REPUBLIC OF SERBIA
MINISTRY OF ECONOMY
DIRECTORATE OF MEASURES AND PRECIOUS METALS
11000 BELGRADE, Mike Alasa 14,
Phone: +381 11 20 24 450, Fax: +381 11 21 81 668
www.dmdm.gov.rs, mail: office@dmdm.rs

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Certification report – CRMs “Ethanol in water solutions” – DMDM-EXX

Jelena Bebić, Ph.D
Head of Group for Metrology in Chemistry
e-mail: jelenabebic@dmdm.rs

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1 SCOPE AND INTRODUCTION

This document describes the characterisation of the Certified Reference Materials (CRMs) „Etanol in water solutions“, which are suitable for type approval and regular calibration or verification of breath analyzers according to OIML R 126[1].

Certified reference materials “Ethanol in water solutions” (CRM “Ethanol in water solutions”) - are solutions of ethanol in water with certified concentration values and respective stated measurement uncertainties. On the basis of testing with CRM “Ethanol in water solutions”, all breathe alcohol analyzers being used in Serbia are type approved and their respective initial, casual and periodic verifications are performed. DMDM has developed the procedure for individual preparation of the CRM “Ethanol in water”. Ten different concentrations of CRM “Ethanol in water solutions” are produced in DMDM. This report includes supporting documentation for the determination of metrological characteristics (measurement uncertainty, stability and homogeneity data) of gravimetricaly produced, by spiking, reference materials "Ethanol in water solutions". Certification is based on a working instruction РУ-X10 “The standard operating procedure for the consistent production of certified reference materials - Ethanol in water". Certified mass concentrations of CRMs DMDM-EXX are in the range of 0 g/L – 5.02 g / L (table 1).

Table 1: Cataloge of CRMs „Ethanol in water“that are produced in DMDM.

CRM code	Mass concentration of solutions at 20 ^{o}C (g/L)	Measurement uncertainty (g/L)
DMDM-E01	0.0000	0.0001
DMDM-E02	0.2573	0.0007
DMDM-E03	0.6432	0.0014
DMDM-E04	1.0292	0.0025
DMDM-E05	1.8011	0.0043
DMDM-E06	2.4443	0.0059
DMDM-E07	3.8594	0.0092
DMDM-E08	5.0172	0.0012
DMDM-E09	1.2252	0.0030
DMDM-E10	0.6126	0.0015

The report is based on study of DMDM-E02, DMDM-E07 and DMDM-E09 and on the subsequent certification of all other reference materials produced. These three solutions are chosen to undergo all tests due to the fact that they are most commonly purchesed and used for calibration of breath analysers by our costumers. Also, DMDM-E02 and DMDM-E07 are solutions with the lowest and highest mass concentration, respectively, that are regularly produced in DMDM. By testing the solutions having the lowest and highest mass concentration, all measurement range is covered, which means that the obtained results for these solutions can be applied on the other solutions with mass concentrations in between tested mass concentrations.

CRMs may also be used for analytical method validation. Production of Reference Material was done in accordance with ISO 17034:2016 – General requirements for the competence of reference material producers and with working instruction РУ-X10.

2 PRODUCTION PROCEDURE AND EQUIPMENT

Laboratory ambient conditions are maintained to ensure the quality of the environment required for preparation of the solutions ethanol in water and during measurement, ethanol must be kept under temperature below 20 °C in order to prevent its evaporation or the absorption of moisture from the environment. Both, ethanol and water must be under the same laboratory conditions for at least 24 hours.

Ethanol and deionized water are separately weighed using calibrated balances, with stated uncertainty values, ethanol in amber glass vials and water in 1 L dark glass bottles, and then mixed together in glass bottles. After mixing, the bottle containing the CRM solution is manually shaken and left to homogenize for 48 h. Each CRM prepared is submitted to a gas chromatography analysis (HSGC-FID) in order to confirm the concentration of gravimetrically prepared solution. Purity of ethanol is assessed *via* Karl-Fischer titration.

