Organic Acids in Lyophilized Urine
UME CRM 1315

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Date
28/05/2021

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ABBREVIATIONS

Δ_m	Absolute difference between mean measured value and the certified value
ANOVA	Analysis of variance
CRM	Certified Reference Material
HPLC	High Performance Liquid Chromatography
ID-LCMS/MS	Isotope Dilution Liquid Chromatography Tandem Mass Spectrometry
ID-LCMS	Isotope Dilution Liquid Chromatography Mass Spectrometry
IS	Internal Standard
ISO	International Standards Organisation
k	Coverage factor
$MS_{between}$	Mean of squares between-unit from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicate measurements
Q-NMR	Quantitative Nuclear Magnetic Resonance
RSD_{stab}	Relative standard deviation of stability data
s	Standard deviation
s_{bb}	Between-unit standard deviation
SI	International System of Units
SRM	Standard Reference Material
SS	Sum of Squares
u_{Δ}	Combined measurement uncertainty for the difference between measurement result and certified value
U_{Δ}	Expanded measurement uncertainty for the difference between measurement result and certified value.
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability
u_{char}	Standard uncertainty of the material characterisation
u_{CRM}	Combined standard uncertainty of CRM
u_{sts}	Standard uncertainty for short term stability study
u_{lts}	Standard uncertainty for long term stability study
u_{meas}	Measurement uncertainty
U_{CRM}	Expanded uncertainty of CRM
ν_{eff}	Effective degrees of freedom
$\nu_{MS_{within}}$	MS_{within} degrees of freedom
X_i	Time for each repeated analysis
$\overline{X}$	Average of all time periods
x	Selected shelf life

ABSTRACT

The Certified Reference Material (CRM) is utilized in chemical measurements, as a useful tool for proving traceability of measurement result and enhances measurement quality. Organic acid concentrations are frequently measured for treatment and diagnosis purposes. Organic acid metabolism distortedness is evaluated according to the concentration of organic acids metabolites.

Number of available CRMs to be used in these measurements is very limited. The purpose of this project is production and certification of UME CRM 1315 “Organic Acids in Lyophilized Urine”. SI traceability of the related method will be proved by utilization of this CRM in the measurements.

This report includes details for the certification of UME CRM 1314 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 have been presented and the corresponding uncertainties (Table 1) have been calculated in accordance with the ISO Guide 35:2017 [2].

Table 1. Certified values and uncertainties for UME CRM 1315

Parameter (CAS No)	Mass Fraction (mg/kg)		Molar Concentration[3] (µmol/L)	
	Certified Value[1]	Uncertainty[2]	Certified Value	Uncertainty
2-Hydroxyglutaric acid (13095-48-2)	1.51	0.13	10.37	0.84
2-Hydroxyphenylacetic acid (614-75-5)	9.7	2.0	65	13
2-Ketoglutaric acid (328-50-7)	7.56	0.68	52.6	4.7
2-Ketoisocaproic acid (816-66-0)	0.358	0.035	2.80	0.27
2-Ketoisovaleric acid (759-05-7)	0.750	0.040	6.57	0.35
2-Methylcitric acid (6061-96-7)	1.33	0.11	6.56	0.53
2-Methylhippuric acid (42013-20-7)	0.220	0.023	1.16	0.12
2-Keto-3-methylvaleric acid (1460-34-0)	1.67	0.20	13.0	1.6
3-Hydroxyisovaleric acid (625-08-1)	3.30	0.15	28.4	1.3
3-Methylglutaric acid (626-51-7)	0.968	0.076	6.73	0.53
4-Hydroxybenzoic acid (99-96-7)	0.293	0.020	2.16	0.15
5-Hydroxyindole-3-acetic acid (54-16-0)	1.27	0.48	6.7	2.6

[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) through a calibration hierarchy using high purity materials of each parameter that were value-assigned using TÜBİTAK UME qNMR purity assessment procedure.

[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.

Table 1. *Continued* :

Parameter (CAS No)	Mass Fraction (mg/kg)		Molar Concentration[3] (µmol/L)	
	Certified Value[1]	Uncertainty[2]	Certified Value	Uncertainty
Adipic acid (124-04-9)	2.60	0.13	18.11	0.85
Arabitol (7643-75-6)	5.91	0.57	39.5	3.8
Benzoic acid (65-85-0)	5.48	0.37	45.6	3.1
Citric acid (77-92-9)	219	45*	1161	239*
Fumaric acid (110-17-8)	1.52	0.10	13.34	0.86
Glutaric acid (110-94-1)	0.209	0.029	1.61	0.23
Glycolic acid (79-14-1)	2.42	0.22	32.3	2.9
Hippuric acid (495-69-2)	31.2	1.1	177.2	6.2
Kynurenic acid (492-27-3)	0.820	0.056	4.41	0.30
Lactic acid (79-33-4)	10.5	0.61	118.0	7.0
Malic acid (97-67-6)	0.380	0.028	2.88	0.21
Mandelic acid (90-64-2)	8.02	0.28	53.6	1.9
Methylmalonic acid (516-05-2)	1.74	0.19	15.0	1.6
N-Acetylaspartic acid (997-55-7)	5.02	0.30	29.1	1.7
N-Suberylglycine (60317-54-6)	1.299	0.043	5.71	0.19
N-Tiglylglycine (35842-45-6)	0.347	0.026	2.25	0.17
N-(3-Methylcrotonyl)gylcine (33008-07-0)	6.69	0.49	43.3	3.2
N-(3-Phenylpropionyl)glycine (56613-60-6)	3.40	0.15	16.68	0.74
Phenylglyoxylic acid (611-73-4)	0.342	0.021	2.32	0.15
Pimelic acid (111-16-0)	0.098	0.020	0.62	0.13
Sebacic acid (111-20-6)	0.115	0.013	0.579	0.063
Suberic acid (505-48-6)	1.286	0.092	7.50	0.54
Succinic acid (110-15-6)	7.50	0.36	64.6	3.1
Tartaric acid (87-69-4)	24.3	1.6	165	11
Vanillomandelic acid (55-10-7)	1.498	0.076	7.69	0.39

[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) through a calibration hierarchy using high purity materials of each parameter that were value-assigned using TÜBİTAK UME qNMR purity assessment procedure.

[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 $k = 2$ (*except Citric acid, k = 2.32), 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: 0.003263 g/mL, $n = 13$) measured at 22 °C and molecular weight of the analyte.

INTRODUCTION

An inborn error of metabolism (IEM) is a permanent and inherited biochemical disorder generally caused by lack of a functional enzyme, some of the amino and organic acids could not be metabolized, which causes accumulation of toxic intermediate organic compounds and emergence of the diseases. Some of widespread diseases are also caused by organic acid metabolism such as Methylmalonic, Propionic acidemia and Biotinidase deficiency [3]. Detection of IEM through screening is the key to early treatment. Early diagnosis of metabolic diseases is very critical and they should be evaluated through reliable screening tests [4]. Newborn screening (NS) is a public health activity, in terms of secondary prevention, aimed at the early identification of infants affected by certain conditions-genetic, metabolic, infectious that threaten their life and long-term health, for which a timely intervention can lead to a significant reduction of morbidity, mortality and associated disabilities [5].

In order to obtain reliable results from measurements of organic acids, before starting any routine analyses, system calibration and quality control must be conducted. When the expression of measurement results has to be in reliable quantitative values, the use of certified reference materials (CRM) is a powerful tool to ensure the quality of the chemical measurements. Particularly, it is important to use CRMs, having the same chemical compositions (matrix matched CRM), for the proper quantification of subject quantity in the mixtures (matrix), such as body fluids, containing multiple analytes. In this way, through the use of matrix CRMs in measurements, verification of the metrological traceability can be ensured.

Organic acid concentrations in lyophilized urine are certified in UME CRM 1315. This report presents the details of the production and certification stages including the data, utilized techniques and statistical analysis. UME CRM 1315 was produced to be used as a material in proving the traceability and quality of LC-MS and LC-MS/MS measurements of organic acids. Specific properties of organic acids are given in Table 2.

Systematic description for UME CRM 1315 according to ISO 15194 is "Certified reference material UME CRM 1315 - Organic acids in Lyophilized Urine".

Table 2. Specific properties of organic acids

Short Name	IUPAC Name	Chemical Formula	CAS Number	Molecular Weight (g/mol)
2-Methylcitric acid	2-hydroxy-1-methylpropane-1,2,3-tricarboxylic acid	$C_7H_{10}O_7$	6061-96-7	206.1501
3-Hydroxy-3-methylglutaric acid	3-hydroxy-3-methylpentanedioic acid	$C_6H_{10}O_5$	503-49-1	162.1406
N-(3-Methylcrotonyl)gylcine	2-(3-methylbut-2-enamido)acetic acid	$C_7H_{11}NO_3$	33008-07-0	157.1671
3-Phenyllactic acid	(2R)-2-hydroxy-3-phenylpropanoic acid	$C_9H_{10}O_3$	20312-36-1	166.1739
Adipic acid	hexanedioic acid	$C_6H_{10}O_4$	124-04-9	146.1412
2-Hydroxyglutaric acid	(2S)-2-hydroxypentanedioic acid	$C_5H_8O_5$	13095-48-2	148.1140
2-Hydroxyisovaleric acid	2-hydroxy-3-methylbutanoic acid	$C_5H_{10}O_3$	17407-56-6	118.1311

Table 2. (*Continued*) Specific properties of organic acids

Short Name	IUPAC Name	Chemical Formula	CAS Number	Molecular Weight (g/mol)
2-Ketoglutaric acid	2-oxopentanedioic acid	$C_5H_6O_5$	328-50-7	146.0981
3-Hydroxybutyric acid	(3S)-3-hydroxybutanoic acid	$C_4H_8O_3$	300-85-6	104.1045
3-Hydroxyisovaleric acid	3-hydroxy-3-methylbutanoic acid	$C_5H_{10}O_3$	625-08-1	118.1311
Citric acid	2-hydroxypropane-1,2,3-tricarboxylic acid	$C_6H_8O_7$	77-92-9	192.1235
Fumaric acid	(2E)-but-2-enedioic acid	$C_4H_4O_4$	110-17-8	116.0722
Glutaric acid	pentanedioic acid	$C_5H_8O_4$	110-94-1	132.1146
Glyceric acid	(2R)-2,3-dihydroxypropanoic acid	$C_3H_6O_4$	473-81-4	106.0773
Glycolic acid	2-hydroxyacetic acid	$C_2H_4O_3$	79-14-1	76.0514
Isocitric acid	1-hydroxypropane-1,2,3-tricarboxylic acid	$C_6H_8O_7$	320-77-4	192.1235
2-Ketoisovaleric acid	3-methyl-2-oxobutanoic acid	$C_5H_8O_3$	759-05-7	116.1152
Lactic acid	(2S)-2-hydroxypropanoic acid	$C_3H_6O_3$	79-33-4	90.0779
Methylmalonic acid	2-methylpropanedioic acid	$C_4H_6O_4$	516-05-2	118.0880
2-Methylglutaric acid	2-methylpentanedioic acid	$C_6H_{10}O_4$	617-62-9	146.1412
3-Methylglutaric acid	3-methylpentanedioic acid	$C_6H_{10}O_4$	626-51-7	146.1412
N-Acetylaspartic acid	(2S)-2-acetamidobutanedioic acid	$C_6H_9NO_5$	997-55-7	175.1394
Orotic acid	2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylic acid	$C_5H_4N_2O_4$	65-86-1	156.0963
4-Hydroxyphenylacetic acid	2-(4-hydroxyphenyl)acetic acid	$C_8H_8O_3$	156-38-7	152.1473
2-Hydroxyphenylacetic acid	2-(2-hydroxyphenyl)acetic acid	$C_8H_8O_3$	614-75-5	152.1473
4-Hydroxyphenyllactic acid	2-hydroxy-3-(4-hydroxyphenyl)propanoic acid	$C_9H_{10}O_4$	306-23-0	182.1733
Pimelic acid	heptanedioic acid	$C_7H_{12}O_4$	111-16-0	160.1678
Pyruvic acid	2-oxopropanoic acid	$C_3H_4O_3$	127-17-3	88.0621
Sebacic acid	decanedioic acid	$C_{10}H_{18}O_4$	111-20-6	202.2475
Suberic acid	octanedioic acid	$C_8H_{14}O_4$	505-48-6	174.1944
Succinic acid	butanedioic acid	$C_4H_6O_4$	110-15-6	118.0880
N-Suberylglycine	7-[(carboxymethyl)carbamoyl]heptanoic acid	$C_{10}H_{17}NO_5$	60317-54-6	231.2457
N-Tiglylglycine	2-[(2E)-2-methylbut-2-enamido]acetic acid	$C_7H_{11}NO_3$	35842-45-6	157.1671
Mandelic acid	(2S)-2-hydroxy-2-phenylacetic acid	$C_8H_8O_3$	90-64-2	152.1473
Homovanillic acid	2-(4-hydroxy-3-methoxyphenyl)acetic acid	$C_9H_{10}O_4$	306-08-1	182.1733
5-Hydroxyindole-3-acetic acid	2-(5-hydroxy-1H-indol-3-yl)acetic acid	$C_{10}H_9NO_3$	54-16-0	191.1834
N-Hexanoylglycine	2-hexanamidoacetic acid	$C_8H_{15}NO_3$	24003-67-6	173.2096

Table 2. (*Continued*) Specific properties of organic acids

Short Name	IUPAC Name	Chemical Formula	CAS Number	Molecular Weight (g/mol)
N-(3-Phenylpropionyl)glycine	2-(3-phenylpropanamido)acetic acid	$C_{11}H_{13}NO_3$	56613-60-6	207.2258
Mevalonolactone	(4S)-4-hydroxy-4-methyloxan-2-one	$C_6H_{10}O_3$	674-26-0	130.1418
2-Ketobutyric acid	2-oxobutanoic acid	$C_4H_6O_3$	600-18-0	102.0886
2-Ketoisocaproic acid	4-methyl-2-oxopentanoic acid	$C_6H_{10}O_3$	816-66-0	130.1418
Propionylglycine	2-propanamidoacetic acid	$C_5H_9NO_3$	21709-90-0	131.1299
2-Keto-3-methylvaleric acid	3-methyl-2-oxopentanoic acid	$C_6H_{10}O_3$	1460-34-0	130.1418
Malic acid	2-hydroxybutanedioic acid	$C_4H_6O_5$	97-67-6	134.0874
Benzoic acid	benzoic acid	$C_7H_6O_2$	65-85-0	122.1230
Tartaric acid	(2R,3R)-2,3-dihydroxybutanedioic acid	$C_4H_6O_6$	87-69-4	150.0868
Arabitol	(2S,4S)-pentane-1,2,3,4,5-pentol	$C_5H_{12}O_5$	7643-75-6	152.1458
Kynurenic acid	4-oxo-1,4-dihydroquinoline-2-carboxylic acid	$C_{10}H_7NO_3$	492-27-3	189.1675
4-Hydroxybenzoic acid	4-hydroxybenzoic acid	$C_7H_6O_3$	99-96-7	138.1207
Hippuric acid	2-(phenylformamido)acetic acid	$C_9H_9NO_3$	495-69-2	179.1727
2-Methylhippuric acid	2-[(2-methylphenyl)formamido]acetic acid	$C_{10}H_{11}NO_3$	42013-20-7	193.1992
Xanthurenic acid	4,8-dihydroxyquinoline-2-carboxylic acid	$C_{10}H_7NO_4$	59-00-7	205.1669
3-İndoleacetic acid	2-(1H-indol-3-yl)acetic acid	$C_{10}H_9NO_2$	87-51-4	175.1840
Vanillomandelic acid	(2S)-2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)acetic acid	$C_9H_{10}O_5$	55-10-7	198.1727
Tricarballylic acid	propane-1,2,3-tricarboxylic acid	$C_6H_8O_6$	99-14-9	176.1241
Phenylglyoxylic acid	2-oxo-2-phenylacetic acid	$C_8H_6O_3$	611-73-4	150.1314
3-Methylhippuric acid	2-[(3-methylphenyl)formamido]acetic acid	$C_{10}H_{11}NO_3$	27115-49-7	193.1992
Glucaric acid	(2R,3S,4S,5S)-2,3,4,5-tetrahydroxyhexanedioic acid	$C_6H_{10}O_8$	25525-21-7	210.1388
4-Methylhippuric acid	2-[(4-methylphenyl)formamido]acetic acid	$C_{10}H_{11}NO_3$	27115-50-0	193.1992

PARTICIPANTS

All production and certification stages of UME CRM 1315 have been performed at TÜBİTAK UME.

Table 3. Participating institutes and definition of their work

Activity	Laboratory / Organization
Project management and data evaluation	TÜBİTAK UME National Metrology Institute Gebze-Kocaeli, TURKEY
Processing	
Homogeneity study	
Stability studies	
Characterisation study	

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) [6]. After classification into subgroups, samples were stored under selected test conditions. The units allocated for sale were stored at (-45 $\pm$ 5) ^{o}C under controlled conditions, in the dark.

HOMOGENEITY

Homogeneity study between the units is performed to show that the assigned values are valid for all units within the stated uncertainty. Homogeneity study between the units is performed with a number of samples representing the whole batch. In order to determine between unit heterogeneity for certification of organic acids, 15 units of UME CRM 1315 have been chosen by utilizing TRaNS [6]. Measurements were performed under repeatability conditions. The samples to be analysed were introduced to the instruments by random order to find out any trend arising from analytical and/or filling sequences.

Data were visually checked whether all individual data follow a unimodal distribution using histograms and normal probability plots. It was found that the distribution was normal and unimodal (except for citric acid, lactic acid and pyruvic acid). Minor deviations from unimodality of the individual values do not significantly affect the estimate of between-unit standard deviations.

The trend-corrected datasets were tested for consistency using Grubbs outlier tests on a confidence level of 99% on the individual results and the unit means. Some outlying individual results and outlying unit means were detected. Since no technical reason for the outliers could be found, all the data were retained for statistical analysis. The results of all statistical evaluations are given in Table 4.

Table 4. Results of the statistical evaluation of the homogeneity studies at the 99% confidence level

Analyte	Is there a Trend?		Is there an Outlier?		Distribution
	Analytical sequence	Filling sequence	All data	Unit averages	All data
2-Methylcitric acid	No	No	No	No	Normal/unimodal
3-Hydroxy-3-methylglutaric acid	No	No	No	No	Normal/unimodal
N-(3-Methylcrotonyl)gylcine	No	No	No	No	Normal/unimodal
3-Phenyllactic acid	No	No	No	No	Normal/unimodal
Adipic acid	No	No	No	No	Normal/unimodal
2-Hydroxyglutaric acid	No	No	No	No	Normal/unimodal
2-Hydroxyisovaleric acid	No	No	No	No	Normal/unimodal
2-Ketoglutaric acid	No	No	No	No	Normal/unimodal
3-Hydroxybutyric acid	No	No	No	No	Normal/unimodal
3-Hydroxyisovaleric acid	No	No	No	No	Normal/unimodal
Citric acid	No	No	No	No	Not normal /unimodal
Fumaric acid	No	No	No	No	Normal/unimodal
Glutaric acid	No	No	No	No	Normal/unimodal
Glyceric acid	No	No	No	No	Normal/unimodal
Glycolic acid	No	No	No	No	Normal/unimodal
Isocitric acid	No	No	No	No	Normal/unimodal
2-Ketoisovaleric acid	No	No	No	No	Normal/unimodal
Lactic acid	No	No	Yes	Yes	Not normal /unimodal

Table 4. (*Continued*) Results of the statistical evaluation of the homogeneity studies at the 99% confidence level

Analyte	Is there a Trend?		Is there an Outlier?		Distribution
	Analytical sequence	Filling sequence	All data	Unit averages	All data
Methylmalonic acid	No	No	No	No	Normal/unimodal
2-Methylglutaric acid	No	No	No	No	Normal/unimodal
3-Methylglutaric acid	No	No	No	No	Normal/unimodal
N-Acetylaspartic acid	No	No	No	No	Normal/unimodal
Orotic acid	No	No	No	No	Normal/unimodal
4-Hydroxyphenylacetic acid	No	No	No	No	Normal/unimodal
2-Hydroxyphenylacetic acid	No	No	No	No	Normal/unimodal
4-Hydroxyphenyllactic acid	No	No	No	No	Normal/unimodal
Pimelic acid	No	No	No	No	Normal/unimodal
Pyruvic acid	No	No	No	No	Not normal/ unimodal
Sebacic acid	No	No	No	No	Normal/unimodal
Suberic acid	No	No	No	No	Normal/unimodal
Succinic acid	No	No	No	No	Normal/unimodal
N-Suberylglycine	No	No	No	No	Normal/unimodal
N-Tiglylglycine	No	No	No	No	Normal/unimodal
Mandelic acid	No	No	No	No	Normal/unimodal
Homovanillic acid	No	No	No	No	Normal/unimodal
5-Hydroxyindole-3-acetic acid	No	No	No	No	Normal/unimodal
N-Hexanoylglycine	No	No	No	No	Normal/unimodal
N-(3-Phenylpropionyl)glycine	No	No	No	No	Normal/unimodal
Mevalonolactone	No	No	No	No	Normal/unimodal
2-Ketobutyric acid	No	No	No	No	Normal/unimodal
2-Ketoisocaproic acid	No	No	No	No	Normal/unimodal

Table 4. (*Continued*) Results of the statistical evaluation of the homogeneity studies at the 99% confidence level

Analyte	Is there a Trend?		Is there an Outlier?		Distribution
	Analytical sequence	Filling sequence	All data	Unit averages	All data
Propionylglycine	No	No	No	No	Normal/unimodal
2-Keto-3-methylvaleric acid	No	No	No	No	Normal/unimodal
Malic acid	No	No	No	No	Normal/unimodal
Benzoic acid	No	No	No	No	Normal/unimodal
Tartaric acid	No	No	No	No	Normal/unimodal
Arabitol	No	No	No	No	Normal/unimodal
Kynurenic acid	No	No	No	No	Normal/unimodal
4-Hydroxybenzoic acid	No	No	No	No	Normal/unimodal
Hippuric acid	No	No	No	No	Normal/unimodal
2-Methylhippuric acid	No	No	No	No	Normal/unimodal
Xanthurenic acid	No	No	No	No	Normal/unimodal
3-İndoleacetic acid	No	No	No	No	Normal/unimodal
Vanillomandelic acid	No	No	No	No	Normal/unimodal
Tricarballylic acid	No	No	No	No	Normal/unimodal
Phenylglyoxylic acid	No	No	No	No	Normal/unimodal
3-Methylhippuric acid	No	No	No	No	Normal/unimodal
Glucaric acid	No	No	No	No	Normal/unimodal
4-Methylhippuric acid	No	No	No	No	Normal/unimodal

The ANOVA allowed the calculation of the within- (s_{wb}) and between-unit homogeneity (s_{bb}), estimated as standard deviations, according to the following equations:

$$s_{wb} = \sqrt{MS_{within}} \quad (1)$$

MS_{within} : Mean squares within-unit

s_{wb} is equivalent to the *s* of the method, provided that subsamples are representative for the whole unit.

$$s_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n}} \quad (2)$$

$MS_{between}$: Mean squares between-unit

n : Number of replicates per unit

When $MS_{between}$ is smaller than MS_{within}, s_{bb} cannot be calculated. Instead, u^*_{bb}, the heterogeneity that can be hidden by the method repeatability, is calculated, according to the following equation [7] (3):

$$u^*_{bb} = \frac{s_{wb}}{\sqrt{n}} \sqrt[4]{\frac{2}{v_{MSwithin}}} \quad (3)$$

$v_{MSwithin}$: Degrees of freedom of MS_{within}

The occurrence of $MS_{between} < MS_{within}$ can be seen, if material heterogeneity is smaller than that can be detected by the analytical methodology used. For the parameters for which ANOVA was applied, the larger value of s_{bb} or u^*_{bb} is taken as uncertainty contribution for homogeneity, u_{bb} (Table 5).

Table 5. Results of the homogeneity study

Analyte	$s_{wb,rel}$ %	$s_{bb,rel}$ %	$u^*_{bb,rel}$ %	$u_{bb,rel}$ %
2-Methylcitric acid	2.124	0.97	0.91	0.97
3-Hydroxy-3-methylglutaric acid	1.854	$MS_{between} < MS_{within}$	0.79	0.79
N-(3-Methylcrotonyl)gylcine	1.780	$MS_{between} < MS_{within}$	0.76	0.76
3-Phenyllactic acid	1.110	1.33	0.48	1.33
Adipic acid	1.004	1.19	0.44	1.19
2-Hydroxyglutaric acid	1.416	1.36	0.62	1.36
2-Hydroxyisovaleric acid	2.062	1.22	0.88	1.22
2-Ketoglutaric acid	1.467	4.15	0.63	4.15
3-Hydroxybutyric acid	1.378	0.81	0.60	0.81
3-Hydroxyisovaleric acid	0.871	1.20	0.38	1.20
Citric acid	3.381	2.53	1.47	2.53
Fumaric acid	2.335	1.33	1.00	1.33
Glutaric acid	1.464	1.16	0.63	1.16
Glyceric acid	3.215	3.04	1.42	3.04
Glycolic acid	3.835	$MS_{between} < MS_{within}$	1.64	1.64
Isocitric acid	7.595	7.17	3.30	7.17
2-Ketoisovaleric acid	1.786	1.04	0.76	1.04
Lactic acid	1.616	2.23	0.69	2.23
Methylmalonic acid	2.582	2.00	1.10	2.00
2-Methylglutaric acid	1.094	1.83	0.47	1.83
3-Methylglutaric acid	1.365	1.36	0.59	1.36
N-Acetylaspartic acid	1.963	1.57	0.85	1.57

Table 5. (*Continued*) Results of the homogeneity study

Analyte	$s_{wb,rel}$ %	$s_{bb,rel}$ %	$u^*_{bb,rel}$ %	$u_{bb,rel}$ %
Orotic acid	2.281	$MS_{between} < MS_{within}$	1.01	1.01
4-Hydroxyphenylacetic acid	3.738	2.74	1.63	2.74
2-Hydroxyphenylacetic acid	1.078	1.37	0.46	1.37
4-Hydroxyphenyllactic acid	1.230	1.37	0.53	1.37
Pimelic acid	2.422	0.66	1.04	1.04
Pyruvic acid	2.465	5.04	1.05	5.04
Sebacic acid	3.030	$MS_{between} < MS_{within}$	1.29	1.29
Suberic acid	3.128	$MS_{between} < MS_{within}$	1.34	1.34
Succinic acid	1.431	1.75	0.61	1.75
N-Suberylglycine	1.032	0.70	0.45	0.70
N-Tiglylglycine	1.297	1.02	0.55	1.02
Mandelic acid	1.818	0.59	0.78	0.78
Homovanillic acid	2.763	2.90	1.20	2.90
5-Hydroxyindole-3-acetic acid	3.534	2.01	1.54	2.01
N-Hexanoylglycine	0.992	1.13	0.42	1.13
N-(3-Phenylpropionyl)glycine	1.217	1.40	0.52	1.40
Mevalonolactone	1.811	1.54	0.80	1.54
2-Ketobutyric acid	3.265	3.76	1.40	3.76
2-Ketoisocaproic acid	1.568	2.54	0.67	2.54
Propionylglycine	1.975	1.32	0.84	1.32
2-Keto-3-methylvaleric acid	2.017	1.69	0.88	1.69
Malic acid	1.895	1.46	0.84	1.46
Benzoic acid	3.104	$MS_{between} < MS_{within}$	1.35	1.35
Tartaric acid	1.760	1.48	0.75	1.48
Arabitol	5.314	$MS_{between} < MS_{within}$	2.27	2.27
Kynurenic acid	1.890	1.19	0.81	1.19
4-Hydroxybenzoic acid	2.115	$MS_{between} < MS_{within}$	0.90	0.90
Hippuric acid	0.872	1.30	0.37	1.30
2-Methylhippuric acid	1.688	0.31	0.72	0.72
Xanthurenic acid	2.637	1.45	1.13	1.45
3-Indoleacetic acid	0.940	1.28	0.40	1.28

Table 5. (*Continued*) Results of the homogeneity study

Analyte	$s_{wb,rel}$ %	$s_{bb,rel}$ %	$u^*_{bb,rel}$ %	$u_{bb,rel}$ %
Vanillomandelic acid	2.374	$MS_{between} < MS_{within}$	1.01	1.01
Tricarballylic acid	2.281	0.74	0.97	0.97
Phenylglyoxylic acid	2.950	2.09	1.26	2.09
3-Methylhippuric acid	2.739	1.58	1.19	1.58
Glucaric acid	3.943	2.35	1.71	2.35
4-Methylhippuric acid	4.051	1.00	1.73	1.73

The plotted data used for the evaluation of homogeneity can be found in Annex 2.

STABILITY

The stability studies were carried out using an isochronous design [8]. In this approach, samples are stored for a certain time at different temperature conditions. Afterwards, the samples are moved to conditions where further degradation can be assumed to be negligible ("reference conditions"), effectively "freezing" the degradation status of the materials. At the end of the isochronous storage, the samples are analysed simultaneously under repeatability conditions. Two different stability tests, for UME CRM 1315, have been conducted. Short term stability test has been performed to simulate transportation conditions and long term stability test to simulate long term storage conditions.

Short term stability study was performed with 10 units of CRM and long term stability study was conducted by 26 units of CRM. Samples were selected by TRaNS software [6].

Short Term Stability Results

For the Short Term Stability (STS) test, three different temperatures (4 ˚C, 18 ˚C and 45 ˚C) and four time points (0, 1, 2, and 4 weeks) were tested. Ten samples were randomly selected. Test samples were moved to -45 ˚C (reference temperature) after completion of the test time. All samples were analysed under repeatability conditions at the same time.

The results obtained from isochronous measurements were first grouped according to the time period and then evaluated for each time point. These evaluations were carried out for three temperatures, separately.

The results were screened for outliers by applying the single Grubbs' test at confidence levels of 95 %. The measured concentration values were plotted against time and the regression lines were calculated to check for significant trends indicating possible changes in the concentrations of the analytes by time. The calculated slope values were tested for significance using a t-test, with $t_{\alpha,df}$ being the critical t-value (two-tailed) for a significance level α = 0.05 (95 % confidence level). The graphs are given in Annex 3. Outliers (shown in Table 6) were identified in the statistical evaluation (Grubbs' test) of the data; nevertheless, as there was no technical reason to exclude them from evaluation, they remained in the

data set. The data evaluation results for the short-term stability at +4 °C, +18 °C and +45 °C are summarised in Table 6. Uncertainty calculations are done using equation (4) [8]. Maximum time for transfer is chosen as one week.

$$u_{sts,rel} = \frac{RSD}{\sqrt{\Sigma(t_i - \bar{t})^2}} \times t \qquad (4)$$

where,

RSD	:	relative standard deviation of the points on the regression line
t_i	:	time point for each replicate
$\bar{t}$	:	mean of all time points
t	:	maximum time suggested for transfer (one week)

Results obtained from short term stability tests are given in Table 6.

Table 6. Results of short term stability tests for 1 week

Analyte	4°C $u_{sts,rel}$ (%)	18°C $u_{sts,rel}$ (%)	45°C $u_{sts,rel}$ (%)	Number of outliers in 95% confidence interval*			Is there a significant trend in 95% confidence interval?		
				4°C	18°C	45°C	4°C	18°C	45°C
2-Methylcitric acid	0.59	**1.27**	1.13	-	-	1	No	No	No
3-Hydroxy-3-methylglutaric acid	0.19	**0.41**	0.29	-	-	-	No	No	No
N-(3-Methylcrotonyl)gylcine	0.24	**0.37**	0.49	-	-	-	No	No	No
3-Phenyllactic acid	2.76	**2.95**	3.01	-	-	-	No	No	No
Adipic acid	1.06	**1.00**	1.02	-	-	-	No	No	No
2-Hydroxyglutaric acid	2.58	**2.82**	2.60	-	-	-	No	No	No
2-Hydroxyisovaleric acid	0.37	**0.10**	0.42	-	-	1	No	No	No
2-Ketoglutaric acid	0.23	**0.43**	16.38	-	-	-	No	No	Yes
3-Hydroxybutyric acid	1.09	**1.45**	1.11	-	-	-	No	No	No
3-Hydroxyisovaleric acid	0.60	**0.96**	0.84	-	-	-	No	No	No
Citric acid	6.22	**8.15**	7.46	-	-	-	No	No	No
Fumaric acid	0.67	**1.10**	2.24	-	-	-	No	No	Yes
Glutaric acid	0.70	**0.62**	0.62	-	1	-	No	No	No
Glyceric acid	2.86	**4.12**	5.81	-	1	1	No	No	No
Glycolic acid	0.66	**0.62**	0.76	-	-	-	No	No	No
Isocitric acid	6.83	**8.21**	7.44	-	-	-	No	No	No
2-Ketoisovaleric acid	0.47	**0.67**	5.35	-	-	-	No	No	Yes
Lactic acid	0.31	**0.45**	0.26	-	-	-	No	No	No
Methylmalonic acid	1.22	**0.69**	0.79	-	-	-	No	No	No
2-Methylglutaric acid	2.71	**3.14**	2.81	-	-	-	No	No	No
3-Methylglutaric acid	1.56	**1.62**	1.66	-	-	-	No	No	No
N-Acetylaspartic acid	1.76	**1.73**	1.98	-	-	-	No	No	No
Orotic acid	0.56	**0.79**	0.81	-	-	-	Yes	No	No
4-Hydroxyphenylacetic acid	5.81	**7.05**	6.28	-	-	-	No	No	No

Table 6. (*Continued*) Results of short term stability tests for 1 week

Analyte	4°C $u_{sts,rel}$ (%)	18°C $u_{sts,rel}$ (%)	45°C $u_{sts,rel}$ (%)	Number of outliers in 95% confidence interval*			Is there a significant trend in 95% confidence interval?		
				4°C	18°C	45°C	4°C	18°C	45°C
2-Hydroxyphenylacetic acid	0.92	**0.98**	1.22	-	-	-	No	No	No
4-Hydroxyphenyllactic acid	0.86	**0.65**	0.54	-	-	-	No	No	No
Pimelic acid	0.91	**0.78**	0.67	-	-	-	Yes	No	No
Pyruvic acid	0.63	**0.81**	26.54	-	-	-	No	No	Yes
Sebacic acid	0.45	**1.12**	0.84	-	-	-	No	No	No
Suberic acid	1.40	**1.47**	0.81	-	-	-	Yes	No	No
Succinic acid	0.87	**0.75**	2.64	-	-	-	No	No	Yes
N-Suberylglycine	0.31	**0.36**	0.24	-	-	-	No	No	No
N-Tiglylglycine	1.27	**0.95**	2.65	-	-	1	No	No	No
Mandelic acid	0.76	**0.91**	0.85	-	-	-	No	No	No
Homovanillic acid	4.49	**5.04**	5.81	-	-	-	No	No	No
5-Hydroxyindole-3-acetic acid	4.71	**4.19**	3.50	-	-	-	No	No	No
N-Hexanoylglycine	0.38	**0.33**	0.19	-	-	-	No	No	No
N-(3-Phenylpropionyl)glycine	0.78	**0.59**	0.65	-	-	-	No	No	No
Mevalonolactone	1.65	**2.08**	2.37	-	-	-	No	No	No
2-Ketobutyric acid	0.87	**0.95**	17.13	-	-	-	No	No	Yes
2-Ketoisocaproic acid	0.68	**0.69**	2.05	-	-	-	No	No	Yes
Propionylglycine	0.70	**0.85**	0.70	-	-	-	No	No	No
2-Keto-3-methylvaleric acid	3.13	**3.75**	4.03	-	-	-	No	No	No
Malic acid	0.54	**0.38**	1.98	-	-	1	No	Yes	No
Benzoic acid	1.59	**1.17**	1.55	-	-	-	No	No	No
Tartaric acid	0.79	**0.77**	0.68	-	-	-	No	No	No
Arabitol	0.75	**1.31**	0.72	-	-	-	No	No	No
Kynurenic acid	0.38	**0.60**	0.49	-	1	1	No	No	No
4-Hydroxybenzoic acid	0.65	**0.90**	0.60	-	-	-	No	No	No
Hippuric acid	0.25	**0.47**	0.18	-	-	-	No	Yes	No
2-Methylhippuric acid	0.28	**0.37**	0.45	-	-	-	No	No	No
Xanthurenic acid	1.05	**0.54**	4.62	-	-	-	No	No	Yes
3-İndoleacetic acid	0.21	**0.24**	0.23	-	-	-	No	No	Yes
Vanillomandelic acid	0.34	**0.70**	2.04	-	-	-	No	No	Yes
Tricarballylic acid	0.72	**0.98**	1.31	-	-	-	No	No	No
Phenylglyoxylic acid	0.38	**0.53**	13.75	-	-	-	No	No	Yes
3-Methylhippuric acid	1.96	**1.92**	2.23	-	-	-	No	No	No
Glucaric acid	3.44	**4.57**	3.27	-	-	-	No	No	No
4-Methylhippuric acid	1.51	**1.58**	1.81	-	-	-	No	No	No

*SGT: Single Grubbs' Test

The material is found to be stable at 18 °C for up to one week. Thus, the samples can be safely dispatched under conditions where the temperatures do not exceed 18 °C for up to one week, i.e. at ambient temperature without applying any cooling elements.

Long Term Stability Results

Shelf life of the CRM has been determined through long term stability measurements. For the measurements, two units for each of the months of 1, 3, 6 and 9 have been stored at -20 °C, +4°C and

+18 °C and transferred to reference temperature (-45 °C) after each period of time to be measured isochronously afterwards.

Two units, designated as reference units, were stored at -45 °C. The data for each time point has been calculated from two replicate measurements for each of two units. Thus, the average of four measurements for each time point is given in Annex 4. The error bars on each time point are calculated as the standard deviation of two unit averages.

No outlying data was detected by Grubbs' test. The graphs were plotted against time and the regression lines were calculated. The relative long term stability uncertainty, $u_{lts,rel}$ for each parameter is then calculated using equation (5) for the required shelf life as [8]:

$$u_{lts,rel} = \frac{RSD}{\sqrt{\Sigma\left(t_i - \bar{t}\right)^2}} \times t \qquad (5)$$

where

RSD *:* the relative standard deviation of the points on the regression line

t_i *:* being the time point for each replicate

$\bar{t}$: being the average of all time points

t : being the proposed shelf life at -20 °C, 4 °C and 18 °C (12 months)

The uncertainty contribution u_{lts} was calculated for nine months (t) at -20 °C. This uncertainty was one of the four parameters of the overall uncertainty budget of the certified values. The results are given in Table 7. The graphs for long term stability are given in Annex 4.

Table 7. Results of the long-term stability tests for 12 months

Analyte	Is the slope significantly different from zero at -20°C?*	$u_{lts,rel}$ [%] for shelf-life of 12 months at -20°C	Is the slope significantly different from zero at 4°C?*	$u_{lts,rel}$ [%] for shelf-life of 12 months at 4°C	Is the slope significantly different from zero at 18°C?*	$u_{lts,rel}$ [%] for shelf-life of 12 months at 18°C
2-Methylcitric acid	No	**2.25**	No	2.51	No	1.97
3-Hydroxy-3-methylglutaric acid	No	**1.39**	No	1.71	No	1.34
N-(3-Methylcrotonyl)gylcine	No	**1.56**	No	1.40	No	0.88
3-Phenyllactic acid	No	**1.50**	No	2.61	No	1.16
Adipic acid	No	**1.02**	No	1.35	No	0.81
2-Hydroxyglutaric acid	No	**2.19**	No	2.03	No	1.33
2-Hydroxyisovaleric acid	Yes	**2.38**	No	1.70	No	1.82
2-Ketoglutaric acid	No	**1.48**	No	1.71	Yes	3.95
3-Hydroxybutyric acid	No	**1.61**	No	1.35	No	1.00
3-Hydroxyisovaleric acid	No	**1.31**	No	1.68	No	1.21
Citric acid	No	**1.60**	No	5.06	No	3.52
Fumaric acid	No	**1.75**	No	1.34	Yes	6.94
Glutaric acid	No	**6.34**	No	12.39	No	7.35
Glyceric acid	No	**3.25**	No	7.09	No	3.54
Glycolic acid	No	**3.77**	No	3.48	No	3.52
Isocitric acid	No	**5.29**	No	15.31	No	5.32
2-Ketoisovaleric acid	No	**1.67**	No	1.35	Yes	2.88
Lactic acid	No	**1.41**	No	2.12	No	1.26
Methylmalonic acid	No	**4.52**	No	2.97	No	3.50
2-Methylglutaric acid	No	**1.75**	No	2.42	No	1.66
3-Methylglutaric acid	No	**1.77**	No	1.75	No	1.66
N-Acetylaspartic acid	No	**1.06**	No	2.01	No	1.29
Orotic acid	No	**1.38**	No	1.23	No	1.25
4-Hydroxyphenylacetic acid	No	**10.61**	No	11.49	No	9.17
2-Hydroxyphenylacetic acid	No	**9.31**	No	8.62	No	8.00
4-Hydroxyphenyllactic acid	No	**1.65**	No	1.92	No	0.71
Pimelic acid	No	**2.07**	No	2.12	No	1.19
Pyruvic acid	No	**2.46**	No	1.75	Yes	14.46
Sebacic acid	No	**3.57**	Yes	5.21	No	3.02
Suberic acid	No	**2.28**	No	2.22	No	2.14

Table 7. (*Continued*) Results of the long-term stability tests for 12 months

Analyte	Is the slope significantly different from zero at -20°C?*	$u_{lts,rel}$ [%] for shelf-life of 12 months at -20°C	Is the slope significantly different from zero at 4°C?*	$u_{lts,rel}$ [%] for shelf-life of 12 months at 4°C	Is the slope significantly different from zero at 18°C?*	$u_{lts,rel}$ [%] for shelf-life of 12 months at 18°C
Succinic acid	No	**0.96**	No	1.27	No	0.99
N-Suberylglycine	No	**1.13**	No	1.81	No	1.15
N-Tiglylglycine	No	**2.82**	No	3.51	No	2.84
Mandelic acid	No	**1.05**	No	2.02	No	1.58
Homovanillic acid	No	**1.72**	No	3.76	No	1.44
5-Hydroxyindole-3-acetic acid	No	**18.29**	No	17.89	No	18.07
N-Hexanoylglycine	No	**1.43**	No	1.18	No	0.67
N-(3-Phenylpropionyl)glycine	No	**1.42**	No	1.39	No	0.80
Mevalonolactone	No	**1.51**	No	2.86	No	1.40
2-Ketobutyric acid	No	**4.67**	No	4.09	Yes	9.53
2-Ketoisocaproic acid	No	**1.93**	No	1.49	Yes	3.29
Propionylglycine	No	**1.33**	No	2.33	No	2.07
2-Keto-3-methylvaleric acid	No	**2.92**	No	4.44	No	2.02
Malic acid	No	**2.06**	No	1.73	No	1.60
Benzoic acid	No	**2.35**	No	4.02	No	3.53
Tartaric acid	No	**1.41**	No	1.54	No	1.87
Arabitol	No	**3.79**	No	3.91	No	4.45
Kynurenic acid	No	**1.91**	No	1.49	No	1.70
4-Hydroxybenzoic acid	No	**1.31**	No	1.48	No	1.28
Hippuric acid	No	**0.98**	No	0.96	No	0.69
2-Methylhippuric acid	No	**1.29**	No	1.22	No	1.54
Xanthurenic acid	No	**3.11**	No	3.32	No	1.73
3-Indoleacetic acid	No	**0.84**	No	1.05	No	0.83
Vanillomandelic acid	No	**1.21**	No	1.18	No	0.91
Tricarballylic acid	No	**1.42**	No	4.30	No	1.35
Phenylglyoxylic acid	No	**1.81**	No	1.60	Yes	5.36
3-Methylhippuric acid	No	**4.34**	No	4.67	No	4.55
Glucaric acid	No	**8.68**	No	10.55	No	10.71
4-Methylhippuric acid	No	**3.45**	No	3.50	No	3.85

*Data are evaluated at confidence level of 95%.

Based on the results obtained, -20 °C was found to be suitable to keep the samples for up to 12 months. In addition, to ensure stability beyond the initially determined shelf life, stability will be re-evaluated in certain periods, based on the results of post-certification monitoring.

CHARACTERIZATION

According to ISO 17034, the characterization and the value assignment can be carried out in different ways. In this project, characterization study for UME CRM 1315 were performed with ID-LCMS which is a single reference measurement procedure applied in one laboratory.

Two units of the candidate CRM were measured with the ID-LCMS technique. Isotope labelled organic acids were used as internal standards for ID-LCMS method. Method details are presented in Annex 1. Three replicate measurements were performed from each unit on two different days. Units to be analyzed were selected randomly by TRaNS. Standards used for the calibration of the method were obtained from commercial suppliers and purity values were determined by Q-NMR analysis at TÜBİTAK UME. Data obtained from characterization study revealed normal distribution and measurement uncertainities were calculated according to the "Guide to the Expression of Uncertainty in Measurements (GUM)" and "EURACHEM/CITAC Guide Quantifying Uncertainty in Analytical Measurement" documents.

$$u_{char} = \sqrt{u_{LC-IDMS}{}^2} \quad (6)$$

where,

u_{char} : standard uncertainty of the characterization study

$u_{LC\text{-}IDMS}$: standard uncertainty of the LC-IDMS method.

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. Applied method was validated in respect to the quality system set up at TÜBİTAK UME.

The uncertainty 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} .

