UME CRM 1315: ORGANIC ACIDS IN LYOPHILIZED URINE
A New Certified Reference Material For Newborn Screening

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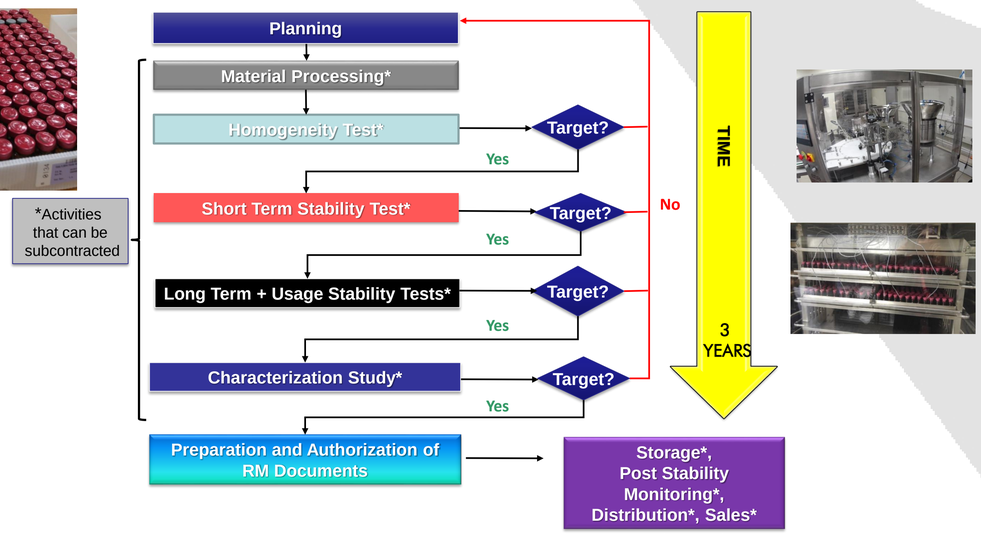
INTRODUCTION

Organic acid and amino acid concentrations are frequently measured for the treatment and diagnosis purposes of inborn errors of metabolism (IEM) which is a permanent and inherited biochemical disorder generally caused by organic acid and amino acid metabolism distortedness. While some of these diseases are life-threatening, some of them cause mental retardation, effect physical developments and lead to lifelong problems. Some of widespread diseases are caused by organic acid metabolism such as Methylmalonic Acidemia, Propionic Acidemia and biotinidase deficiency. Early diagnosis of metabolic diseases is very critical and they should be evaluated through reliable screening tests.
The Certified Reference Material (CRM) is utilized in chemical measurements as a useful tool for proving traceability of measurement result and enhances measurement quality. Particularly, it is important to use CRMs, having the same chemical compositions (matrix matched CRM), for the detection of subject quantity in the mixtures (matrix), such as body fluids, containing more than one metabolite. In this way, through the use of CRMs in measurements, metrological traceability chain can be ensured.

In this study, the production and the certification of the CRMs were carried out according to the technical requirements of ISO Guide 35, ISO 17034, ISO 15194 and quality management system based on ISO/IEC 17025. ID-LC-MS method was applied as primary method of measurement for the characterisation of the materials. The study includes details for the certification of “UME CRM 1315 Organic acid concentrations in lyophilized urine” in accordance with the requirements of ISO 17034:2016 [1]. The production facilities, chemical analyses, results of homogeneity assessment, stability and characterization studies, statistical evaluation of data, and conclusions were presented in the Certification Report. The corresponding uncertainties have been calculated in accordance with the ISO Guide 35:2017 [2].

REFERENCE MATERIAL PRODUCTION PROCESS



MATERIAL PROCESSING

UME CRM 1315 was prepared by adding organic acid standards into the urine containing changing quantities. Synthetic urine was purchased from Dyna-Tek, Inc (USA) and pure standards were purchased from Acros Organics, Fluka, Santa Cruz Biotechnology and Sigma-Aldrich (USA), Toronto Research Chemicals (Canada) and Enamine (Ukraine).
The homogenised solution was filled into amber glass vials using an automated filling machine (FARMATEK, FTED 1-150, Turkey) as approximately 3 mL for each unit. Filling order was recorded by labelling after filling. A total of 1500 units were prepared. The vials were first lyo-capped in the lyophilizer (Millrock Technology, USA) under slightly vacuum-nitrogen atmosphere and then crimp capped after being removed from the lyophilizer.
Units were classified in respect to CRM production stages (homogeneity, stability and characterization) with the random stratified sample selection approach by a software developed at TÜBİTAK UME (TRaNS). After classification into subgroups, samples were stored under selected test conditions. The units allocated for sale were stored at (-45 ± 5) ºC under controlled conditions, in the dark.

PROPERTY VALUE AND UNCERTAINTY ASSIGNMENT

Assigned values and uncertainities of the CRM were evaluated by applying approach in the characterization and uncertainty data that contribute to the homogeneity and stability assessments.
The certified value is the mean of the ID-LCMS results, which is a reference method traceable to the SI via Q-NMR purity analysis and gravimetric preparation.
The uncertainties of the certified values contains contributions of the characterisation u_{char}, the homogeneity u_{bb},the long-term stability u_{lts} and the short term stability u_{sts} .

TRACEABILITY

Stock solutions and calibrations solutions used in the in-house validated methods were gravimetrically prepared from the solid materials for which the purity values were assigned by qNMR measurements at TÜBİTAK UME. The assigned values of the amino acid calibrants are traceable to SI through qNMR measurements using SI traceable calibrants.