Each bottle has its own lot number and is accompanied by its own Calibration Certificate. The bottles with CRM are marked with a label whose numerical code consists of a date and a serial number **(DMDM-EXX/serial number/YYMMDD).**

2.1 EQUIPMENT FOR PREPARATION AND CHARACTERIZATION OF REFERENCE MATERIALS

No.	EQUIPMENT				Remark
	Name	Type/model	Manufacturer	Serial number	
1.	Non-automatic balance	PM4000	Mettler, Switzerland	F69840	measuring range from 0 kg to 4 kg, resolution 0.01 g
2.	Non-automatic balance	GPC 225-CW	Sartorius, Germany	23708644	measuring range from 0 g to 220 g, resolution 0.00001 g
3.	Non-automatic balance	JL-180	Chyo, Japan	307114	measuring range from 0 g to 180 g, resolution 0.0001 g
4.	Gas chromatography with flame ionization detector (HSGC-FID) and Headspace	GC-2010 AOC-5000	Shimadzu, Japan		
5.	KF Coulometer	831	Metrohm		•**Determination range** - 10 µg to 200 mg H_2O •**Resolution**-0.1 µg H_2O

2.1.1. SUPPORTING EQUIPMENT

No.	Name
1.	Dark glass bottles with threaded plastic closures, 1L
2.	Gas-tight syringe (5 mL)
3.	adjustable-volume pipette (1-10) mL, (100-1000) µL, (10-100) µL

No.	Name
6.	Screw thread glass vials with PTFE caps (4 mL), (10 ml), (24 mL)
9.	Screw thread glass vials with PTFE silicone septa aluminum caps-GC vials (24 mL)
10.	Metal clamp
11.	Glass flasks (250 mL and 100 mL)

2.2 REQUIRED CHEMICALS FOR PREPARATION AND CHARACTERIZATION OF REFERENCE MATERIALS

No.	Name	Manufacturer
1.	≥ 99.9 % Ethanol	Ethanol G CHROMASOLV®, absolute, for gradient elution, HONEYWELL, USA
2.	Deionized water	DMDM- production within the institute
3.	≥ 99.9 % n-Propanol	CHROMASOLV®, HPLC grade, SIGMA-ALDRICH, USA
4.	HYDRANAL®-Coulomat AG reagent	Anolyte solution for coulometric KF titration, HONEYWELL, USA
5.	HYDRANAL®-Coulomat CG reagent	Catholyte solution for coulometric KF titration, HONEYWELL, USA
6.	HYDRANAL® Water Standard 1.0	Water standard for Karl Fischer titration (water content 1 mg/g = 0.1 %)

3 CERTIFICATION

3.1 CALCULATION OF THE ETHANOL CONCENTRATION

Calculation of the certified ethanol mass concentration is performed according to following equation:

$$\gamma_{aq} = c_m . \rho(c_m) = \frac{m_e . p . f_{eva}}{m_e + m_w} . \rho(c_m)$$

γ_{aq} = mass concentration (at 20 °C) of ethanol in the solution
ρ = density of the ethanol/water solution at 20 °C
m_e = mass of ethanol (with buoyancy correction)
m_w = mass of water (with buoyancy correction)
p = purity of ethanol as an ethanol mass fraction
f_{eva} = correction factor to ethanol loss

The density of the solution at 20 °C as a function of ethanol mass fraction is taken from PTB report W-46, 2nd edition [5] or international alcoholometric tables [6].

3.2 MEASUREMENT UNCERTAINTY CALCULATION

Uncertainties of the ethanol mass concentrations $u_c(w)$ were estimated by propagating the standard uncertainties $u(x_i)$ of the input quantities x_i (x_i i: m_e, m_w, p, $\rho(c_m)$, fe_{va}) according to ISO GUM[2].

$$u_c^{\,2}(w) = \sum \left(\frac{\partial w}{\partial X_i} \right)^2 \cdot \left(u(x_i)\right)^2$$

$$w_i = \left(\frac{\partial w}{\partial x_i} \right)$$

Standard uncertainty of masses of ethanol and water m_e and m_w

The load depended balance reproducibility calculated from the long term control charts for the balances were taken as an estimate for the uncertainty of the masses. The uncertainty of the buoyancy correction was taken into consideration, although its influence is negligible when compared with the remainder of uncertainty contributions. The uncertainty of the balance was taken from the Calibration certificates of the balances. The uncertainty of the balance resolution was assumed from the rectangular distribution.