The different contributions to the CRM uncertainty are combined using equation (7):

$$U_{\text{CRM}} = k \cdot \sqrt{u_{\text{char}}^2 + u_{\text{bb}}^2 + u_{\text{lts}}^2 + u_{\text{sts}}^2} \quad (7)$$

The expanded uncertainty of the certified value U_{CRM} is calculated with a coverage factor of k = 2, representing a confidence level of approximately 95 % considering the effective degrees of freedom values which were found to be greater than 10 for all analytes (except Citric acid) using Welch‑Satterthwaite formula (8) [9].

$$\nu_{eff} = \frac{u_{CRM}^4}{\frac{u^4 char}{n_{char}-1} + \frac{u^4 bb}{n_{bb}-1} + \frac{u^4 lts}{n_{lts}-1} + \frac{u^4 sts}{n_{sts}-1}} \quad (8)$$

The certified and assigned values and uncertainties are summarised in Table 8 and Table 9

Table 8. Summary of certified values and their uncertainties

Analyte	Certified Value Mass fraction (mg/kg)	U_{CRM} (mg/kg) (k = 2) *(k = 2.32)	$U_{CRM,rel}$ (%) (k = 2)	$u_{char,rel}$ (%)	$u_{bb,rel}$ (%)	$u_{sts,rel}$ (%)	$u_{lts,rel}$ (%)
2-Hydroxyglutaric acid	**1.51**	**0.13**	**8.08**	1.31	1.36	2.82	2.19
2-Hydroxyphenylacetic acid	**9.7**	**2.0**	**20.04**	3.29	1.37	0.98	9.31
2-Ketoglutaric acid	**7.56**	**0.68**	**8.92**	0.54	4.15	0.43	1.48
2-Ketoisocaproic acid	**0.358**	**0.035**	**9.51**	3.46	2.54	0.69	1.93
2-Ketoisovaleric acid	**0.750**	**0.040**	**5.27**	1.62	1.04	0.67	1.67
2-Methylcitric acid	**1.33**	**0.11**	**8.04**	2.93	0.97	1.27	2.25
2-Methylhippuric acid	**0.220**	**0.023**	**10.30**	4.92	0.72	0.37	1.29
2-Keto-3-methylvaleric acid	**1.67**	**0.20**	**11.99**	3.24	1.69	3.75	2.92
3-Hydroxyisovaleric acid	**3.30**	**0.15**	**4.34**	0.79	1.2	0.96	1.31
3-Methylglutaric acid	**0.968**	**0.076**	**7.76**	2.73	1.36	1.62	1.77
4-Hydroxybenzoic acid	**0.293**	**0.020**	**6.77**	2.85	0.90	0.90	1.31
5-Hydroxyindole-3-acetic acid	**1.27**	**0.48**	**37.86**	1.49	2.01	4.19	18.29
Adipic acid	**2.60**	**0.13**	**4.64**	1.39	1.19	1.00	1.02
Arabitol	**5.91**	**0.57**	**9.49**	1.13	2.27	1.31	3.79
Benzoic acid	**5.48**	**0.37**	**6.75**	1.64	1.35	1.17	2.35
Citric acid	**219**	**45***	**17.39**	0.46	2.53	8.15	1.60
Fumaric acid	**1.52**	**0.10**	**6.45**	2.09	1.33	1.10	1.75
Glutaric acid	**0.209**	**0.029**	**13.89**	2.52	1.16	0.62	6.34
Glycolic acid	**2.42**	**0.22**	**8.79**	1.42	1.64	0.62	3.77
Hippuric acid	**31.2**	**1.1**	**3.47**	0.38	1.30	0.47	0.98
Kynurenic acid	**0.820**	**0.056**	**6.78**	2.47	1.19	0.60	1.91
Lactic acid	**10.5**	**0.61**	**5.80**	1.12	2.23	0.45	1.41
Malic acid	**0.380**	**0.028**	**7.24**	2.57	1.46	0.38	2.06
Mandelic acid	**8.02**	**0.28**	**3.49**	0.71	0.78	0.91	1.05
Methylmalonic acid	**1.74**	**0.19**	**10.49**	1.61	2.00	0.69	4.52

***** For Citric acid, covarage factor; k=2.32 is used for the calculation of expanded uncertainty (ν_{eff} = 9)

Table 8. (*Continued*) Summary of certified values and their uncertainties

Analyte	Certified Value Mass fraction (mg/kg)	U_{CRM} (mg/kg) (k = 2)	$U_{CRM,rel}$ *(%)* (k = 2)	$u_{char,rel}$ (%)	$u_{bb,rel}$ (%)	$u_{sts,rel}$ (%)	$u_{lts,rel}$ (%)
N-Acetylaspartic acid	**5.02**	**0.30**	**5.83**	1.39	1.57	1.73	1.06
N-Suberylglycine	**1.299**	**0.043**	**3.31**	0.91	0.70	0.36	1.13
N-Tiglylglycine	**0.347**	**0.026**	**7.31**	1.86	1.02	0.95	2.82
N-(3-Methylcrotonyl)gylcine	**6.69**	**0.49**	**7.33**	3.21	0.76	0.37	1.56
N-(3-Phenylpropionyl)glycine	**3.40**	**0.15**	**4.39**	0.72	1.40	0.59	1.42
Phenylglyoxylic acid	**0.342**	**0.021**	**6.14**	1.23	2.09	1.81	0.53
Pimelic acid	**0.098**	**0.020**	**20.29**	9.85	1.04	0.78	2.07
Sebacic acid	**0.115**	**0.013**	**10.75**	3.64	1.29	1.12	3.57
Suberic acid	**1.286**	**0.092**	**7.13**	1.88	1.34	1.47	2.28
Succinic acid	**7.50**	**0.36**	**4.71**	1.01	1.75	0.75	0.96
Tartaric acid	**24.3**	**1.6**	**6.22**	2.22	1.48	0.77	1.41
Vanillomandelic acid	**1.498**	**0.076**	**5.07**	1.86	1.01	0.70	1.21

Table 9. Summary of assigned values and their uncertainties

Analyte	Assigned Value Mass fraction (mg/kg)	U_{CRM} (mg/kg) (k = 2)	$U_{CRM,rel}$ *(%)* (k = 2)	$u_{char,rel}$ (%)	$u_{bb,rel}$ (%)	$u_{sts,rel}$ (%)	$u_{lts,rel}$ (%)
2-Hydroxyisovaleric	**0.939**	**0.061**	**6.47**	1.81	1.22	0.10	2.38
2-Ketobutyric acid	**0.436**	**0.068**	**15.39**	4.73	3.76	0.95	4.67
2-Methylglutaric acid	**1.00**	**0.10**	**9.46**	2.47	1.83	3.14	1.75
3-Hydroxybutyric acid	**1.565**	**0.078**	**4.95**	0.89	0.81	1.45	1.61
3-Hydroxy-3-methylglutaric acid	**2.828**	**0.102**	**3.60**	0.72	0.79	0.41	1.39
3-Indoleacetic acid	**1.333**	**0.046**	**3.39**	0.69	1.28	0.24	0.84
3-Methylhippuric acid	**0.385**	**0.075**	**19.42**	8.32	1.58	1.93	4.34
3-Phenyllactic acid	**10.26**	**0.83**	**8.06**	1.87	1.33	2.95	1.50
4-Hydroxyphenylacetic acid	**5.7**	**1.5**	**26.34**	1.93	2.74	7.05	10.61
4-Hydroxyphenyllactic acid	**1.78**	**0.13**	**7.05**	2.72	1.37	0.65	1.65
4-Methylhippuric acid	**0.356**	**0.076**	**21.37**	9.84	1.73	1.58	3.45
Glyceric acid	**0.602**	**0.081**	**13.46**	2.92	3.04	4.12	3.25
Glucaric acid	**4.13**	**0.84**	**20.30**	1.15	2.35	4.57	8.68
Homovanillic acid	**3.09**	**0.42**	**13.51**	2.98	2.90	5.04	1.72
Isocitric acid	**17.5**	**4.3**	**24.26**	0.60	7.17	8.21	5.29

Table 9. (*Continued*) Summary of assigned values and their uncertainties

Analyte	Assigned Value Mass fraction (mg/kg)	U_{CRM} (mg/kg) (k = 2)	$U_{CRM,rel}$ (%) (k = 2)	$u_{char,rel}$ (%)	$u_{bb,rel}$ (%)	$u_{sts,rel}$ (%)	$u_{lts,rel}$ (%)
Mevalonolactone	**0.140**	**0.015**	**10.69**	4.43	1.54	2.08	1.51
N-Hexanoylglycine	**0.360**	**0.020**	**5.50**	2.03	1.13	0.33	1.43
Propionylglycine	**0.226**	**0.017**	**7.42**	3.09	1.32	0.85	1.33
Pyruvic acid	**3.99**	**0.46**	**11.54**	1.09	5.04	0.81	2.46
Orotic acid	**2.16**	**0.10**	**4.48**	1.21	1.01	0.79	1.38
Tricarballylic acid	**0.631**	**0.030**	**4.72**	1.28	0.97	0.98	1.42
Xanthurenic acid	**0.144**	**0.027**	**18.19**	8.41	1.45	0.54	3.11

INFORMATIVE VALUES

For twelve of the analytes (presented in Table 10), internal standards which are not the isotope labeled molecules of these analytes were used for the measurements. Therefore these values are reported as informative.

Table 10. Informative values and uncertainties for UME CRM 1315

Parameter (CAS No)	Mass Fraction (mg/kg)		Molar Concentration[3] (µmol/L)	
	Assigned Value[1]	Uncertainty[2]	Assigned Value	Uncertainty
2-Hydroxyisovaleric acid (17407-56-6)	0.939	0.061	8.09	0.53
2-Ketobutyric acid (600-18-0)	0.436	0.068	4.34	0.67
2-Methylglutaric acid (617-62-9)	1.00	0.10	6.98	0.67
3-Hydroxybutyric acid (300-85-6)	1.565	0.078	15.29	0.76
3-Hydroxy-3-methylglutaric acid (503-49-1)	2.828	0.102	17.74	0.64
3-Indoleacetic acid (87-51-4)	1.333	0.046	7.74	0.27
3-Methylhippuric acid (27115-49-7)	0.385	0.075	2.02	0.40

[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 (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.

Table 10. (*Continued)* Informative values and uncertainties for UME CRM 1315

Parameter (CAS No)	Mass Fraction (mg/kg)		Molar Concentration[3] (µmol/L)	
	Assigned Value[1]	Uncertainty[2]	Assigned Value	Uncertainty
3-Phenyllactic acid (20312-36-1)	10.26	0.83	62.79	5.1
4-Hydroxyphenylacetic acid (156-38-7)	5.7	1.5	38.2	10.1
4-Hydroxyphenyllactic acid (306-23-0)	1.78	0.13	9.95	0.71
4-Methylhippuric acid (27115-50-0)	0.356	0.076	1.87	0.41
Glyceric acid (473-81-4)	0.602	0.081	5.77	0.78
Glucaric acid (25525-21-7)	4.13	0.84	20.0	4.1
Homovanillic acid (306-08-1)	3.09	0.42	17.3	2.4
Isocitric acid (320-77-4)	17.5	4.3	92.4	22.5
Mevalonolactone (674-26-0)	0.140	0.015	1.09	0.12
N-Hexanoylglycine (24003-67-6)	0.360	0.020	2.11	0.12
Propionylglycine (21709-90-0)	0.226	0.017	1.75	0.13
Pyruvic acid (127-17-3)	3.99	0.46	46.0	5.4
Orotic acid (65-86-1)	2.16	0.10	14.08	0.64
Tricarballylic acid (99-14-9)	0.631	0.030	3.64	0.18
Xanthurenic acid (59-00-7)	0.144	0.027	0.71	0.13

[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 (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.

For 2-Hydroxyisovaleric acid, 2-Ketobutyric acid, 3-Hydroxybutyric acid, 3-Hydroxy-3-methylglutaric acid, 3-Indoleacetic acid, Mevalonolactone, N-Hexanoylglycine, Propionylglycine, Pyruvic acid and Orotic acid purity assessment by Q-NMR could not be achieved. Therefore these values are reported as informative.

COMMUTABILITY

The intended use of this reference material is to check method performance and validation of organic acid measurements in urine with LC-MS and LC-MS/MS methods.

Commutability of the material with routine *in vitro* diagnostic organic acid kit methods has not been assessed. The user would need to assess the commutability, if UME CRM 1315 is going to be used for evaluating the accuracy of routine *in vitro* diagnostic organic acid kit methods.

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 Q-NMR measurements at TÜBİTAK UME. All weighing operations were performed with balances calibrated at TUBITAK UME with E2 Class weigh sets traceable to national standards. The assigned values of the organic acid calibrants are traceable to SI through Q-NMR measurements using SI traceable calibrants. Purity assessment capability of TÜBİTAK UME by Q-NMR was demonstrated with several international comparison studies and publications [10].

INSTRUCTIONS FOR USE

Intended Use

This material is intended to be used for method performance check and validation purposes of organic acid measurements in urine by LC-MS and LC-MS/MS methods.

Scope of Application

UME CRM 1315 is suitable for use in evaluating the accuracy of procedures for determination of organic acid concentrations in urine using LC-MS and LC-MS/MS methods.

The material is not suitable for evaluating the accuracy of organic acid measurements in urine using routine *in vitro* diagnostic organic acid kit methods unless the commutability of the material is proven by the user.

Safety Precautions

Raw material: synthetic urine (origin: USA) was produced by the manufacturer (Dyna-Tek. Inc). The usual laboratory safety measures apply. The material is suitable for *in-vitro* use only. Safety Data Sheet (SDS) should be read before use.

Storage Conditions

The material should be stored at temperatures equal to or lower than -20 ^{o}C in a dry and dark place. Solutions of UME CRM 1315 should not be exposed to direct sunlight or UV light. User should take necessary precautions against evaporation or sublimation of the sample.

TUBITAK UME cannot be held responsible for changes that might happen to the material at customer's premises due to noncompliance with the instructions for use, and the storage conditions given in the certificate.

Reconstitution of the Material

All content of the unit should be reconstituted at once according to the recommended protocol presented below:

- UME CRM 1315 and deionized water should be kept at room temperature at least for one hour in the room with balance before weighing.

- Before opening, the vial should be tapped gently to table or benchtop in order to collect the whole content at the bottom of the vial.

- Crimp cap is opened. The vial with the inner cap is placed to the balance and tare is pressed. Unit is opened gently at vertical position by balancing the inner pressure with outer pressure. Care should be taken in order not to lose any sample on cap or elsewhere. 2900 µL of water is then added gently with a calibrated pipette into the sample. Then the inner cap is closed.

- Vial, with its content and inner cap is weighed (*m*).

- The average amount of water added to the analysed units at TUBITAK UME was

$$m_{avg} = (2.90686 \pm 0.001287)\ g\ (k=2)$$

If m is deviating from m_{avg}, then the corrected value of the analyte concentrations can be calculated as:

$$\text{Corrected analyte concentration} = \text{Certified value} \times \frac{m_{avg}}{m}$$

The vial with added water should be gently shaken with its cap and inner cap closed. If material is stacked to the inner cap, user can gently rotate the vial upside down several times to dissolve those particles. Solid particles should be inspected, and mixing should be continued until all of the content is dissolved. User should perform the measurement on the dissolved material as quickly as possible. If it is necessary to keep the dissolved material for later measurements, it should be portioned into the smaller volumes, and stored at temperatures equal to or lower than -20 °C in dark conditions. Subsamples should be stored in the vials resistive to evaporation and sun light.

Minimum Sample Intake

The minimum sample intake suggested for the reconstituted sample is 300 µL.

Use of the Certified Value

For assessing the method performance, the measured values of the CRMs are compared with the certified values [11]. The procedure can be described briefly as follows:

- Calculate the absolute difference between mean measured value and the certified value (Δ_m).

- Combine measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}):

$$u_{\Delta} = \sqrt{u_{meas}^2 + u_{CRM}^2}$$

- Calculate the expanded uncertainty (U_{Δ}) from the combined uncertainty (u_{Δ}) using a coverage factor of two (k = 2), corresponding to a confidence level of approximately 95%.

If $\Delta_m \leq U_\Delta$, then it is assumed that there is no significant difference between the measurement result and the certified value at 95% confidence level.

An online application: CRM Result Evaluation-CRM RE to evaluate your measurement results and automatically create quality control charts is available through the link https://rm.ume.tubitak.gov.tr/en/crm_re/

ACKNOWLEDGMENT

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

Murat Çelik and Gökhan Günay (former Zivak Technologies employees) are gratefully acknowledged for their contribution to the project.

We would like to thank to Dr. Byungjo Kim for valuable discussions and comments during the ISO 17034 accreditation assessment of the project.

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11. For more information about comparison of a measurement result with the certified value please see https://ec.europa.eu/jrc/sites/jrcsh/files/erm_application_note_1_en.pdf

REVISION HISTORY

Date	Remarks
31.05.2019	First issue.
28.05.2021	CRM certified values and uncertainty values are revised. Intended use, commutability and traceability information is revised. U_{CRM} for citric acid is recalculated for k = 2.32. 2-Ketobutyric acid, 3-Hydroxybutyric acid, 3-Hydroxy-3-methylglutaric acid, 3-Indoleacetic acid, *N*-Hexanoylglycine, Mevalonolactone, Orotic acid, Propionylglycine, Pyruvic acid and Xanthurenic acid are moved from Certified Values to the Informative Values section. More information about the method performance is added to Annex 1.

ANNEXES

Annex 1. Details of ID-LCMS Technique

Sample was dissolved with 2900 µL of deionized water (≥18 MΩ). 300 µL portion of this solution was transferred into an Eppendorf tube by weighing. 100 µL isotopes labeled internal standards (IS) DL-2-Methylcitric acid(Methyl-D3,98%) (7.70 mg/kg), 3-Hydroxy-3-methylglutaric acid (Methyl-D3, 98%) (19.47 mg/kg), 3-Methylcrotonylgylcine (Glycine-13C2, 98%; 15N, 98%) (15.31 mg/kg), Adipic acid (13C6) (16.32 mg/kg), 2-Hydroxyglutaric acid, disodium salt (2,3,3-D3) (61.53 mg/kg), 3-Hydroxyisovaleric acid (3-4-methyl-13C3,99%) (16.86 mg/kg), α-Ketoglutaric acid (13C5,99%) (38.30 mg/kg), Sodium (+/-)-3-Hydroxybutyrate-3,4,4,4-d4 (9.91 mg/kg), 3-Hydroxyisovaleric acid (3-4-methyl-13C3,99%) (16.86 mg/kg), Citric acid (2,2,4,4-D4) (987.25 mg/kg), Fumaric acid (2,3-D2) (7.42 mg/kg), Glutaric acid (2,2,4,4-D4) (1.52 mg/kg), DL-Tartaric acid (2,3-D2) (85.66 mg/kg), Glycolic acid (2,2-D2) (21.71 mg/kg), Alpha-Ketoisovaleric acid, sodium salt (U-13C5) (6.11 mg/kg), Sodium L-Lactate (3,3,3-D3) (12.15 mg/kg), Methylmalonic acid (methyl-D3) (10.35 mg/kg), 3-Methylglutaric acid (2,2,4,4-D4) (6.16 mg/kg), Dl-aspartic acid,N-acetyl (aspartate-2,3,3-D3) (30.72 mg/kg), Orotic acid:H2O (1,3-15N2) (12.41 mg/kg), 2-Hydroxyphenylacetic acid-d4 (8.16 mg/kg), Pimelic acid (2,2,6,6-D4) (0.61 mg/kg), Pyruvic acid (1-13C) (24.58 mg/kg), Sebacic acid (D16) (1.37 mg/kg), Suberic acid (2,2,7,7-D4) (6.92 mg/kg), Succinic acid (1,4-13C2) (47.36 mg/kg), N-Suberylglycine (2,2-D2) (4.64 mg/kg), Tiglylglycine (GLYCINE-13C2, 98%; 15N, 98%) (2.36 mg/kg), Sodium mandelate (phenyl-D5) (49.32 mg/kg), DL-Vanillomandelic acid-d3(99%) (9.20 mg/kg), 5-Hydroxyindole-3-Acetic acid (2,4,6,7, α - α -D6) (7.35 mg/kg), Glycine,N-Hexanoyl (2,2-D2) (1,53 mg/kg), N-(3-Phenylpropionyl)glycine (2,2-D2) (24.31 mg/kg), (+/-)-Mevalonolactone-4,4,5,5-d4 (0.72 mg/kg), α -Ketobutyric acid,sodium salt (13C4,3,3-D2) (3.03 mg/kg), α -Ketoisocaproic acid, sodium salt (methyl-D3) (3.36 mg/kg), Glycine, N-Propionyl (2,2-D2) (1.53 mg/kg), 3-methyl-2-oxovaleric acid-d8 sodium salt (5.11 mg/kg), L-malic acid (13C4) (2.45 mg/kg), Benzoic acid (Carboxyl-13C) (37.61 mg/kg), D-Arabinitol (U-13C5) (37.23 mg/kg), Kynurenic-3,5,6,7,8-d5 Acid (5.48 mg/kg), 4-Hydroxybenzoic acid (Carboxyl-13C) (1.50 mg/kg), Glycine,N-Benzoyl (hippuric acid)(15N) (178.91 mg/kg), 2-methylhippuric acid (Glycine-2,2-D2) (1.41 mg/kg), Xanthurenic acid (D4) (1.30 mg/kg), Indole-3-Acetic acid (2,2-D2) (8.10 mg/kg) and Phenylglyoxylic acid-d5 (Benzoylformic acid-d5) (6.13 mg/kg) was added. The content was mixed with vortex for 10 s. 50 µL 1M HCl : Methanol 10% (1:1) solution was added and was mixed with vortex for 10 s. 2 µL of this solution was injected to LC-HRMS.

The parameters of LC-MS instrument, LC parameters and MS properties are given in Table A1, Table A2, Table A3 and Table A4.

Table A1. LC-MS Instrument

Name of the Component	Producer	Model
HRMS	Thermo	Q Exactive Orbitrap (Germany)
HPLC	Thermo	Dionex Ultimate 3000 (Germany)
HPLC column	Phenomenex	Phenomenex EZ:faast 4u AAA-MS (250 x 2.0 mm)

Table A2. LC parameters

Name	Value
Column Temperature	40° C
Mobile Phase	A: Methanol: Water (5:95) (3 mM Ammonium acetate, 0.1% Formic acid) B: Methanol (1 M Ammonium acetate 0.3%, Formic acid 0.1%)
Flow rate	0.25 mL/min, Gradient
Gradient Pump Program	00:00 min 0 % B 14:00 min 100 % B 16:00 min 100 % B 16:01min 0 % B 20:00 min 0 % B

Table A3. MS Parameters

Parameter	Value
Ionization Mode	ESI positive + ESI negative
Sheath Gas Flow Rate	35
Aux Gas Flow	10
Sweep Gas	0
Discharge Current (µA)	5

Table A4. MS Parameters

Analyte	MH+- [m/z]	RT [min]	Internal Standard (IS)	MH+- [m/z]	RT [min]
2-Methylcitric acid	205.034	5.58	DL-2-Methylcitric acid(Methyl-D3,98%)	208.053	5.56
3-Hydroxy-3-methylglutaric acid	161.044	5.45	3-Hydroxy-3-methylglutaric acid (Methyl-D3, 98%)	164.063	5.41
N-(3-Methylcrotonyl)gylcine	156.065	10.85	3-Methylcrotonyl Gylcine (Glycine-13C2, 98%; 15N, 98%)	159.068	10.85
3-Phenyllactic acid	165.054	15.96	Adipic acid (13C6)	151.069	10.25
Adipic acid	145.049	10.25	Adipic acid (13C6)	151.069	10.25
2-Hydroxyglutaric acid	147.028	3.38	(RS)-2-Hydroxyglutaric acid, disodium salt (2,3,3-D3)	150.047	3.36
2-Hydroxyisovaleric acid	117.054	10.56	3-Hydroxyisovaleric acid (3-4-methyl-13C3,99%)	120.064	8.48
2-Ketoglutaric acid	145.01257	2.88	α-Ketoglutaric acid (13C5,99%)	150.02914	2.88
3-Hydroxybutyric acid	103.038	5.14	Sodium (+/-)-3-Hydroxybutyrate-3,4,4,4-d4	107.063	5.05
3-Hydroxyisovaleric acid	117.054	8.48	3-Hydroxyisovaleric acid (3-4-methyl-13C3,99%)	120.064	8.48
Citric acid	191.018	3.32	Citric acid (2,2,4,4-D4)	195.043	3.29
Fumaric acid	115.002	4.10	Fumaric acid (2,3-D2)	117.014	4.10
Glutaric acid	131.033	7.52	Glutaric acid (2,2,4,4-D4)	135.058	7.44
Glyceric acid	105.01755	2.37	DL-Tartaric acid (2,3-D2)	151.0196	2.31
Glycolic acid	75.007	2.47	Glycolic acid (2,2-D2)	77.01955	2.43
Isocitric acid	191.01831	2.65	Citric acid (2,2,4,4-D4)	195.043	3.29
2-Ketoisovaleric acid	115.038	7.21	Alpha-Ketoisovaleric acid, sodium salt (U-13C5)	120.055	7.21
Lactic acid	89.02254	3.38	Sodium L-Lactate (3,3,3-D3)	92.0414	3.34
Methylmalonic acid	117.017	4.79	Methylmalonic acid (methyl-D3)	120.036	4.75
2-Methylglutaric acid	145.049	10.71	3-Methylglutaric acid (2,2,4,4-D4)	149.074	10.37
3-Methylglutaric acid	145.049	10.46	3-Methylglutaric acid (2,2,4,4-D4)	149.074	10.37
N-Acetylaspartic acid	174.039	3.21	Dl-aspartic acid,N-acetyl (aspartate-2,3,3-D3)	177.058	3.19
Orotic acid	155.008	2.78	Orotıc acid:H2O (1,3-15N2)	157.00217	2.78

Table A4. (Continued) MS Parameters

Analyte	MH+- [m/z]	RT [min]	Internal Standard (IS)	MH+- [m/z]	RT [min]
4-Hydroxyphenylacetic acid	151.038	11.87	2-Hydroxyphenylacetic acid-d4	155.064	11.04
2-Hydroxyphenylacetic acid	151.038	11.12	2-Hydroxyphenylacetic acid-d4	155.064	11.04
4-Hydroxyphenyllactic acid	181.049	10.50	Adipic acid (13C6)	151.069	10.25
Pimelic acid	159.065	12.93	Pimelic acid (2,2,6,6-D4)	163.089	12.83
Pyruvic acid	87.00694	2.68	Pyruvic acid (1-13C)	88.0102	2.68
Sebacic acid	201.112	17.35	Sebacic acid (D16)	217.212	17.33
Suberic acid	173.080	16.38	Suberic acid (2,2,7,7-D4)	177.105	16.39
Succinic acid	117.017	4.48	Succinic acid (1,4-13C2)	119.024	4.48
N-Suberylglycine	230.102	12.39	N-Suberylglycine (2,2-D2)	232.114	12.36
N-Tiglylglycine	158.081	10.63	Tiglylglycine (Glycine-13C2, 98%; 15N, 98%)	161.085	10.63
Mandelic acid	151.038	11.12	Sodium mandelate (phenyl-D5)	156.069	11.02
Homovanillic acid	181.049	12.83	DL-Vanillomandelic acid-d3(99%)	200.063	6.58
5-Hydroxyindole-3-acetic acid	192.065	11.03	5-Hydroxyindole-3-Acetic acid (2,4,6,7, α - α -D6)	198.102	10.94
N-Hexanoylglycine	172.096	16.39	Glycine,N-Hexanoyl (2,2-D2)	174.109	16.37
N-(3-Phenylpropionyl)glycine	206.081	16.37	N-(3-Phenylpropionyl)glycine (2,2-D2)	208.093	16.35
Mevalonolactone	131.070	6.79	(+/-)-Mevalonolactone-4,4,5,5-d4	135.095	6.67
2-Ketobutyric acid	101.023	4.02	α -Ketobutyric acid,sodium salt (13C4,3,3-D2)	107.049	4.00
2-Ketoisocaproic acid	129.054	11.62	α -Ketoisocaproic acid, sodium salt (methyl-D3)	132.073	11.56
Propionylglycine	130.049	4.69	Glycine, N-Propionyl (2,2-D2)	132.062	4.67
2-Keto-3-methylvaleric acid	129.054	11.04	3-methyl-2-oxovaleric acid-d8 sodium salt	137.104	10.89
Malic acid	133.012	2.68	L-malic acid (13C4)	137.026	2.68
Benzoic acid	121.028	16.97	Benzoic acid (Carboxyl-13C)	122.031	16.97
Tartaric acid	149.007	2.31	DL-Tartaric acid (2,3-D2)	151.0196	2.31
Arabitol	153.075	2.17	D-Arabinitol (U-13C5)	158.092	2.17
Kynurenic acid	188.034	11.54	Kynurenic-3,5,6,7,8-d5 Acid	193.065	11.48

Table A4. (Continued) MS Parameters

Analyte	MH+- [m/z]	RT [min]	Internal Standard (IS)	MH+- [m/z]	RT [min]
4-Hydroxybenzoic acid	137.023	11.81	4-Hydroxybenzoic acid (Carboxyl-13C)	138.026	11.81
Hippuric acid	178.049	11.87	Glycine,N-Benzoyl (hippuric acid)(15N)	179.046	11.87
2-Methylhippuric acid	192.065	13.38	2-methylhippuric acid (Glycine-2,2-D2)	194.078	13.34
Xanthurenic acid	204.029	11.06	Xanthurenic acid (D4)	208.054	10.99
3-İndoleacetic acid	176.070	16.66	Indole-3-Acetic acid (2,2-D2)	178.083	16.64
Vanillomandelic acid	197.044	7.06	DL-Vanillomandelic acid-d3(99%)	200.063	6.58
Tricarballylic acid	175.023	4.48	Succinic acid (1,4-13C2)	119.024	4.48
Phenylglyoxylic acid	149.022	9.59	Phenylglyoxylic acid-d5 (Benzoylformic acid-d5)	154.054	9.49
3-Methylhippuric acid	192.065	16.08	2-methylhippuric acid (Glycine-2,2-D2)	194.078	13.34
Glucaric acid	209.028	2.16	Succinic acid (1,4-13C2)	119.024	4.48
4-Methylhippuric acid	192.065	16.00	2-methylhippuric acid (Glycine-2,2-D2)	194.078	13.34

Table A5. Method Validation Spike Recovery Results

Analyte	Spiked Mass Fraction (mg/kg)	Measured Mass Fraction (mg/kg)	Recovery (%)
2-Methylcitric acid	5.390	5.386	99.9
2-Ketoisovaleric acid	2.650	2.621	98.9
2-Hydroxyphenylacetic acid	26.670	26.513	99.4
2-Methylglutaric acid	2.660	2.632	98.9
2-Methylhippuric acid	0.59	0.586	99.3
3-Hydroxy-3-methylglutaric acid	7.810	7.799	99.9
3-Indoleacetic acid	3.540	3.510	99.2
3-Methyl-2-oxovaleric acid	5.340	5.263	98.6
3-Methylcrotonyl Gylcine	19.770	19.585	99.1
3-Methylglutaric acid	2.670	2.669	99.9
3-Methylhippuric acid	1.010	1.001	99.1
3-Phenyllactic acid	26.640	26.307	98.8
4-Hydroxy-3-methoxymandelic acid	4.000	3.860	96.5
4-Hydroxybenzoic acid	0.82	0.810	98.8
4-Hydroxyphenylacetic acid	16.040	15.930	99.3
4-Methylhippuric acid	1.000	1.001	100.1
5-Hydroxyindole-3-acetic acid	3.060	3.044	99.5
Adipic acid	6.850	6.756	98.6
2-Hydroxyglutaric acid	5.450	5.425	99.5

Table A5. (Continued) Method Validation Spike Recovery Results

Analyte	Spiked Mass Fraction (mg/kg)	Measured Mass Fraction (mg/kg)	Recovery (%)
2-Ketobutyric acid	1.320	1.323	100.2
2-Ketoisocaproic acid	1.410	1.388	98.4
Arabitol	16.330	16.305	99.8
Benzoic acid	15.030	14.966	99.6
Benzoylformic acid	1.050	1.048	99.8
Citric acid	591.000	574.242	97.2
Fumaric acid	3.980	3.959	99.5
Glucaric acid	15.210	15.030	98.8
Glutaric acid	0.540	0.537	99.4
Glyceric acid	4.560	4.606	101.0
Glycolic acid	9.260	9.256	100.0
Hexanoyl glycine	1.030	1.015	98.6
Hippuric acid	85.740	84.200	98.2
Homovanillic acid	7.290	7.114	97.6
Isocitric acid	87.670	86.392	98.5
Kynurenic acid	2.350	2.323	98.9
Lactic acid	30.300	30.169	99.6
Malic acid	1.060	1.054	99.5
Mandelic acid	21.570	20.885	96.8
Methylmalonic acid	4.490	4.449	99.1
Mevalonolactone	0.390	0.385	98.8
N-(3-Phenylpropionyl)glycine	9.320	9.196	98.7
N-Acetyl-L-aspartic acid	13.320	13.191	99.0
Orotic acid	5.360	5.333	99.5
p-Hydroxyphenyllactic acid	5.350	5.273	98.6
Pimelic acid	0.260	0.258	99.4
Propionyl Glycine	0.650	0.651	100.2
Pyruvic acid	11.560	11.539	99.8
Sebacic acid	0.310	0.307	99.2
Suberic acid	3.350	3.333	99.5
Suberyl glycine	3.540	3.490	98.6
Succinic acid	20.660	20.460	99.0
Tartaric acid	65.190	63.818	97.9
Tiglyl glycine	1.010	0.988	97.9
Tricarballylic acid	4.00	3.860	96.5
Xanthurenic acid	0.56	0.563	100.5
α -Ketoglutarıc acid	20.670	20.471	99.0
α-Hydroxyisovaleric acid	2.680	2.686	100.2
β-Hydroxybutyric acid	4.160	4.140	99.5
β-Hydroxyisovaleric acid	8.790	8.670	98.6