CERTIFIED VALUES AND UNCERTAINTIES

Parameter (CAS No)	Mass Fraction (mg/kg)		Molar Concentration[3] (µmol/L)	
	Certified Value[1]	Uncertainty[2]	Certified Value	Uncertainty
2-Methylcitric acid (6061-96-7)	1.64	0.12	8.09	0.60
3-Hydroxy-3-methylglutaric acid (503-49-1)	2.88	0.10	18.06	0.62
N-(3-Methylcrotonyl)gylcine (33008-07-0)	6.52	0.25	42.2	1.7
Adipic acid (124-04-9)	2.62	0.11	18.23	0.77
2-Hydroxyglutaric acid (13095-48-2)	1.54	0.12	10.57	0.81
2-Ketoglutaric acid (328-50-7)	7.58	0.68	52.8	4.8
3-Hydroxybutyric acid (300-85-6)	1.623	0.077	15.82	0.76
3-Hydroxyisovaleric acid (625-08-1)	3.36	0.15	28.9	1.3
Citric acid (77-92-9)	221	39	1170	210
Fumaric acid (110-17-8)	1.53	0.08	13.4	0.7
Glutaric acid (110-94-1)	0.210	0.027	1.62	0.21
Glycolic acid (79-14-1)	2.64	0.23	35.3	3.1
2-Ketoisovaleric acid (759-05-7)	0.830	0.035	7.27	0.31
Lactic acid (79-33-4)	11.16	0.61	126.0	6.9
Methylmalonic acid (516-05-2)	1.71	0.18	14.7	1.6
3-Methylglutaric acid (626-51-7)	0.969	0.054	6.75	0.38
N-Acetylaspartic acid (997-55-7)	4.96	0.26	28.8	1.6
Orotic acid (65-86-1)	2.047	0.082	13.35	0.54
2-Hydroxyphenylacetic acid (614-75-5)	9.9	2.1	66.2	14.1
Pimelic acid (111-16-0)	0.088	0.005	0.559	0.032
Pyruvic acid (127-17-3)	3.91	0.50	45.1	5.8
Sebacic acid (111-20-6)	0.110	0.009	0.553	0.046
Suberic acid (505-48-6)	1.27	0.08	7.41	0.47
Succinic acid (110-15-6)	7.29	0.40	62.8	3.5
N-Suberylglycine (60317-54-6)	1.29	0.04	5.67	0.18
N-Tiglylglycine (35842-45-6)	0.370	0.024	2.39	0.16
Mandelic acid (90-64-2)	7.99	0.32	53.4	2.2
5-Hydroxyindole-3-acetic acid (54-16-0)	0.87	0.34	4.60	1.81
N-Hexanoylglycine (24003-67-6)	0.373	0.015	2.19	0.09
N-(3-Phenylpropionyl)glycine (56613-60-6)	3.43	0.15	16.83	0.74
Mevalonolactone (674-26-0)	0.146	0.010	1.14	0.08
2-Ketobutyric acid (600-18-0)	0.439	0.055	4.38	0.55
2-Ketoisocaproic acid (816-66-0)	0.500	0.034	3.91	0.27
Propionylglycine (21709-90-0)	0.232	0.010	1.799	0.078
2-Keto-3-methylvaleric acid (1460-34-0)	1.69	0.20	13.2	1.6
Malic acid (97-67-6)	0.395	0.021	3.00	0.16
Benzoic acid (65-85-0)	5.53	0.34	46.0	2.9
Tartaric acid (87-69-4)	24.0	1.1	162.6	7.5
Arabitol (7643-75-6)	6.21	0.60	41.5	4.1
Kynurenic acid (492-27-3)	0.87	0.05	4.68	0.27
4-Hydroxybenzoic acid (99-96-7)	0.297	0.013	2.19	0.10
Hippuric acid (495-69-2)	32.9	1.2	187	7
2-Methylhippuric acid (42013-20-7)	0.217	0.008	1.142	0.043
Xanthurenic acid (59-00-7)	0.165	0.013	0.818	0.065
3-Indoleacetic acid (87-51-4)	1.35	0.05	7.84	0.30
Vanillomandelic acid (55-10-7)	1.47	0.06	7.54	0.31
Phenylglyoxylic acid (611-73-4)	0.374	0.022	2.53	0.15

[1] Certified values are the mean of 6 measurement results obtained from two units of the CRM by ID-LC-MS technique. The certified values and the uncertainties are traceable to the International System of Units (SI).
[2] The expanded uncertainty of certified value includes characterization, homogeneity, stability components and is stated as the standard uncertainty of measurement multiplied by the coverage factor k = 2, which for a normal distribution corresponds to a coverage probability of approximately 95%. The standard uncertainty of measurement has been determined in accordance with GUM “Guide to the Expression of Uncertainty in Measurement”.
[3] Certified values and the uncertainties in molar concentrations are calculated from the mass fraction (mg/kg) using density of the reconstituted material (mean: 1.01680 g/mL, SD: 0.00326 g/mL, n = 13) measured at 22 °C and molecular weight of the analyte.

REFERENCES

[1] ISO 17034. General requirements for the competence of reference materials producers. International Organization for Standardization. 2016.
[2] ISO Guide 35. Reference materials- Guidance for characterization and assessment of homogeneity and stability. International Organization for Standardization. 2017.
For further information please visit: https://rm.ume.tubitak.gov.tr/

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