Purity p

The standard deviation of a triangular distribution $1 \geq p \geq 0.999$ was taken as an estimate for the uncertainty of p.

Correction factor for evaporation f_{eva}

The loss of ethanol due to evaporation can occur during the transfer of the vial with ethanol into the bottle with water and during the removal of the vial from the bottle. The transfer of the ethanol vial takes about 3 s. To obtain an estimate of the loss of ethanol within this period, the mass reduction in an open vial with ethanol was measured on a balance as a function of that time. The uncertainty of the loss of ethanol during the removal of the vial from the bottle was estimated in the same way. The sum of all possible losses of ethanol was taken as an estimate for $u(f_{eva})$.

Density of the ethanol/water solution $\rho(c_m)$

The density $\rho(c_m)$ at 20 °C as a function of ethanol mass fraction c_m was calculated using the equation:

$$u(\rho) = \frac{\rho_- - \rho_+}{2}$$

where ρ - and ρ_+ are the densities of solutions with ethanol mass fractions $(c_m - u(c_m))$ and $(c_m + u(c_m))$, respectively.

The standard uncertainty $u(c_m)$ was calculated using the equations in document A1.1.2/A1.1.4 A protocol for the preparation of ethanol in water solutions including cleaning and drying procedures.

Exmple of uncertainty budget calculation is given for DMDM-E09 CRM.

Table 2: The uncertainty budget of DMDM-E09

Quantity (x_i)	Uncertainty $u(y)$				Contribution $\lvert c_i \rvert \cdot u(x)$ g/L
	Source	Distribution	Standard uncertainty	Combined standard uncertainty	
Mass ethanol	Weighing	Normal, A	0.00007 g	0.00008 g	0.00002

Quantity (x_i)	Uncertainty $u(y)$				Contribution $u(x)$ $\lvert c_i \rvert \cdot u(x)$ g/L
	Source	Distribution	Standard uncertainty	Combined standard uncertainty	
m_e	Balance calibration	Normal, B	0,00003 g		
Mass water M_w	Weighing	Normal, A	0.05 g	0,055 g	0.0002
	Balance calibration	Normal, B	0.02 g		
Purity p	inpurities	Triangular, B	0.0005	0.0005	0.00024
	In ethanol				
Evaporation f_{eva}	Transfer of the vial	B	0.00005	0.00005	0.00004
	Evaporation from the bottle	B	0.00001		
Density, ρ	Table calculation	B	0.0001 g/L	0.0001 g/L	$1 \cdot 10^{-6}$
Ethanol mass concentration at 20 °C	1.0292 g/L	0.0025			

Ethanol-water CRMs are always produced in the same way by spiking. Slight changes in the ethanol concentration from bottle to bottle are caused by fluctuations in the weighed-in mass of ethanol. The uncertainty budget is transferable to all solutions of the same reference material code. Uncertainty of the blank value DMDM-E01 is defined by the rounded detection limit of the analytical method as a measure of the expanded uncertainty of the blank value.

4 VERIFICATION OF THE CERTIFIED VALUE

Headspace gas chromatography with flame-ionization detection (HSGC–FID) is chosen to be used for CRM analysis because of its ease of automation, sensitivity, accuracy, and relative specificity. This method is based on determination of the concentration of ethanol in CRM using n-propanol as the internal standard. Measurement of CRM samples is done using method "CRM20072018". The quantification of ethanol in CRM sample is performed by comparing the value of the peak area of the analyzed substance ethanol and n-propanol (internal standard) using the internal software. Prior to HSGC-FID analysis, the internal standard solution is prepared and added in all samples. The concentration of internal standard is approximately 100 mg/g.