Annex 2. Graphs for Homogeneity Studies

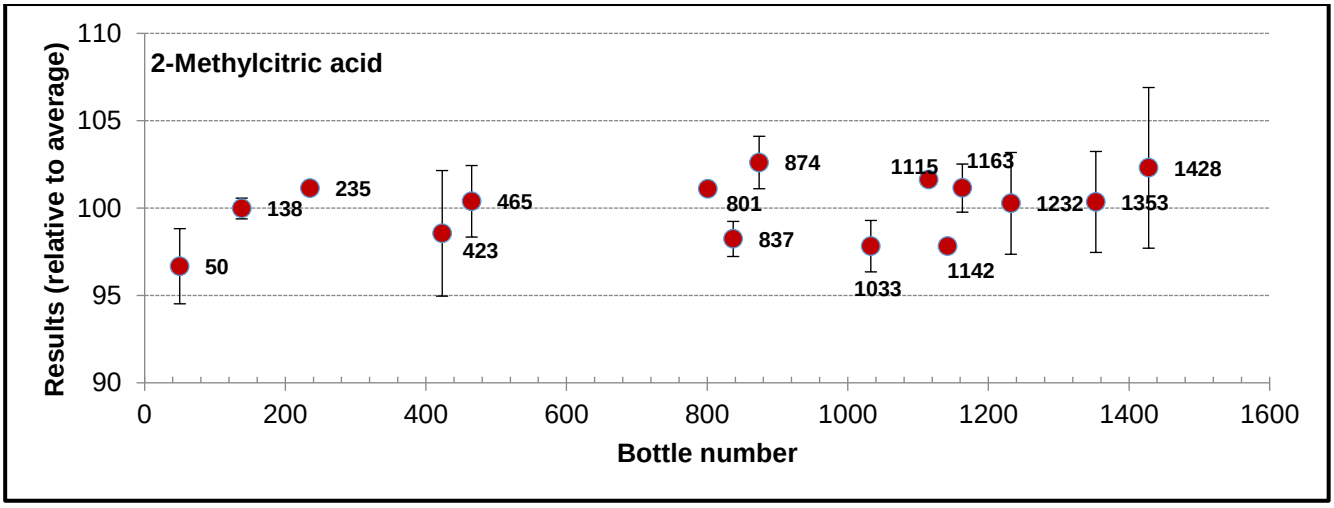

Figure A1. Homogeneity plot for 2-Methylcitric acid

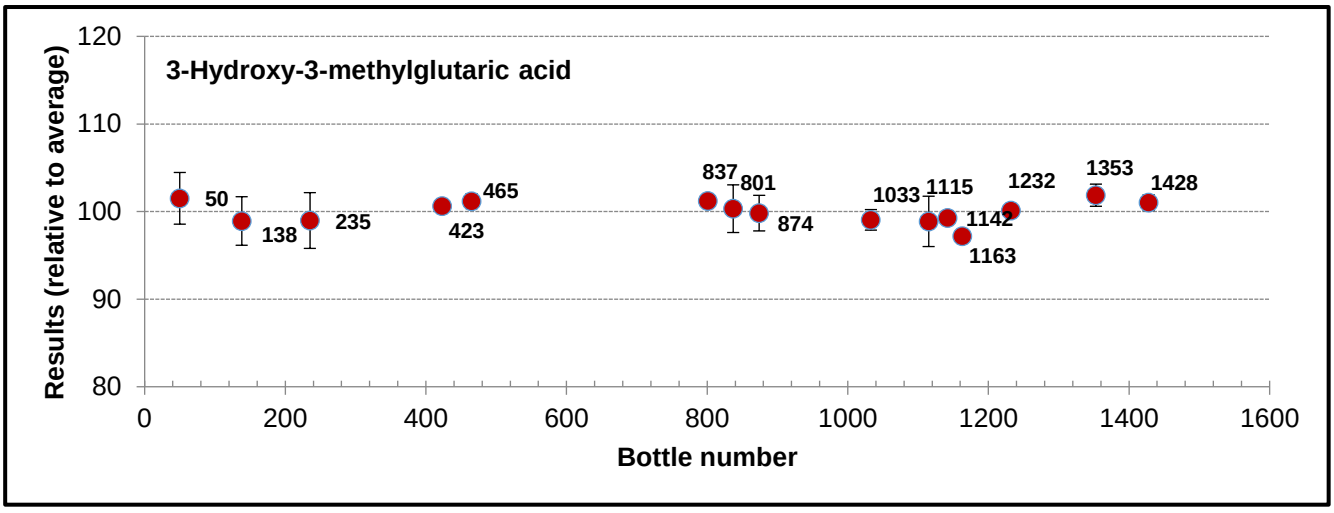

Figure A2. Homogeneity plot for 3-Hydroxy-3-methylglutaric acid

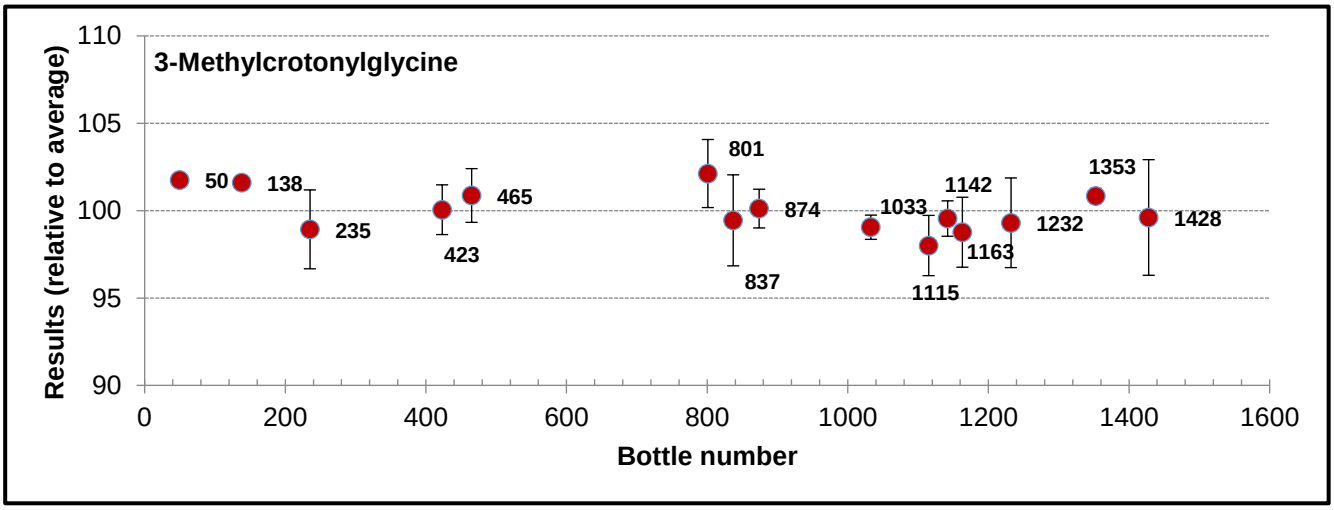

Figure A3. Homogeneity plot for 3-Methylcrotonylglycine

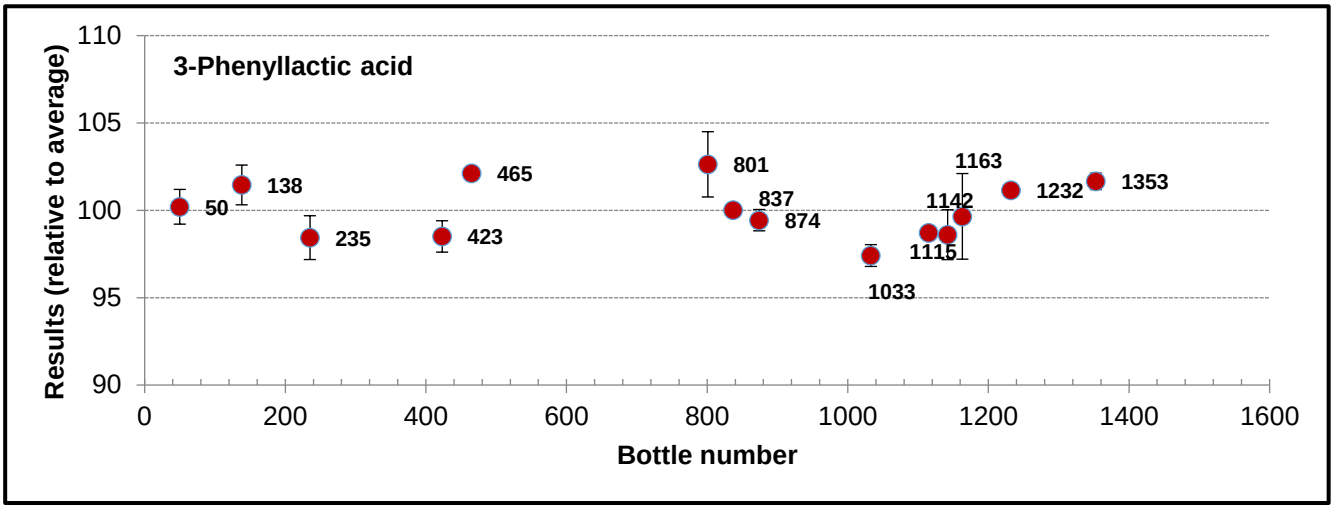

Figure A4. Homogeneity plot for 3-Phenyllactic acid

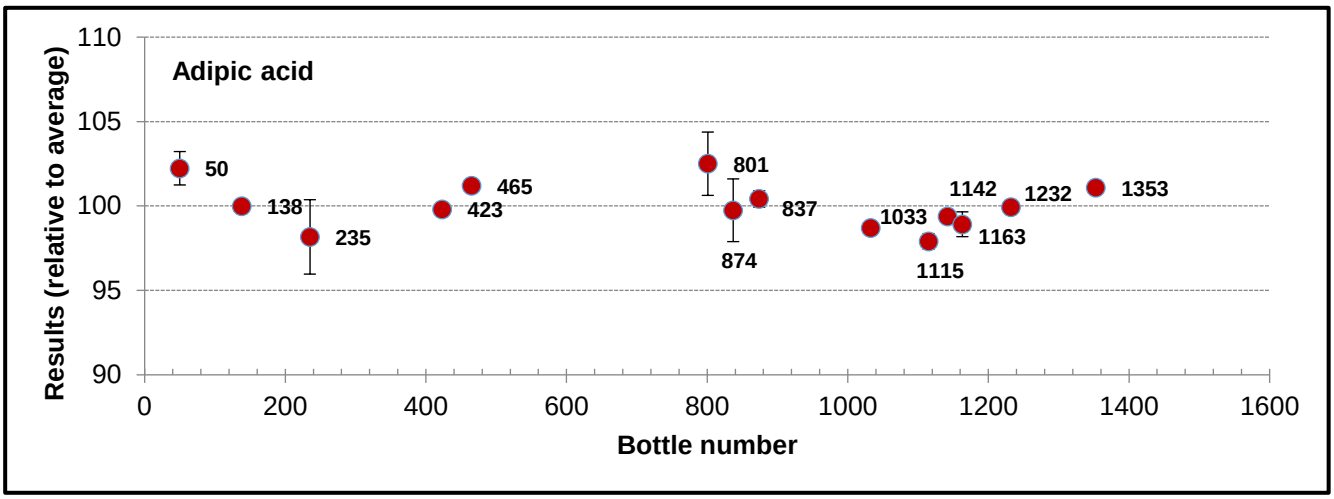

Figure A5. Homogeneity plot for Adipic acid

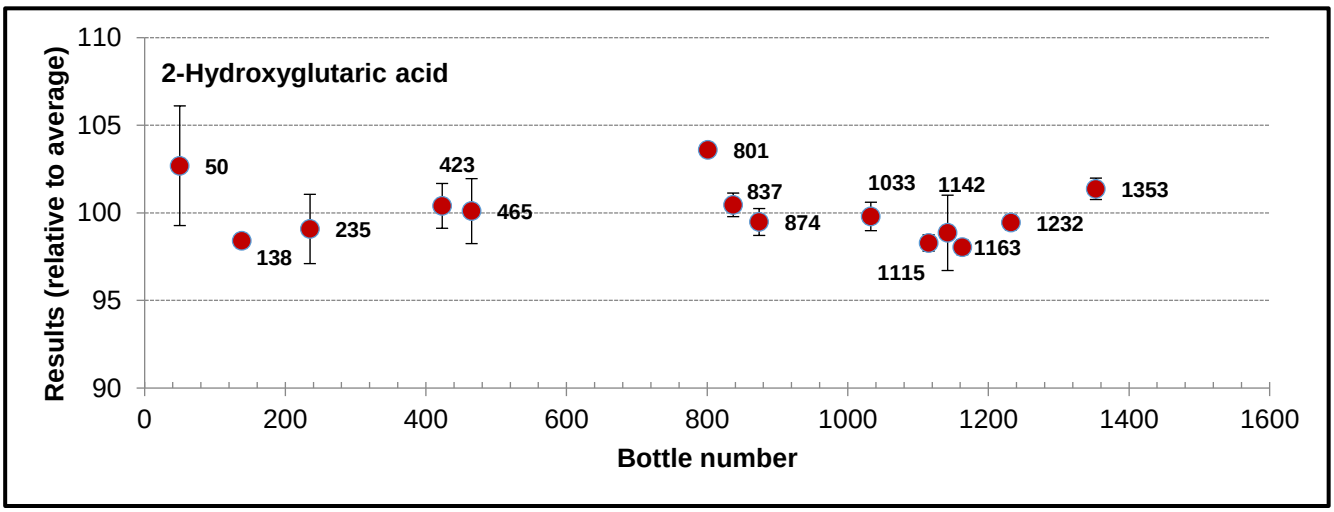

Figure A6. Homogeneity plot for 2-Hydroxyglutaric acid

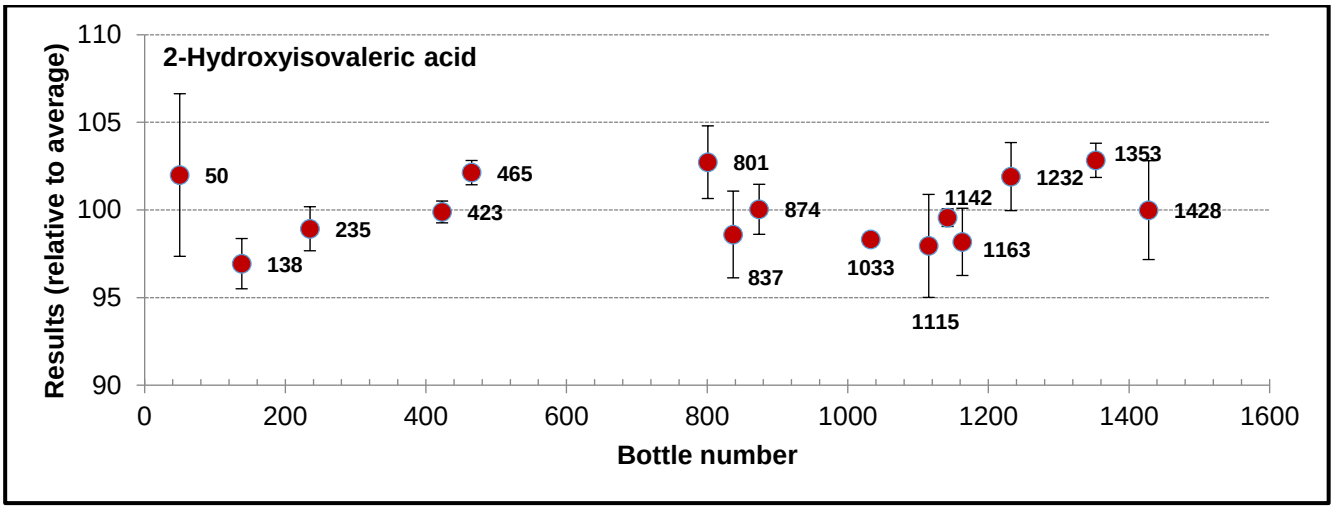

Figure A7. Homogeneity plot for 2-Hydroxyisovaleric acid

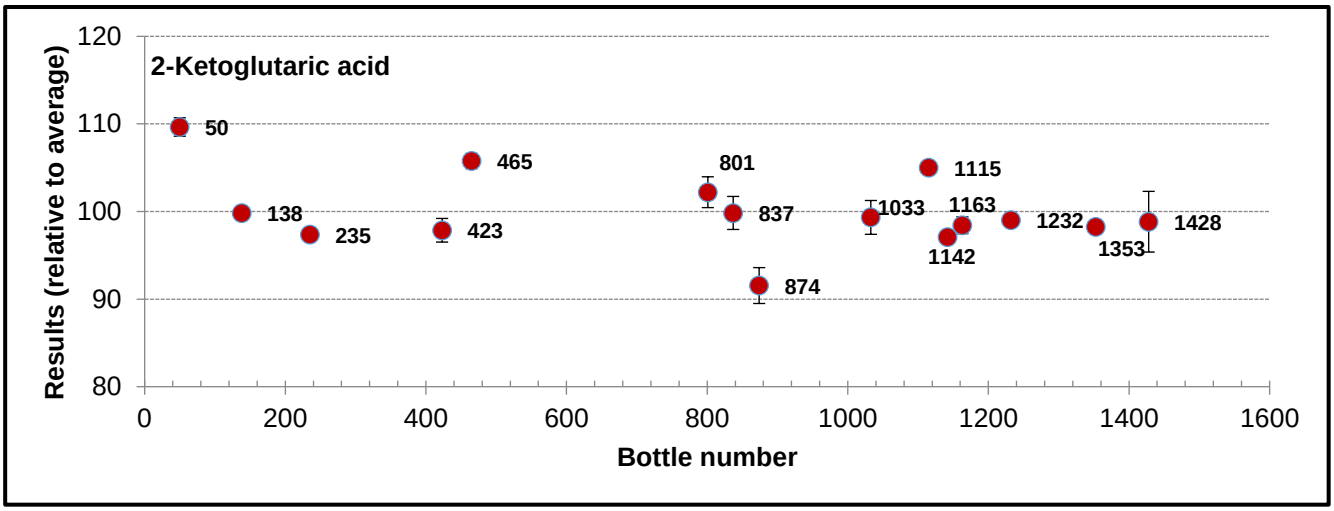

Figure A8. Homogeneity plot for 2-Ketoglutaric acid

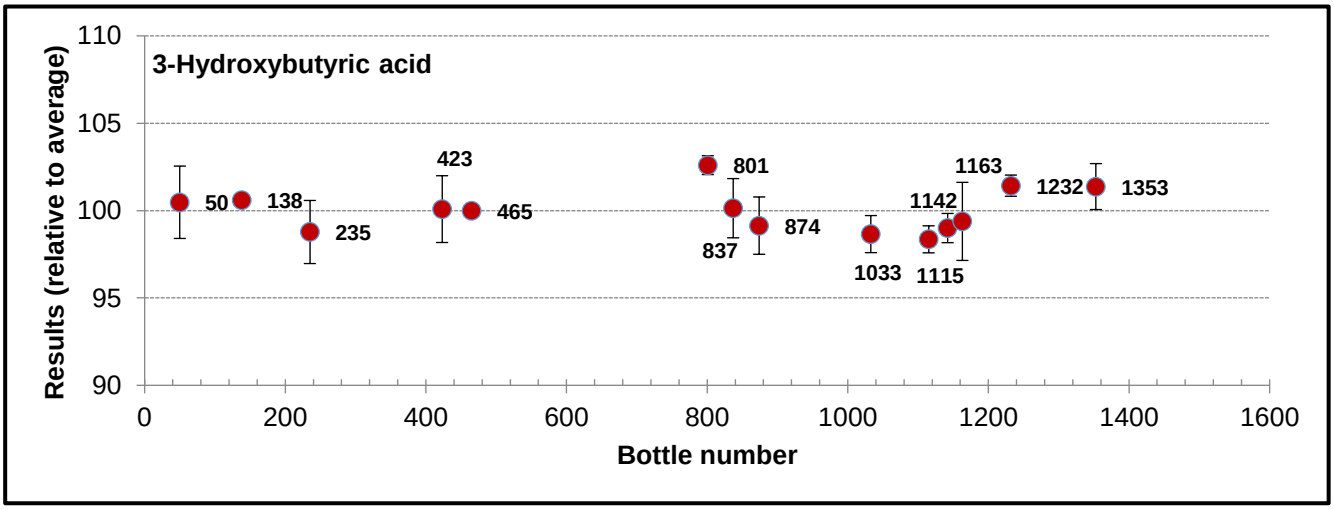

Figure A9. Homogeneity plot for 3-Hydroxybutyric acid

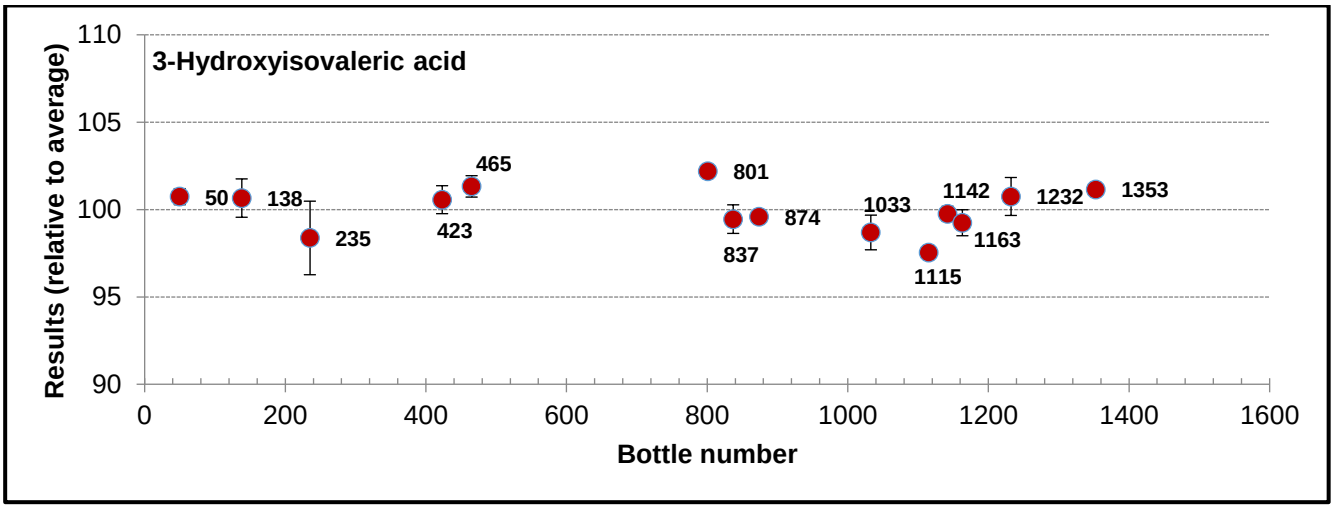

Figure A10. Homogeneity plot for 3-Hydroxyisovaleric acid

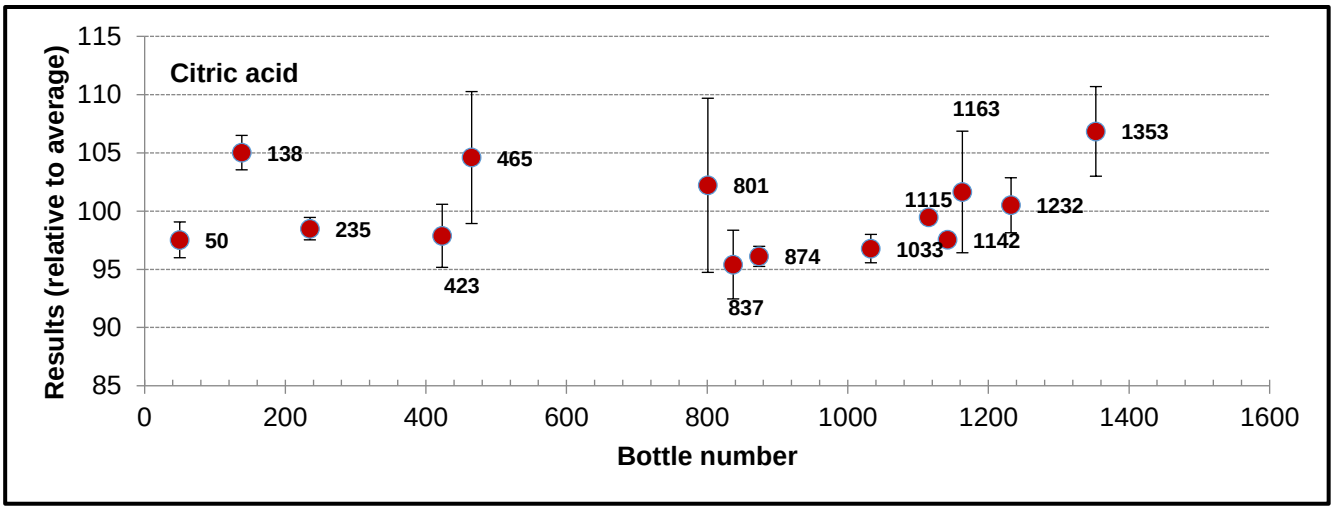

Figure A11. Homogeneity plot for Citric acid

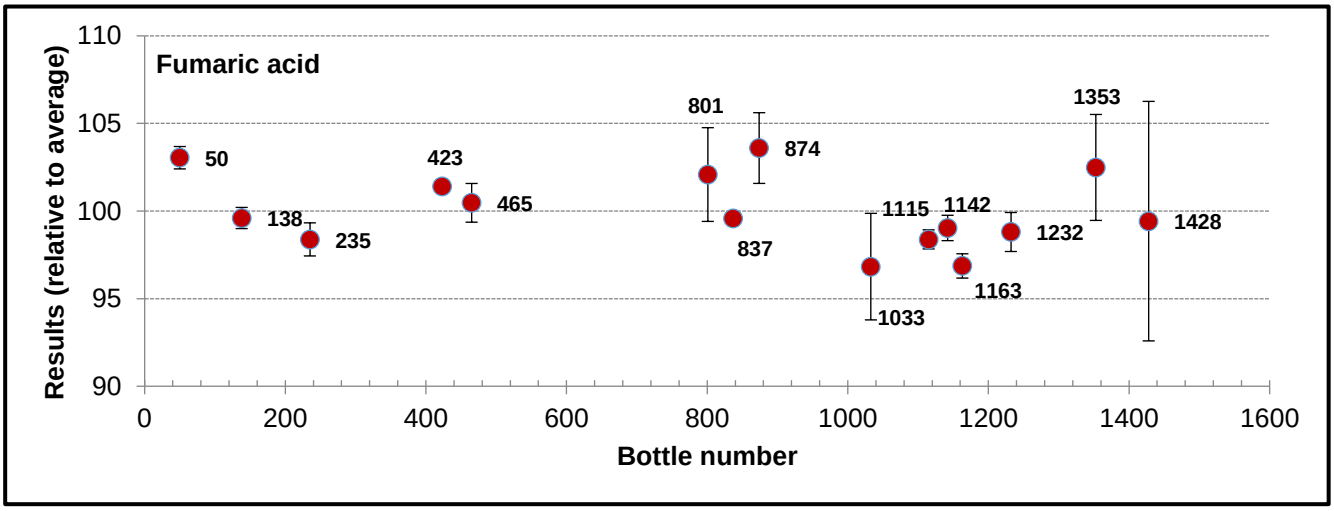

Figure A12. Homogeneity plot for Fumaric acid

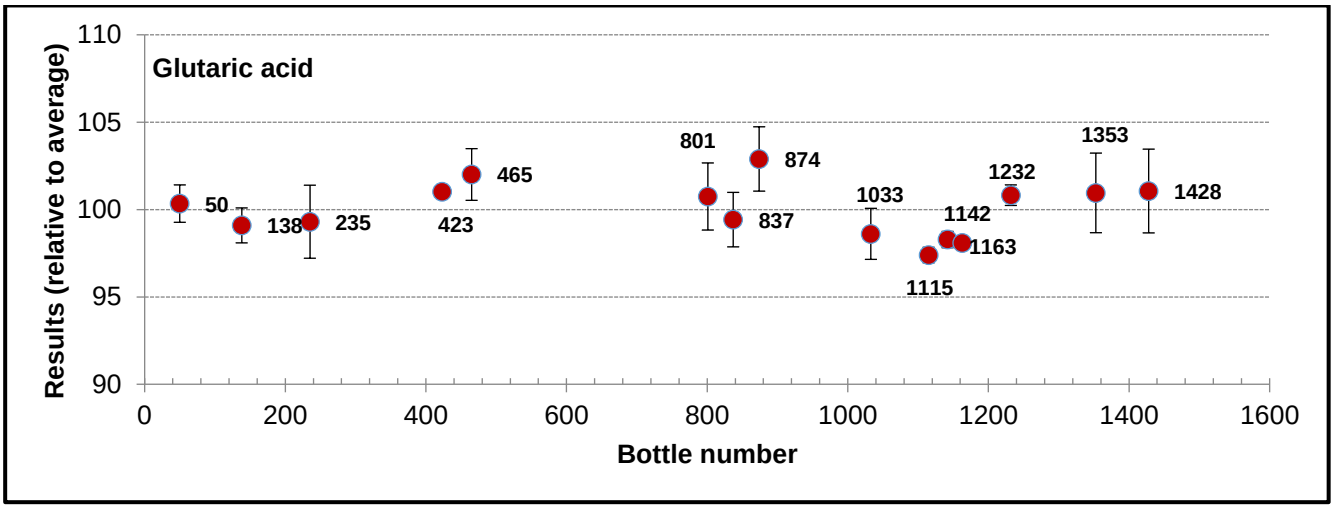

Figure A13. Homogeneity plot for Glutaric acid

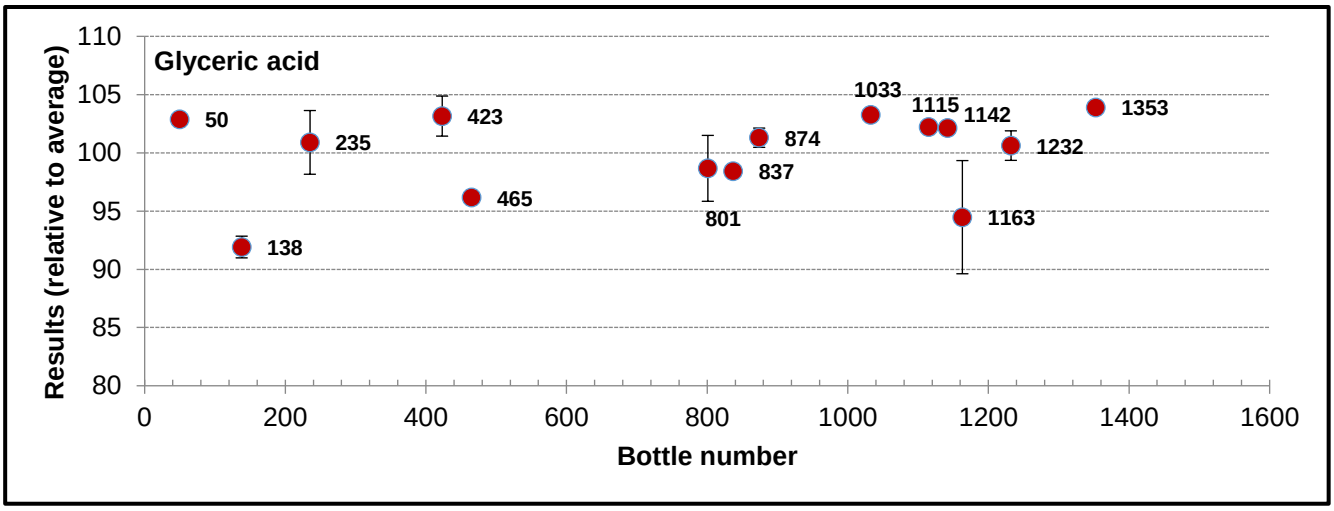

Figure A14. Homogeneity plot for Glyceric acid

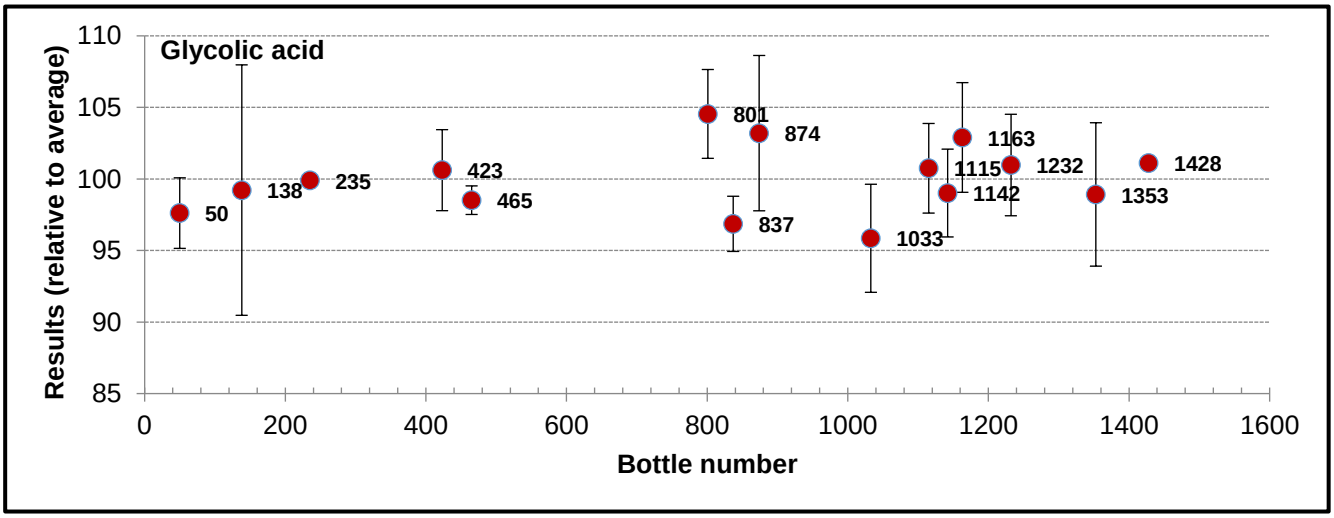

Figure A15. Homogeneity plot for Glycolic acid

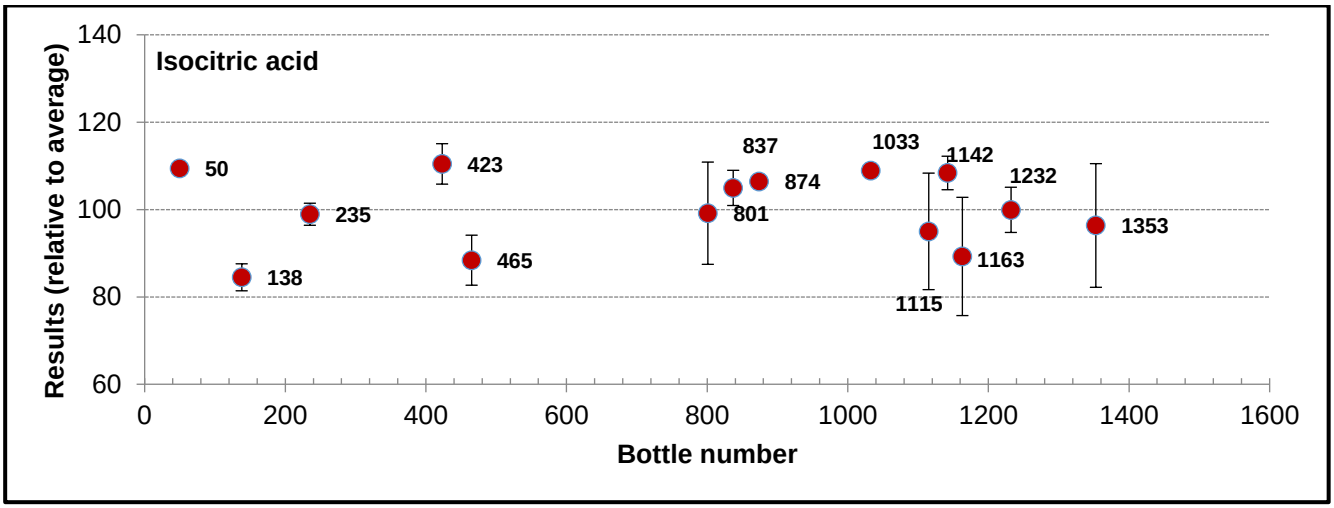

Figure A16. Homogeneity plot for Isocitric acid

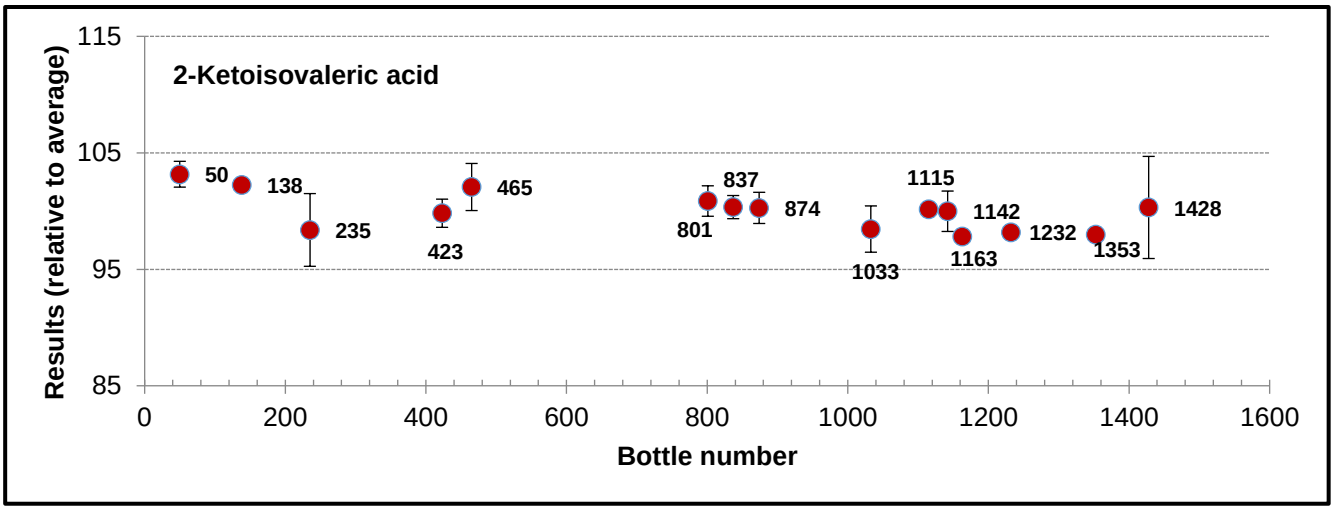

Figure A17. Homogeneity plot for 2-Ketoisovaleric acid

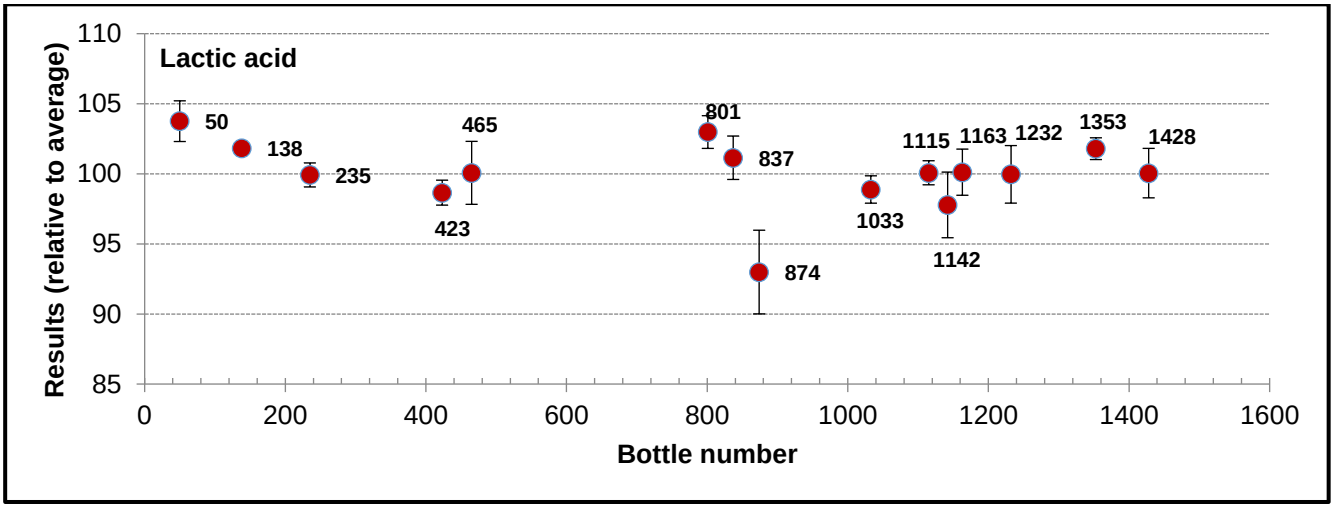

Figure A18. Homogeneity plot for Lactic acid

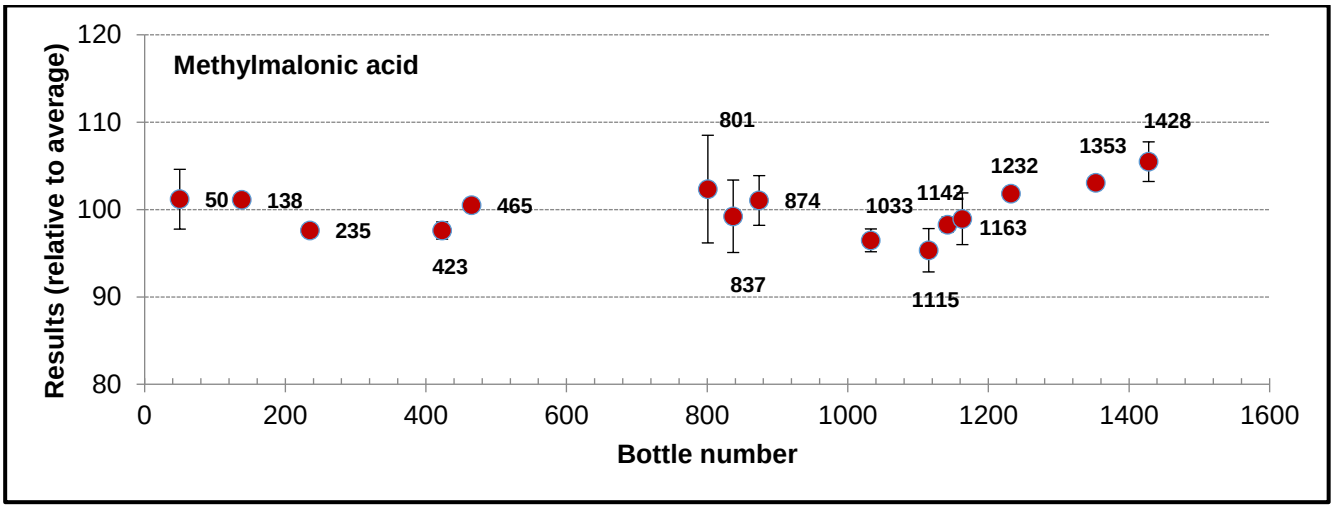

Figure A19. Homogeneity plot for Methylmalonic acid

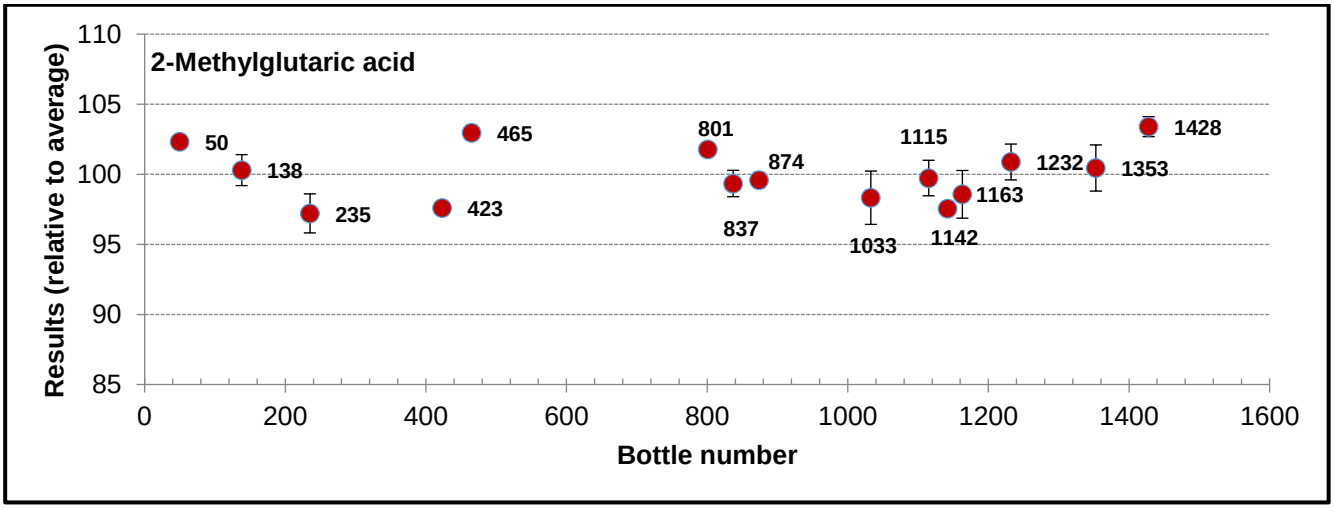

Figure A20. Homogeneity plot for 2-Methylglutaric acid

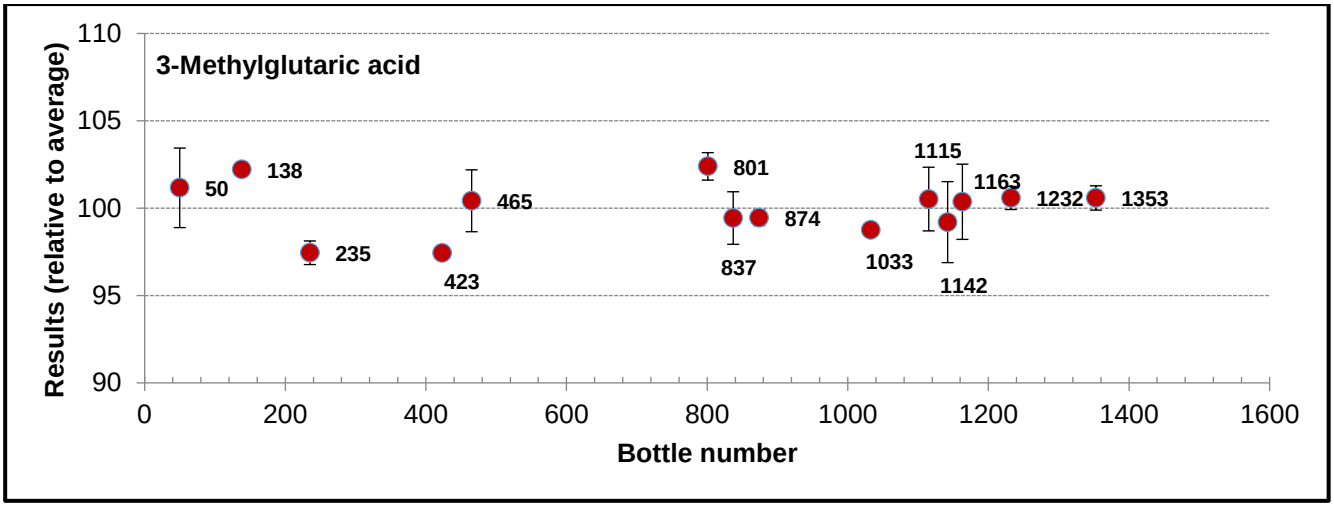

Figure A21. Homogeneity plot for 3-Methylglutaric acid

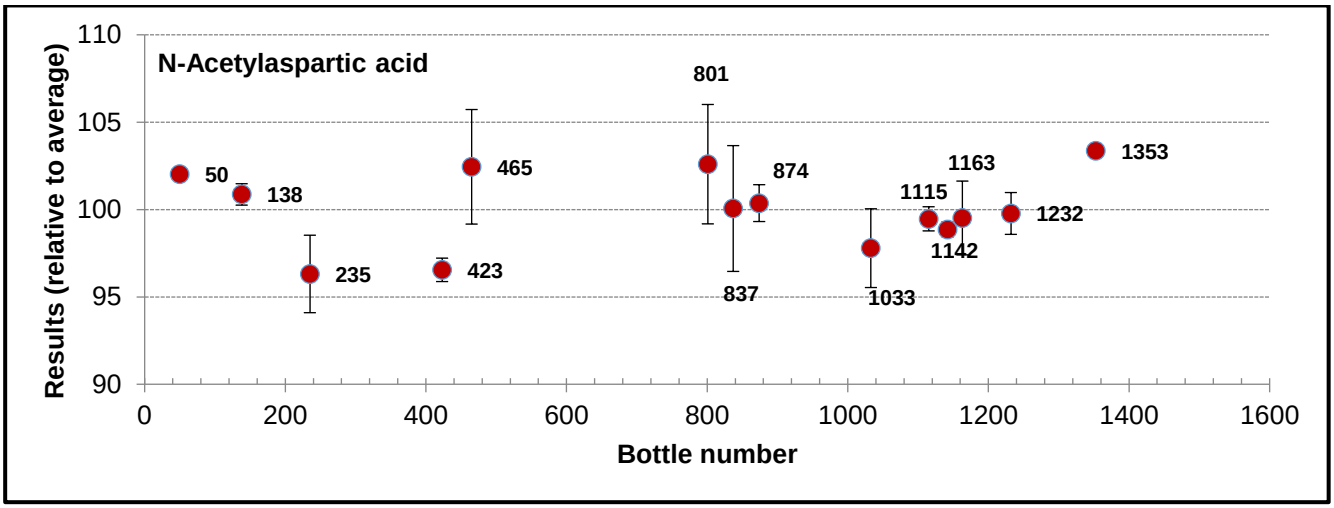

Figure A22. Homogeneity plot for N-acetylaspartic acid

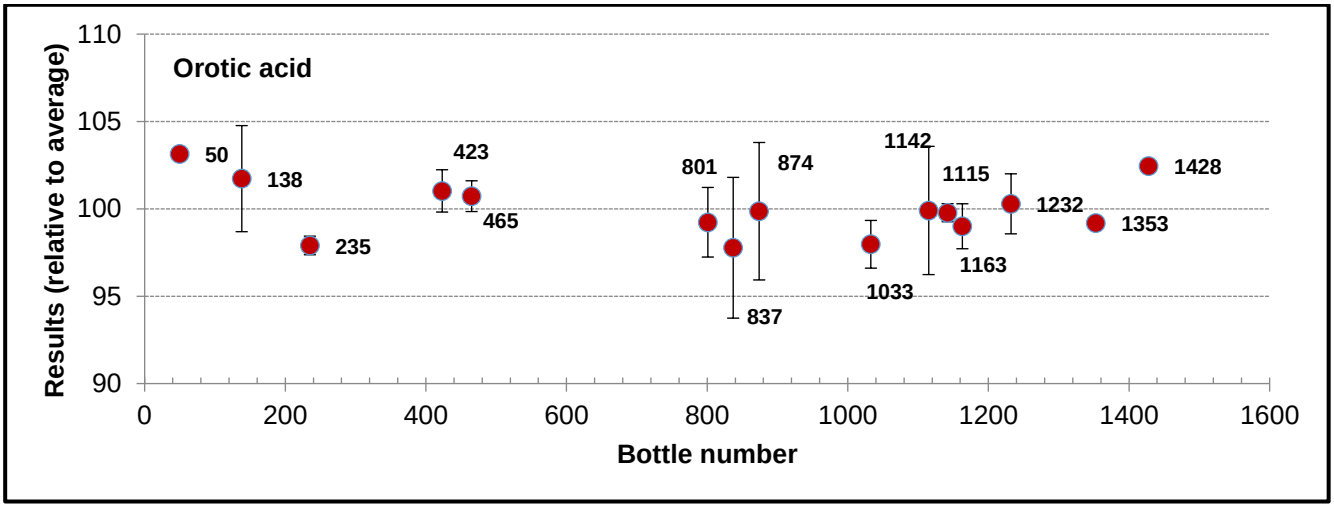

Figure A23. Homogeneity plot for Orotic acid

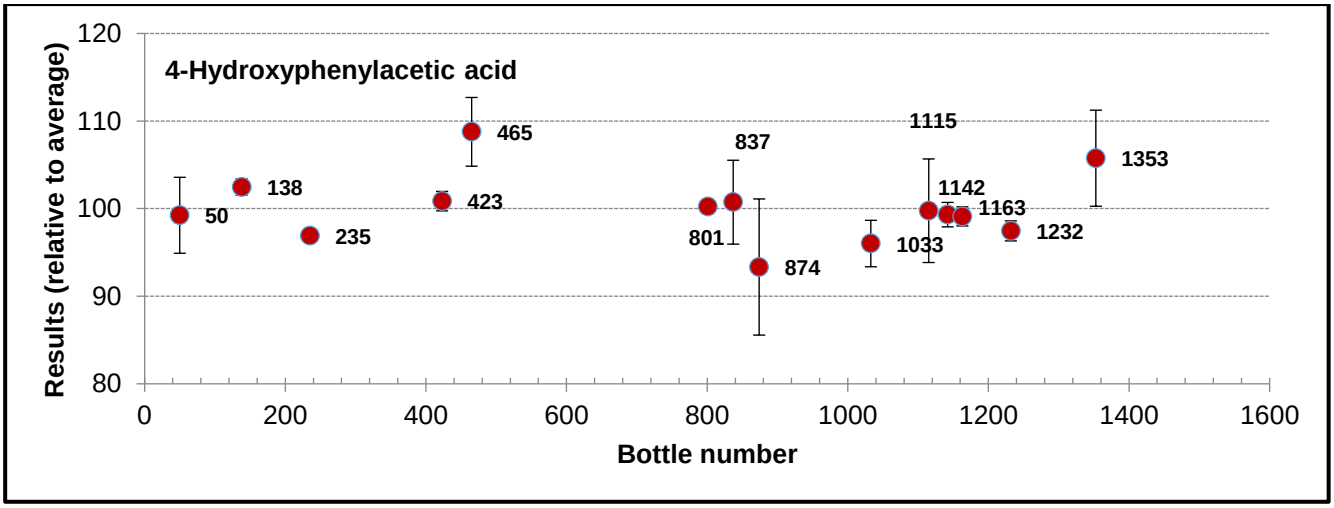

Figure A24. Homogeneity plot for 4-Hydroxyphenylacetic acid

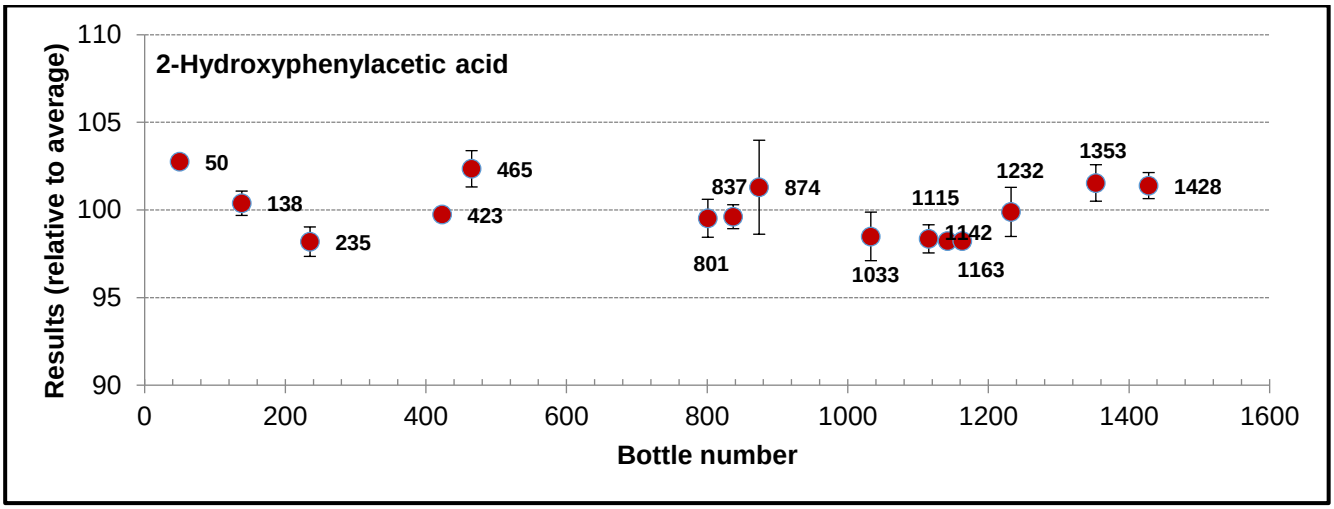

Figure A25. Homogeneity plot for 2-Hydroxyphenylacetic acid

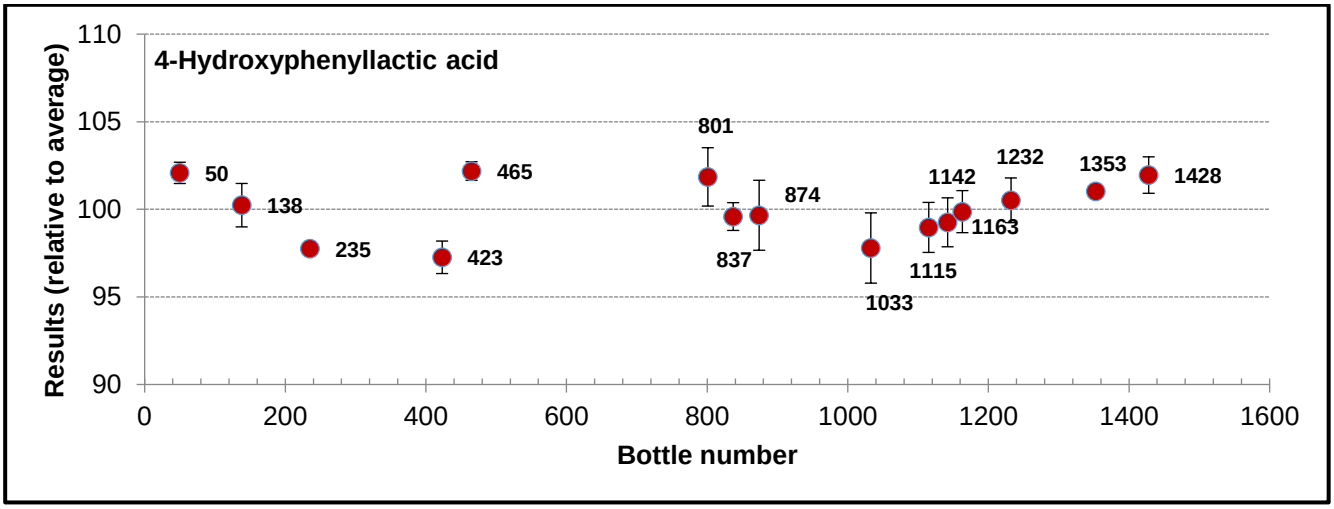

Figure A26. Homogeneity plot for 4-Hydroxyphenylactic acid

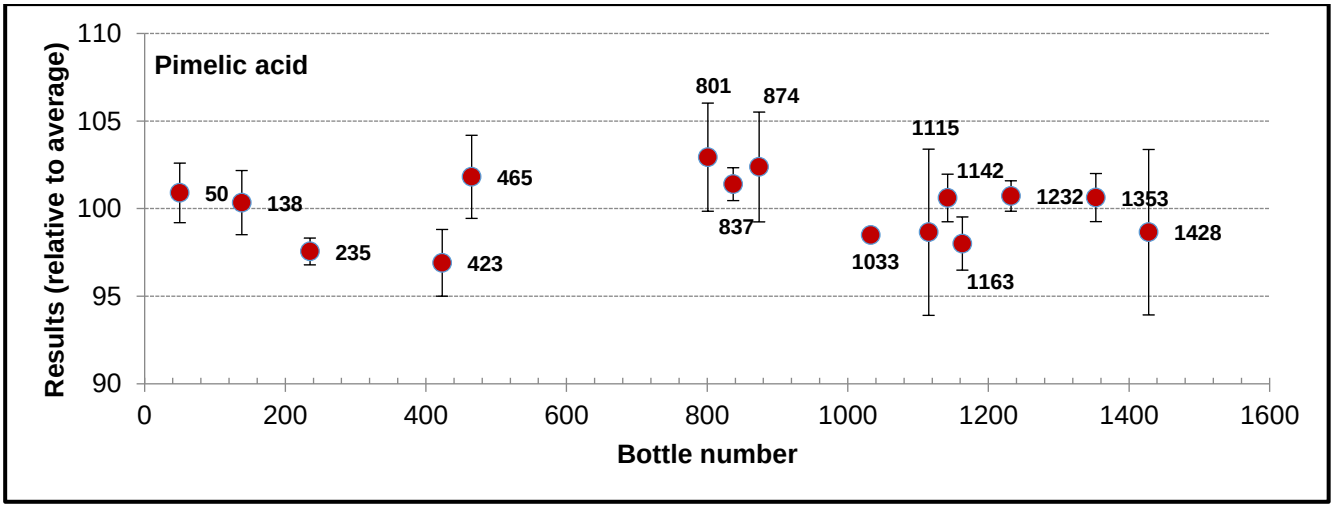

Figure A27. Homogeneity plot for Pimelic acid

