensions), properly sealed, containing the test items to be analyzed. The PT coordination is responsible for the test item dispatch to the participant laboratories, which will be made via Sedex. Regarding criterion 5 of the "Participation" item, Inmetro will send to KTR a polystyrene box with the 10 kits, containing the test items to be analyzed by the indicated laboratories. It will be KTR responsibility the distribution to the indicated participants.

Upon receipt of the test item, the laboratory shall perform an inspection to verify the existence of any leak or damage to the bottle that may invalidate the measurement results. The inspection result shall be registered in the receipt item form (English or Portuguese version) available on Inmetro website.

CONFIDENTIALITY

Each participant will be identified by an individual code which will be known just by the participant and by this PT coordination, except laboratories accredited by Inmetro's General Accreditation Coordination (Cgcre), which

¹ These parameters are set out in Annex "REGULAMENTO TÉCNICO PARA FIXAÇÃO DOS PADRÕES DE IDENTIDADE E QUALIDADE PARA AGUARDENTE DE CANA E PARA CACHAÇA" of the referred Normative Instruction and can be accessed at the address <http://extranet.agricultura.gov.br/sislegis-consulta/servlet/VisualizarAnexo?id=14175>.

will have their identification code available. The participant will receive, by email, the identification code corresponding to its PT participation. This code shall be used as identification in filling out the results form. The results can be used by Inmetro in studies and publications, respecting the confidentiality.

As established in item 4.10.4 of ISO/IEC 17043:2010, in exceptional circumstances, a regulatory authority may request the PT provider the results and the participants' identification. When this occurs, the PT provider will notify this action to the participants.

At the end of this PT it will be provided to those who sent their results a participation certificate. This certificate will contain the participant identification code.

COMPLAINS AND APPEALS

In case the participant wishes to formalize a complaint or an appeal regarding the proficiency test an email must be sent to pep-inmetro@inmetro.gov.br.

ADDITIONAL INFORMATION

Through this PT protocol the participants will have the information and rules. The contact with this PT Coordination or Committees can be made by the phone number (21) 2145-3002 or via email: pep-inmetro@inmetro.gov.br.

PROGRAM DOCUMENTS

At Inmetro's website (<http://www.inmetro.gov.br/metcientifica/ensaio-proficiencia/profiCachaca.asp>) all necessary documents for this program it will be available, which are:

- Proficiency Testing Protocol (English and Portuguese version);
- Registration Form (only in Portuguese version);
- Receipt test item Form (English and Portuguese version);
- Report Form (English and Portuguese version).

The English version Forms should be filled only by the non-Brazilian laboratories.

SCHEDULE

Description	Initial Date	End Date / Deadline
Registration period.	19/October/2016	28/October/2016
Shipping the test items to KTR in Korea.	-	11/November/2016
Dispatch the test items to laboratories by Inmetro.	-	16/November/2016
Sending the identification code of each Brazilian participant by email.	-	17/November/2016
Sending the identification code of each Korean participant by email.		05/December/2016
Results Record Form submission to PT coordination by the Brazilian participants.	-	16/December/2016
Results Record Form submission to PT coordination by the Korean participants.		23/December/2016
Sending the draft report to participants by email.		06/April/2017
Deadline for the participant to submit the considerations on the draft report to the PT coordination.	-	20/April/2017

Description	Initial Date	End Date / Deadline
Final report approval by the head of the Technical Division and the PT Coordinator.	-	16/May/2017
Final report available on Inmetro's website for download and communication to participants by email.	-	18/May/2017
Sending the PT participation certificate.	-	19/May/2017

MÉTODOS DE MEDIÇÃO

PT participants laboratories shall use one of the routine methods and must perform 3 (three) readings per parameter in each bottle, 2 (two) bottles per parameter, totaling 6 readings (see Report Form). Laboratories that do not send the 6 readings of each parameter will be **EXCLUDED** from de PT.

Table 1 presents the recommended measurement techniques, as well as the measurement range for each parameter and some reference standards. The use of these standards is not mandatory and each laboratory shall use its routine method, recording details of it in the results registration form available by this EP coordination.

Table 1: Parameters range for the test items and recommended methodologies for analysis

Batch	Parameter	Range	Recommended analytical technique	Reference Standards
1	Methanol	2 e 30 mg/100g	Gas chromatography with flame ionization detection (GC-FID) or gas chromatography mass spectrometry (GC-MS)	AOAC Official Method 972.11 (CG-DIC): methanol; AOAC Official Method 968.09 (CG-DIC) or 972.10 (CG-DIC): other alcohols
	2-butanol	1 e 20 mg/100g		
	1-butanol	0,1 a 10mg/100g		
	Isobutanol	5 a 100 mg/100g		
	1-propanol	5 a 100 mg/100g		
2	Ethyl carbamate	50 e 300 ng/g	Gas chromatography with mass spectrometry (GC-MS)	AOAC Official Method 994.07 (CG-EM)

MEASUREMENTS RECORDING AND RESULTS SUBMISSION

Laboratories shall make the measurement records in an excel file named:

- in Portuguese, “Formulário de registro de resultados”;
- in English, “Report Form”.