Ethanol elutes with a retention time of 2.407 minutes, and the n-propanol (internal standard) elutes with a retention time of 2.894 minutes. The total analysis time of one CRM sample is 7.33 minutes. The measured values of CRM sample solutions are recorded on GC device. Each CRM sample are measured in triplicate.

The parameters of the GC-FID method are:

-Pre-treatment (Headspace autosampler parameters): heat the GC-vial with sample (10 ml of CRM sample and 100 µ of internal standard) for 3 minutes at 70 °C with constant agitation (Agitation speed 500 rpm).

- Capillary column InterCap® FFAP (Inner diameter 0.25 mm x Length 30 m, Film thickness 0.25 µm, Max. Temp. 240 °C), GL Sciences Inc, Tokyo, Japan

- The column temperature program: holds at 70 °C for 1 minutes, afterwards linearly increase temperature from 70 to 120 °C at 15 °C / min., holds 120 °C for 3 min.

-The injection temperature: 150 °C, Injection speed 500 µL/s, Sample volume: 500 µL.

-The carrier gas: nitrogen with total flow of 50 mL/min and purge flow 3 ml/min.
-The detector temperature: 250 °C

Method validation parameters are given in the table:

Parameters that are validated	***Obtained values***
LOD	0,018 g/l
LOQ	0,06 g/l
Repeatability	Standard deviation of replicate injections < 0.5 %
Within-lab reproducibility	<0.1 %
Trueness/Recovery	Comparison with NIST SRM 2895, control chart, measurement results are within the uncertainty of certified value
Measurement uncertainty	*U =4,4 % (0,129 g/kg ethanol), U=0,56 % (5,05 g/kg ethanol)*

5 METROLOGICAL TRACEABILITY

To ensure the traceability of the product, the high purity of ethanol and calibration of the balances are required. The balances are calibrated in one year interval internally within DMDM. Each balance is checked prior to each preparation of CRMs. Detailed weighing procedure and regular calibration regime for balances are described in document ALCOREF DMDM Short report A.1.1.5.

Ethanol used for preparation of solutions is Chromasolv G, SIGMA-ALDRICH® for liquid chromatography, purity ≥ 99,9 %. Purity of ethanol is confirmed *via* Karl-Fischer titration. At the start of CRM production process, a sample of ethanol (Ethanol G CHROMASOLV®, purity of 99.9 %) is taken for determination of the water content *via* Karl Fischer titration, based on a procedure ALCOREF DMDM Short report A.1.1.6. If the water content is above the limit defined by the manufacturer's specification, that ethanol is replaced with a new one.
Certified value of concentration of gravimetrically prepared solutions is traceable to international mass standard that represents realisation of a mass unit in accordance with International System of Units (SI). Traceability was realised through the non-automatic balance, producer Sartorius, Germany, type GPC 225-CW, serial number 23708644, measuring range from 0 g to 220 g, resolution 0,01 mg; as well as non-automatic balance, producer Mettler, Switzerland, type PM4000, serial number F69840, measuring range from 0 kg to 4 g, resolution 0.01 g, calibrated internally at DMDM.

6 HOMOGENITY STUDY

Three CRMs with different mass concentrations (DMDM-E02, DMDM-E09 and DMDM-E07) were chosen to undergo the homogenization test. Before final sealing of bottles with CRMs, in the spare glass vials are taken samples of prepared CRMs. The vials with samples are labelled and then used for analysis on GC-FID.

The homogeneity was tested by taking the sample from the bottom and the top of each bottle with prepared CRM (homogeneity within bottle). Also, the influence of time on homogenization process was examined, for some bottles with prepared CRM the homogenization period lasted 24 h and for some 48 h. Additionally, half of bottles with prepared CRMs were kept standing upright while the other half of bottles with CRMs were kept standing upside down during homogenization (24 and 48

h). The criterion for evaluation of results regarding homogeneity is established according ISO Guide 35:2017 [4]. Results for DMDM-E09 are given in a table.