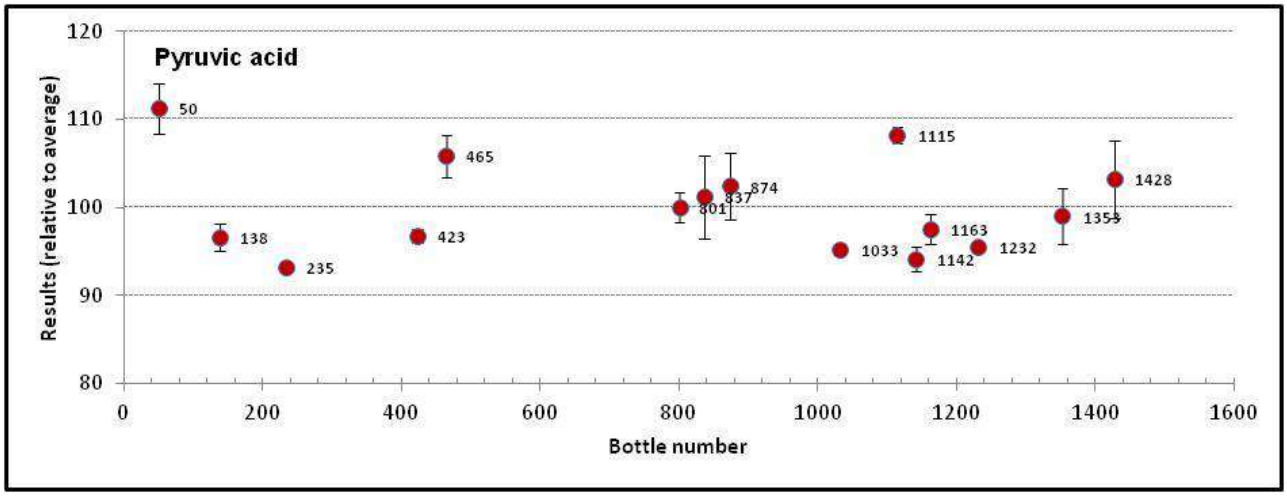

Figure A28. Homogeneity plot for Pyruvic acid

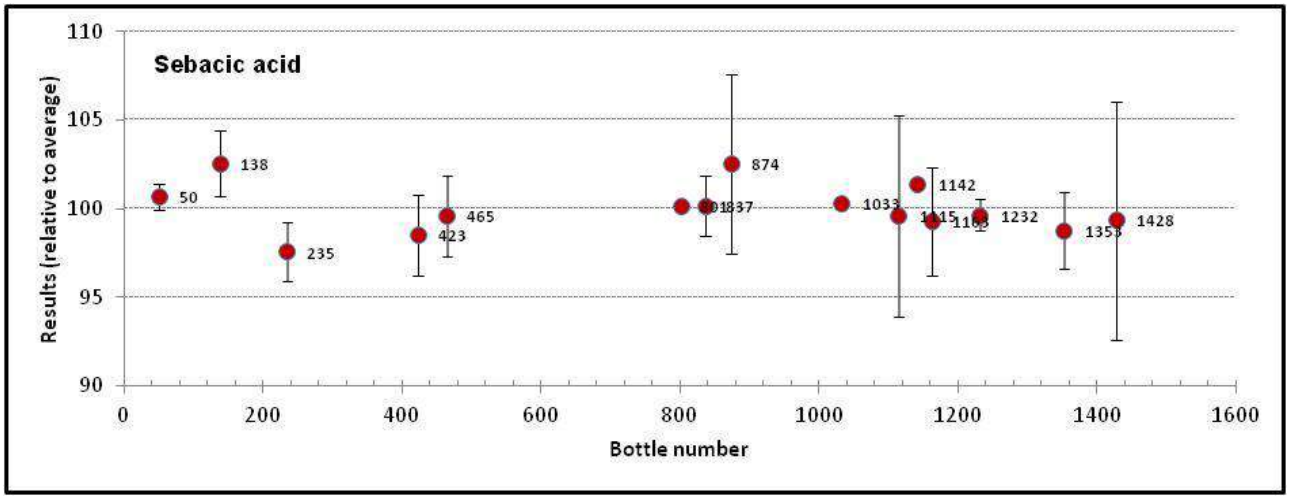

Figure A29. Homogeneity plot for Sebacic acid

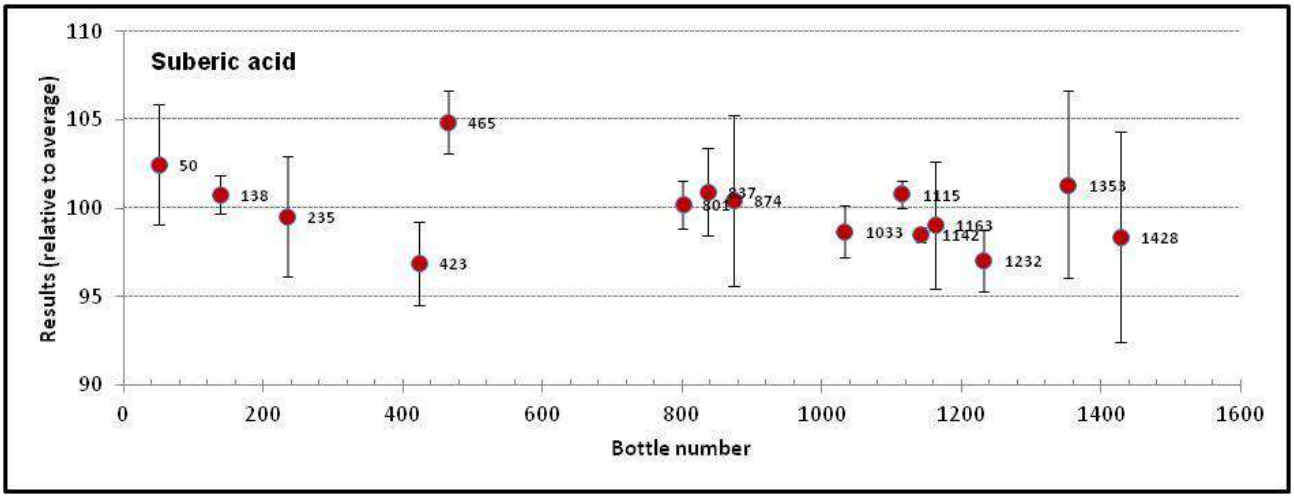

Figure A30. Homogeneity plot for Suberic acid

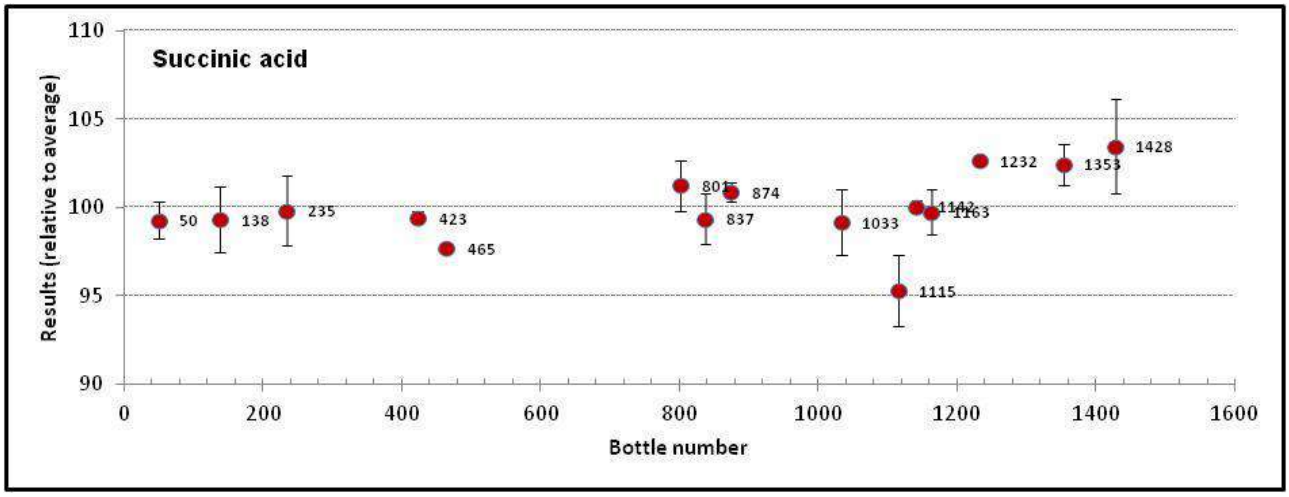

Figure A31. Homogeneity plot for Succinic acid

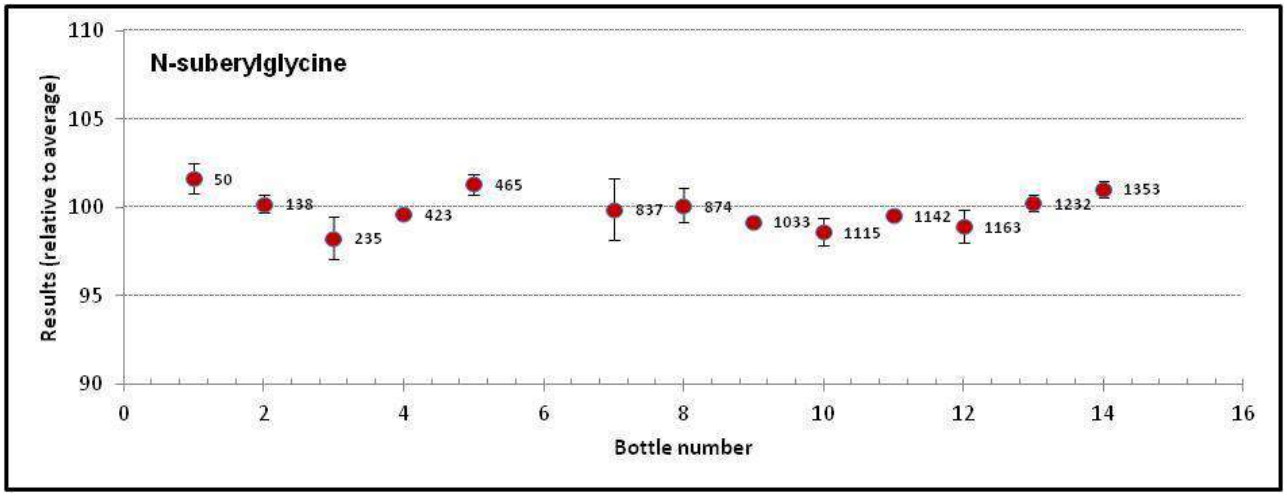

Figure A32. Homogeneity plot for N-Suberylglycine

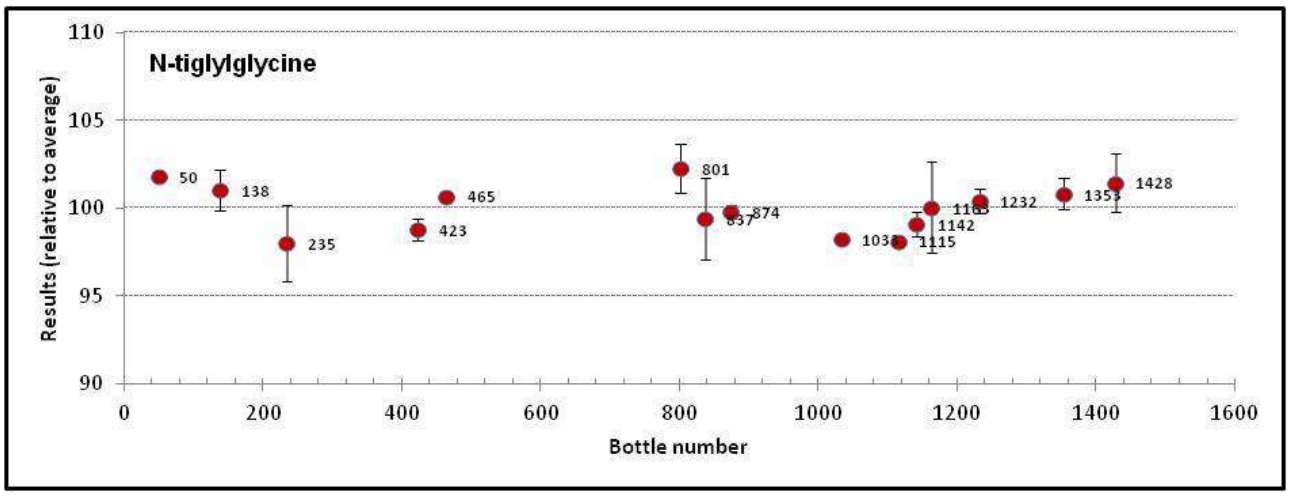

Figure A33. Homogeneity plot for N-Tiglylglycine

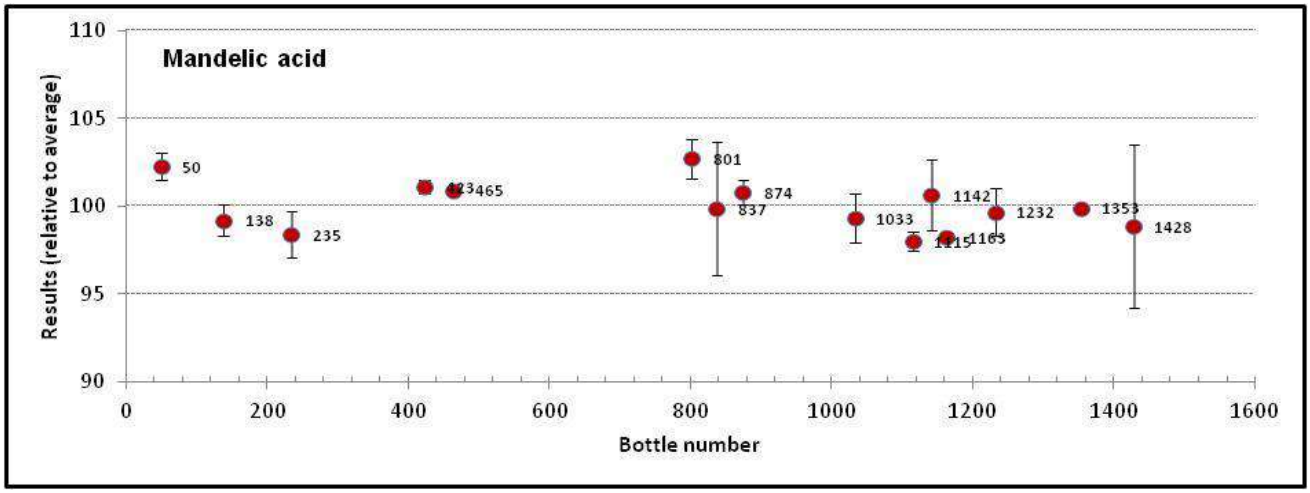

Figure A34. Homogeneity plot for Mandelic acid

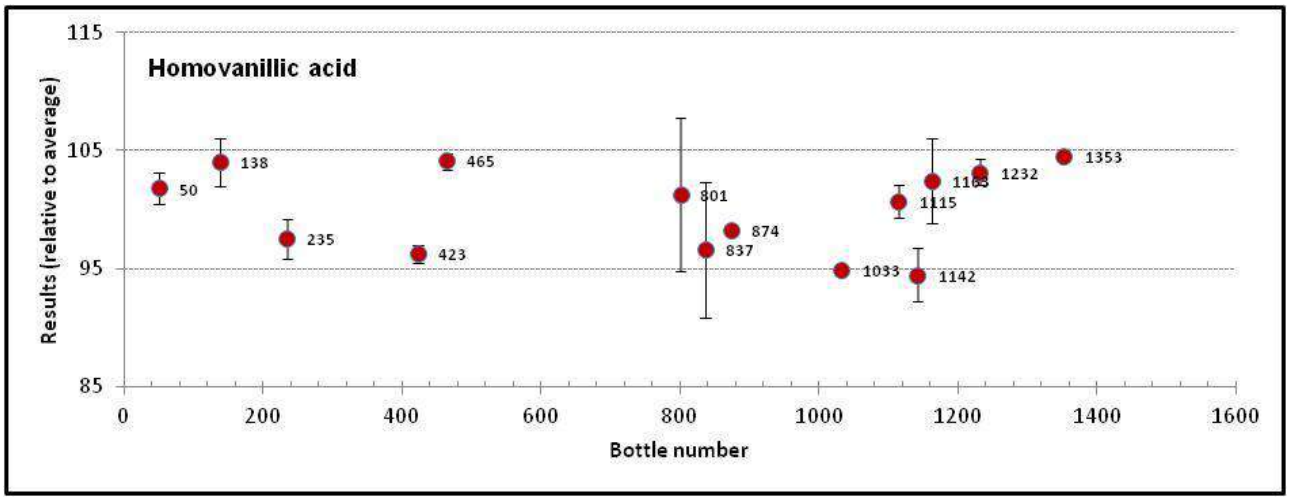

Figure A35. Homogeneity plot for Homovanillic acid

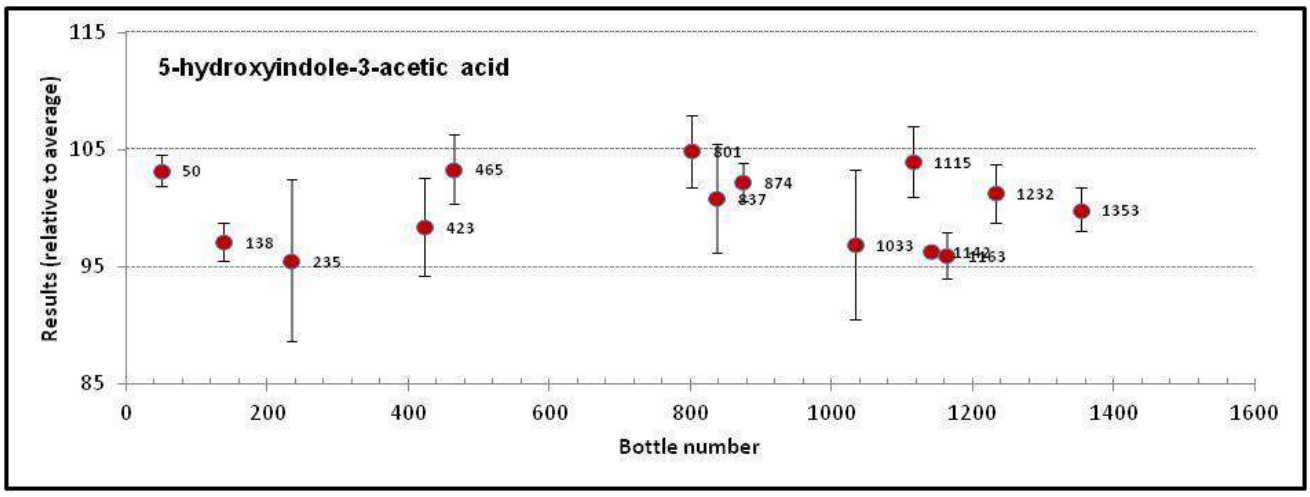

Figure A36. Homogeneity plot for 5-hydroxyindole-3-acetic acid

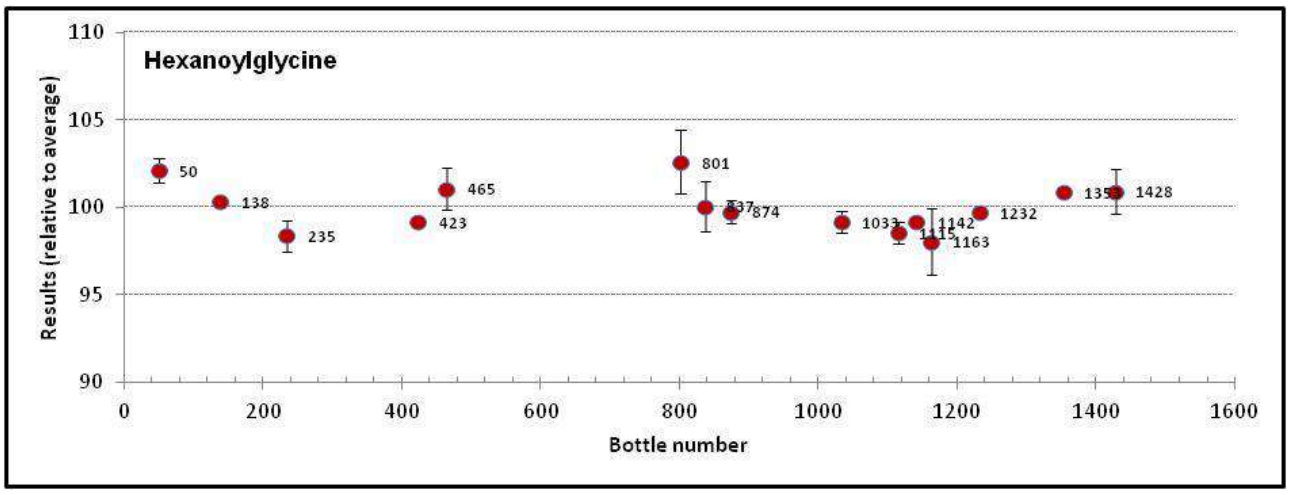

Figure A37. Homogeneity plot for Hexanoylglycine

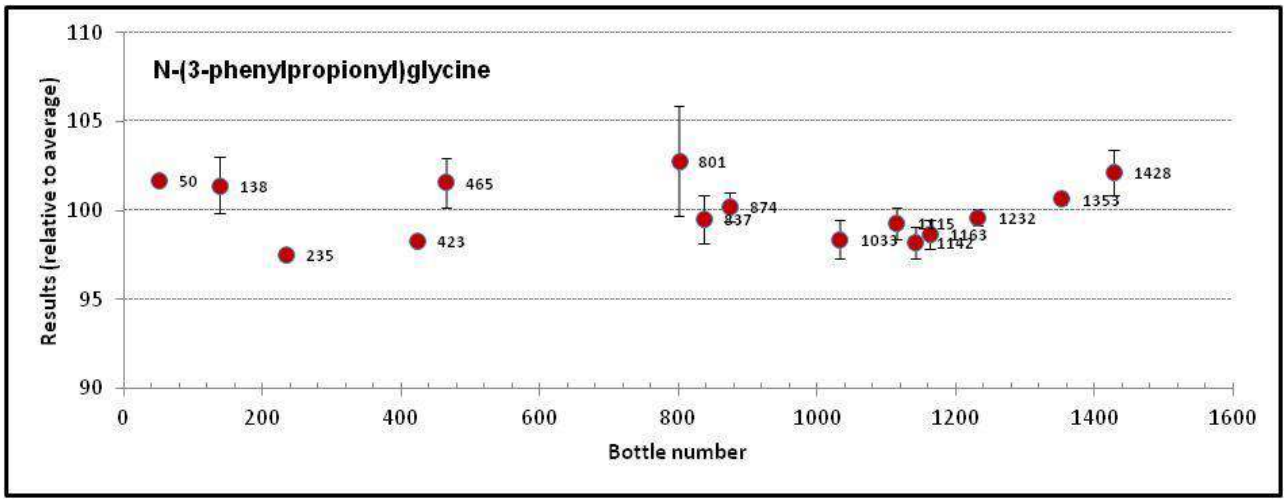

Figure A38. Homogeneity plot for N-(3-phenylpropionyl)glycine

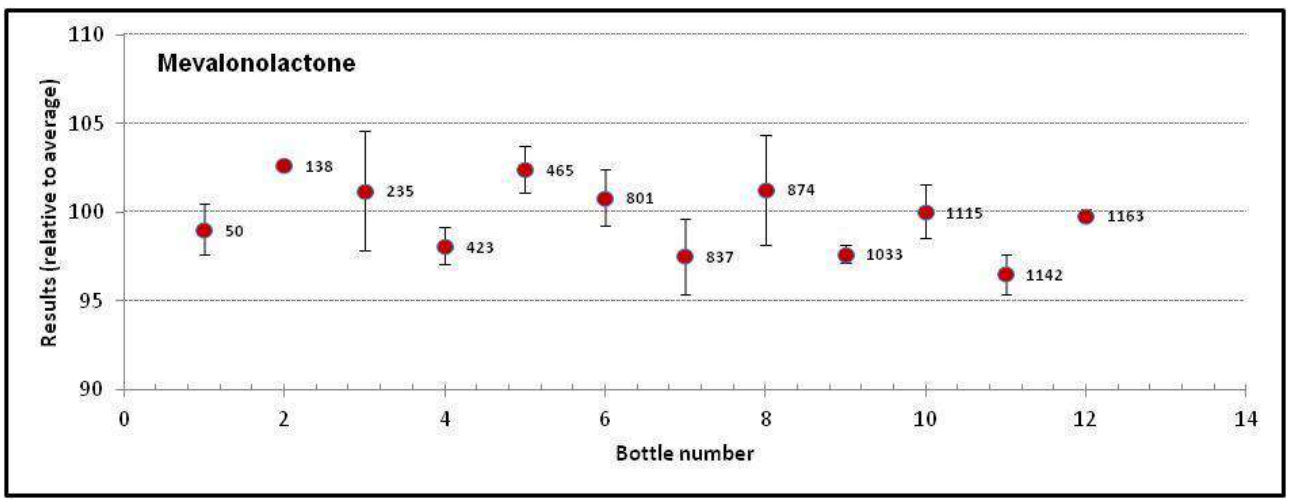

Figure A39. Homogeneity plot for Mevalonolactone

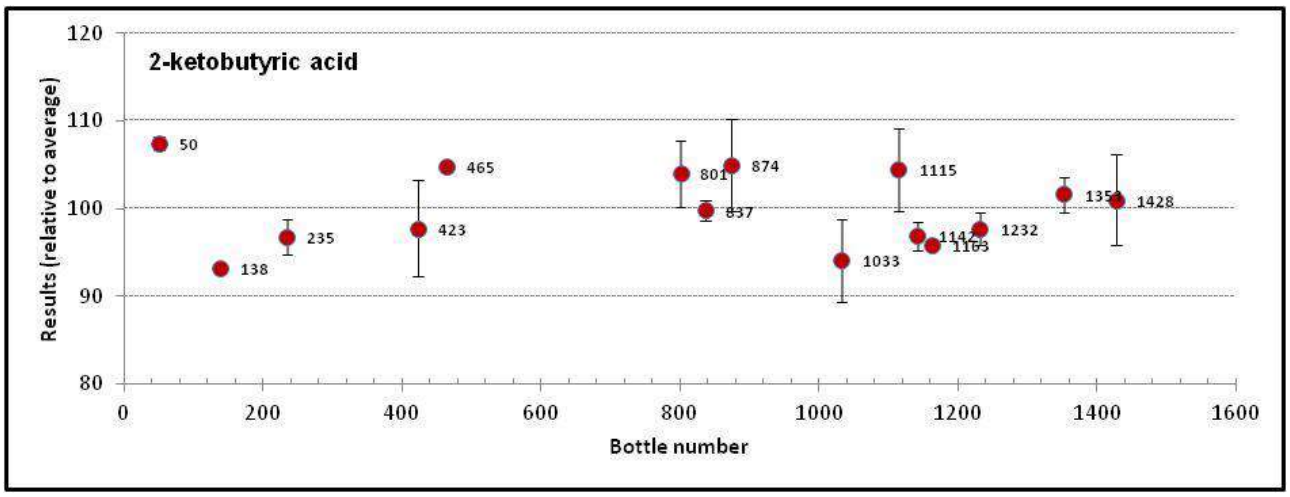

Figure A40. Homogeneity plot for 2-ketobutyric acid

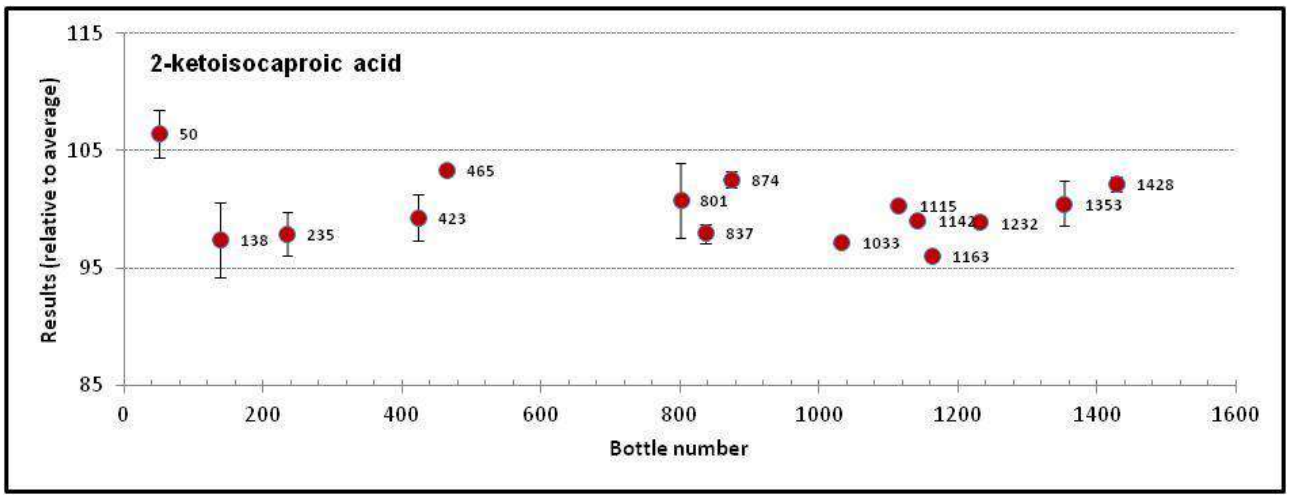

Figure A41. Homogeneity plot for 2-ketoisocaproic acid

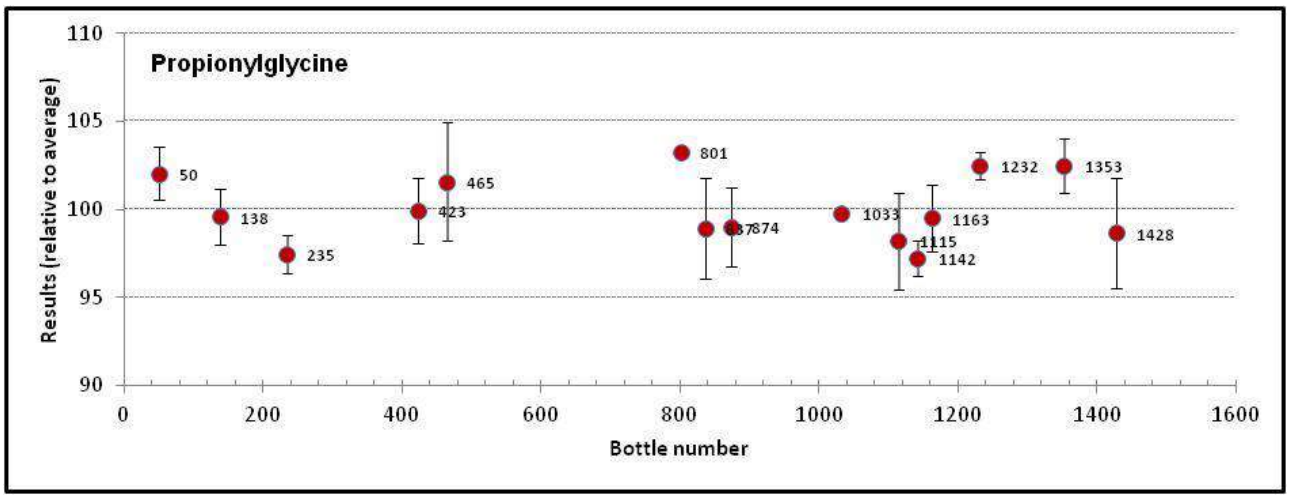

Figure A42. Homogeneity plot for Propionylglycine

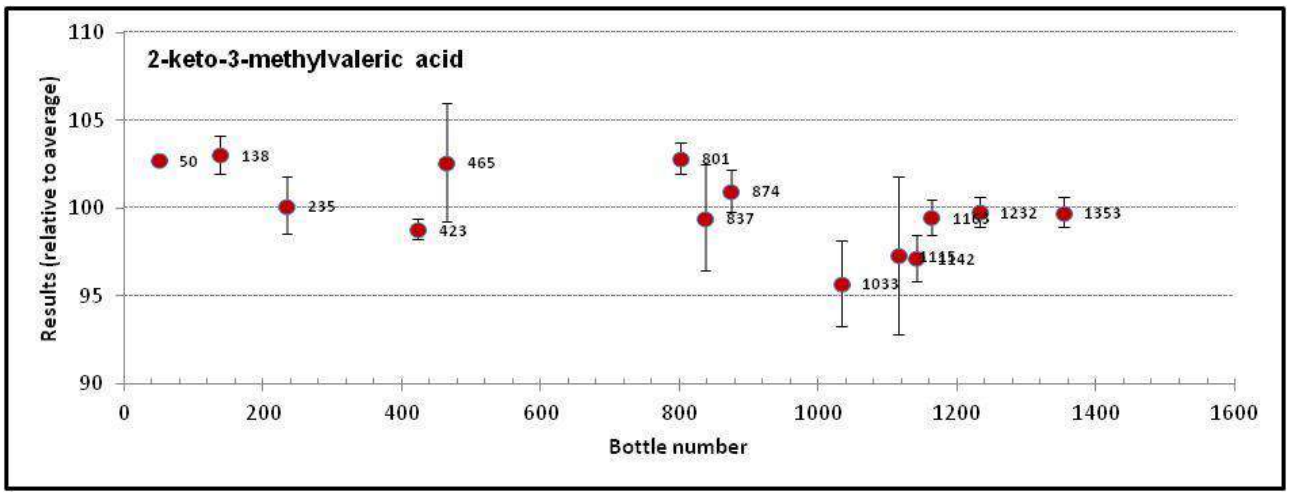

Figure A43. Homogeneity plot for 2-keto-3-methylvaleric acid

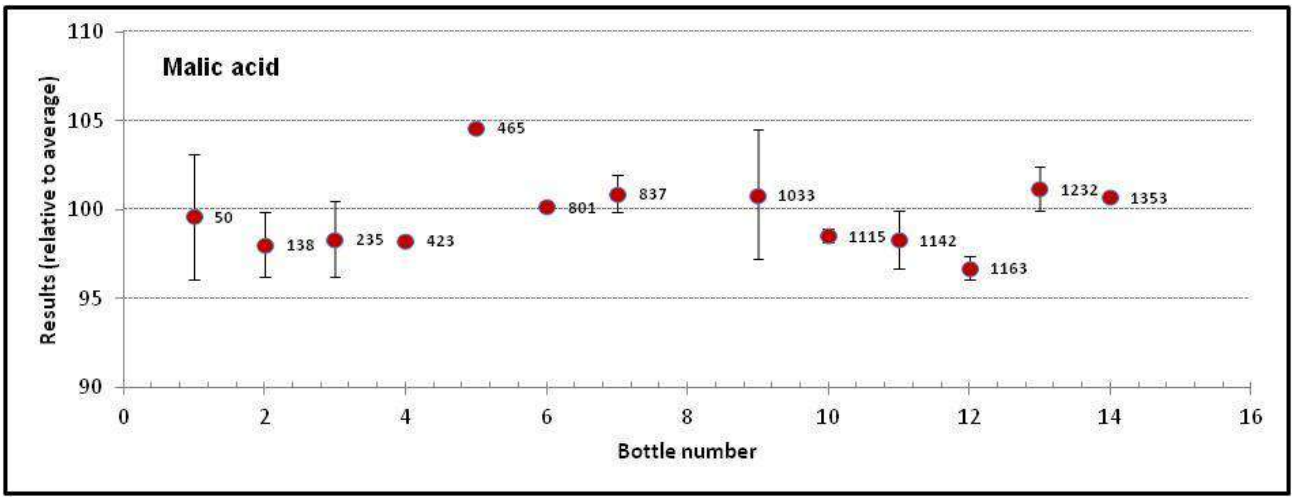

Figure A44. Homogeneity plot for Malic acid

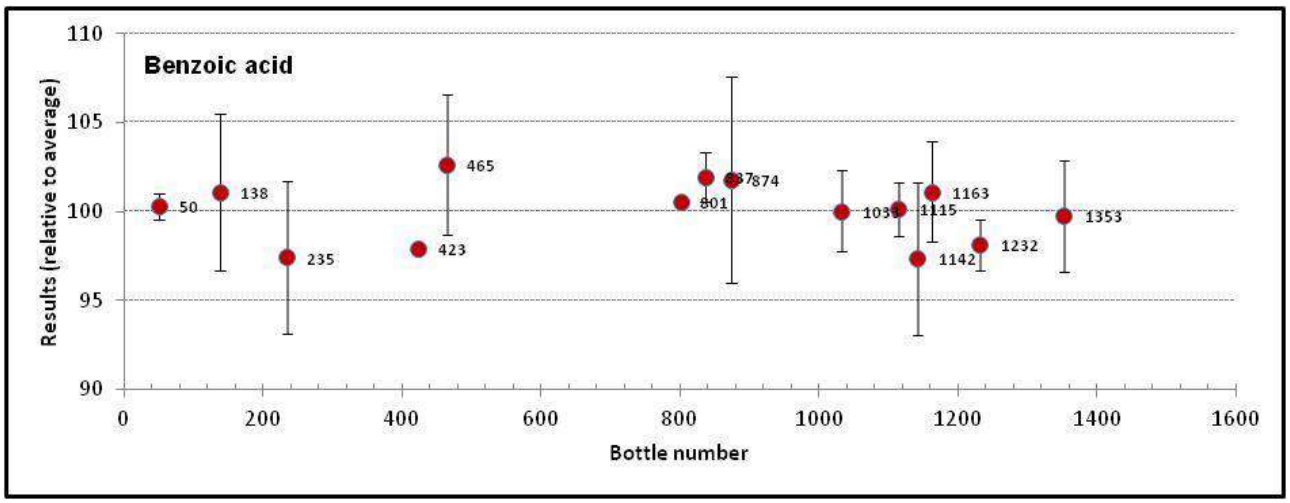

Figure A45. Homogeneity plot for Benzoic acid

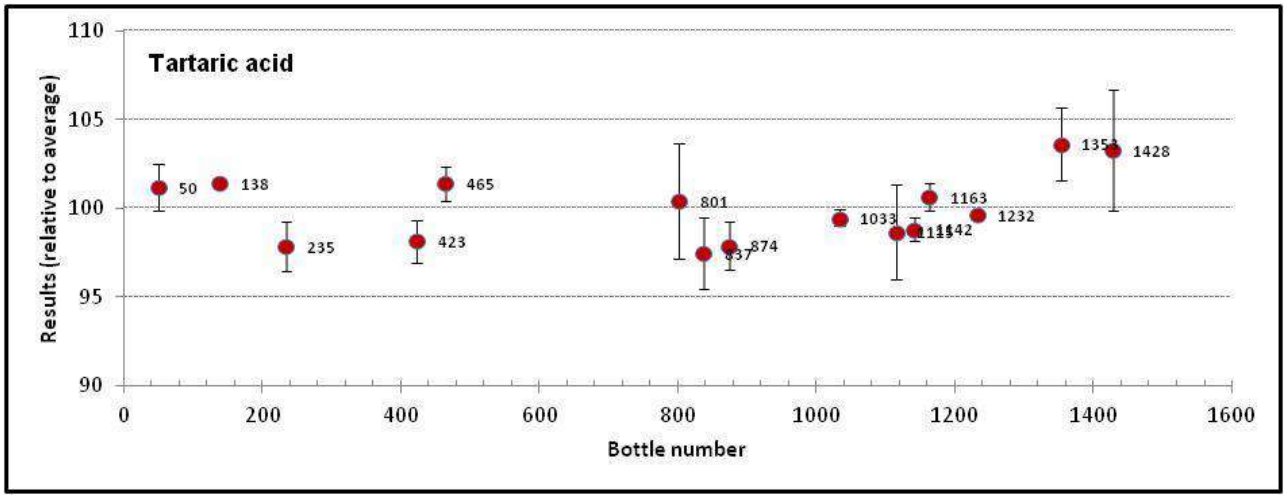

Figure A46. Homogeneity plot for Tartaric acid

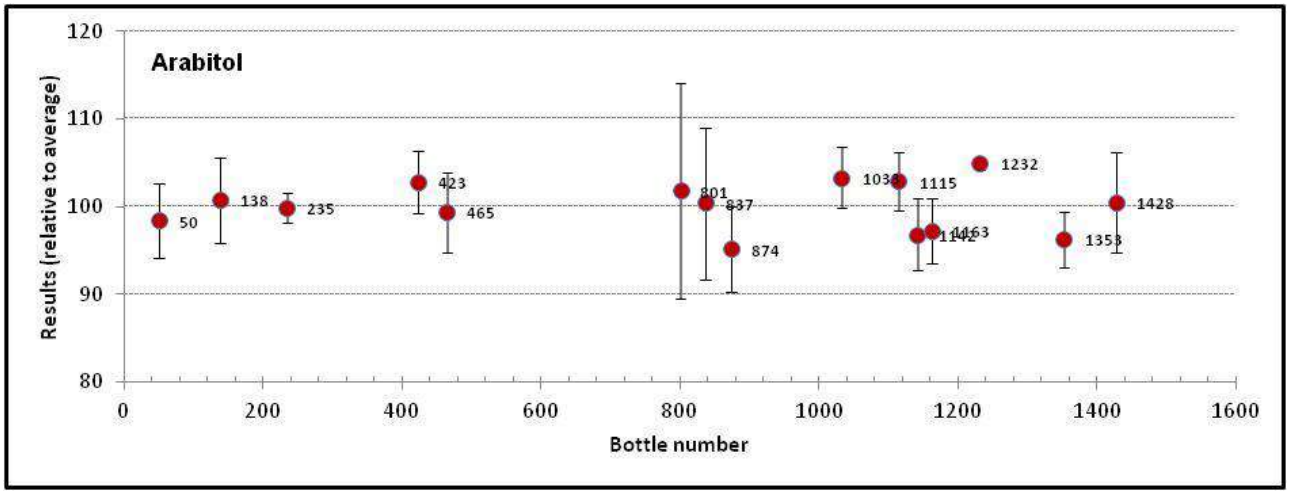

Figure A47. Homogeneity plot for Arabitol

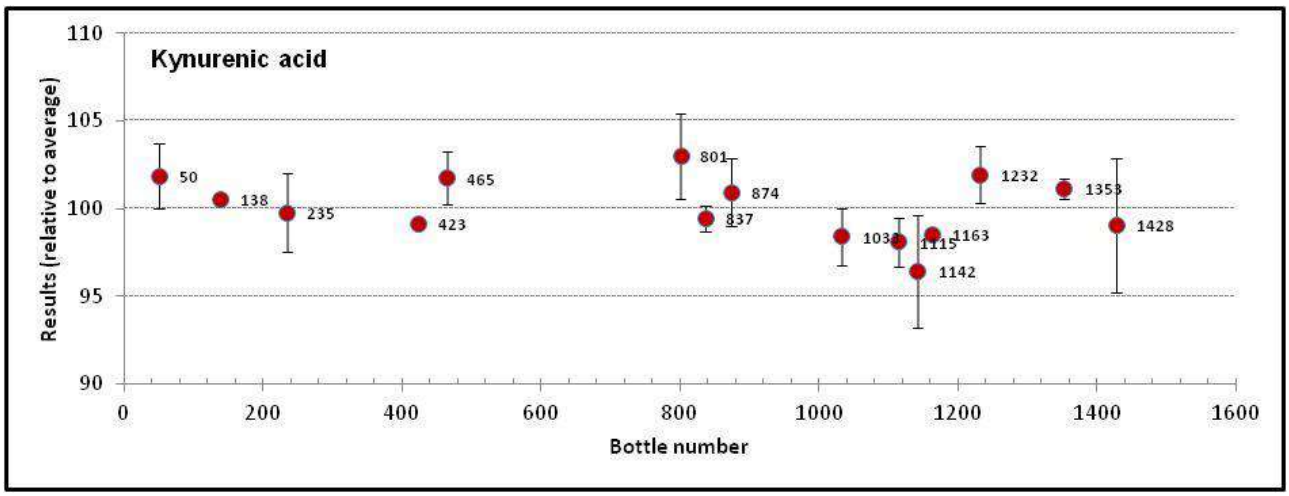

Figure A48. Homogeneity plot for Kynurenic acid

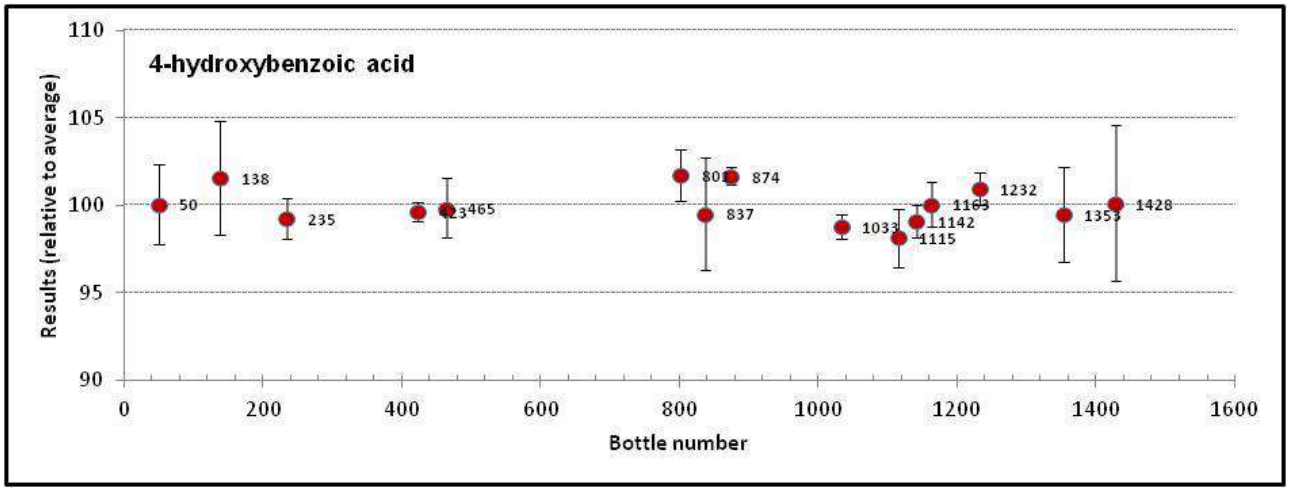

Figure A49. Homogeneity plot for 4-hydroxybenzoic acid

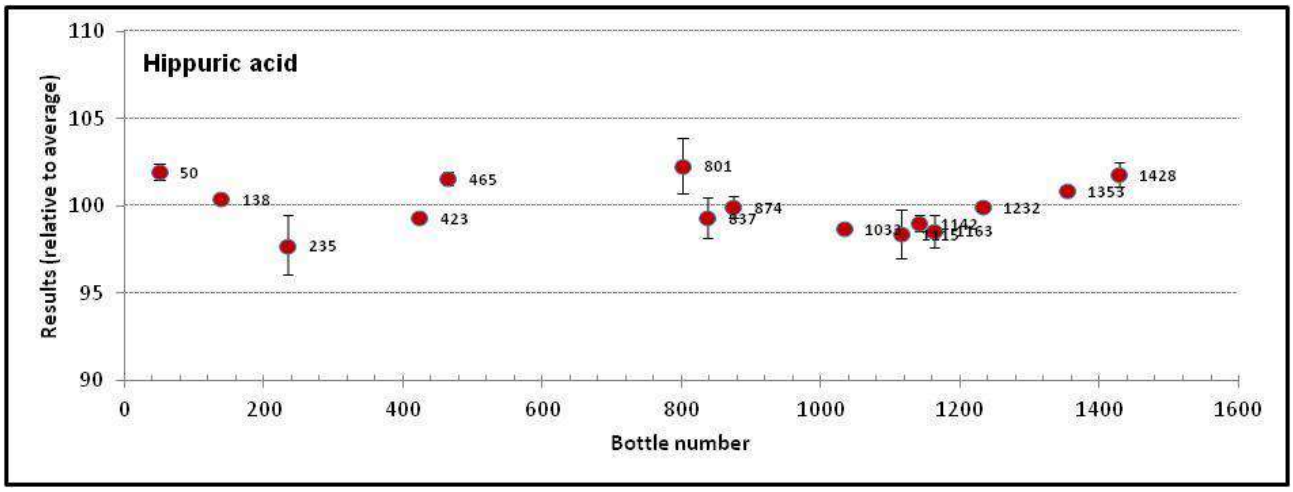

Figure A50. Homogeneity plot for Hippuric acid

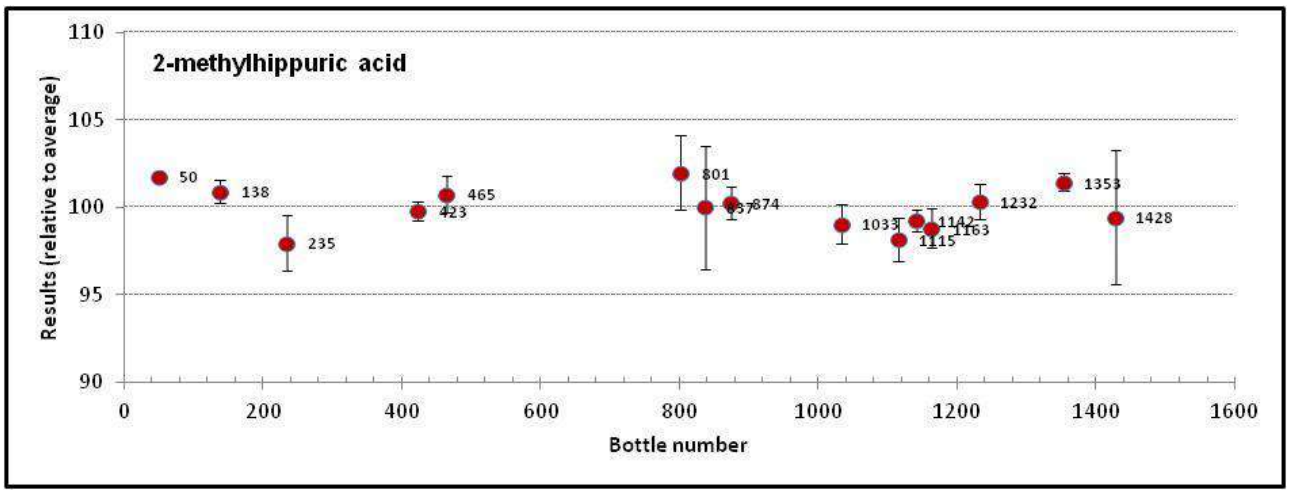

Figure A51. Homogeneity plot for 2-methylhippuric acid

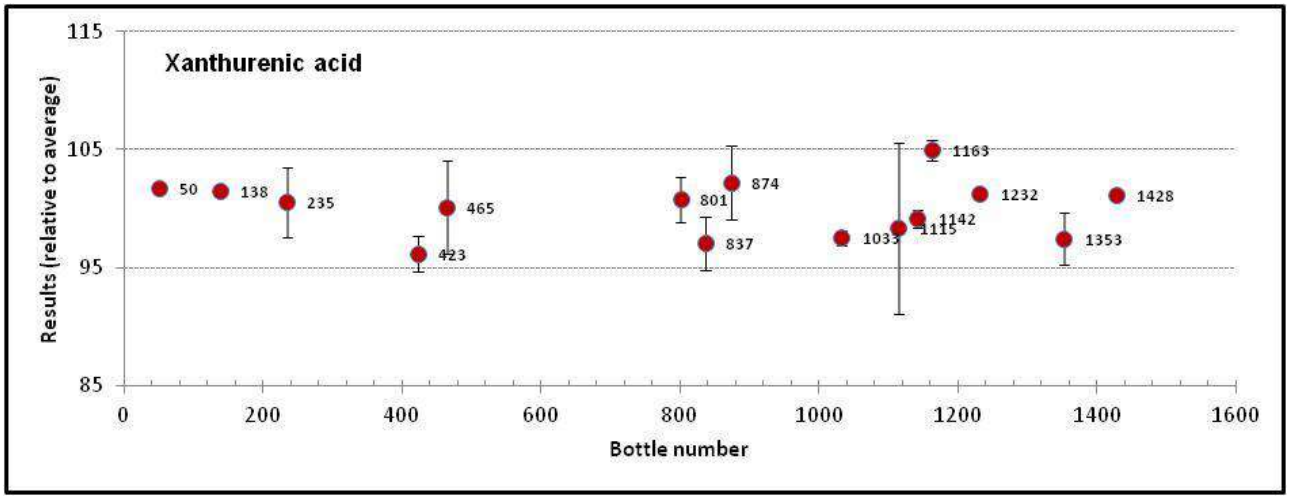

Figure A52. Homogeneity plot for Xanthurenic acid

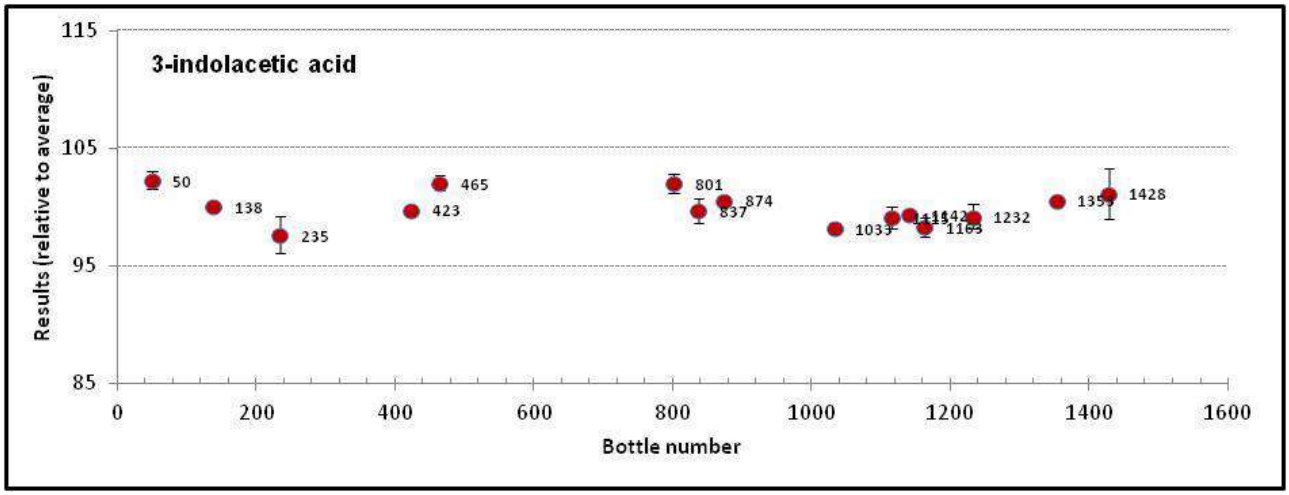

Figure A53. Homogeneity plot for 3-indolacetic acid

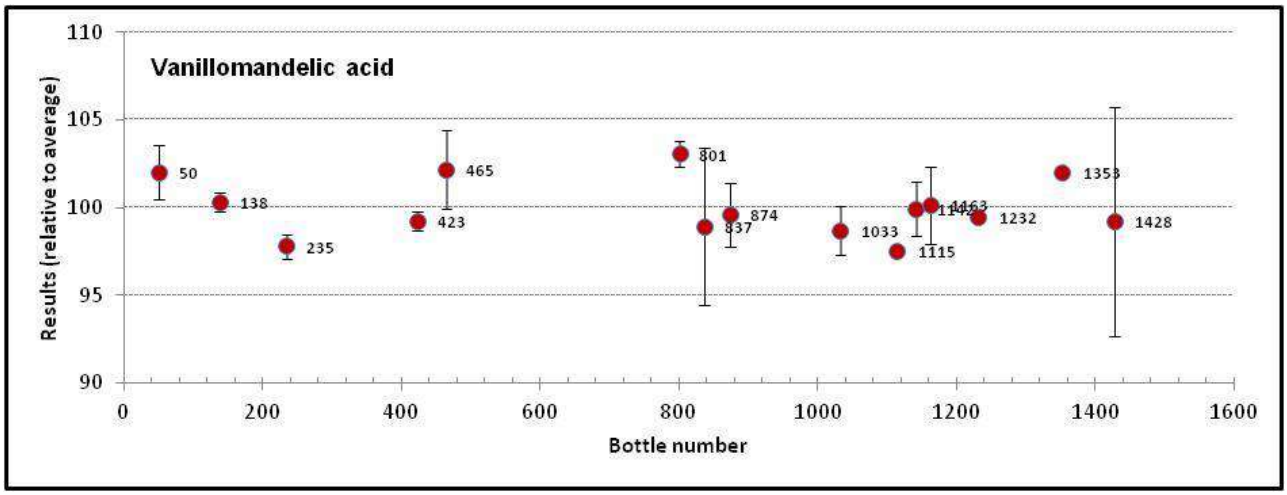

Figure A54. Homogeneity plot for Vanillomandelic acid

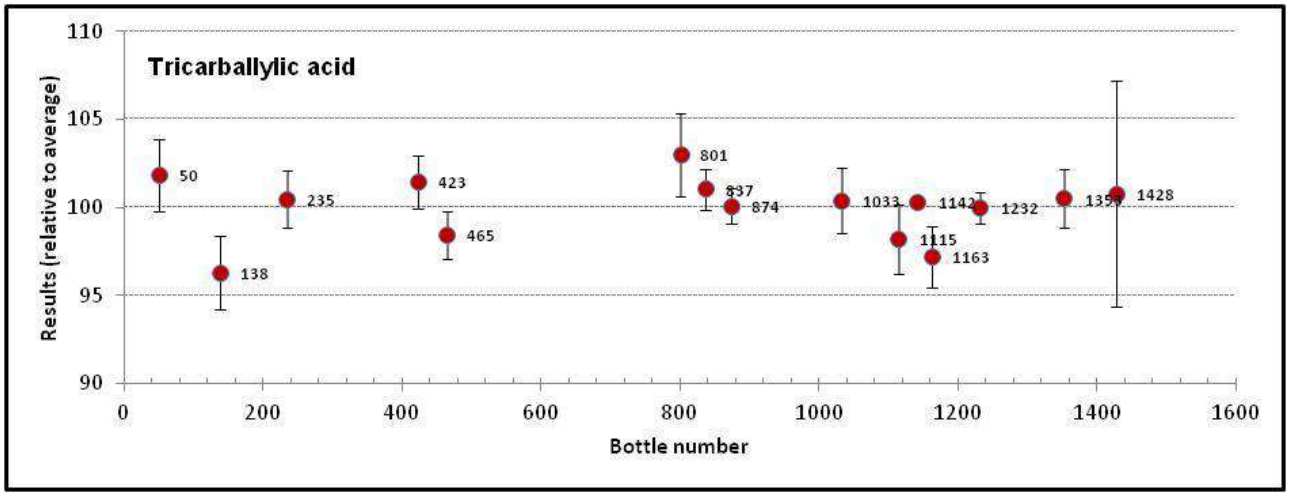

Figure A55. Homogeneity plot for Tricarballylic acid

