UME CRM 1314: AMINO ACIDS IN LYOPHILIZED PLASMA
A New Certified Reference Material For Newborn Screening

Seda Damla Hatipoglu[1], Simay Gunduz[1], Alper Isleyen[1], Ilker Un[1], Ali Enis Sadak[1], Nihal Zorlu[1], Gokhan Aktas[1], Murat Celik[2], Gokhan Gunay[2], Gokhan Bilsel[1], Ahmet C. Goren[1]

[1]TUBITAK National Metrology Institute (UME), Gebze/KOCAELİ, TURKEY
[2]Zivak Technologies, 34400 Kağıthane, Istanbul, TURKEY
simay.gunduz@tubitak.gov.tr

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. Phenylketonuria, Maple Syrup Urine and Tyrosinemia are common diseases that are generated from amino acid metabolism disorders. 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 1314 Amino acid concentrations in lyophilized human plasma” 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 1314 was prepared by adding amino acid standards into the human plasma containing amino acids endogenously. 3-methylhistidine, 2-Aminoadipic acid, 2-Aminobutyric acid, 2-Aminopimelic acid, Anserin, Arginine, Argininosuccinic acid, Asparagine, Aspartic acid, Beta alanine, 3-Aminoisobutyric acid, Citrulline, Cystathionine, 4-Aminobutyric acid, Glutamic acid, Glycine, Histamine, Histidine, Hydroxyproline, Methionine, *N*-Methylhistidine, Ornithine, Phenylalanine, Tryptophan, Tyrosine, Ethanolamine, Creatinine and *N*-Acetyltyrosine was added to the human plasma obtained from 48 healthy men donors purchased from Türk Kızılay (Turkey) and amino acid standards were purchased from Medical Isotopes. Acros Organics. Santa Cruz Biotechnology and Sigma-Aldrich (USA).
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. 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 screw 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.

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.
As a quality control for the amino acids quantification in plasma samples conducted at TUBITAK UME, NIST SRM 1950 “Metabolites in Frozen Human Plasma” was used.

CERTIFIED VALUES AND UNCERTAINTIES

	Mass Fraction (mg/kg)		Molar Concentration[3] (µmol/L)	
Parameter (CAS No)	**Certified Value[1]**	**Uncertainty[2]**	**Certified Value**	**Uncertainty**
Alanine (56-41-7)	32.6	1.7	383	20
2-Aminoadipic acid (1118-90-7)	5.18	0.90	33.7	5.9
2-Aminobutyric acid (1492-24-6)	1.65	0.21	16.8	2.2
Anserine (584-85-0)	20.1	4.2	87.6	18.4
Arginine (74-79-3)	15.13	0.59	90.8	3.7
Argininosuccinic acid (2387-71-5)	21.7	3.4	78	13
Asparagine (70-47-3)	39.9	7.5	316	60
Aspartic acid (56-84-8)	2.42	0.28	19.0	2.2
Beta-alanine (107-95-9)	8.5	1.4	100	17
Citrulline (372-75-8)	15.8	1.2	94.4	7.2
Cystathionine (56-88-2)	2.94	0.10	13.85	0.48
4-Aminobutyric acid (56-12-2)	1.57	0.19	15.9	2.0
Glutamic acid (56-86-0)	42.7	6.4	304	46
Glycine (56-40-6)	21.2	1.5	296	21
Histamine (51-45-6)	1.31	0.13	12.34	1.23
Histidine (71-00-1)	17.7	1.2	119.4	8.1
Hydroxyproline (51-35-4)	5.24	0.90	41.8	7.2
Isoleucine (73-32-5)	9.94	1.80	79.3	14.4
Leucine (61-90-5)	24.6	1.3	196	11
Lysine (56-87-1)	25.7	3.6	184	26
Methionine (63-68-3)	7.52	0.97	52.8	6.9
N-methylhistidine (332-80-9)	7.2	1.2	44.4	7.5
Ornithine (70-26-8)	19.7	1.5	156	12
Phenylalanine (63-91-2)	13.56	0.90	86.2	5.8
Proline (147-85-3)	29.4	2.3	267	21
Serine (56-45-1)	23.5	1.5	234	15
Threonine (72-19-5)	12.3	0.7	108.1	6.2
Tyrosine (60-18-4)	5.46	0.20	31.6	1.2
Valine (72-18-4)	31.1	1.4	278	13
Ethanolamine (141-43-5)	1.81	0.54	31.0	9.3
Creatinine (60-27-5)	12.50	0.54	115.7	5.6
Sarcosine (107-97-1)	0.94	0.12	11.05	1.42
3-Methylhistidine (368-16-1)	9.6	1.6	59.4	9.9
2-Aminopimelic acid (3721-85-5)	1.06	0.10	6.34	0.60
3-Aminoisobutyric acid (144-90-1)	1.78	0.37	18.1	3.8
Tryptophan (73-22-3)	7.1	2.2	36.4	11.3
N-Acetyltyrosine (537-55-3)	60.1	9.3	282	44

[1] Assigned values are the mean of 6 measurement results obtained from two units of the CRM by LC-MS technique.
[2] The expanded uncertainty of assigned 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] Assigned values and the uncertainties in molar concentrations are calculated from the mass fraction (µg/kg) using density of the reconstituted material (mean: 1.04702 g/mL, SD: 0.00481 g/mL, n = 15) 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/

ACKNOWLEDGEMENT

This study is part of the project SBAG-213S172 which was funded by the Scientific and Technological Research Council of Turkey (TUBITAK) within the framework of the priority areas of research for development of reference materials for clinical diagnostics.