Sample ID	*Measurement uncertainty, mg/g*	*Deviation from gravimetric value, % (Allowed deviation 0.5 %)*	*Homogeneity within bottle (difference between top and bottom), mg/g*	*Difference whether the bottle is standing upright or upside down), mg/g*
DMDM-E09/1/251218	*0.0030*	*0.075*	***0.0017***	***0.0021***
DMDM-E09/2/251218	*0.0030*	*-0.097*	***0.0001***	
DMDM-E09/1/111218	*0.0030*	*0.059*	***0.0005***	***0.0010***
DMDM-E09/2/111218	*0.0030*	*0.124*	***0.0008***	

The obtained results for homogenization demonstrated good homogeneity for the period homogenization of 48 h, with bottles standing upright and occasionally shaking, with samples taken frome either top or the bottom of the bottle, for all CRMs tested. The difference between bottles and within bottle is within stated criterion.

7 STABILITY STUDY

7.1 SHORT TERM STABILITY STUDY

Three different CRMs were selected to undergo short term stability test, DMDM-E02, DMDM-E09 and solution DMDM-E07. Selected CRMs solutions were prepared in duplicate by means of gravimetry. Before final sealing of CRMs bottles, the sample of each prepared CRM is taken for HSGC-FID analysis (initial analysis). The prepared CRMs (6 bottles of each selected solution) were kept at 45 °C for different time periods (1, 2 and 4 weeks) based on Isochronous scheme, and then the bottles were transferred to the reference temperature (room temperature of 18 °C to 22 °C). Within DMDM, it is estimated that temperature in bottle of CRM during transport at hot summer day should not exceed 45 °C, so it was chosen to conduct short term stability test at this temperature. Additionally, two bottles of each selected solution (DMDM-E02, DMDM-E07 and DMDM-E09) are kept at reference temperature for 4 weeks. When transfer of all solutions to the reference temperature is completed (after 4 weeks), all prepared solutions (in total 24 bottles) were analysed on HSGC-FID. The obtained results are then compared and statistically evaluated.
In order to assess if selected samples of CRMs can meet requirements with regard to stability, the following criterion is established (ISO Guide 35:2017):

$$|X_{CRM} - X_{meas}| \leq k\sqrt{u_{CRM}^2 + u_{meas}^2}$$ (ISO Guide 35:2017)

If this criterion is met, where XCRM is the gravimetric concentration of the CRM, Xmeas the concentration of solution measured on HSGC-FID, and k is an appropriate coverage factor at a level of confidence of 95 %, then the CRM may be considered to be sufficiently stable, and the stability is demonstrated (provided that the method of measurement is unbiased).