The file contains seven spreadsheets. In “Formulário de Registro de Resultados”: “Instruções”, “Metanol”, “1-butanol”, “2-butanol”, “Isobutanol”, “1-propanol” e “Carbamato de Etila”. In “Report Form”: “Instructions”, “Methanol”, “1-butanol”, “2-butanol”, “Isobutanol”, “1-propanol” and “Ethyl carbamate”.

Before filling out the information and the results in the spreadsheets, it is important to read the guidelines in the instruction spreadsheet.

The concentration of the analytes reported by the laboratory should be in mg/kg with at least three decimal places. Each laboratory is responsible for the conversion of the concentration unit according to the value of the density of the cachaça samples for the determination of alcohols and the value of the density of the water-alcohol solution samples (simulated cachaça) for determination of ethyl carbamate. The density values are informed in table 1, on the “Instructions” (English version) or “Instruções” (Portuguese version) spreadsheet. The results reported in units other than mg/kg will be not evaluated.

After filling in the data, each spreadsheet shall be protected with a password. The password is the digital signature and will ensure data integrity. If the laboratory uses Microsoft Office 2007 software or 2010, click the “Review” tab and then “Protect spreadsheet”. That will open a dialog box where it will be necessary to define a password known only to the participant. After that, click “OK” and save the file. If the participant uses a Microsoft Office version prior to 2007, click menu “Tools”, then “Protect” and “Protect spreadsheet”. Define a password known only to the participant and click “OK”, and then save the file.

It's important to follow the protection instructions described above for the Technical Committee to access the data sent by the laboratories. In case of doubt, contact the PT coordination (e-mail: pep@inmetro.gov.br). The file with the password protected spreadsheet shall be sent to the PT coordination through the email pep-inmetro@inmetro.gov.br until **16/December/2016** (Brazilian laboratories) and **23/December/2016** (KTR indicated laboratories).

Remarks:

Only the results reported in the “Report Form”, identified by the laboratory code (submitted to the PT coordination), protected with password and in the time period established in the schedule will be analyzed.

The results shall only be reported with a minimum of three decimal places.

ASSIGNED VALUES AND ITS UNCERTAINTY

According to the procedures stated in NBR ISO/IEC 17043:2010, the determination of the assigned value of this PT will be through the use of certificated reference values.

The assigned value (X) and the standard uncertainty (u_X) of the test item will be obtained according to the approach of the ISO/IEC GUIDE 35 standard. The assigned value will be the result of the CRM characterization study, which will be performed by the primary method of isotope dilution mass spectrometry (IDMS) for the ethyl carbamate and by two independent methods for the other parameters.

EVALUATION OF PERFORMANCE

For the performance evaluation of the participant laboratories, according to ISO/IEC 17043:2010, the z-score (z) statistic method will be applied, in which the estimate of the variability (s) is the uncertainty of the reference value, determined by the reference laboratory. Laboratories who also inform the result measurement uncertainty (U_{Lab}) and the coverage factor (k), which are optional, it will also be applied the Normalized error (En) for their performance evaluation.

FINAL REPORT

The Technical Committee will prepare the draft and final reports of this PT, in Portuguese and English. Participating laboratories will receive the PT draft report and may send considerations (suggestions, doubts, etc.) to the PT coordination, by means of the e-mail pep-inmetro@inmetro.gov.br. The steps dates are included in the schedule of this protocol. The considerations will be evaluated by the Technical Committee and, when considered pertinent, will be incorporated in the final report. The final report will be emitted by Inmetro and published at Inmetro's website (<http://www.inmetro.gov.br/metcientifica/ensaioProficiencia.asp>).

The preliminary and final reports will contain information such as:

- Date of issue of the report;
- Identification of the test item;
- List of participating laboratories;
- Procedures used for the statistical analysis of data;
- Laboratories results, identified only by their codes;
- Comment on the participants' performance.

REVISION HISTORY

- Changing dates and steps inclusion in schedule;
- Changing date for sending the spreadsheet with the results on item “Measurements Recording and Results Submission”.
- Changing the sentence related to the dates for receiving and sending considerations to the draft report by the participants to the PT coordination on item “Final Report”.