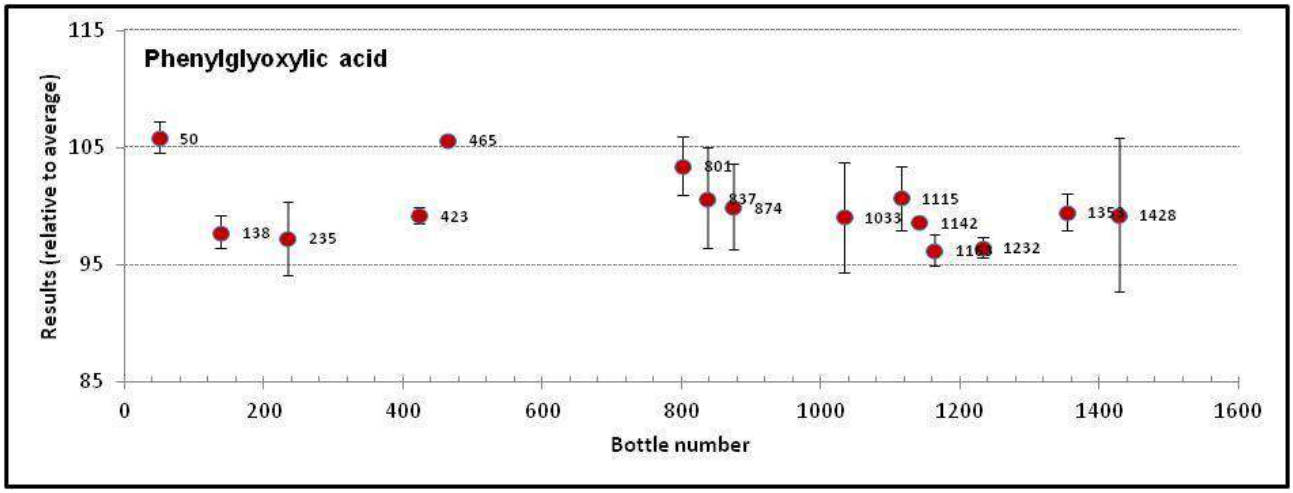

Figure A56. Homogeneity plot for Phenylglyoxylic acid

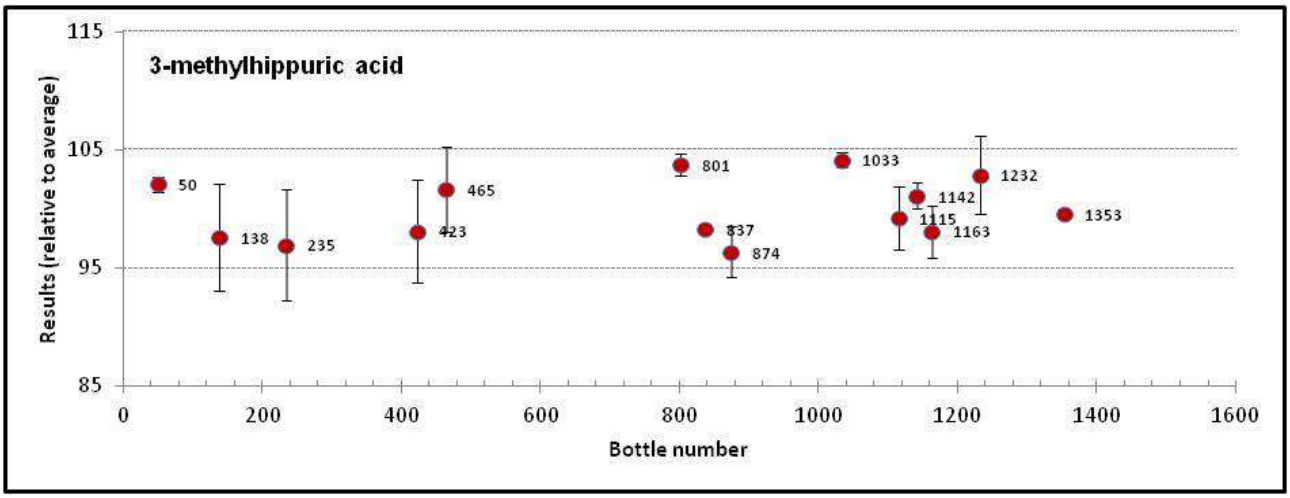

Figure A57. Homogeneity plot for 3-methylhippuric acid

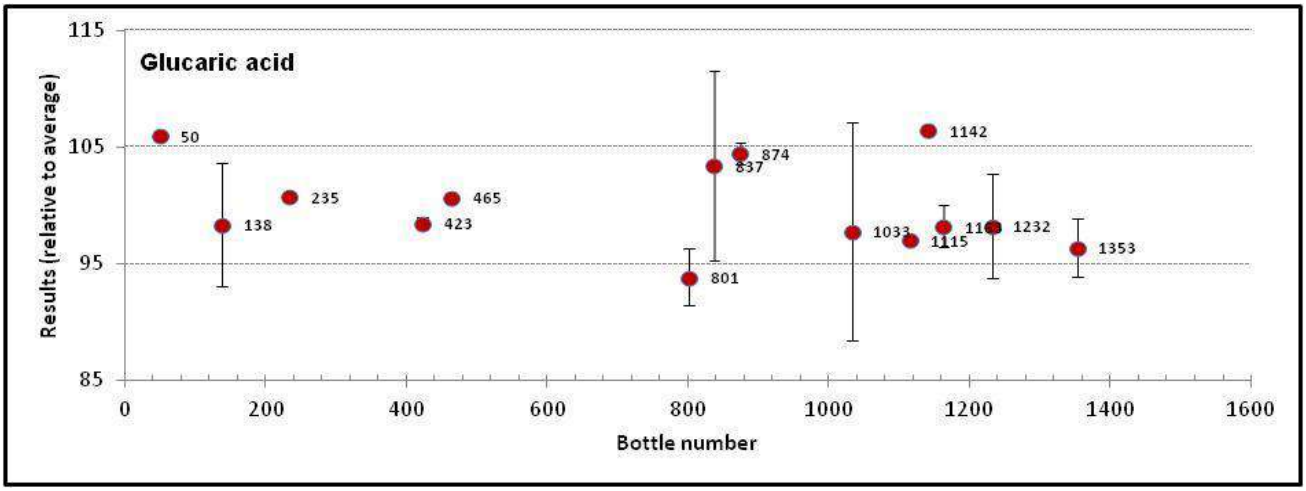

Figure A58. Homogeneity plot for Glucaric acid

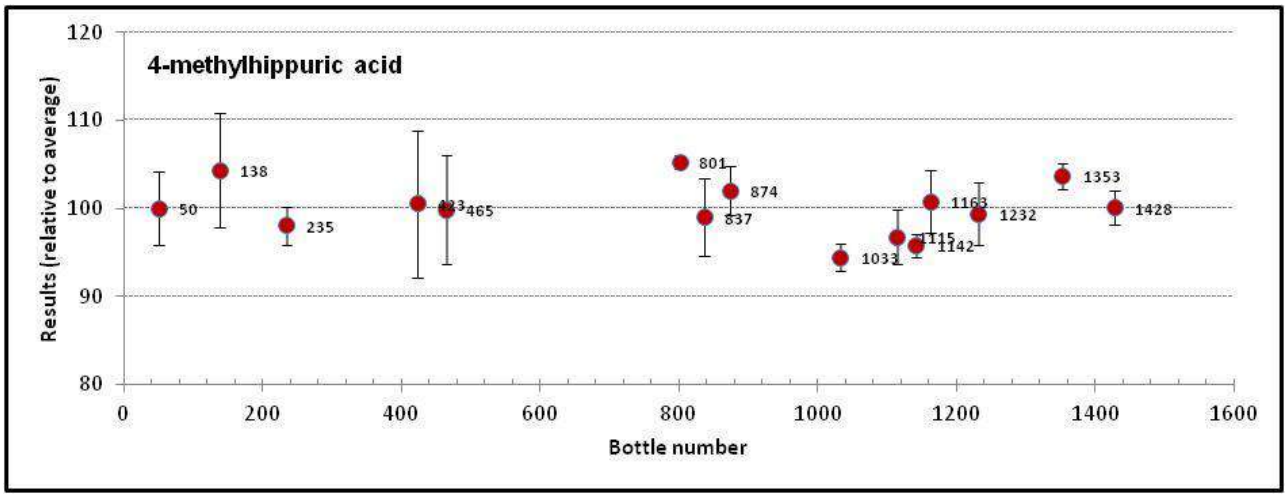

Figure A59. Homogeneity plot for 4-methylhippuric acid

Annex 3. Graphs for Short Term Stability Studies

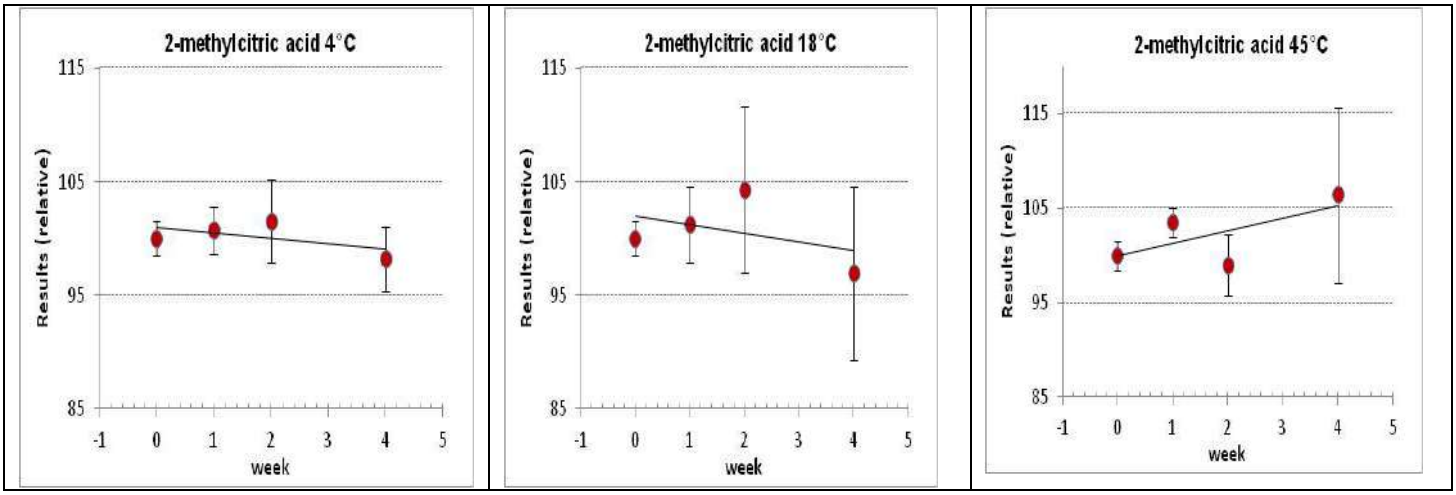

Figure A60. Short Term Stability Plot for 2-methylcitric acid at 4°C, 18 °C and 45°C

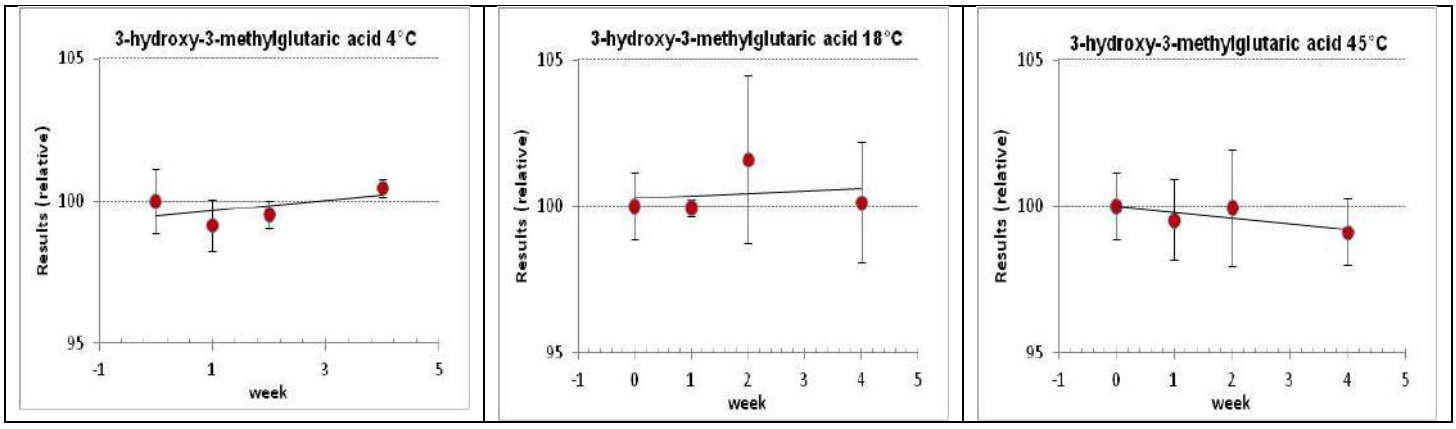

Figure A61. Short Term Stability Plot for 3-hydroxy3-methylglutaric acid at 4°C, 18 °C and 45°C

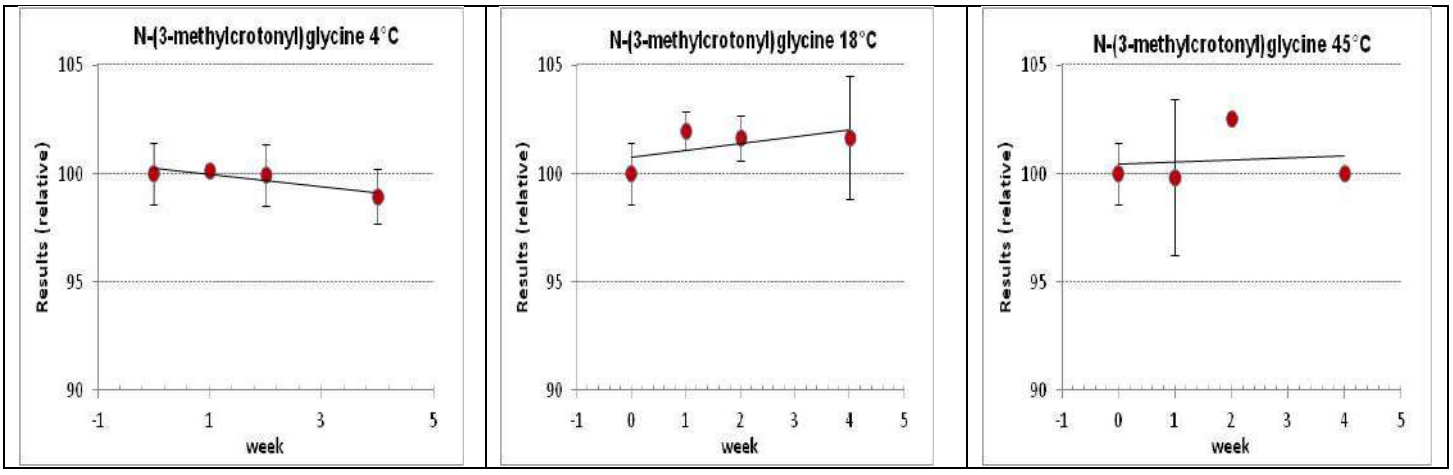

Figure A62. Short Term Stability Plot for N-(3-methylcrotonyl)glycine acid at 4°C, 18 °C and 45°C

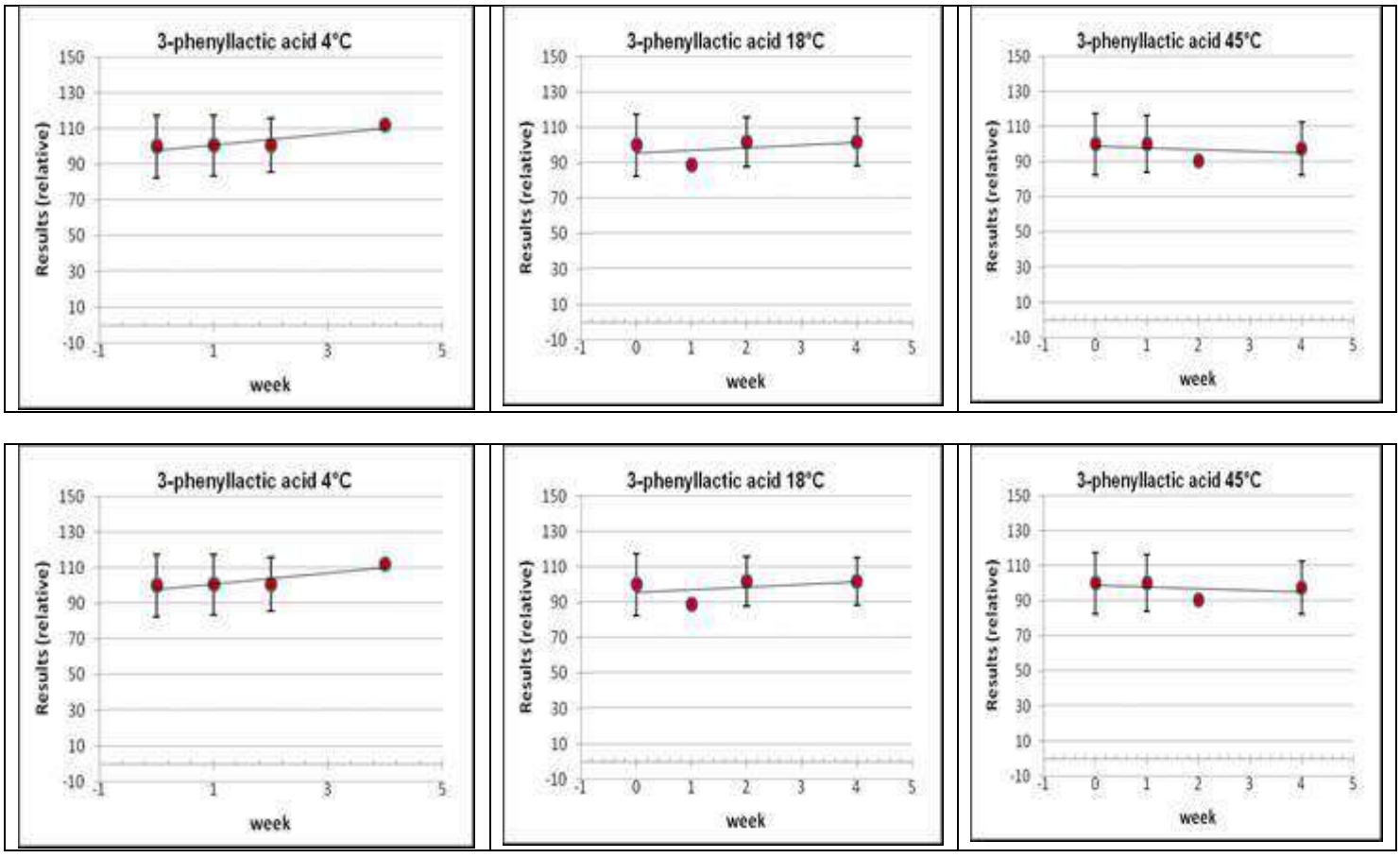

Figure A63. Short Term Stability Plot for 3-phenyllactic acid at 4°C, 18 °C and 45°C

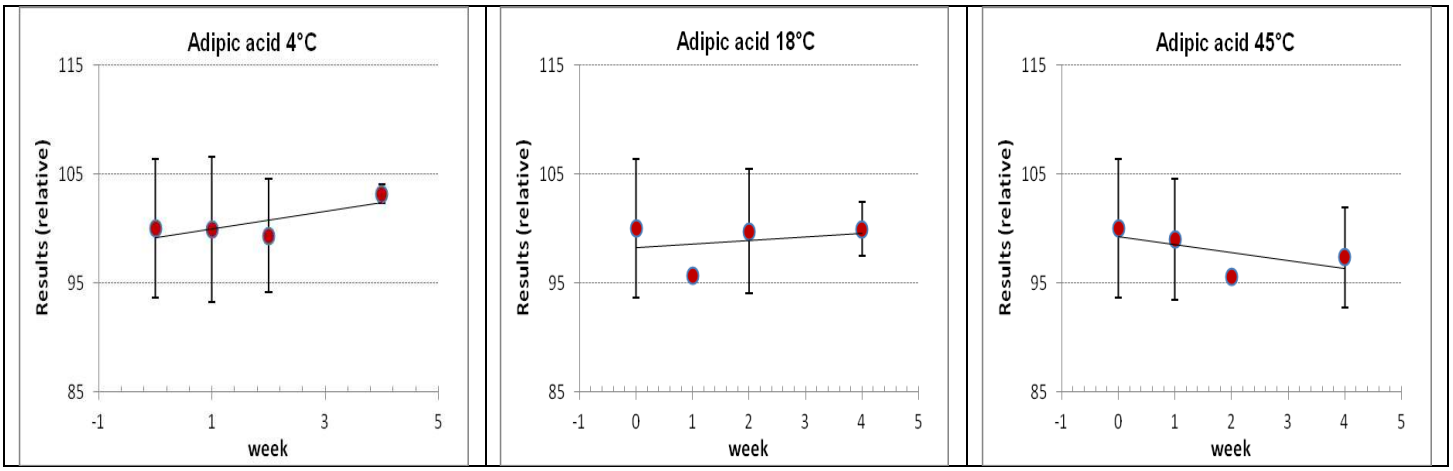

Figure A64. Short Term Stability Plot for Adipic acid at 4°C, 18 °C and 45°C

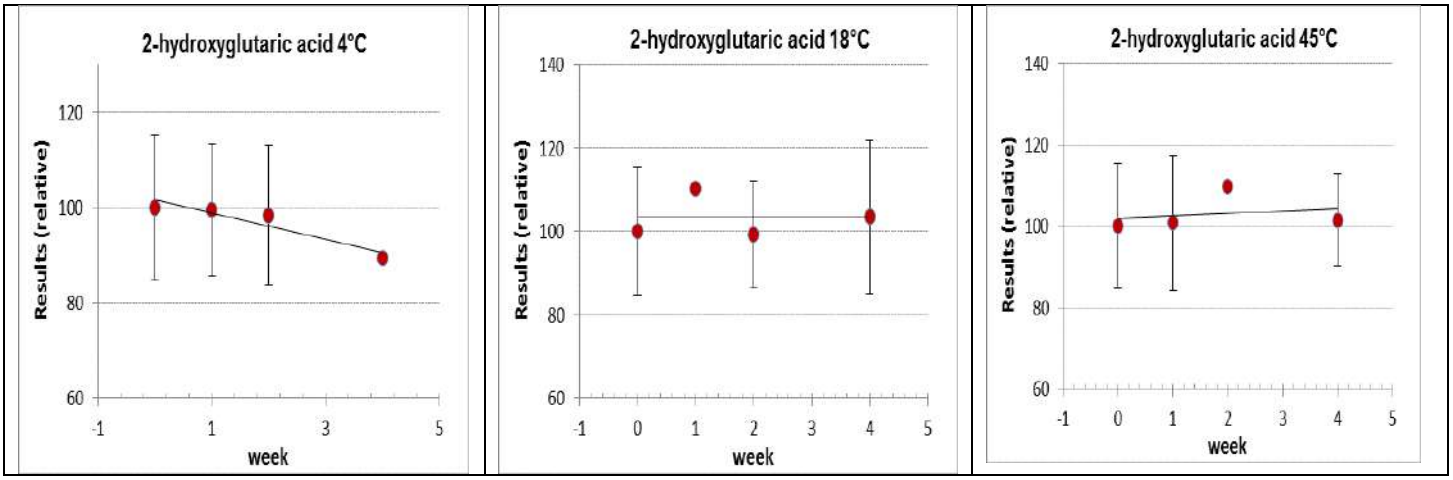

Figure 65. Short Term Stability Plot for 2-hydroxyglutaric acid at 4°C, 18 °C and 45°C

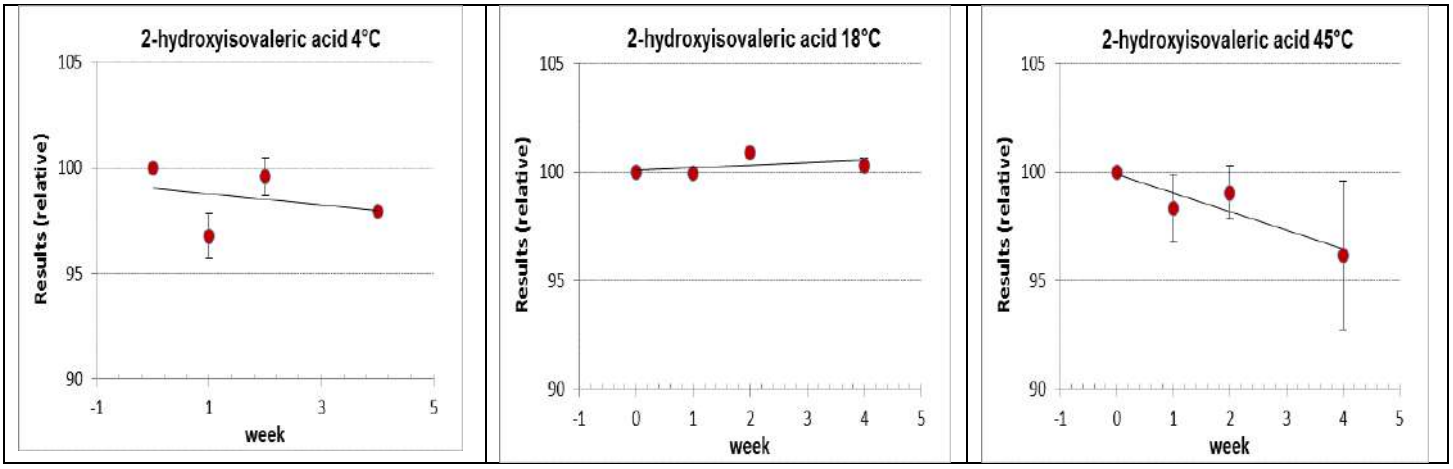

Figure A66. Short Term Stability Plot for 2-hydroxyisovaleric acid at 4°C, 18 °C and 45°C

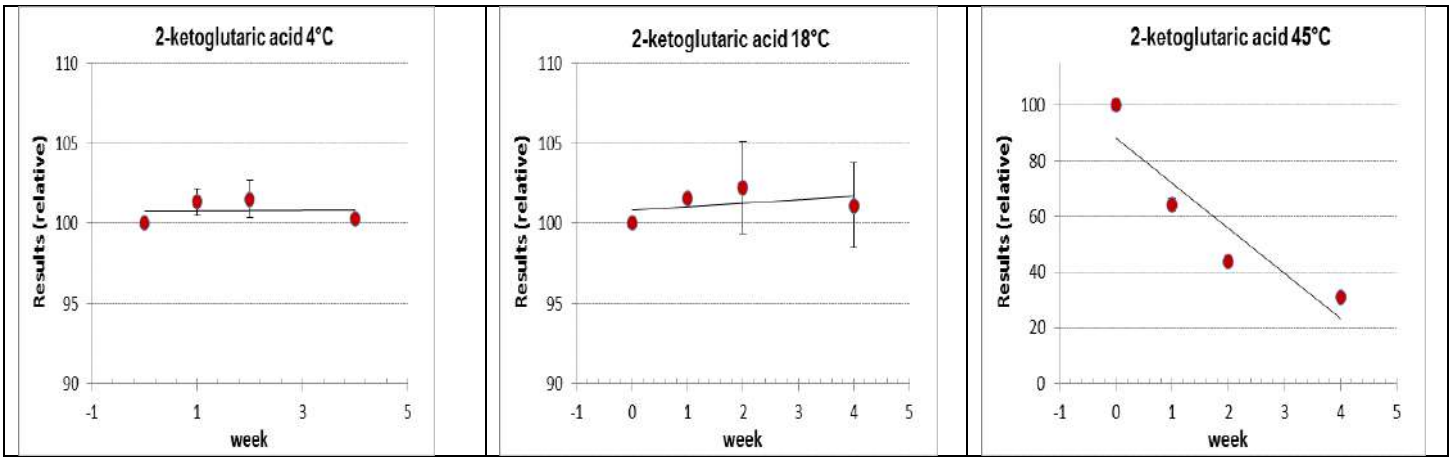

Figure A67. Short Term Stability Plot for 2-ketoglutaric acid at 4°C, 18 °C and 45°C

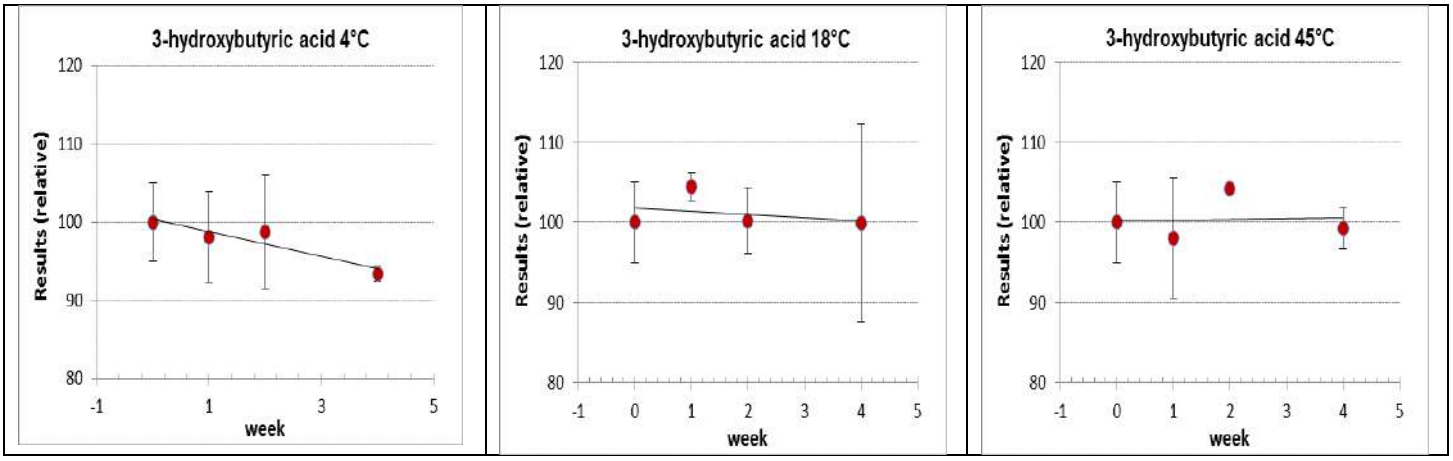

Figure A68. Short Term Stability Plot for 3-hydroxybutyric acid at 4°C, 18 °C and 45°C

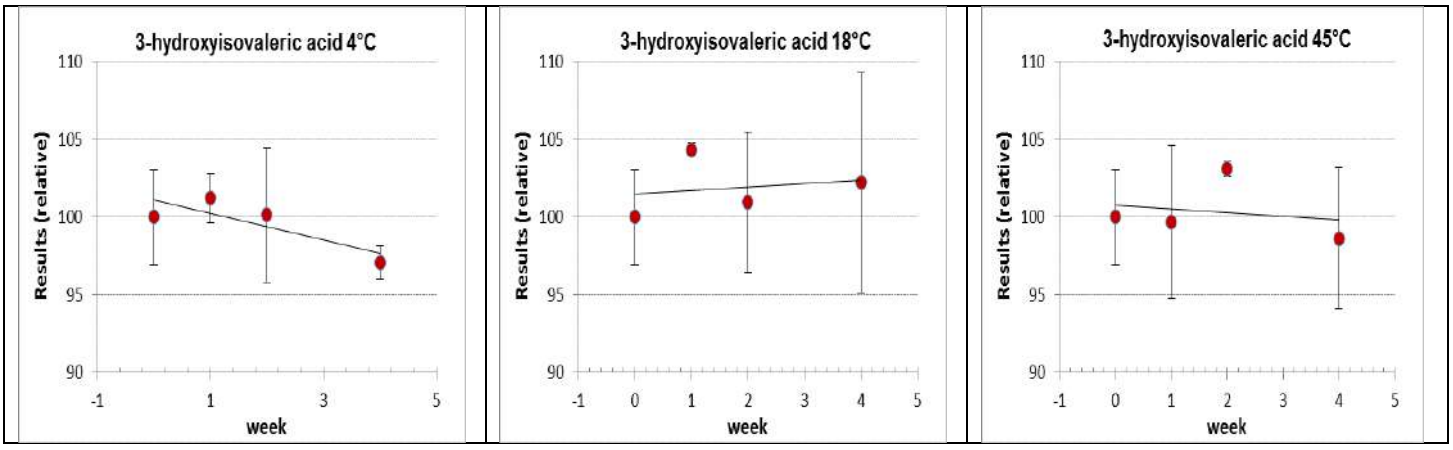

Figure A69. Short Term Stability Plot for 3-hydroxyisovaleric acid at 4°C, 18 °C and 45°C

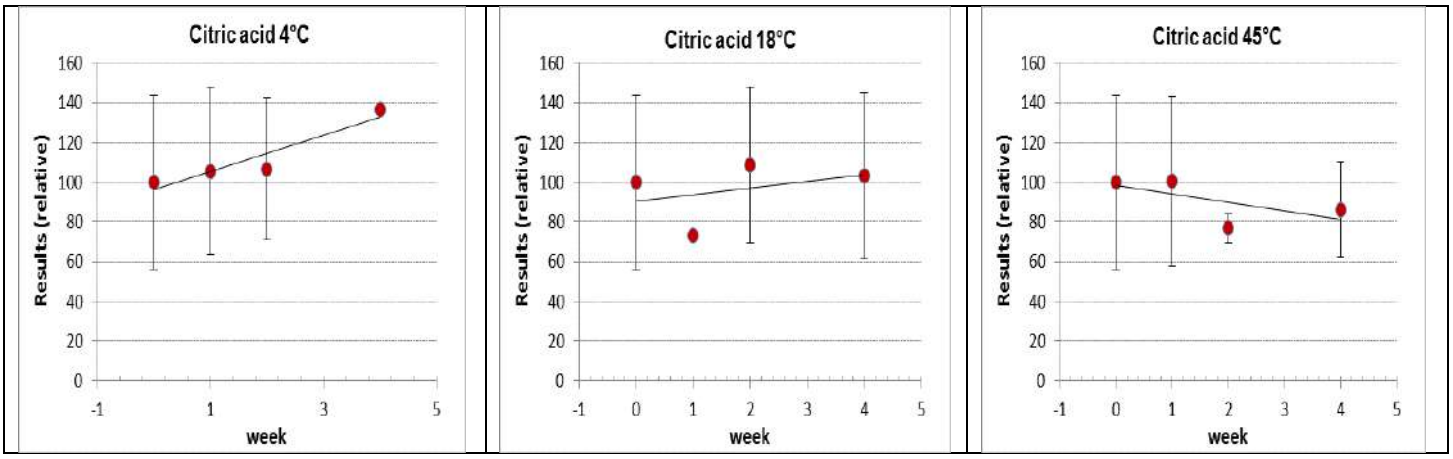

Figure A70. Short Term Stability Plot for Citric acid at 4°C, 18 °C and 45°C

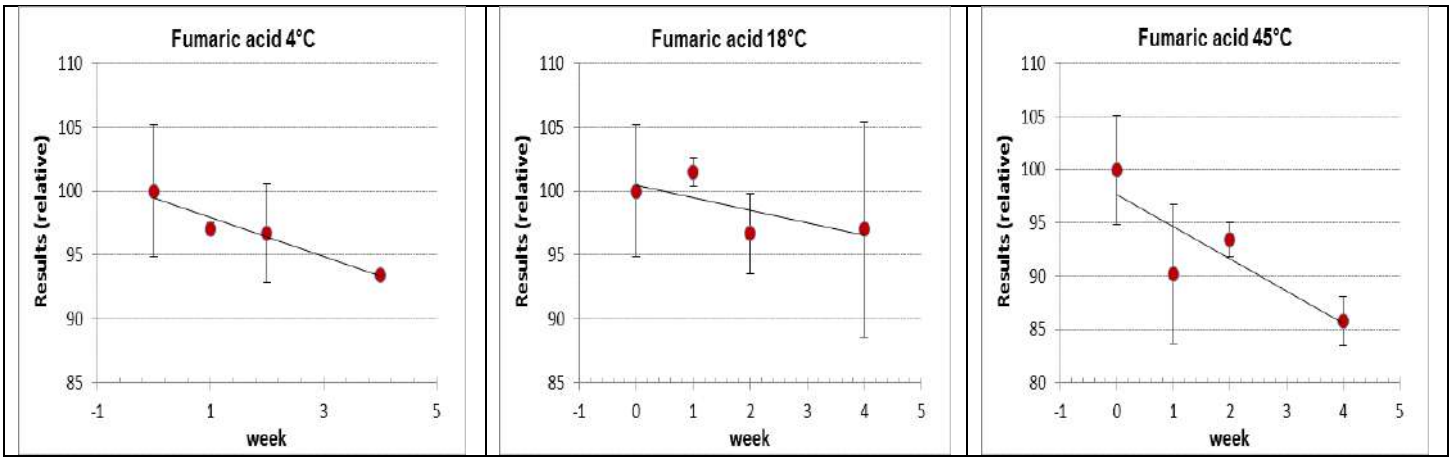

Figure A71. Short Term Stability Plot for Fumaric acid at 4°C, 18 °C and 45°C

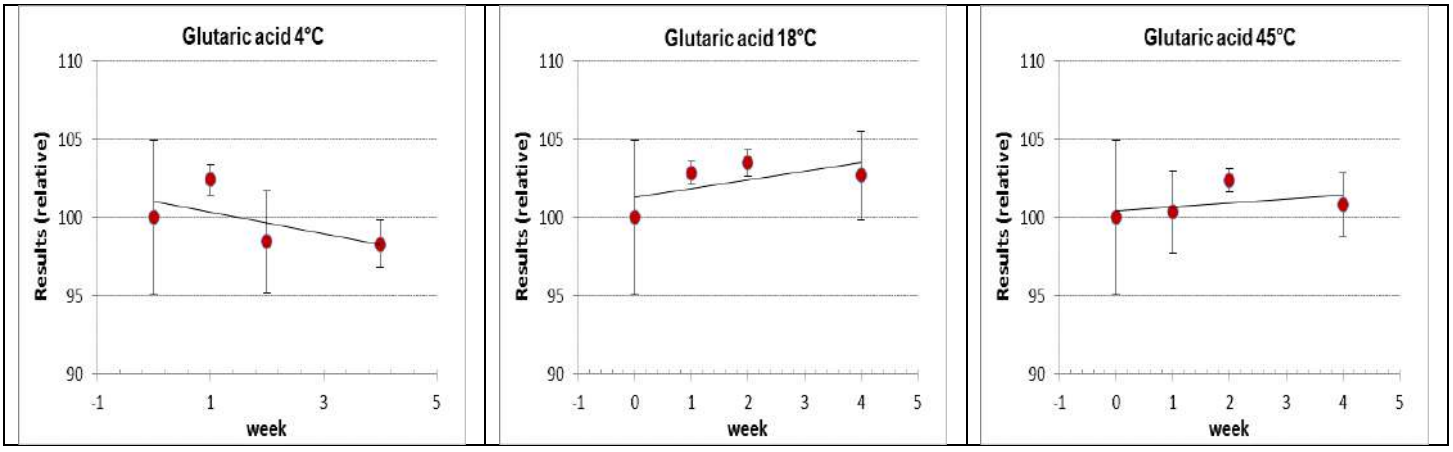

Figure A72. Short Term Stability Plot for Glutaric acid at 4°C, 18 °C and 45°C

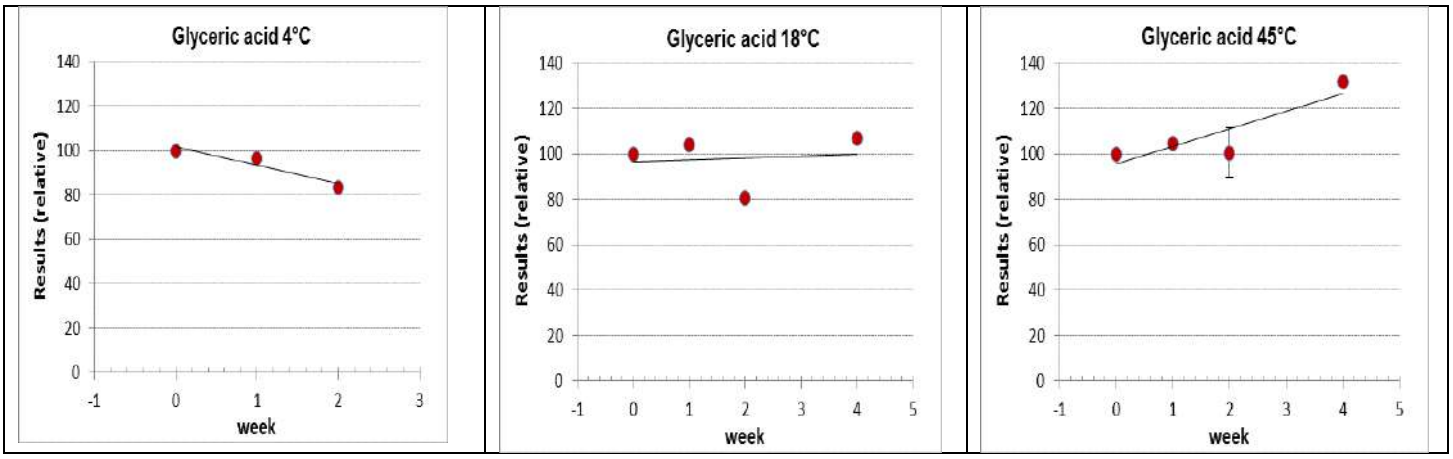

Figure A73. Short Term Stability Plot for Glyceric acid at 4°C, 18 °C and 45°C

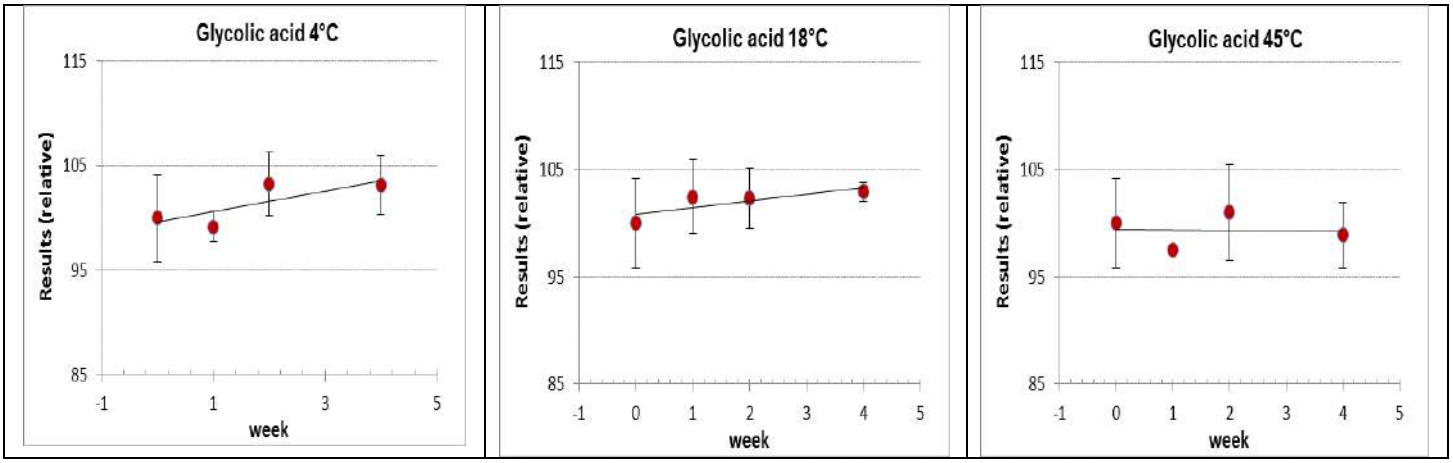

Figure A74. Short Term Stability Plot for Glycolic acid at 4°C, 18 °C and 45°C

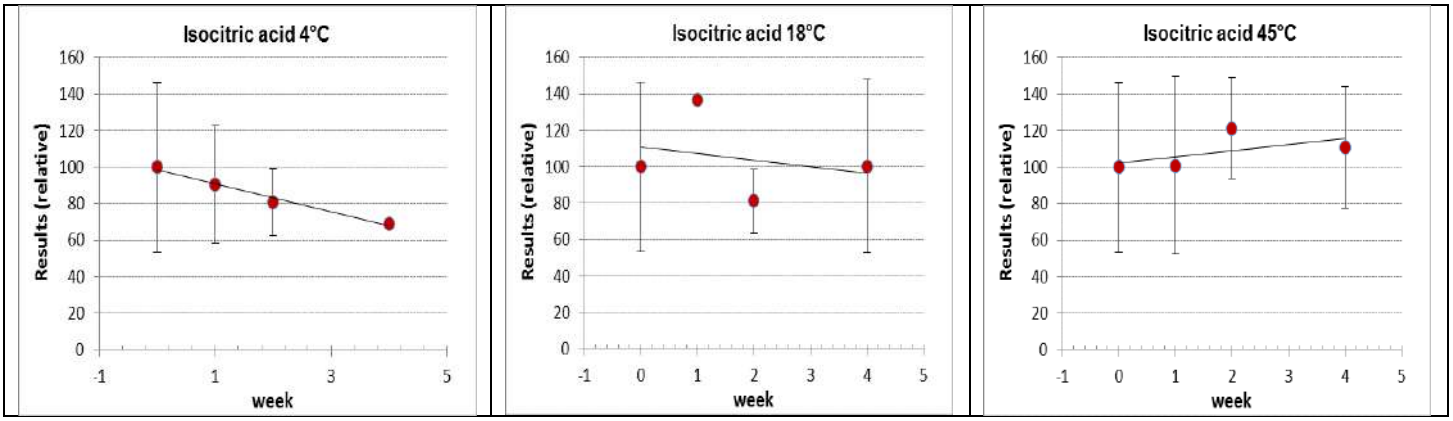

Figure A75. Short Term Stability Plot for Isocitric acid at 4°C, 18 °C and 45°C

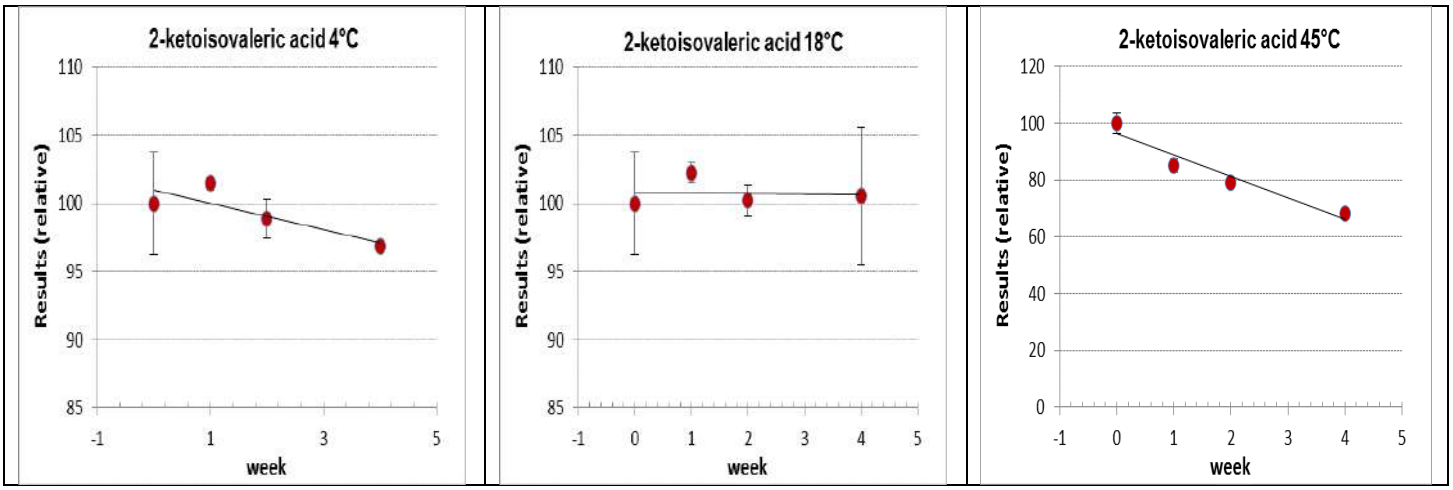

Figure A76. Short Term Stability Plot for 2-ketoisovaleric acid at 4°C, 18 °C and 45°C