The results for DMDM-E07 are given below

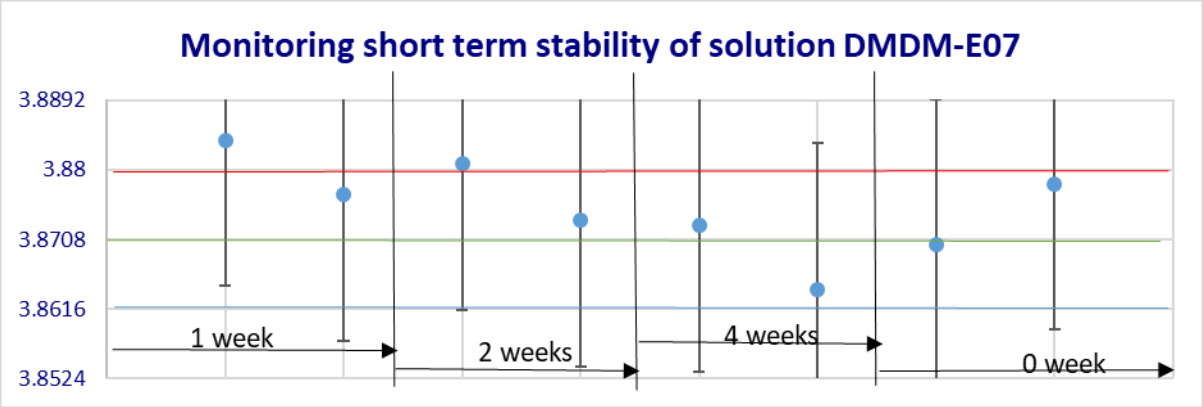


Figure legend: *Green line-gravimetric value, mg/g, red line-maximum allowed gravimetric value according to measurement uncertainty of gravimetric method, mg/g, blue line- minimum allowed gravimetric value according to measurement uncertainty of gravimetric method, mg/g, blue dots-measured value on GC, mg/g, error bars-measurement uncertainty of GC method, umeas, mg/g.*

As it can be seen from the graph., all tested solutions DMDM-E07 met the stated requirement with regard to stability. Difference between XCRM and Xmeas, t=4weeks was lower then 0.0428 mg/g which was assigned criterion. By comparing the results obtained during initial GC analysis and GC analysis after 4 weeks, it is noticable that difference between this results is also lower than assigned criterion. So it can be concluded that the reference material DMDM-E07 is sufficiently stable, and its stability is demonstrated during exposure on 45 °C for 4 weeks.

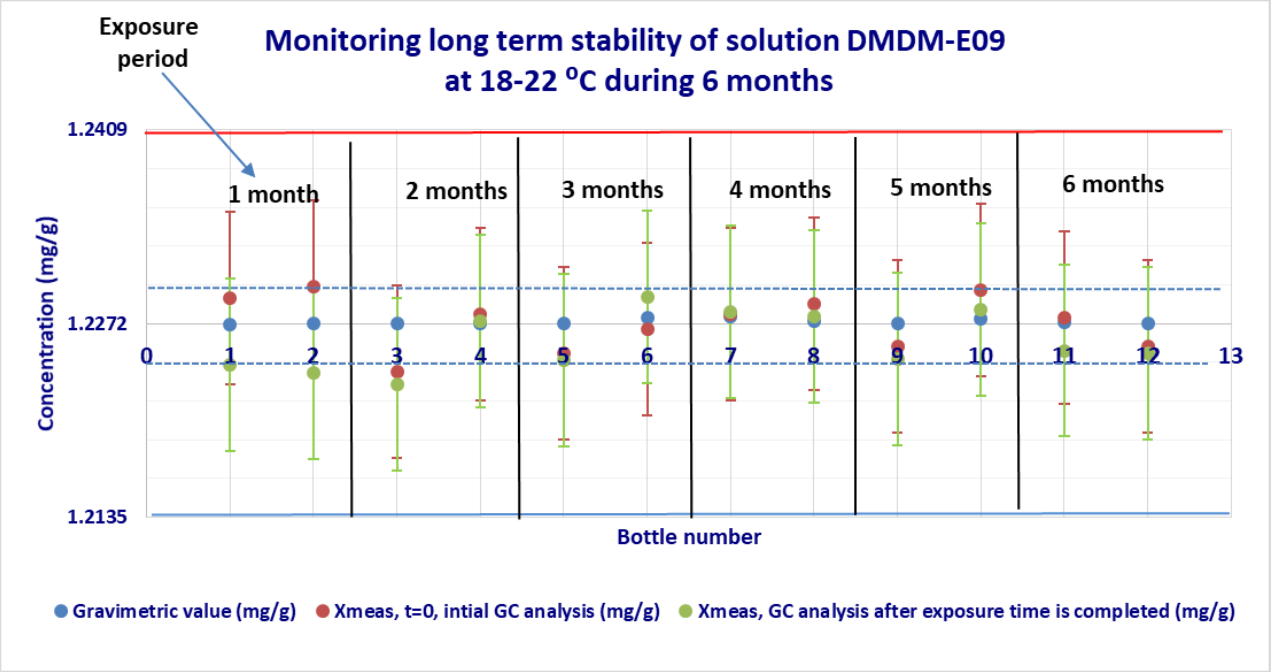
Similar results were obtained for all other CRMs investigated, so the short term stability, as a simulation of transport conditions, was demonstrated to be satisfactory, for the period of four weeks.