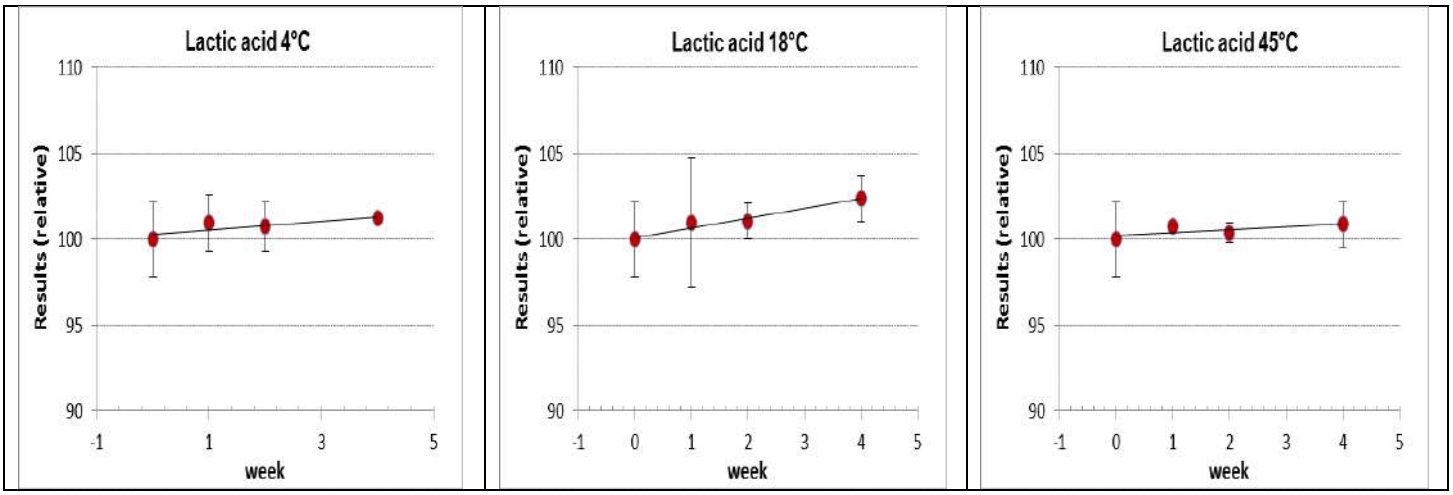

Figure A77. Short Term Stability Plot for Lactic acid at 4°C, 18 °C and 45°C

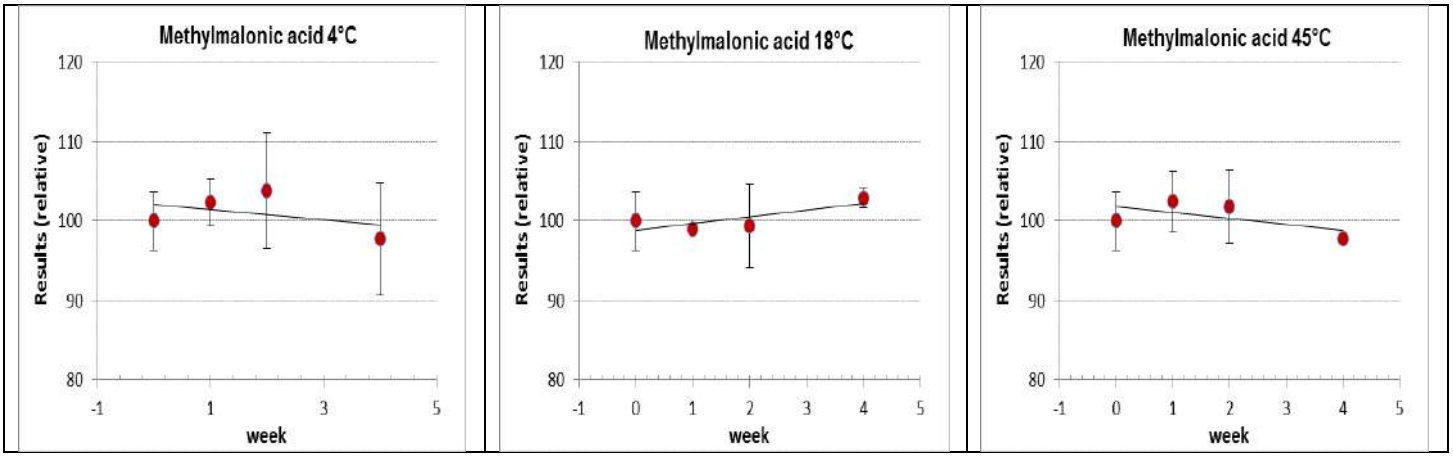

Figure A78. Short Term Stability Plot for Methylmalonic acid at 4°C, 18 °C and 45°C

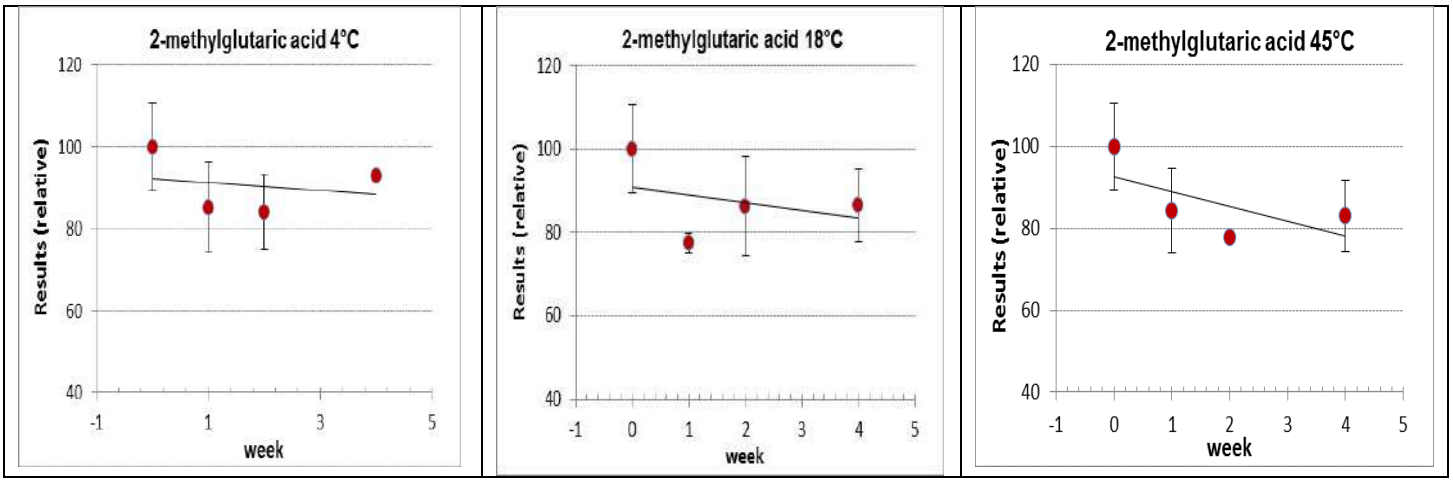

Figure A79. Short Term Stability Plot for 2-methyglutaric acid at 4°C, 18 °C and 45°C

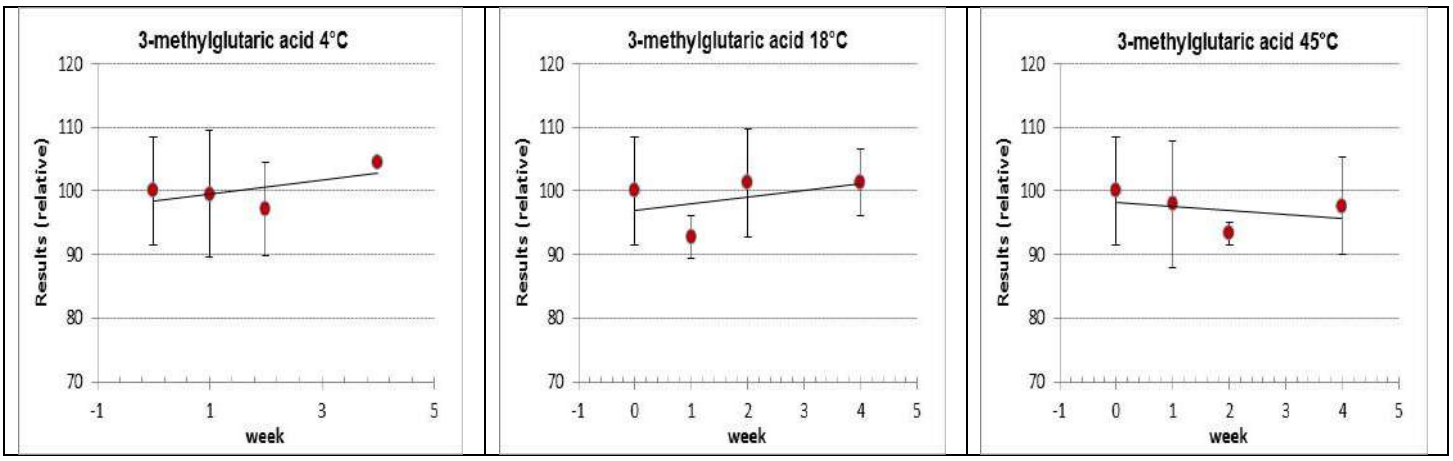

Figure A80. Short Term Stability Plot for 3-methylglutaric acid at 4°C, 18 °C and 45°C

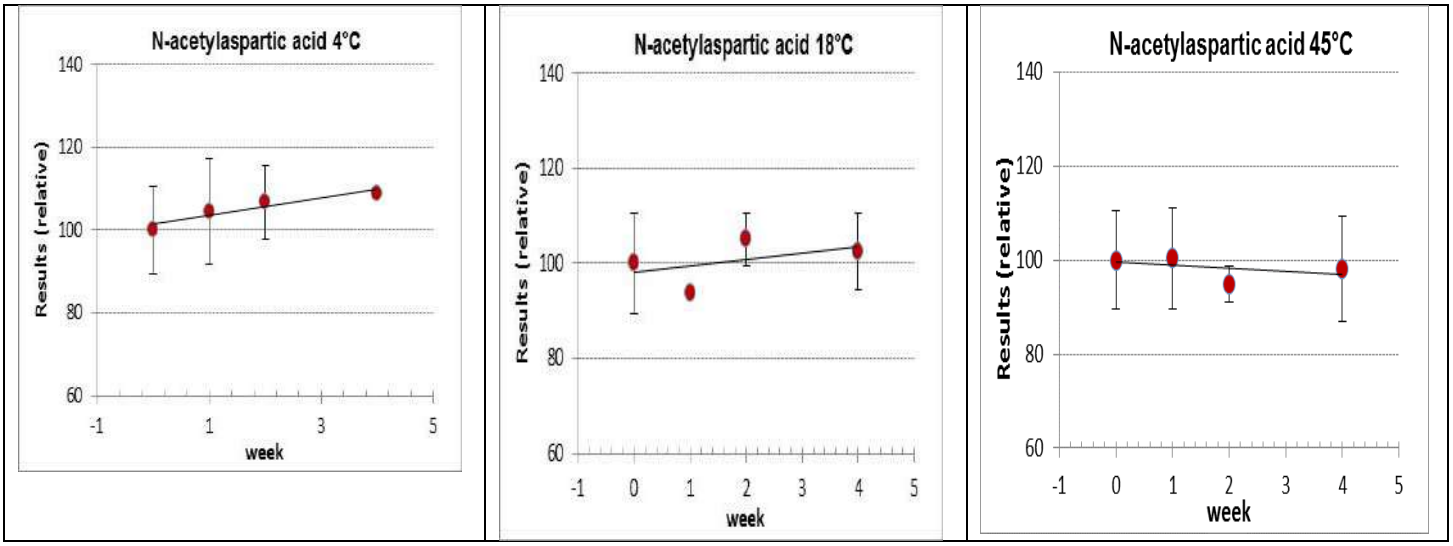

Figure A81. Short Term Stability Plot for N-acetylaspartic acid at 4°C, 18 °C and 45°C

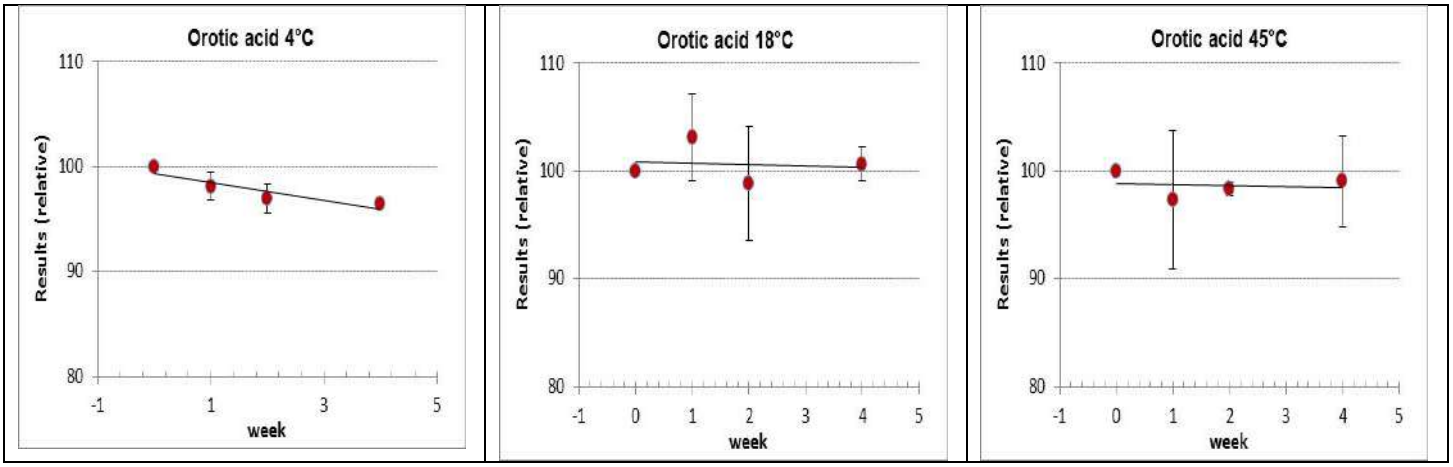

Figure A82. Short Term Stability Plot for Orotic acid at 4°C, 18 °C and 45°C

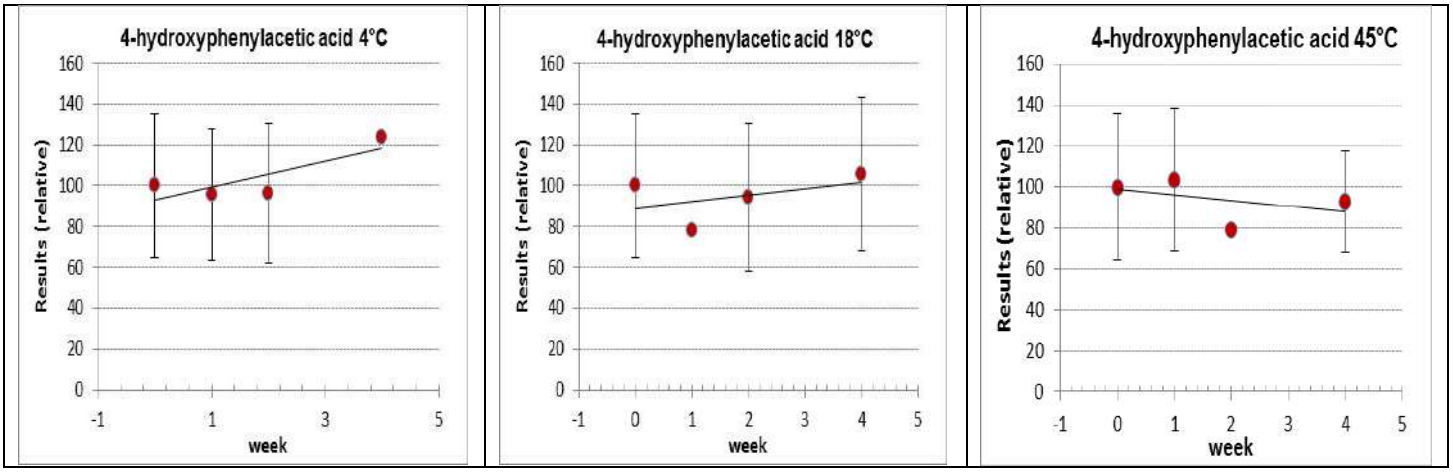

Figure A83. Short Term Stability Plot for 4-hydroxyphenylacetic acid at 4°C, 18 °C and 45°C

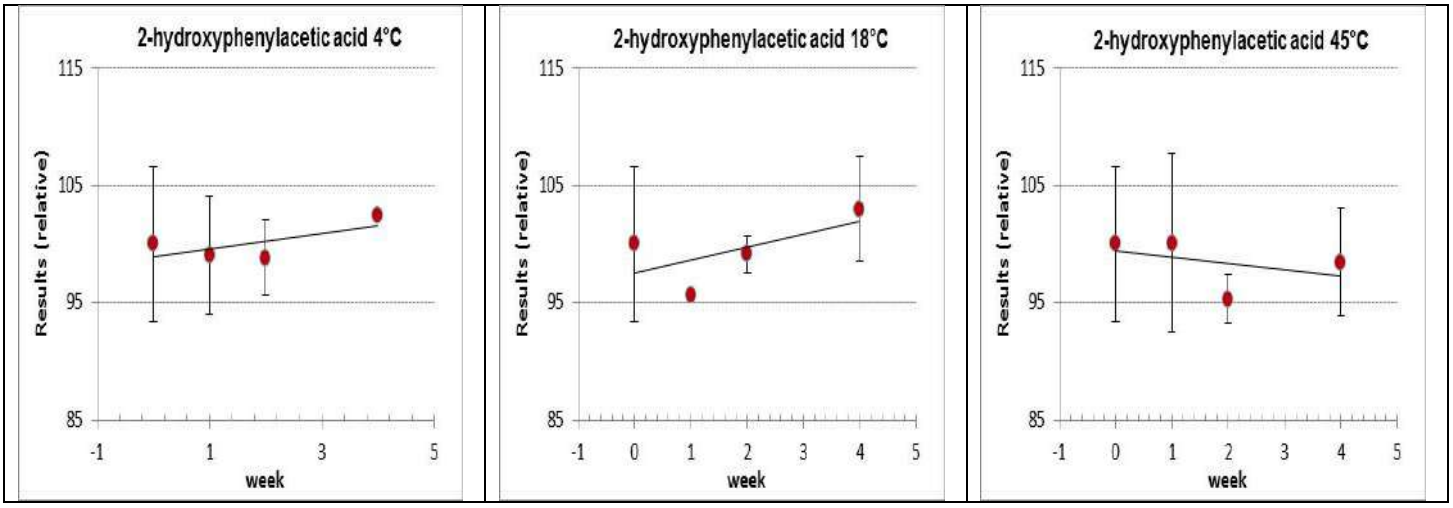

Figure A84. Short Term Stability Plot for 2-hydroxyphenylacetic acid at 4°C, 18 °C and 45°C

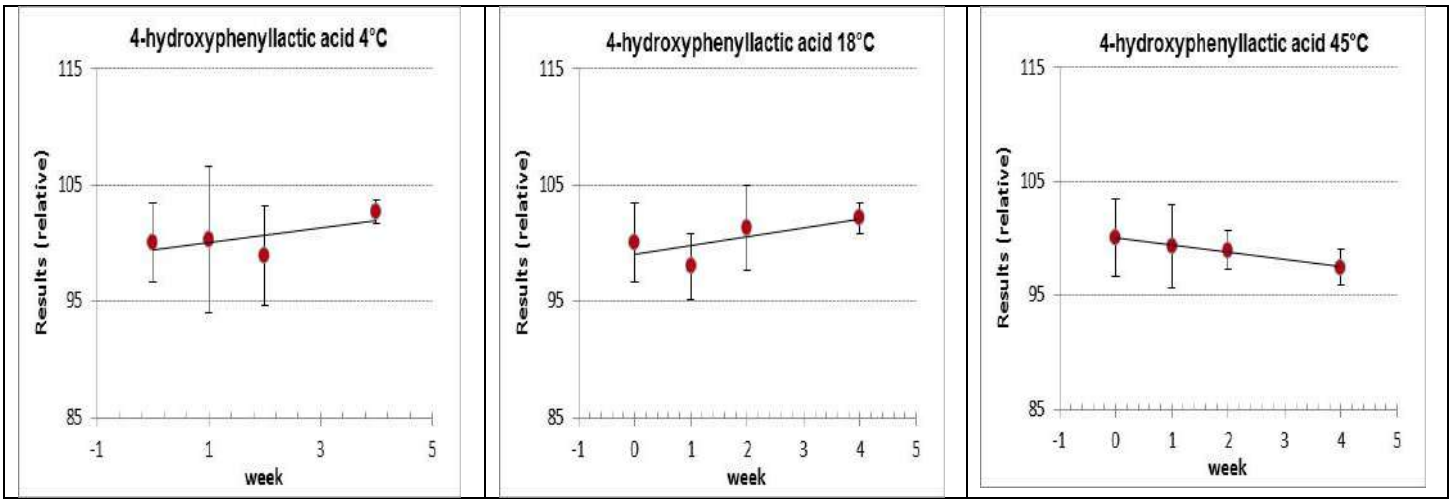

Figure A85. Short Term Stability Plot for 4-hydroxyphenyllactic acid at 4°C, 18 °C and 45°C

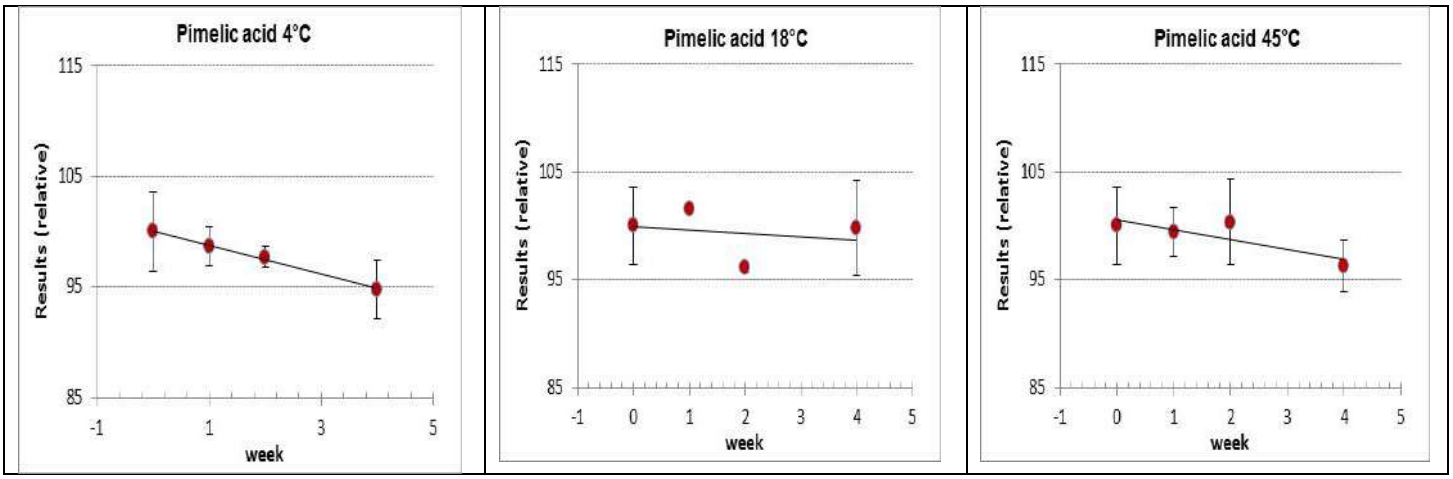

Figure A86. Short Term Stability Plot for Pimelic acid at 4°C, 18 °C and 45°C

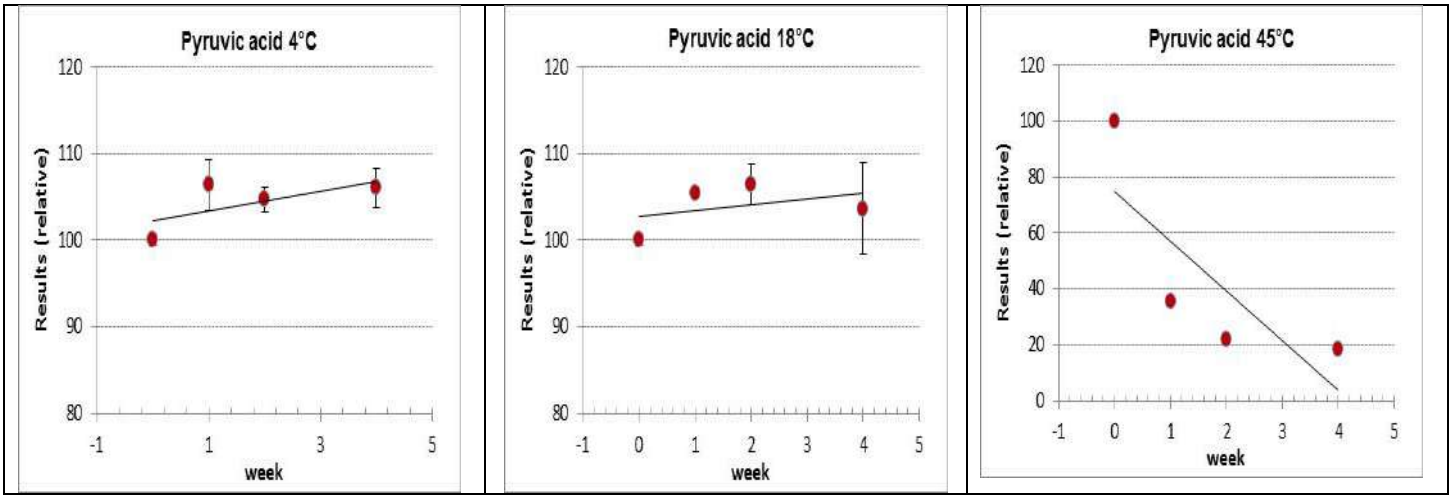

Figure A87. Short Term Stability Plot for Pyruvic acid at 4°C, 18 °C and 45°C

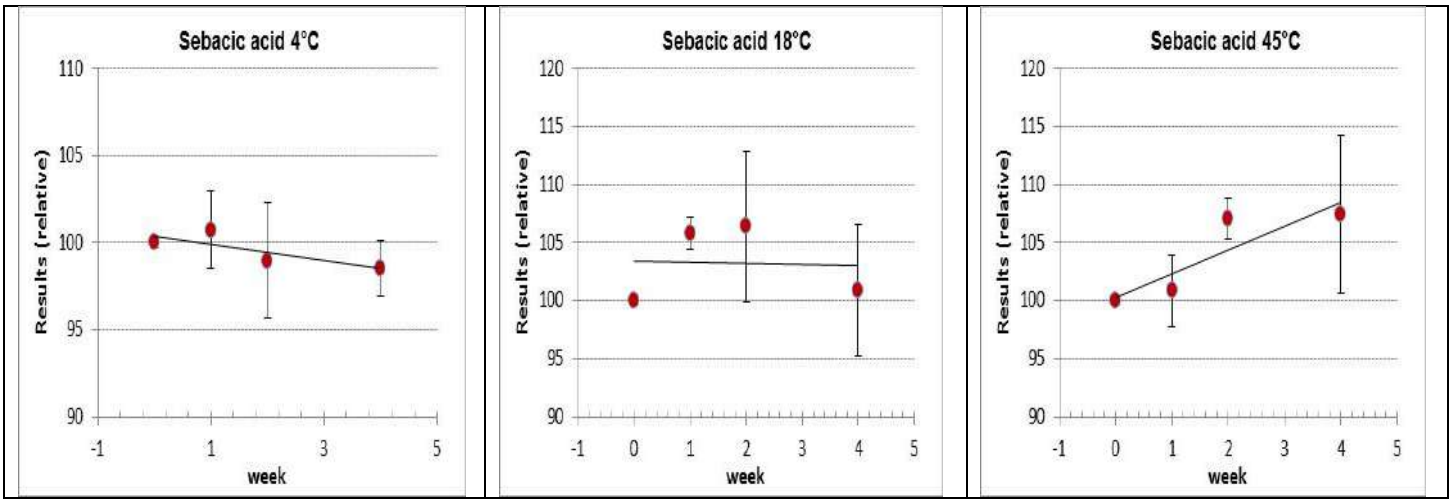

Figure A88. Short Term Stability Plot for Sebacic acid at 4°C, 18 °C and 45°C

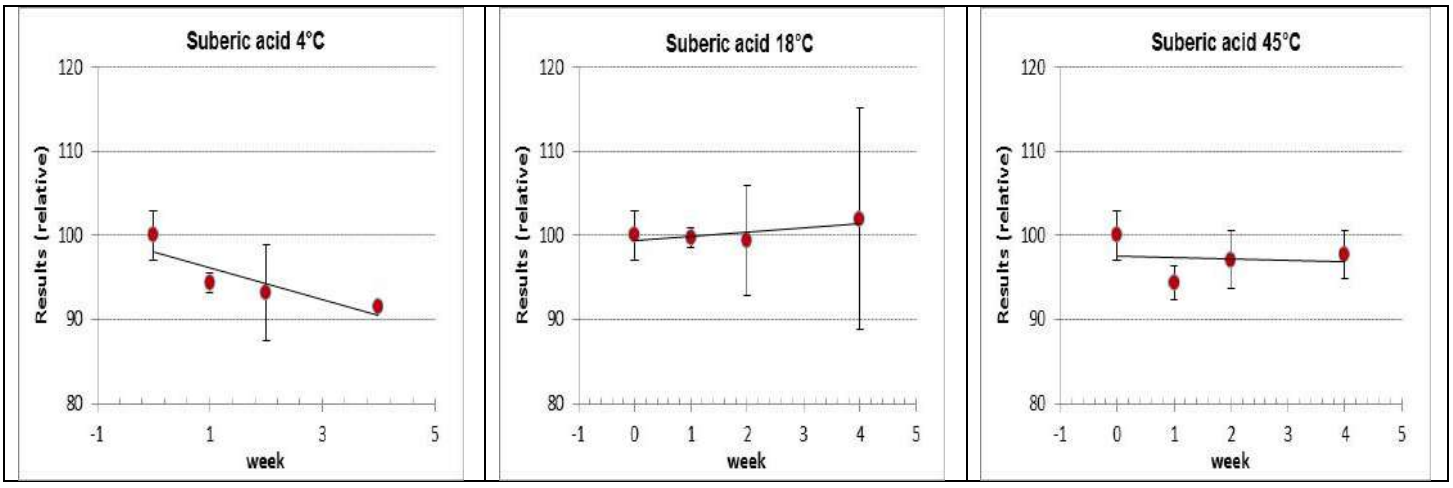

Figure A89. Short Term Stability Plot for Suberic acid at 4°C, 18 °C and 45°C

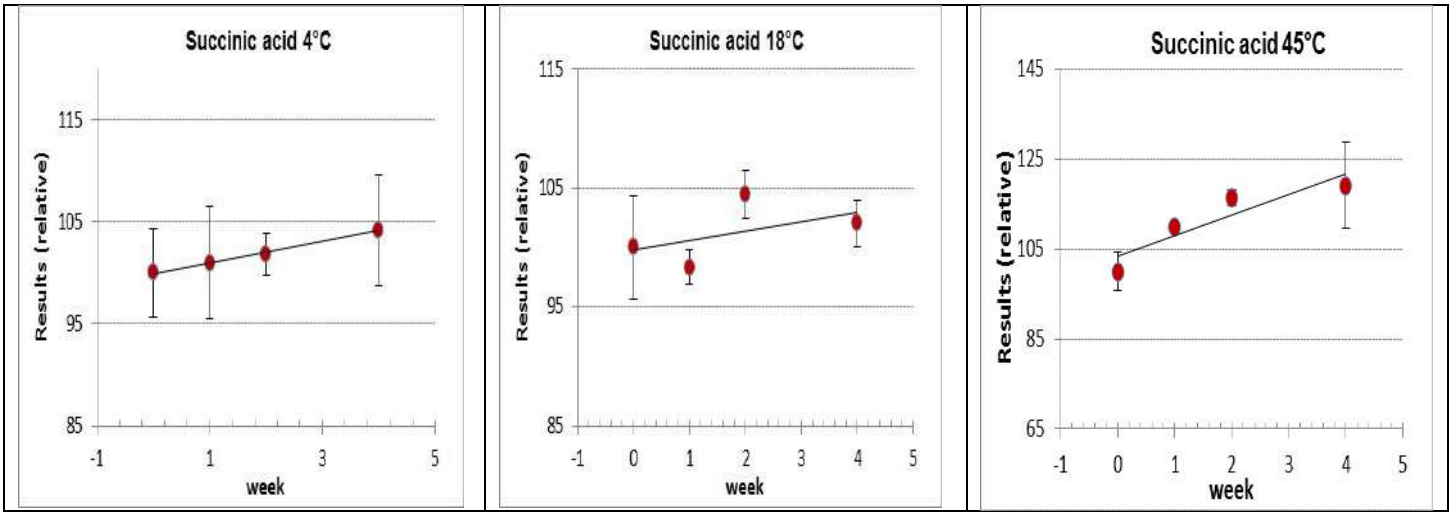

Figure A90. Short Term Stability Plot for Succinic acid at 4°C, 18 °C and 45°C

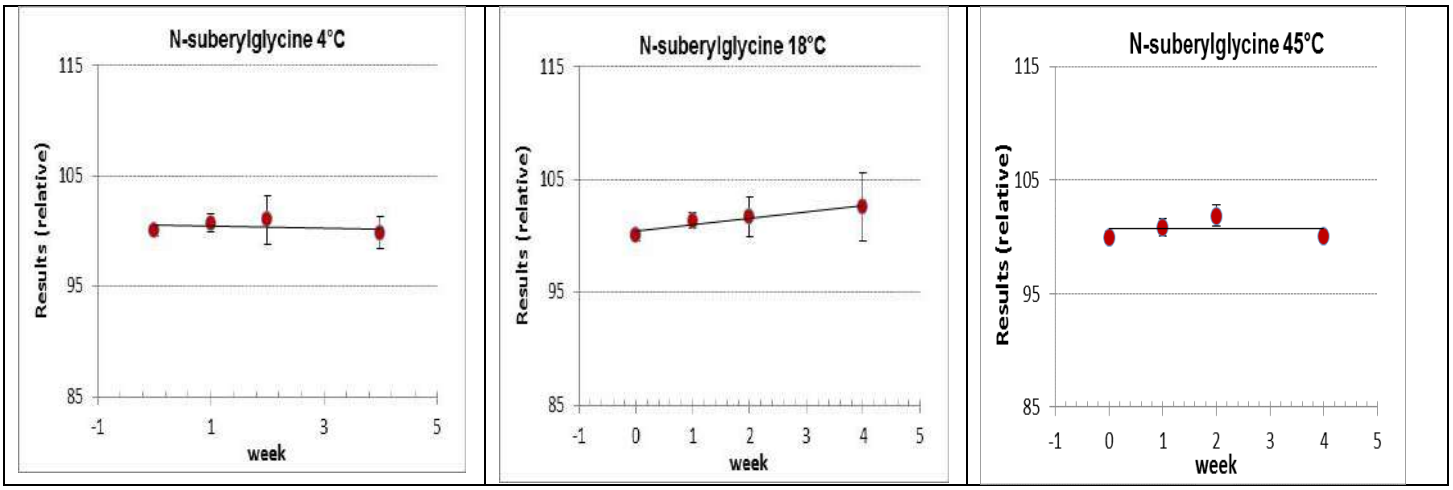

Figure A91. Short Term Stability Plot for N-suberylglycine at 4°C, 18 °C and 45°C

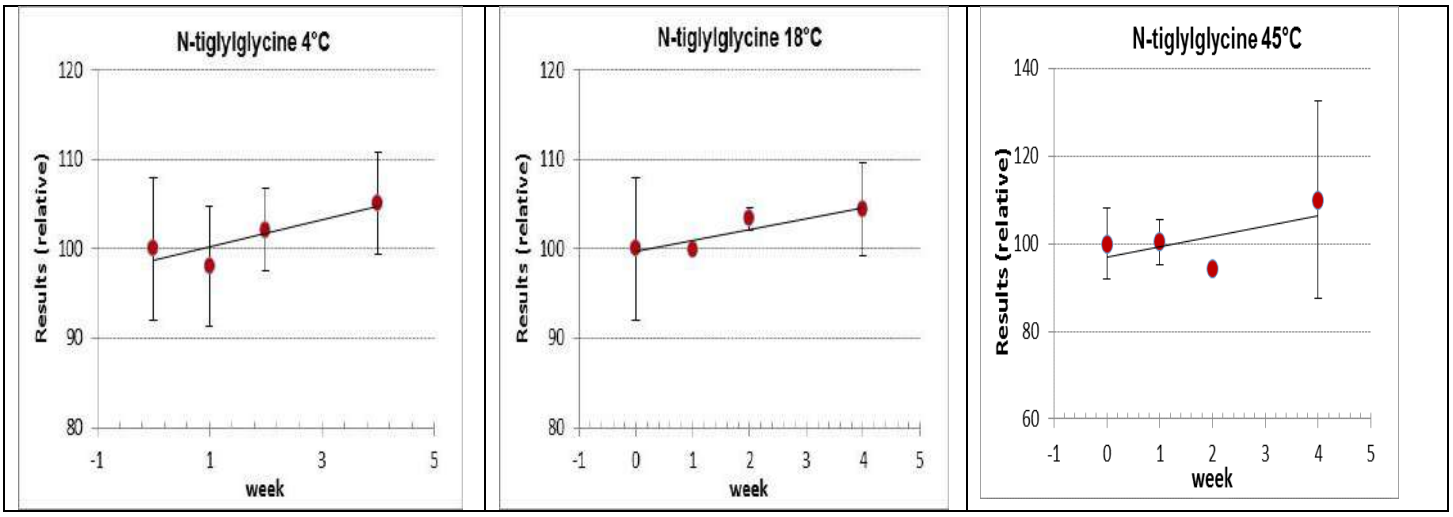

Figure A92. Short Term Stability Plot for N-tiglylglycine at 4°C, 18 °C and 45°C

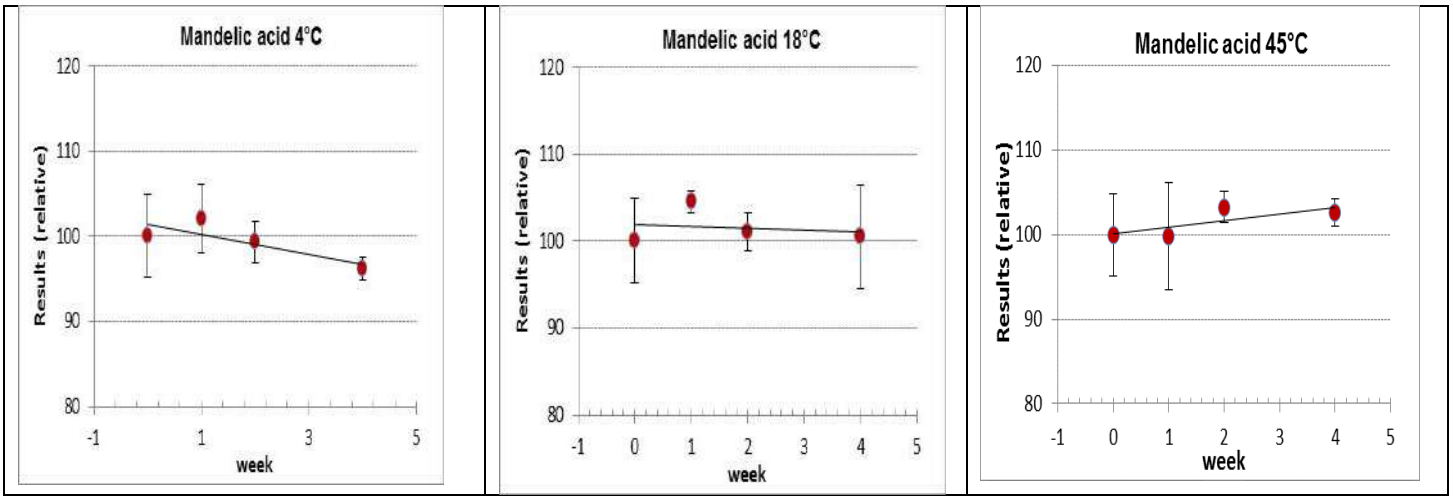

Figure A93. Short Term Stability Plot for Mandelic acid at 4°C, 18 °C and 45°C

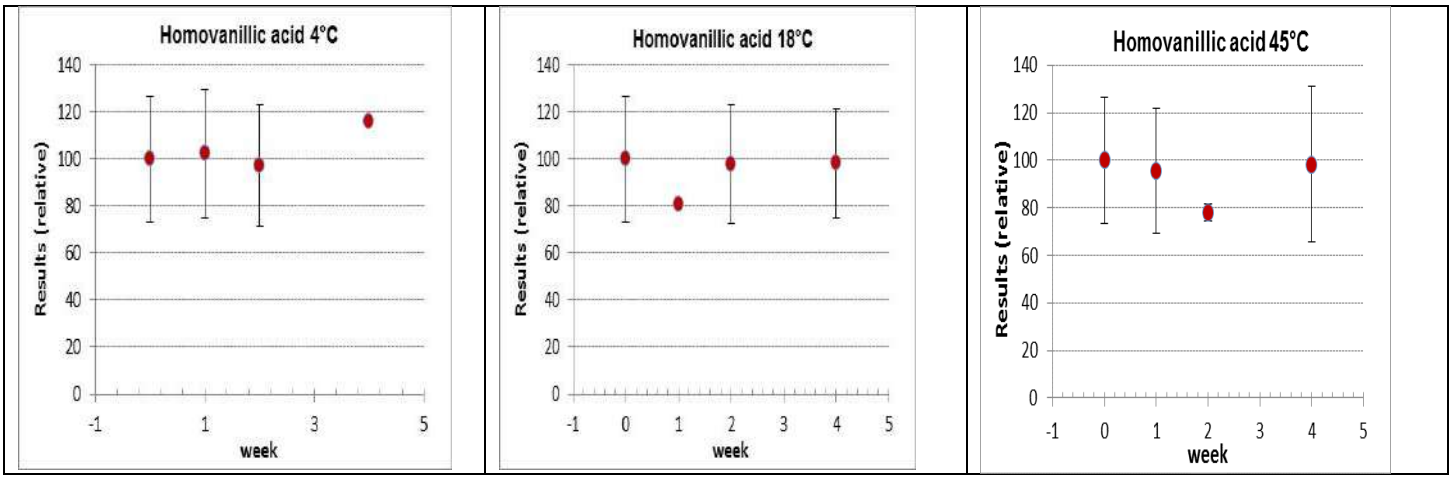

Figure A94. Short Term Stability Plot for Homovanillic acid at 4°C, 18 °C and 45°C

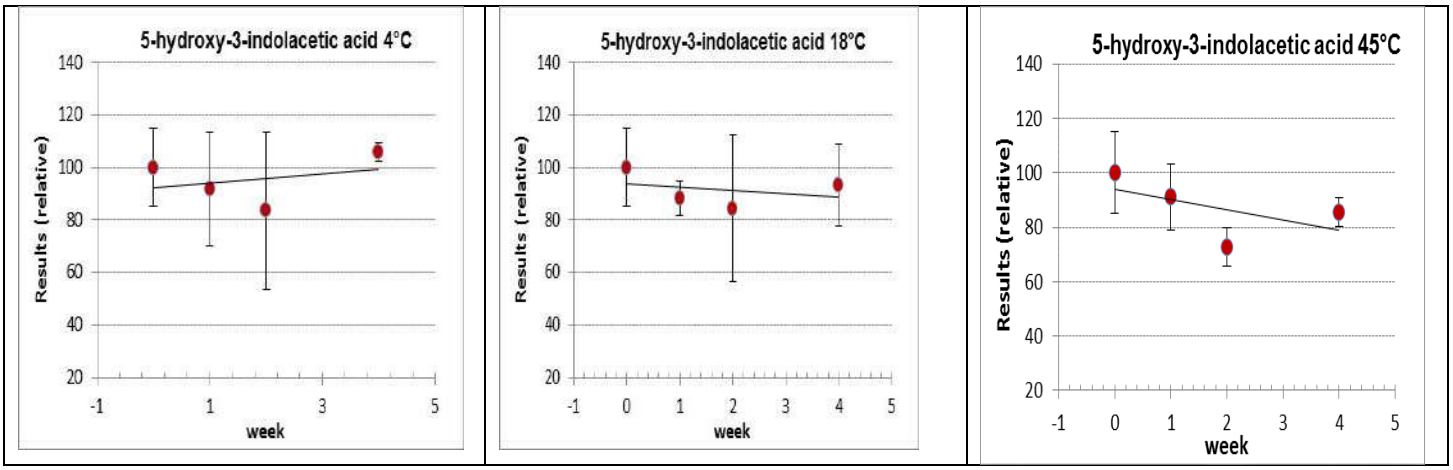

Figure A95. Short Term Stability Plot for 5-hydroxy-3-indolacetic acid at 4°C, 18 °C and 45°C

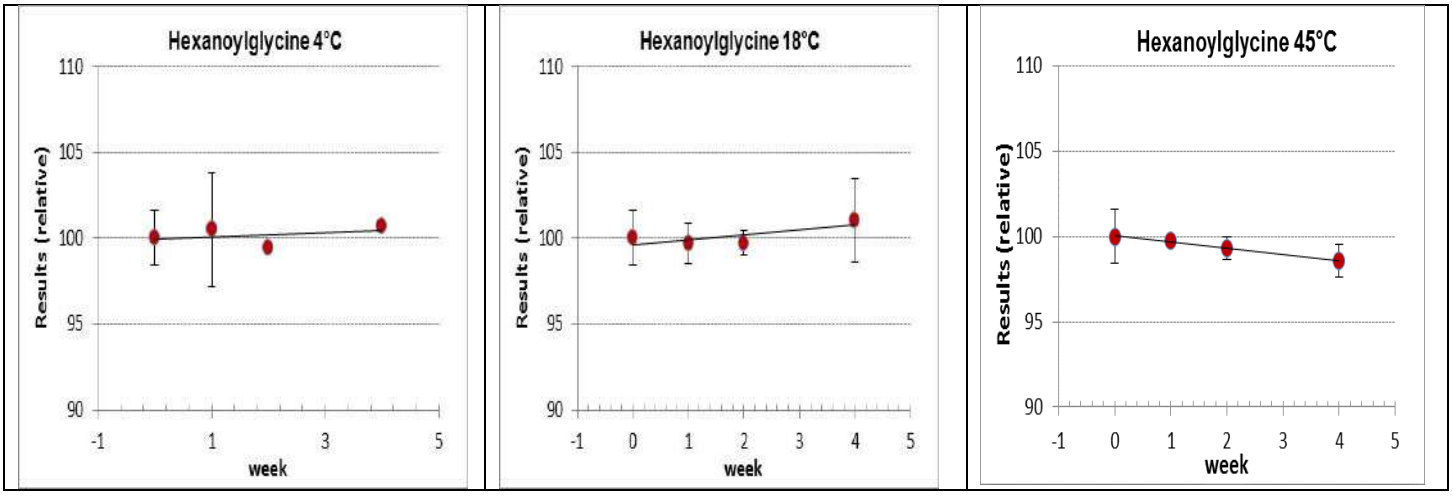

Figure A96. Short Term Stability Plot for Hexanoylglycine at 4°C, 18 °C and 45°C

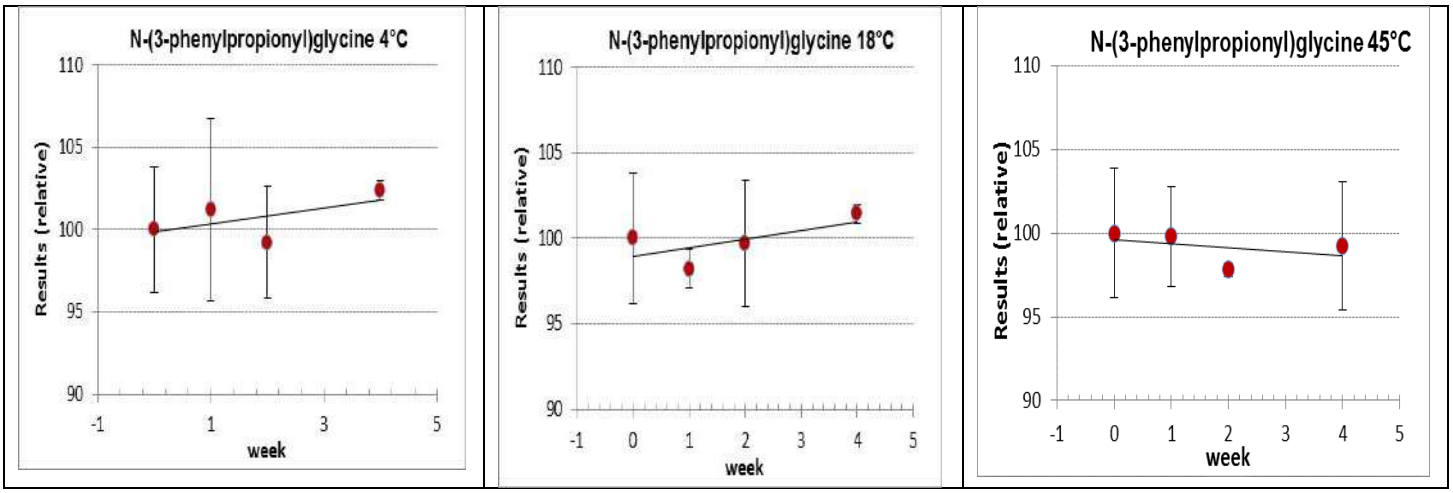

Figure A97. Short Term Stability Plot for N-(3-phenylproionyl)glycine at 4°C, 18 °C and 45°C

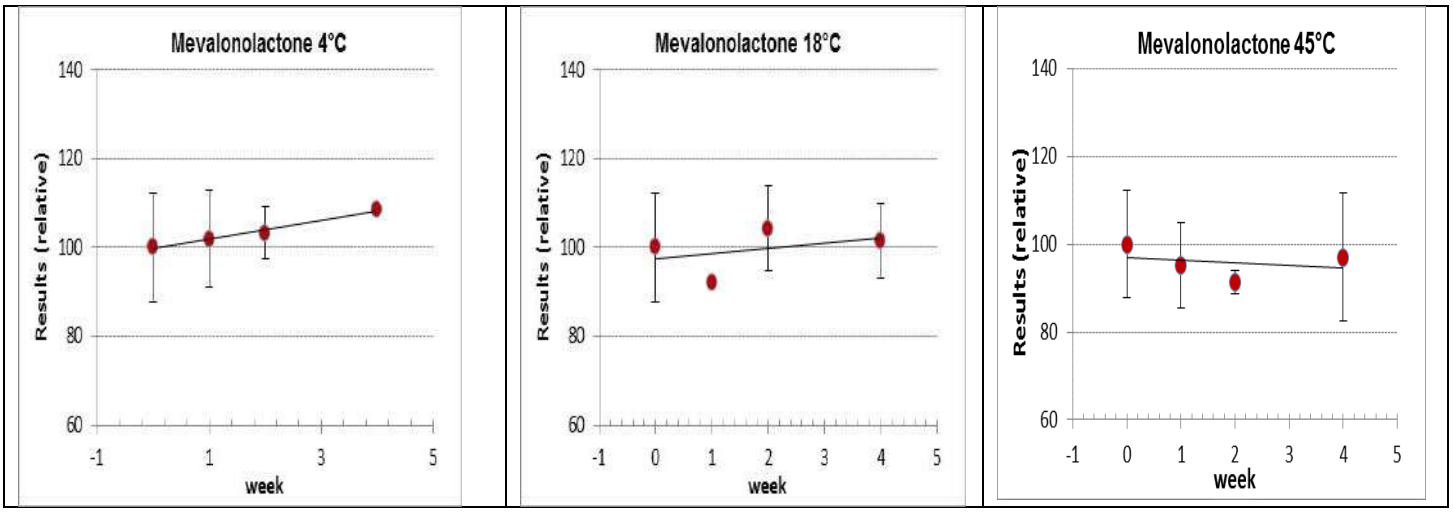

Figure A98. Short Term Stability Plot for Mevalonolactone at 4°C, 18 °C and 45°C

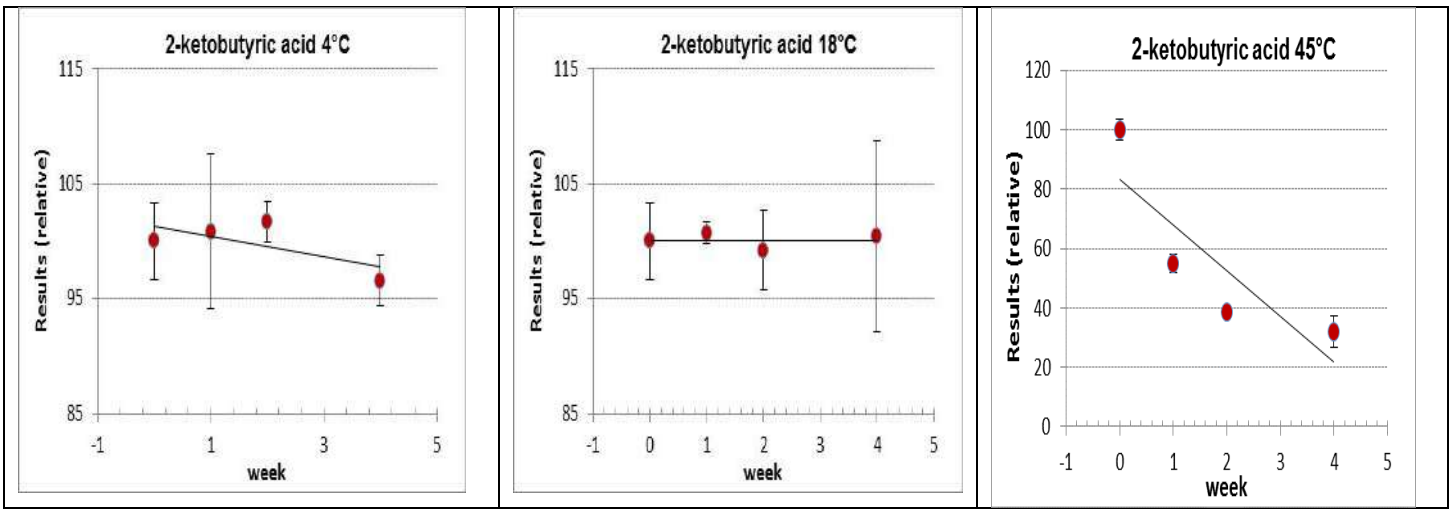

Figure A99. Short Term Stability Plot for 2-ketobutyric acid at 4°C, 18 °C and 45°C

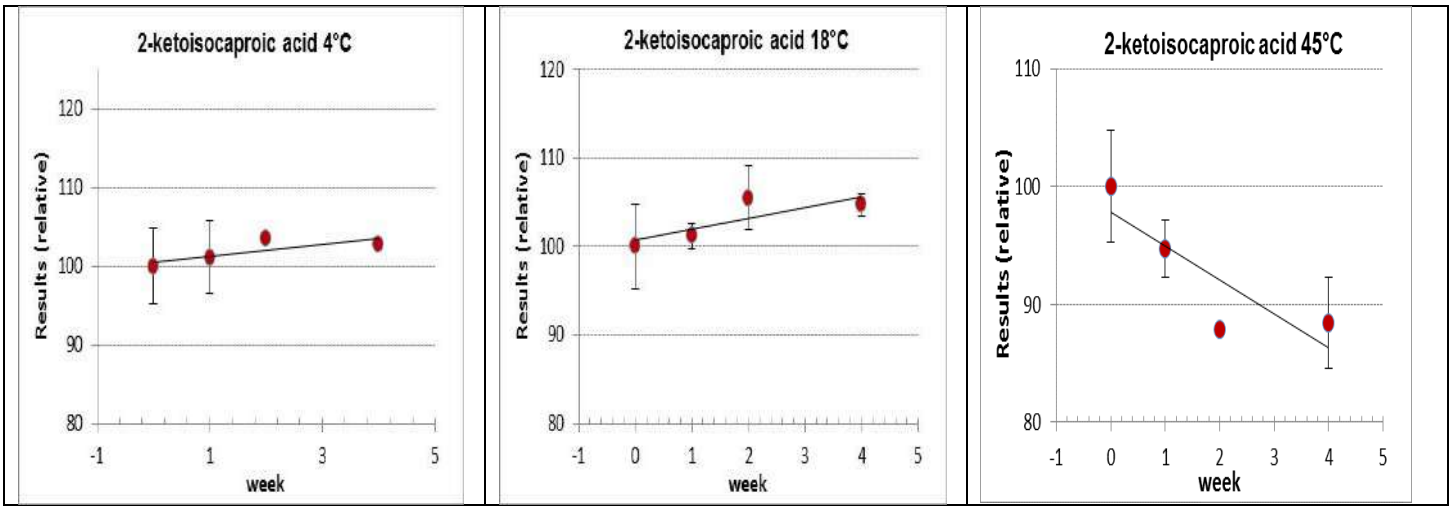

Figure A100. Short Term Stability Plot for 2-ketoisocaproic acid at 4°C, 18 °C and 45°C

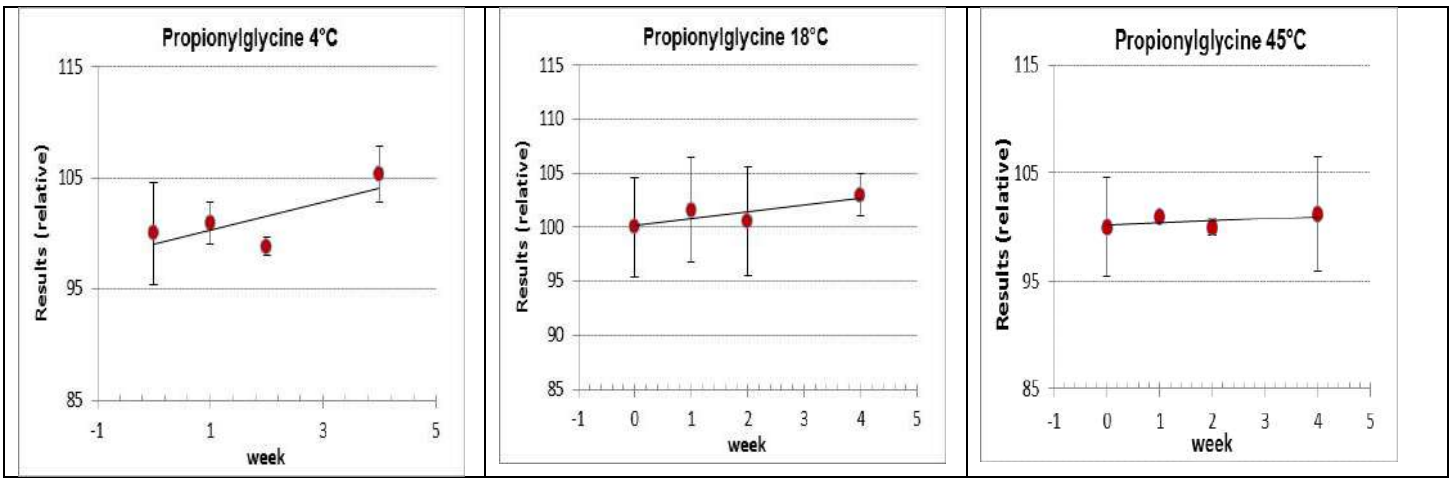

Figure A101. Short Term Stability Plot for Propionylglycine acid at 4°C, 18 °C and 45°C

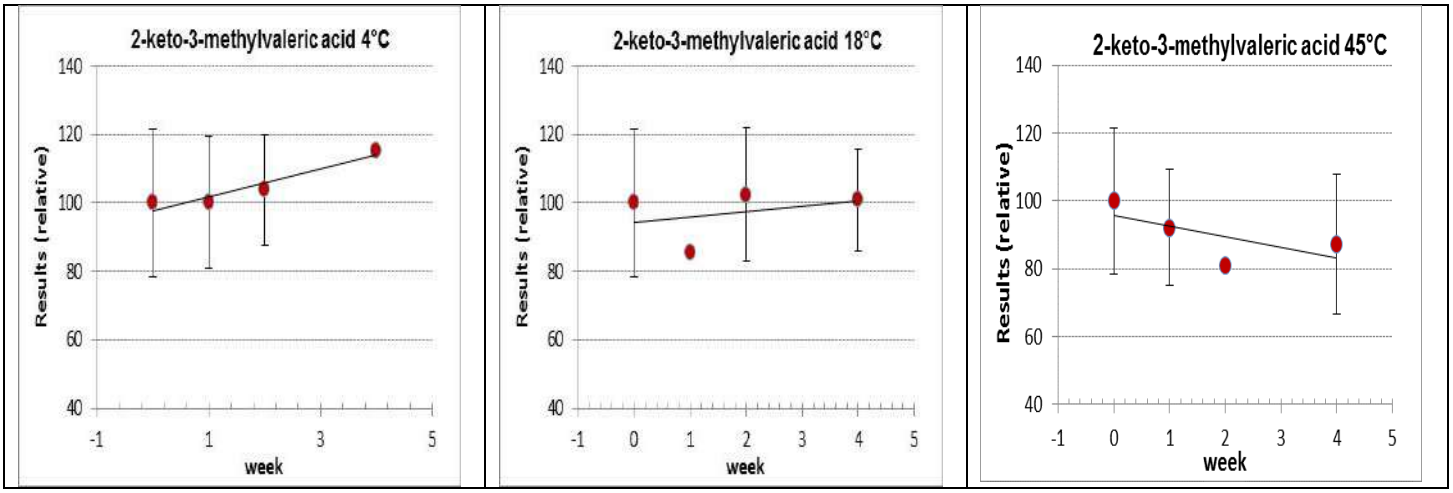

Figure A102. Short Term Stability Plot for 2-keto-3-methylvaleric acid at 4°C, 18 °C and 45°C

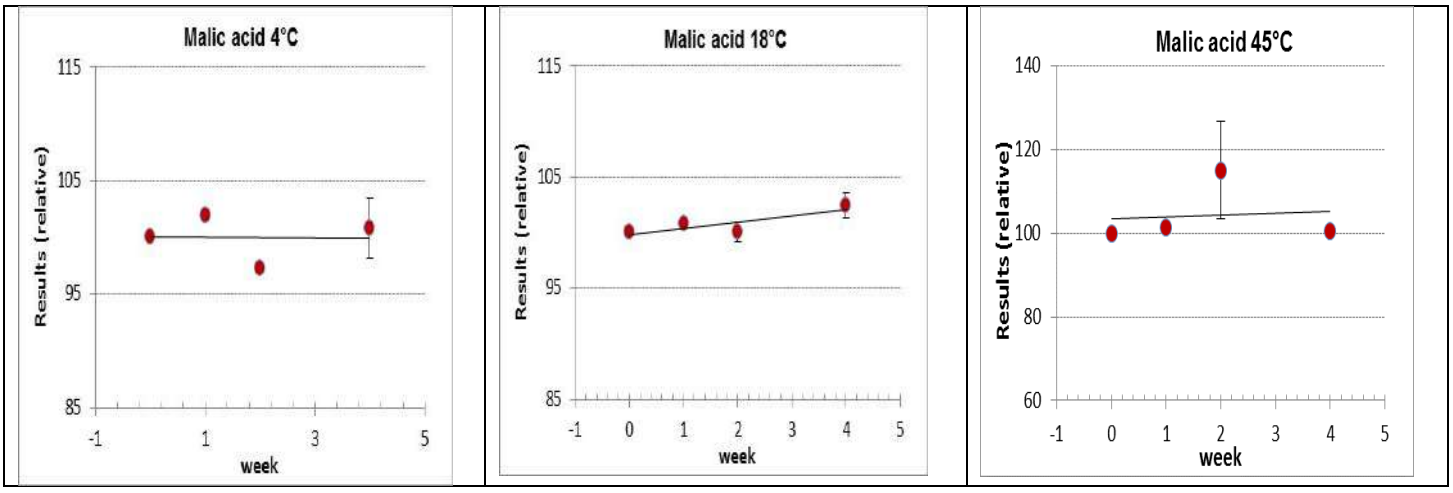

Figure A103. Short Term Stability Plot for Malic acid at 4°C, 18 °C and 45°C

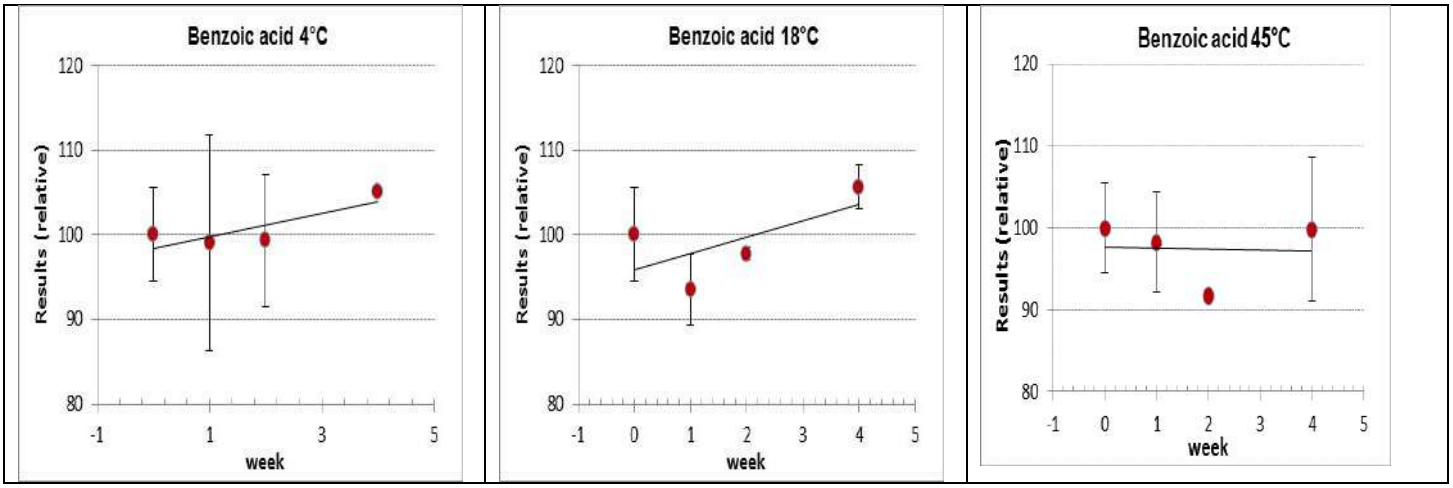

Figure A104. Short Term Stability Plot for Benzoic acid at 4°C, 18 °C and 45°C

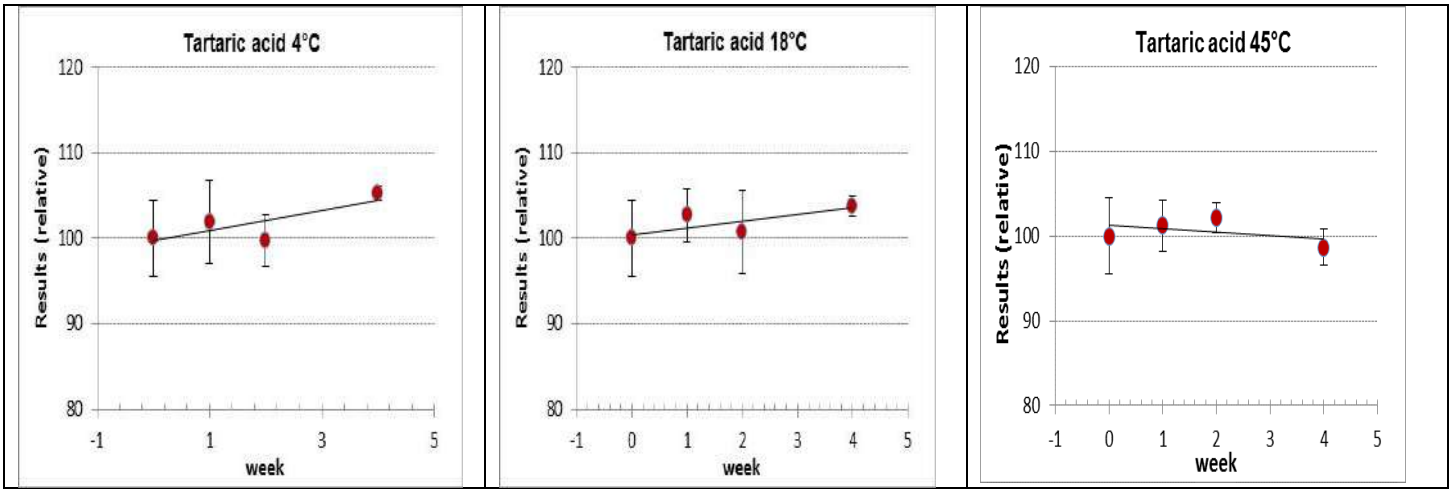

Figure A105. Short Term Stability Plot for Tartaric acid at 4°C, 18 °C and 45°C

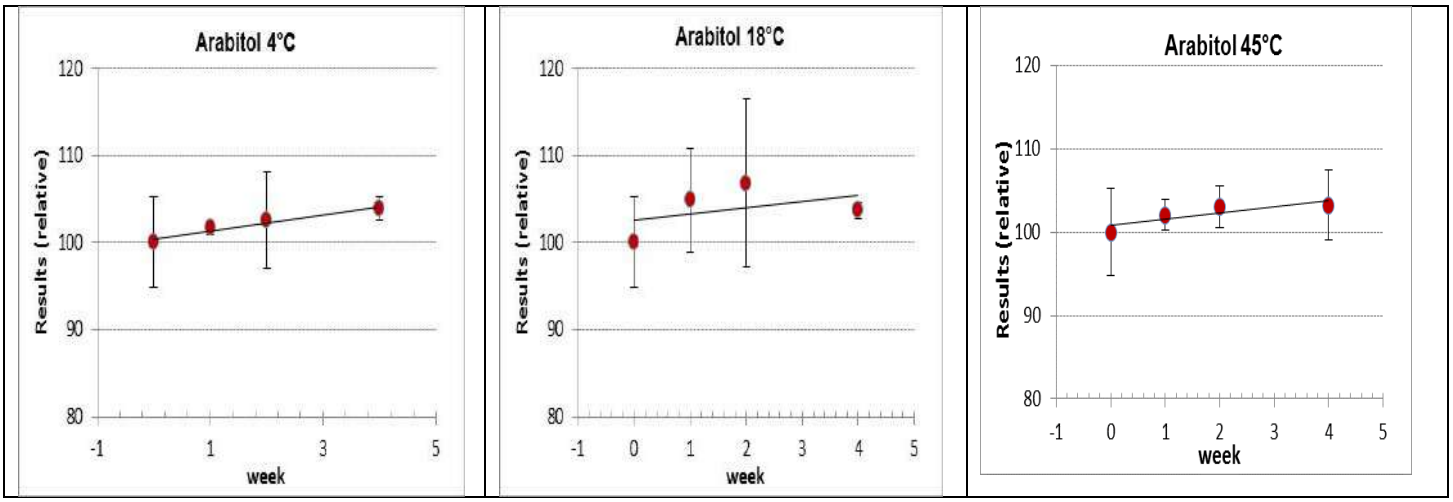

Figure A106. Short Term Stability Plot for Arabitol acid at 4°C, 18 °C and 45°C

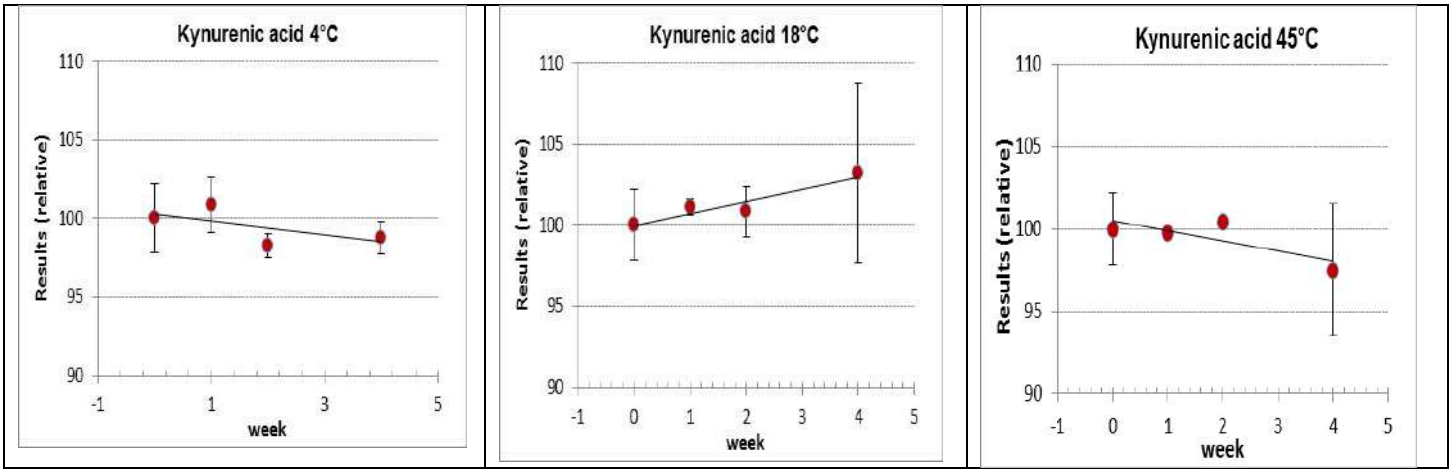

Figure A107. Short Term Stability Plot for Kynurenic acid at 4°C, 18 °C and 45°C

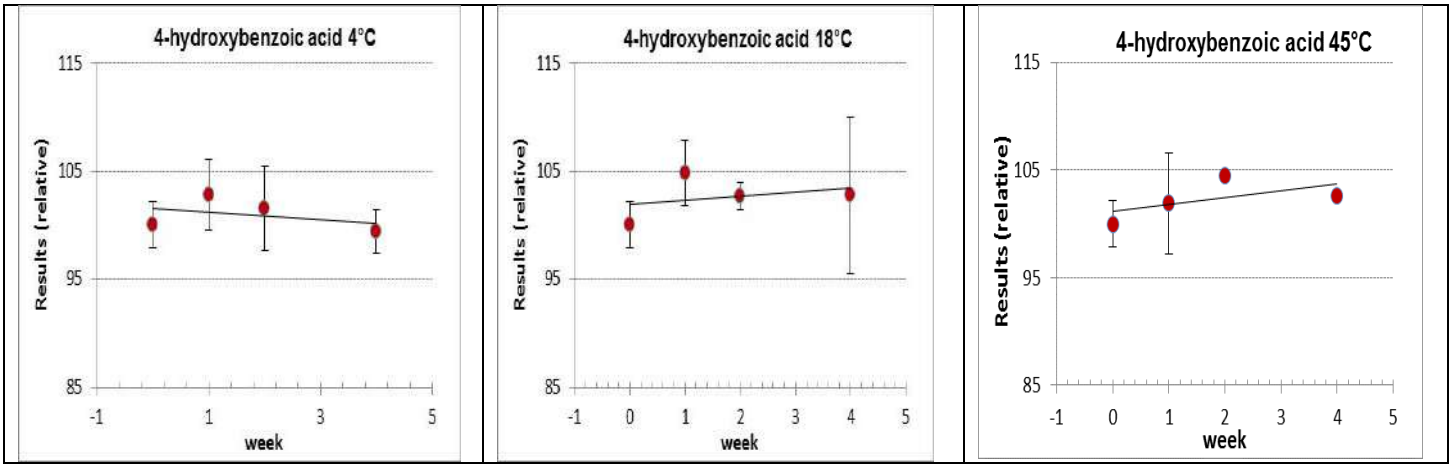

Figure A108. Short Term Stability Plot for 4-hydroxybenzoic acid at 4°C, 18 °C and 45°C

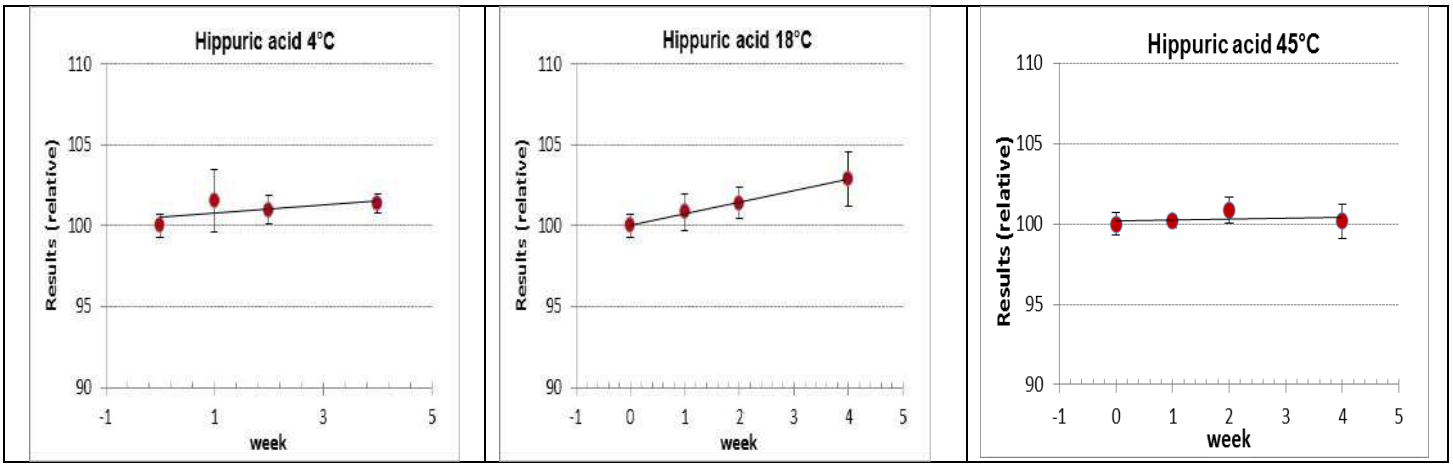

Figure A109. Short Term Stability Plot for Hippuric acid at 4°C, 18 °C and 45°C

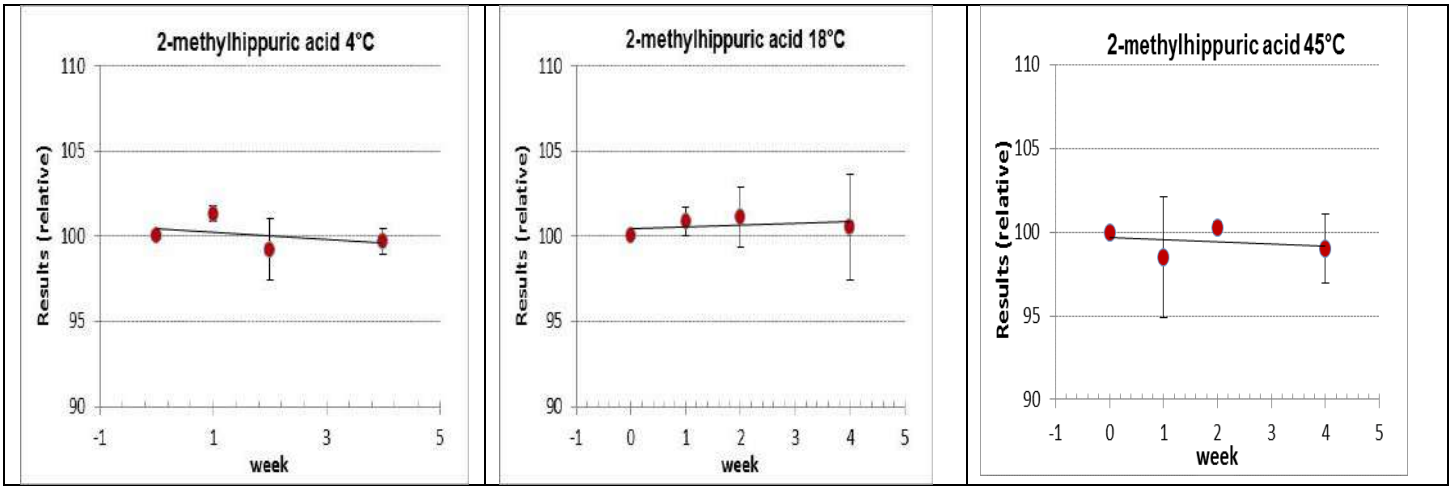

Figure A110. Short Term Stability Plot for 2-methylhippuric acid at 4°C, 18 °C and 45°C

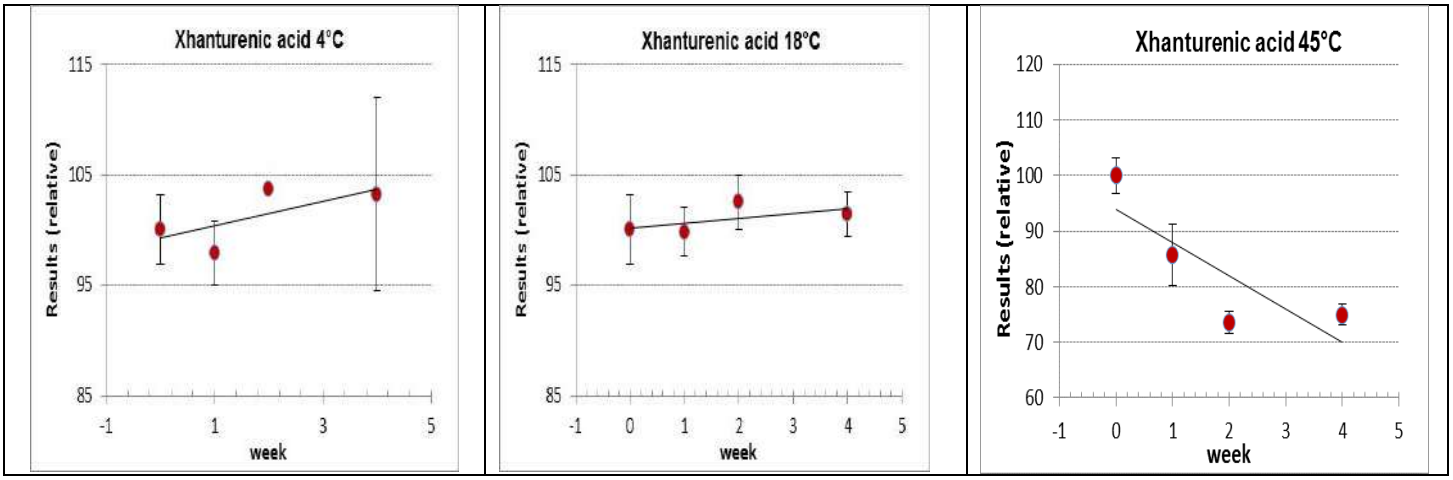

Figure A111. Short Term Stability Plot for Xanthurenic acid at 4°C, 18 °C and 45°C

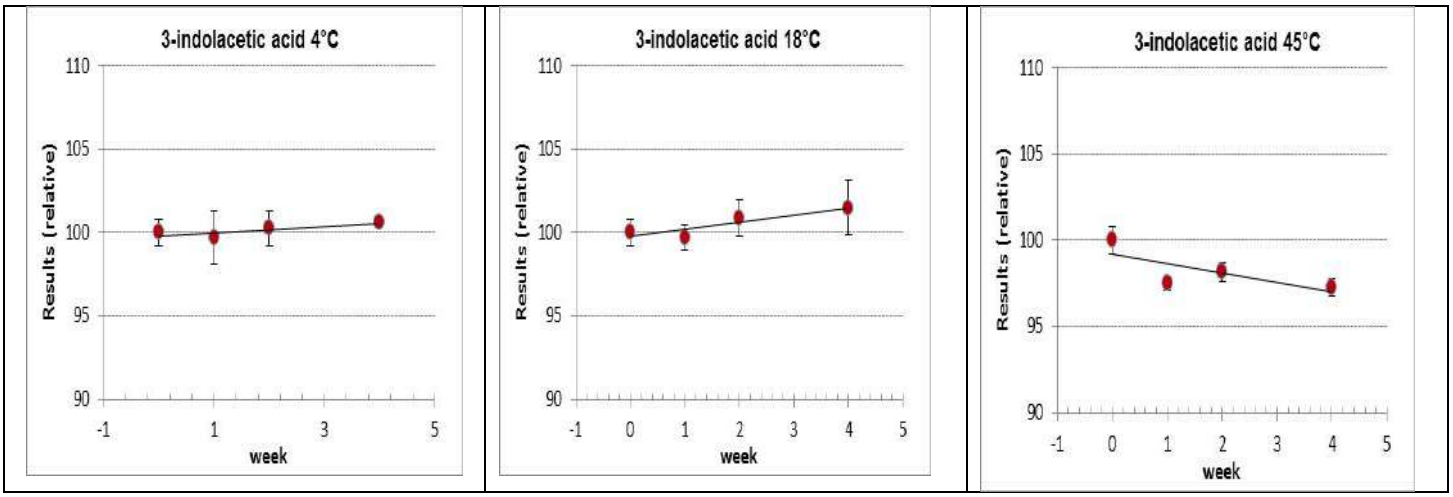

Figure A112. Short Term Stability Plot for 3-indolacetic acid at 4°C, 18 °C and 45°C

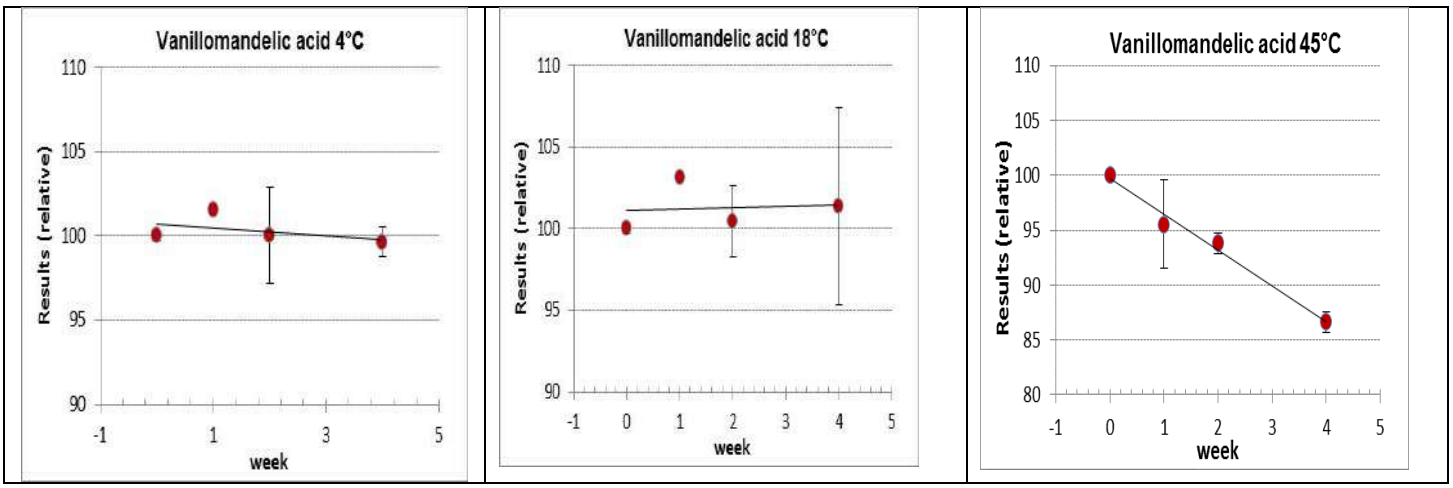

Figure A113. Short Term Stability Plot for Vanillomandelic acid at 4°C, 18 °C and 45°C

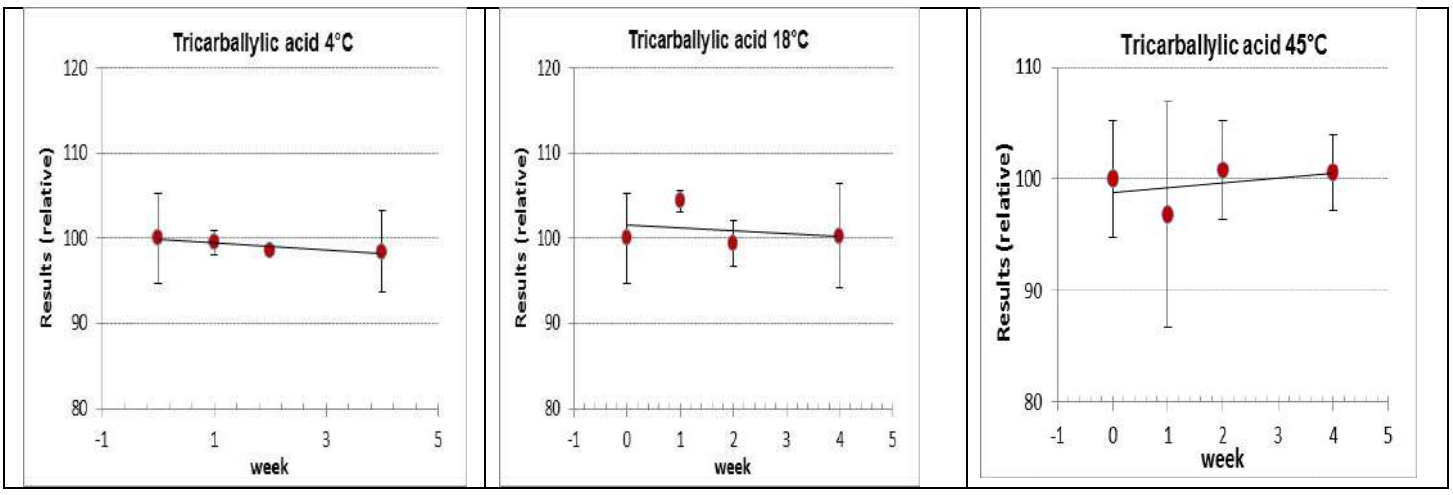

Figure A114. Short Term Stability Plot for Tricarballylic acid at 4°C, 18 °C and 45°C

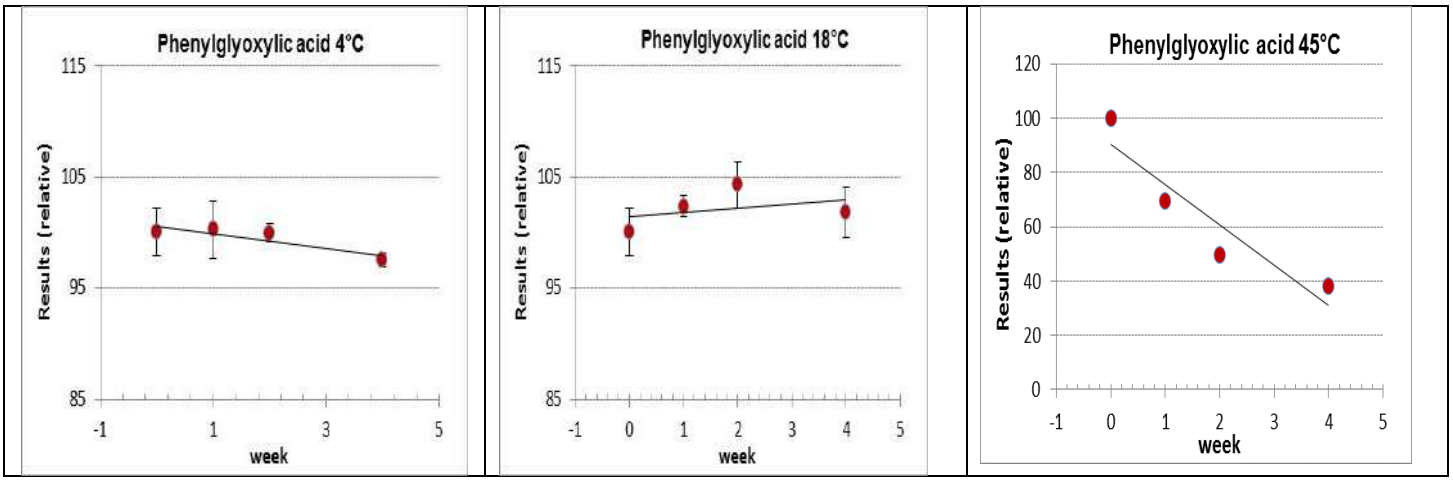

Figure A115. Short Term Stability Plot for Phenylglyoxylic acid at 4°C, 18 °C and 45°C

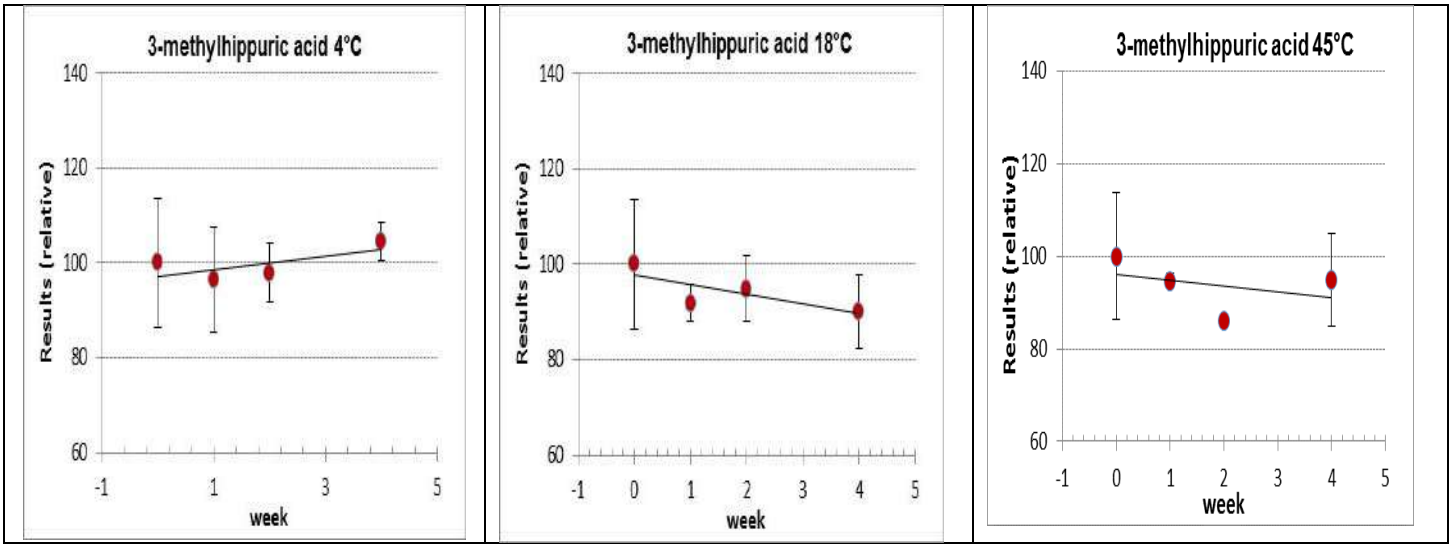

Figure A116. Short Term Stability Plot for 3-methylhippuric acid at 4°C, 18 °C and 45°C