7.2 LONG TERM STABILITY STUDY

Three different CRMs were selected to undergo long term stability test DMDM-E02 ,DMDM-E09 and solution DMDM-E07. Selected CRMs solutions were prepared in duplicate by means of gravimetry. Before final sealing of CRMs bottles, the sample of each prepared CRM is taken for HSGC-FID analysis (initial analysis). The prepared CRMs (12 bottles of each selected solution were kept at reference temperature (18-22 °C) for different time periods (1-6 months).

All solutions (in total 36 bottles) were analysed on HSGC-FID after different time period. The obtained results are then compared and statistically evaluated.

In order to assess if selected samples of CRMs can meet requirements with regard to stability, the same criterion as for the short term stability is established based on ISO Guide 35:2017 [4].



According to the obtained results of this study, it is determined that shelf life for the CRMs produced in DMDM (DMDM-E0X) is 6 months. The certified values of the CRMs are valid within the stated uncertainty for 6 months period. However, due to the production process requirements and frequency of production, at DMDM, based on the monthly production, and on the fact that DMDM does not provide transportation of CRMs, the shelf life of CRMs is stated as three months, in the Certificate.

8 HANDLING AND STORAGE

CRMs ethanol-water produced in DMDM are packed in glass bottles, stored in cardboard boxes, in a separate storage room, with room temperature of (20 ± 2) °C.

CRMs are accompanied with MSDS sheet, issued by authorized legal entity, as well as with Cerificates (the Calibration report).

DMDM does not perform a distribution of a CRMs to end users. Instead, customers pick up the CRMs at DMDM, and organize transportation by themselves. During the pickup of CRMs, customers sign a dated dispatch note, that is kept at DMDM documentation for a defined time period.

CRM solutions should be stored at room temperature (between 15 °C and 25 °C), with no direct exposure to light. Period of validity of Reference Material to the first opening of the bottle is 3 months from the production date. After opening the bottle, the solutions are used at once. The homogeneity of the material is guaranteed if the subsample removed from the bottle is greater than or equal to 10 ml. After a subsample has been removed, the certified value for the solution remaining in the bottle can no longer be guaranteed.

9 PROCEDURE FOR ORDERING CRMS BY CUSTOMERS

The buyer starts purchase of CRMs from DMDM by submitting the printed version of request for purchase through DMDM Administrative Office (the request could be downloaded from web page http://www.dmdm.rs/Obrasci/DP-11_A 03_Zahtev_za_etalononiranje_RM.pdf).

The request includes information about the customer-buyer, as well as which and how much CRMs they want to buy. During submission of a request, the payment instruction are given to the buyer. If necessary, the customer can give additional comments. After that the order is released.

A delivery process takes place when preparation of CRMs are finished. The DMDM informs the buyer via e-mail when he can come to take the prepared CRMs. Payment of the order is done electronically within a week.

The production of CRMs at DMDM is done once a month.

10 REFERENCES

[1] OIML R126:2012 Evidental breath analyzers, https://www.oiml.org/en/files/pdf_r/r126-e12.pdf

[2] JCGM (2008), *Evaluation of measurement data — Guide to the expression of uncertainty in measurement*, 1st edn. JCGM 100:2008.

[3] ISO Guide 30:2015, Terms and definitions used in connection with reference materials; ISO Guide 31:2015, Reference materials Contents of certificates and labels; ISO Guide 33:2015, Uses of certified reference materials; ISO Guide 34:2009, General requirements for the competence of reference material producers; ISO Guide 35:2006, Reference materials General and statistical principles for certification, ISO, Geneva, Switzerland.

[4] ISO GUIDE 35:2006 Reference materials – General and statistical principles for certification. https://www.iso.org/standard/39269.html