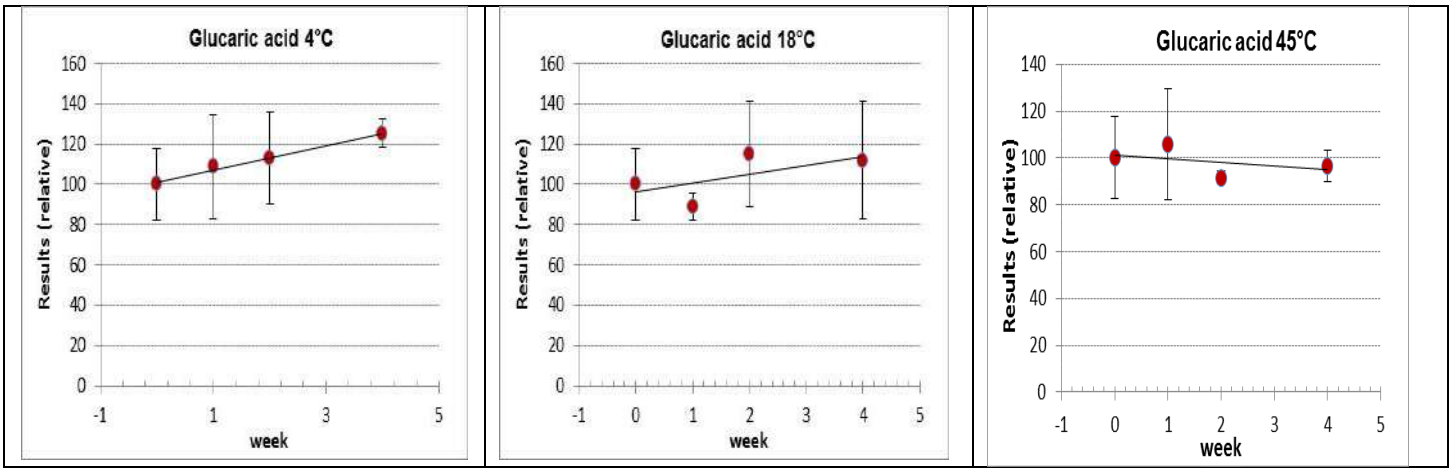

Figure A117. Short Term Stability Plot for Glucaric acid at 4°C, 18 °C and 45°C

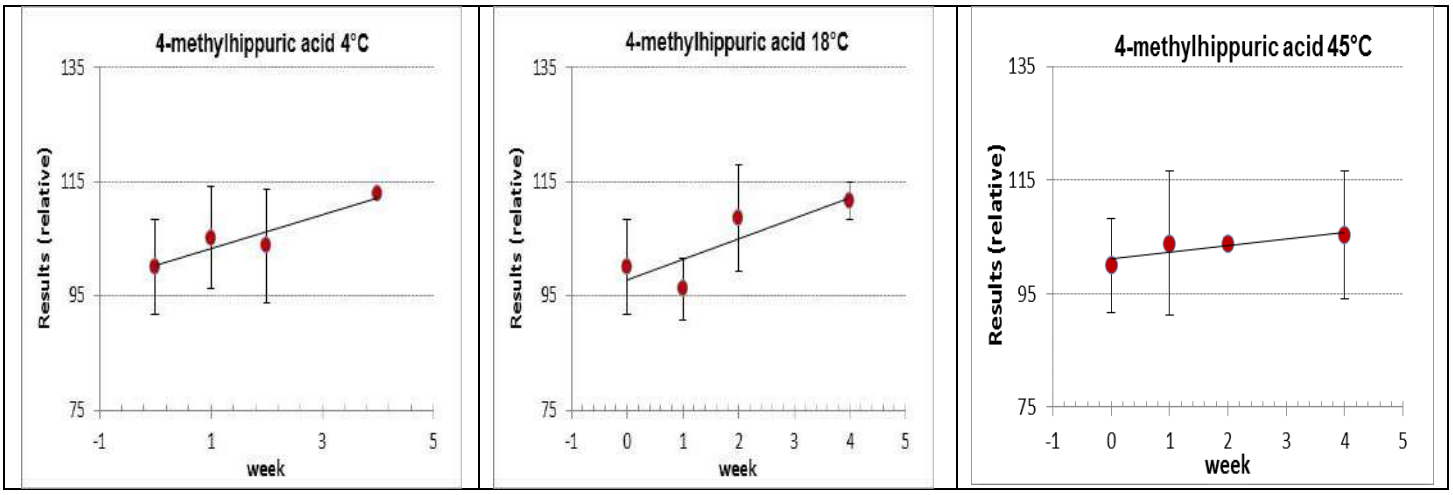

Figure A118. Short Term Stability Plot for 4-methylhippuric acid at 4°C, 18 °C and 45°C

Annex 4. Graphs for Long Term Stability Studies

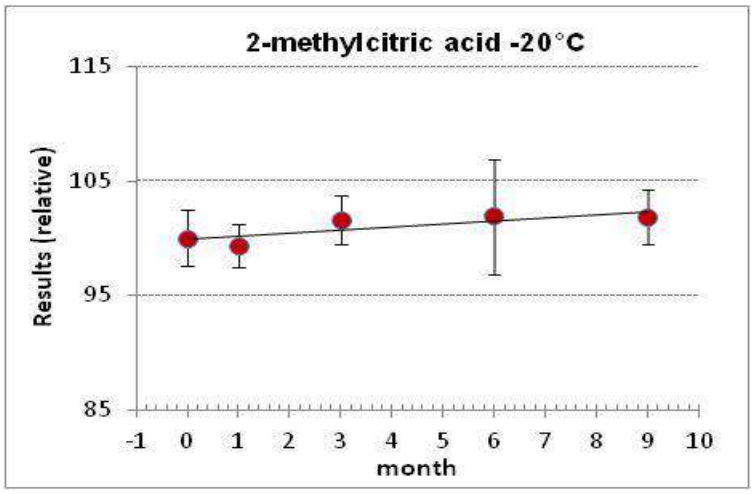

Figure A119. Long Term Stability Plot for 2-methylcitric acid at -20 °C

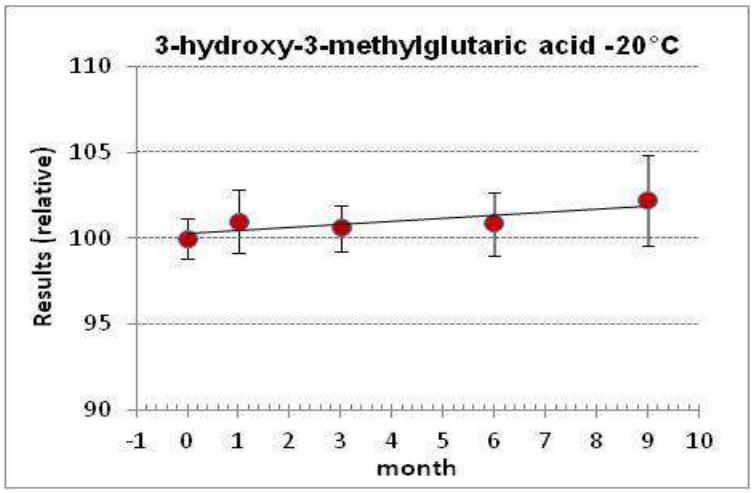

Figure A120. Long Term Stability Plot for 3-hydroxy-3-methylglutaric acid at -20 °C

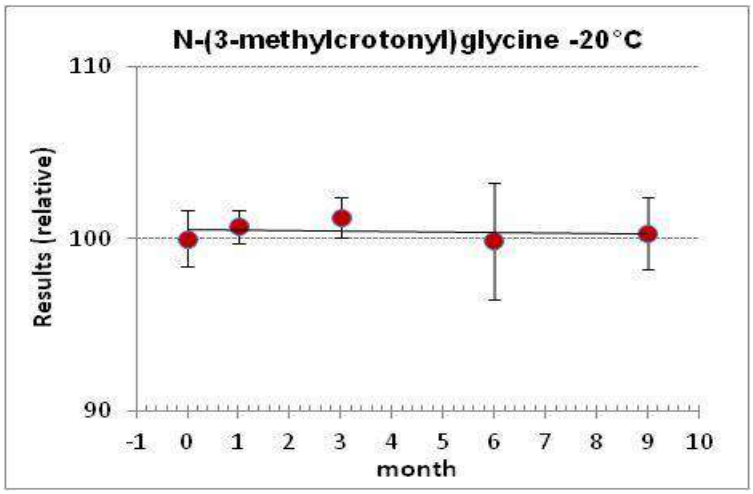

Figure A121. Long Term Stability Plot for N-(3-methylcrotonyl)glycine at -20 °C

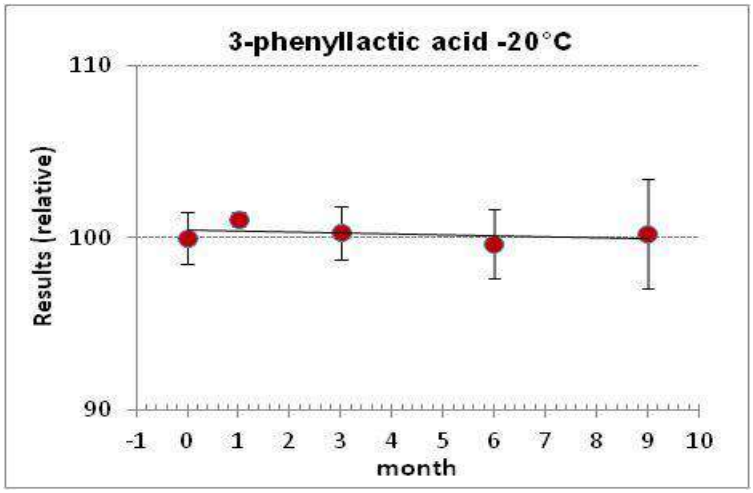

Figure A122. Long Term Stability Plot for 3-phenyllactic acid at -20 °C

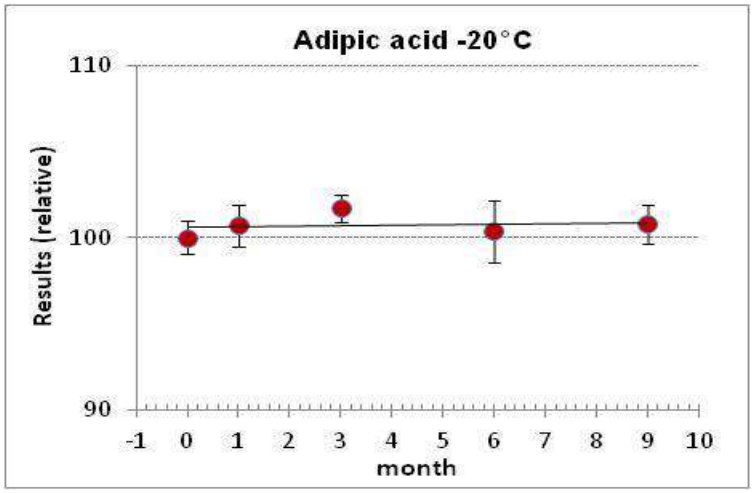

Figure A123. Long Term Stability Plot for Adipic acid at -20 °C

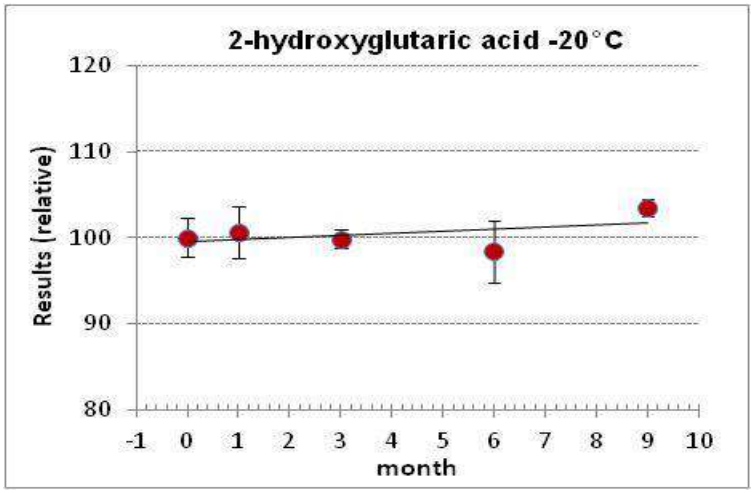

Figure A124. Long Term Stability Plot for 2-hydroxyglutaric acid at -20 °C

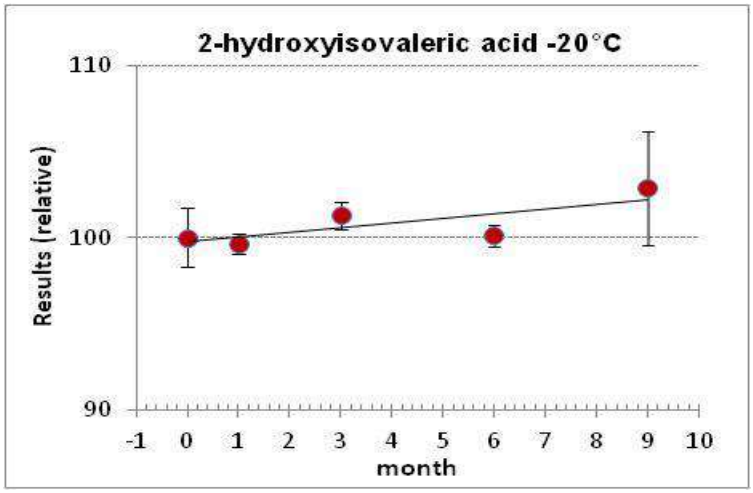

Figure A125. Long Term Stability Plot for 2-hydroxyisovaleric acid at -20 °C

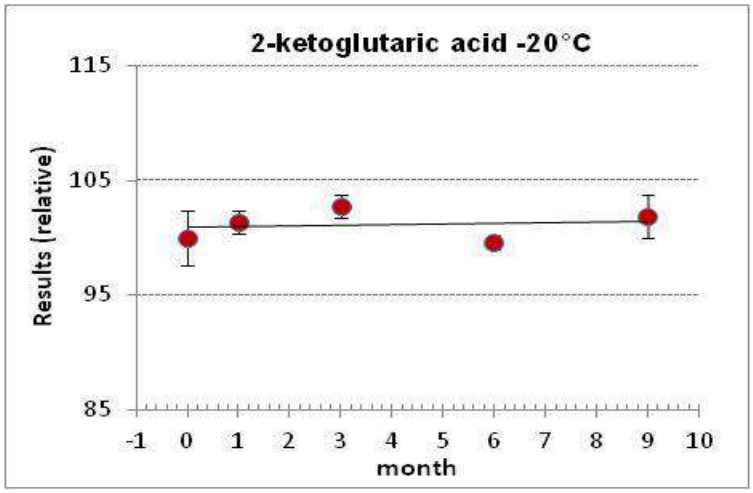

Figure A126. Long Term Stability Plot for 2-ketoglutaric acid at -20 °C

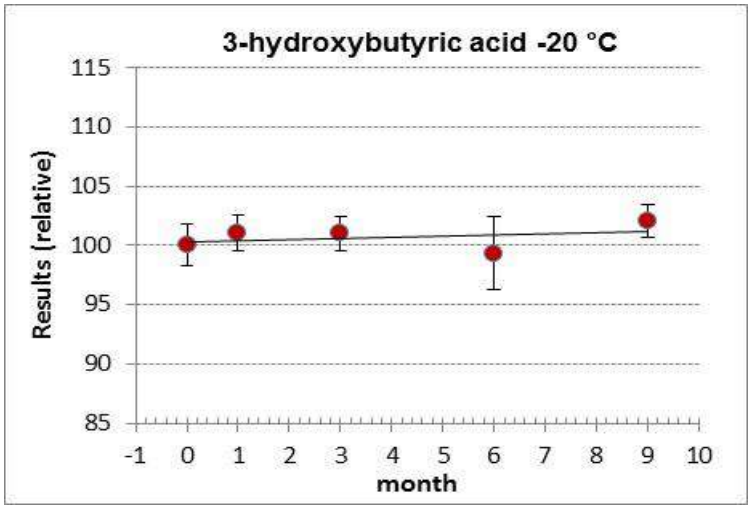

Figure A127. Long Term Stability Plot for 3-hydroxybutyric acid at -20 °C

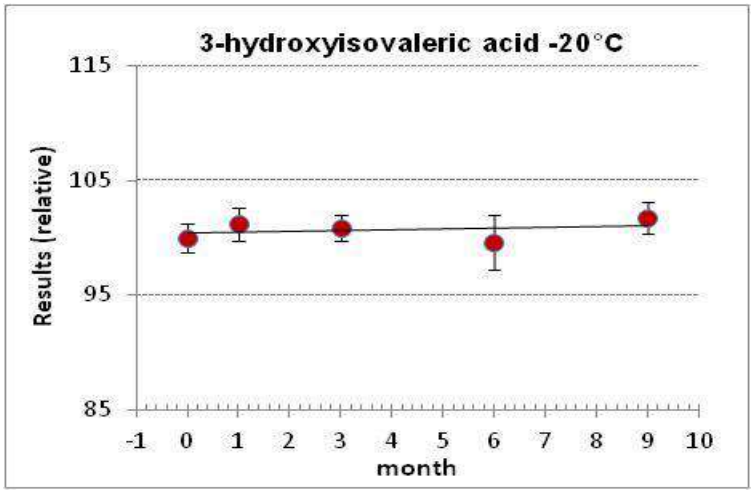

Figure A128. Long Term Stability Plot for 3-hydroxyisovaleric acid at -20 °C

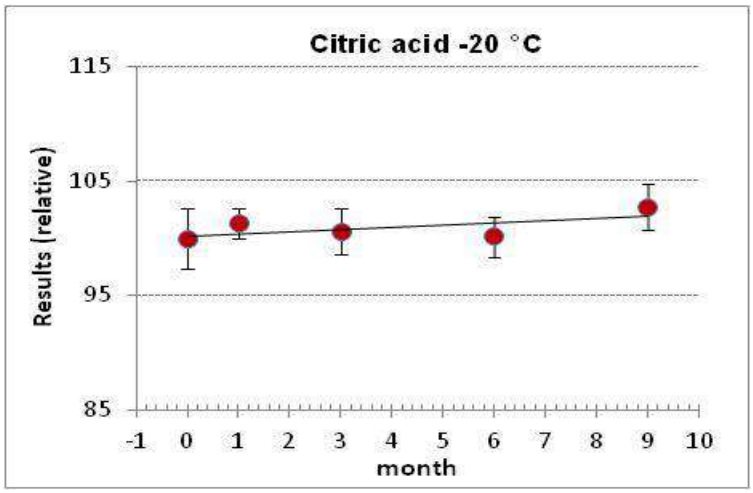

Figure A129. Long Term Stability Plot for Citric acid at -20 °C

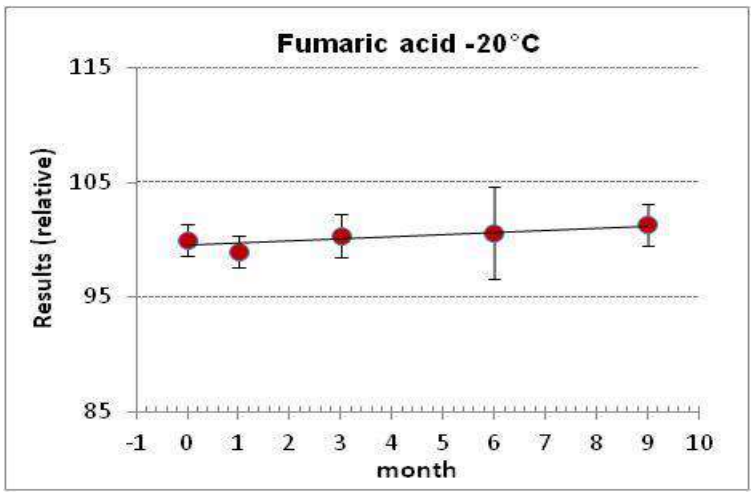

Figure A130. Long Term Stability Plot for Fumaric acid at -20 °C

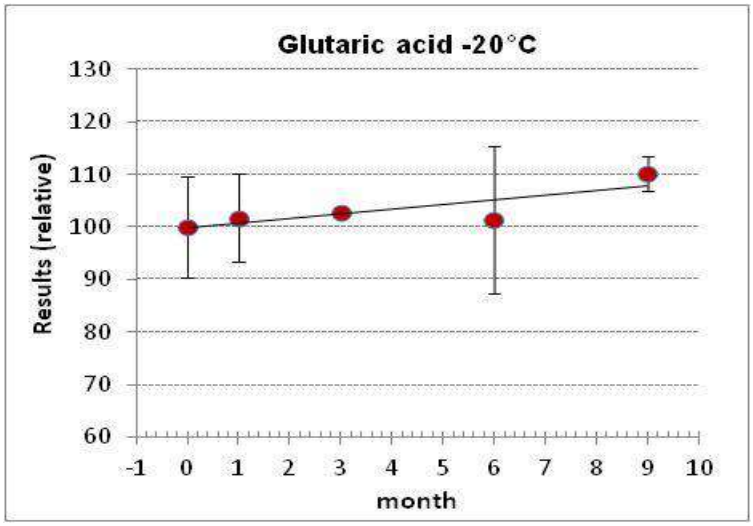

Figure A131. Long Term Stability Plot for Glutaric acid at -20 °C

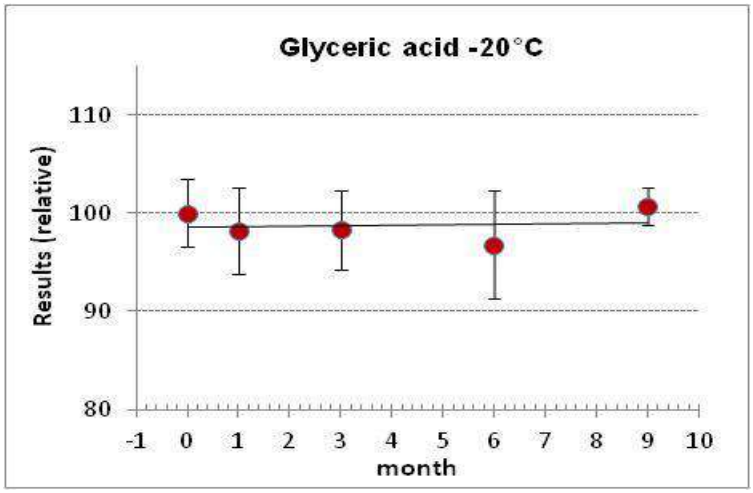

Figure A132. Long Term Stability Plot for Glyceric acid at -20 °C

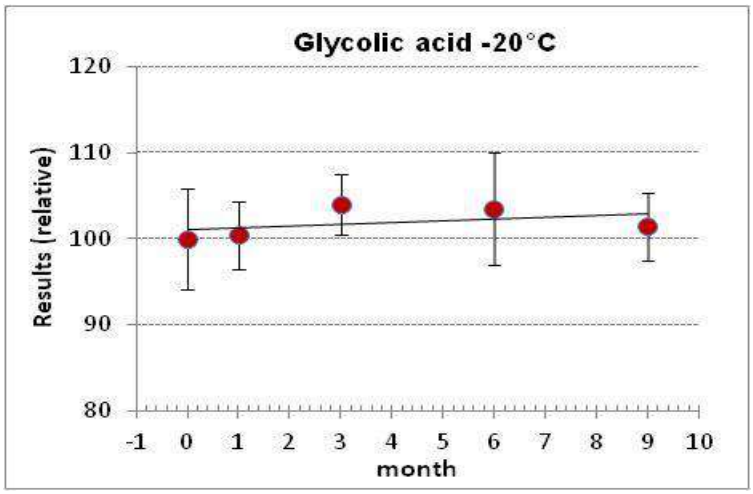

Figure A133. Long Term Stability Plot for Glycolic acid at -20 °C

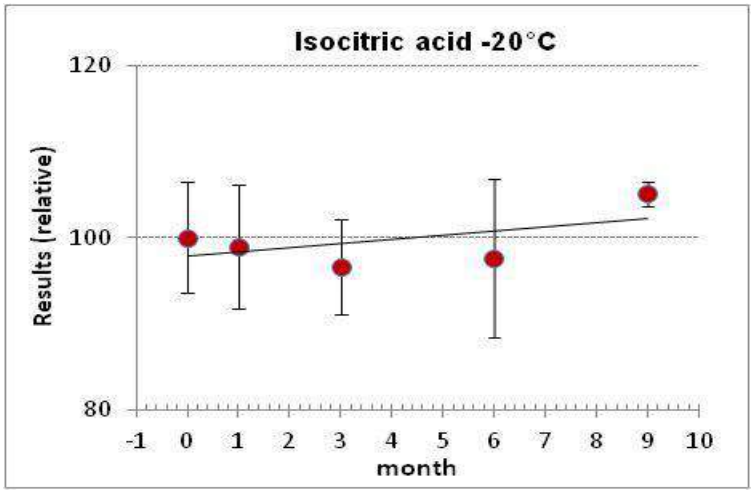

Figure A134. Long Term Stability Plot for Isocitric acid at -20 °C

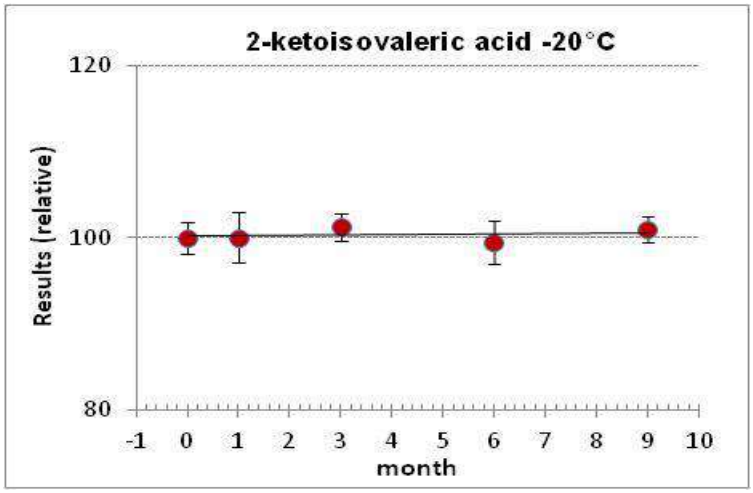

Figure A135. Long Term Stability Plot for 2-ketoisovaleric acid at -20 °C

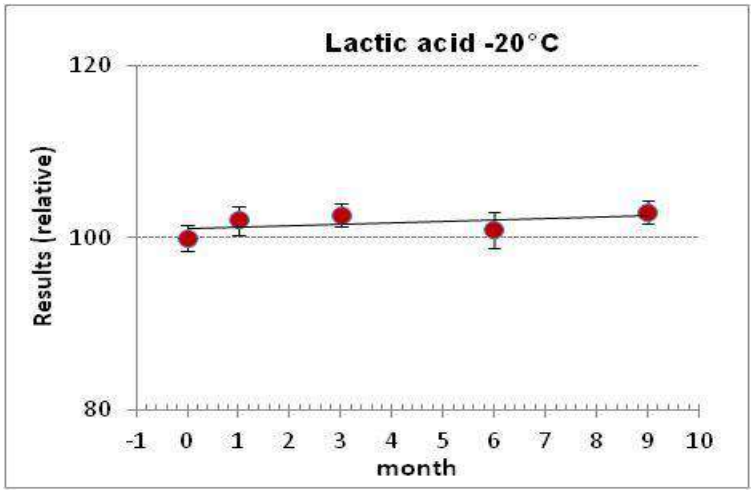

Figure A136. Long Term Stability Plot for Lactic acid at -20 °C

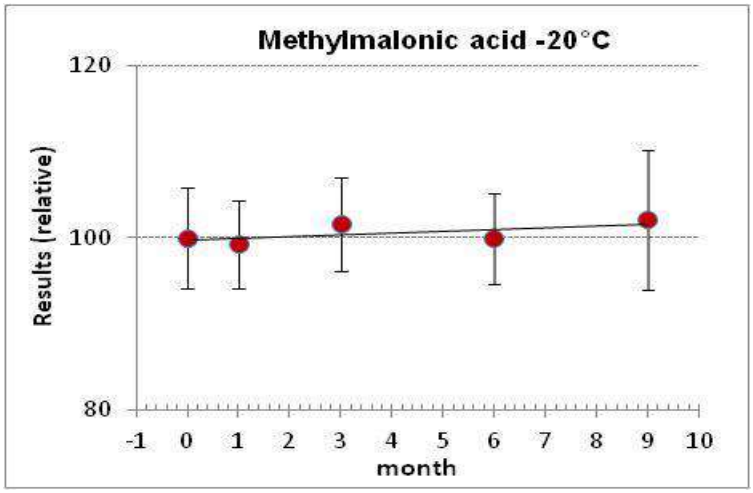

Figure A137. Long Term Stability Plot for Methylmalonic acid at -20 °C

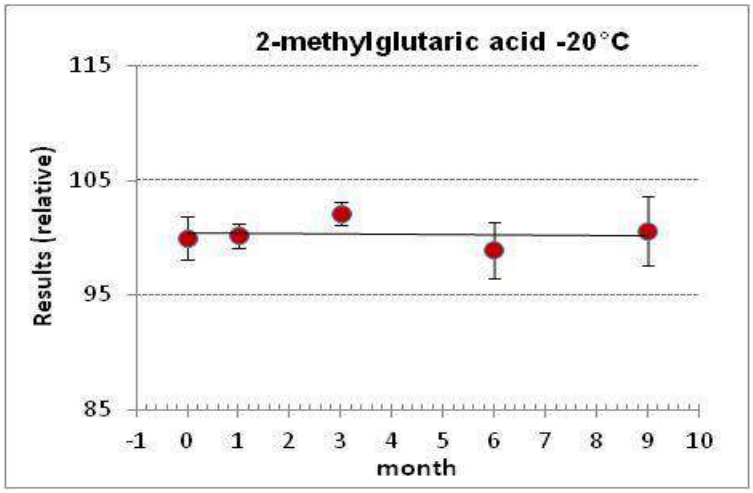

Figure A138. Long Term Stability Plot for 2-methylglutaric acid at -20 °C

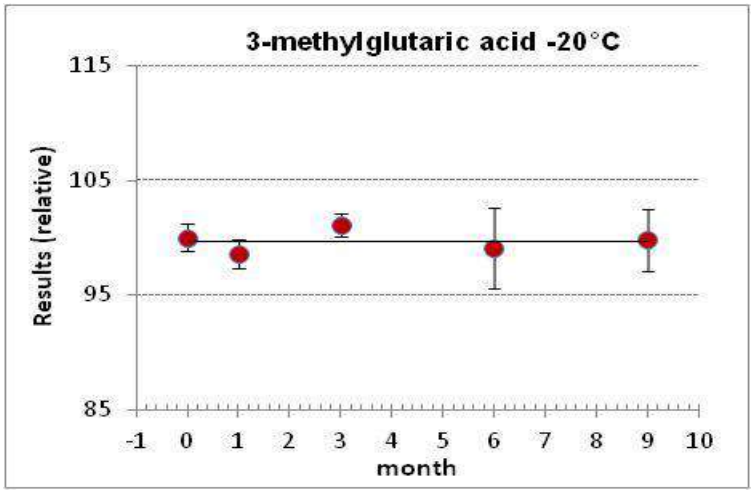

Figure A139. Long Term Stability Plot for 3-methylglutaric acid at -20 °C

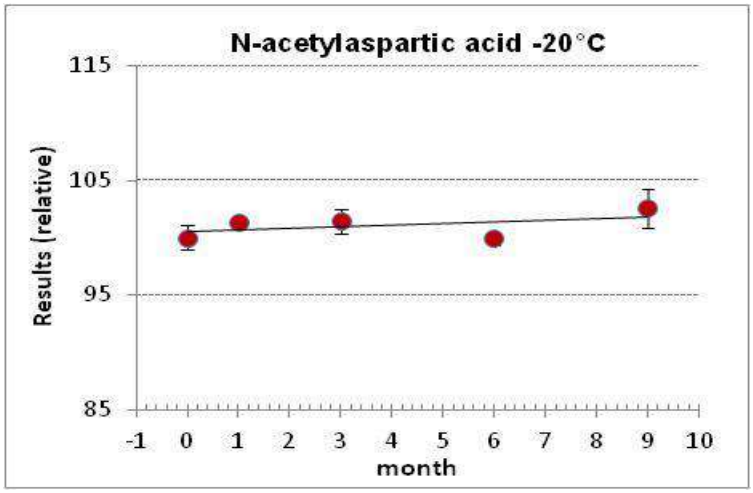

Figure A140. Long Term Stability Plot for N-acetylaspartic acid at -20 °C

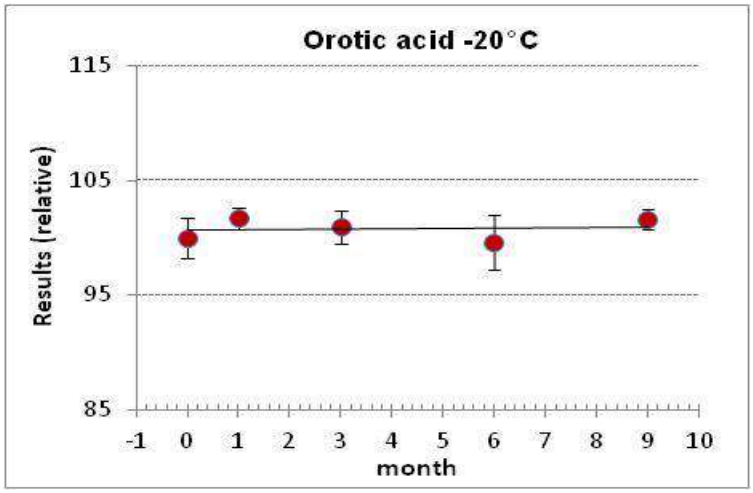

Figure A141. Long Term Stability Plot for Orotic acid at -20 °C

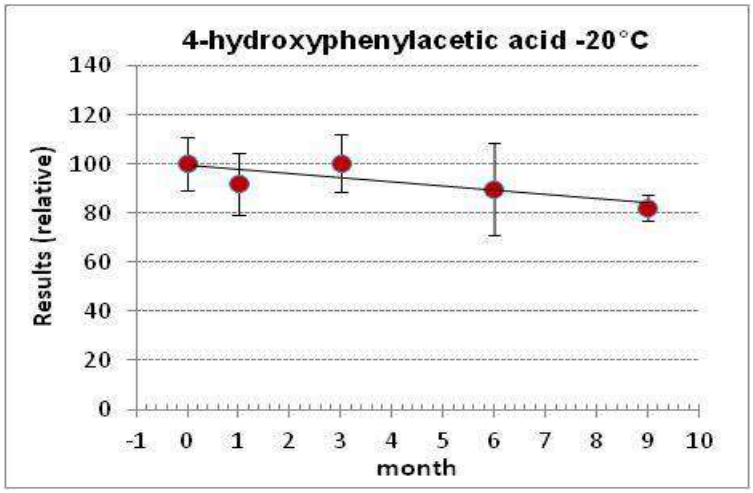

Figure A142. Long Term Stability Plot for 4-hydroxyphenylacetic acid at -20 °C

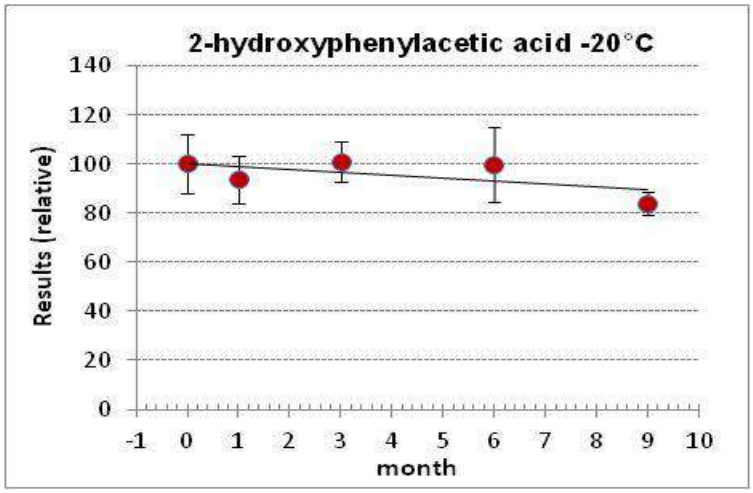

Figure A143. Long Term Stability Plot for 2-hydroxyphenylacetic acid at -20 °C

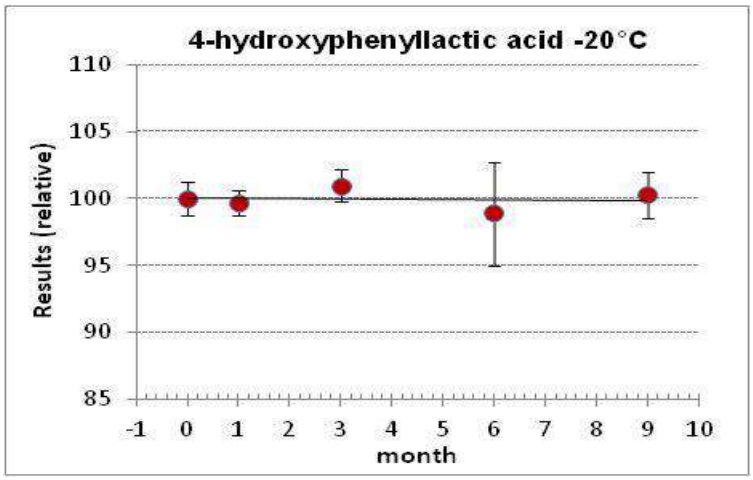

Figure A144. Long Term Stability Plot for 2-hydroxyphenylacetic acid at -20 °C

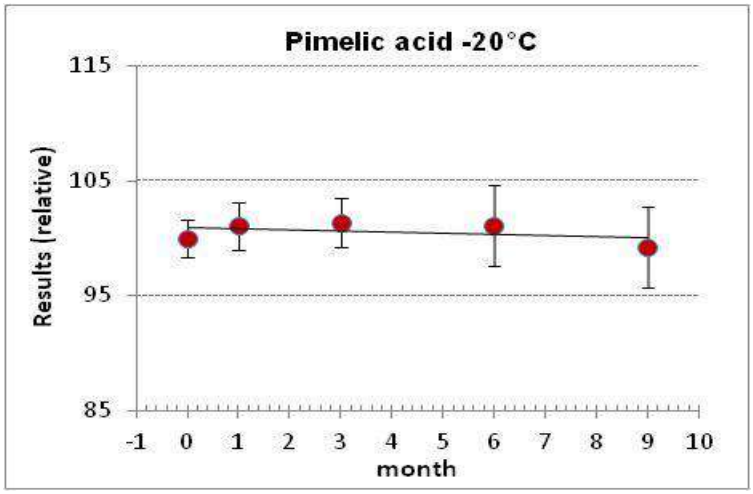

Figure A145. Long Term Stability Plot for Pimelic acid at -20 °C

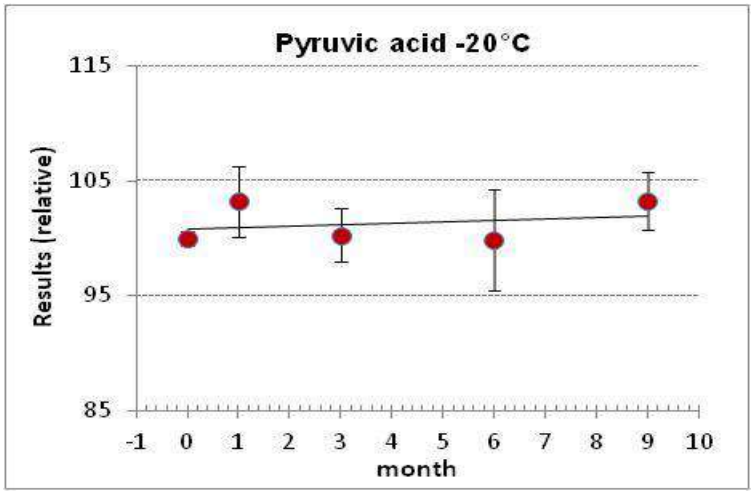

Figure A146. Long Term Stability Plot for Pyruvic acid at -20 °C

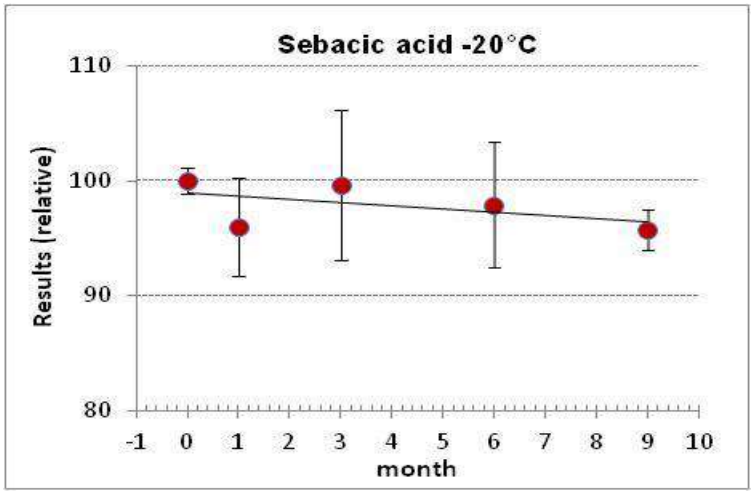

Figure A147. Long Term Stability Plot for Sebacic acid at -20 °C

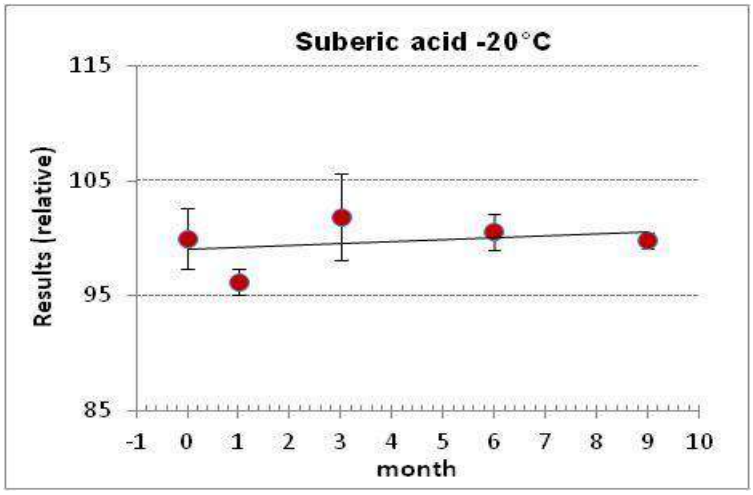

Figure A148. Long Term Stability Plot for Suberic acid at -20 °C

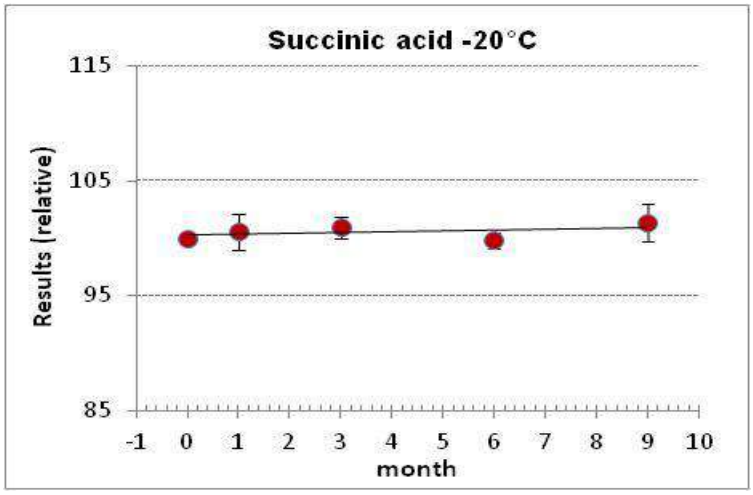

Figure A149. Long Term Stability Plot for Succinic acid at -20 °C

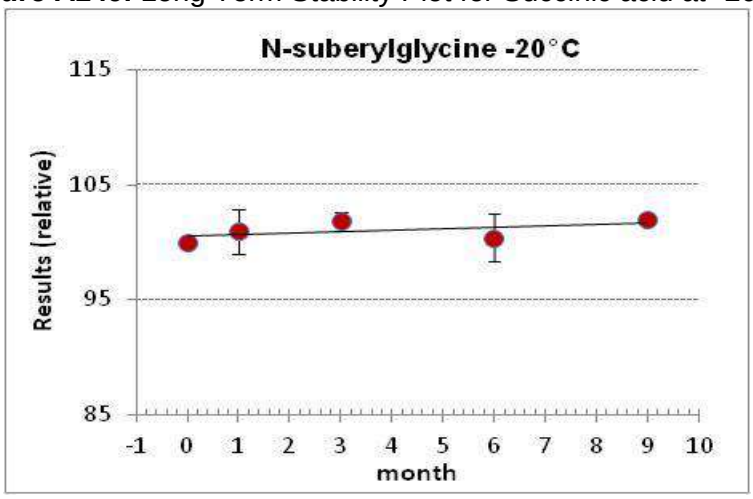

Figure A150. Long Term Stability Plot for N-suberylglycine at -20 °C

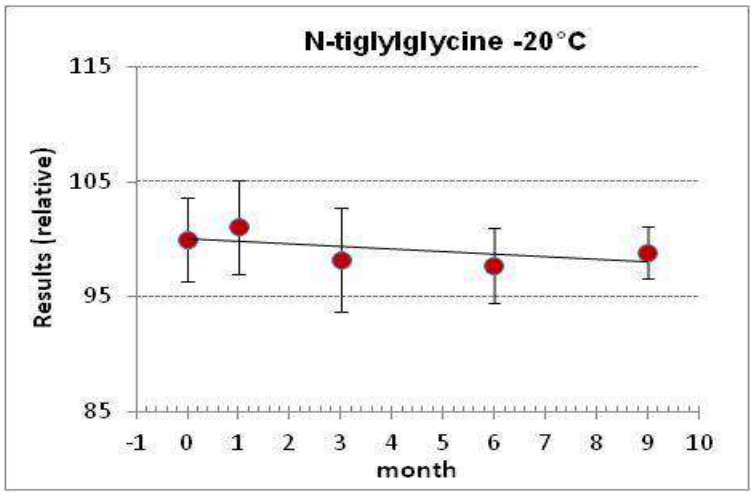

Figure A151. Long Term Stability Plot for N-tiglylglycine at -20 °C

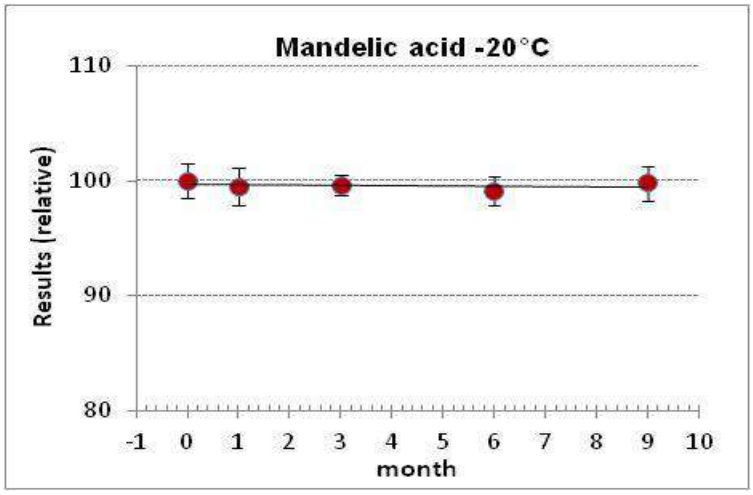

Figure A152. Long Term Stability Plot for Mandelic at -20 °C

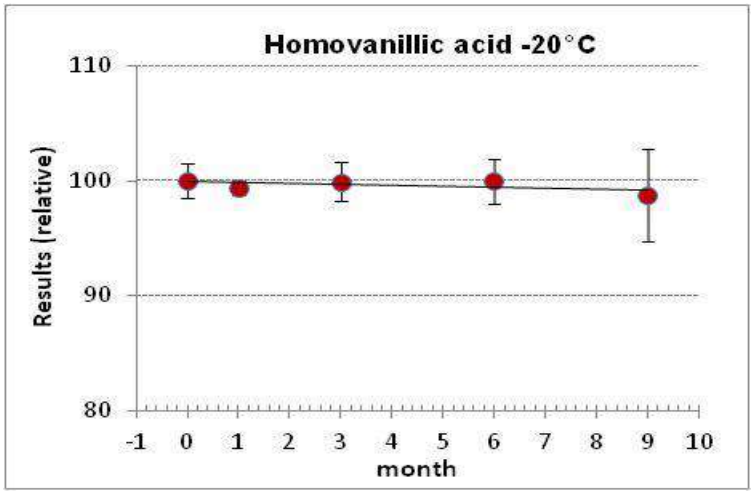

Figure A153. Long Term Stability Plot for Homovanillic at -20 °C

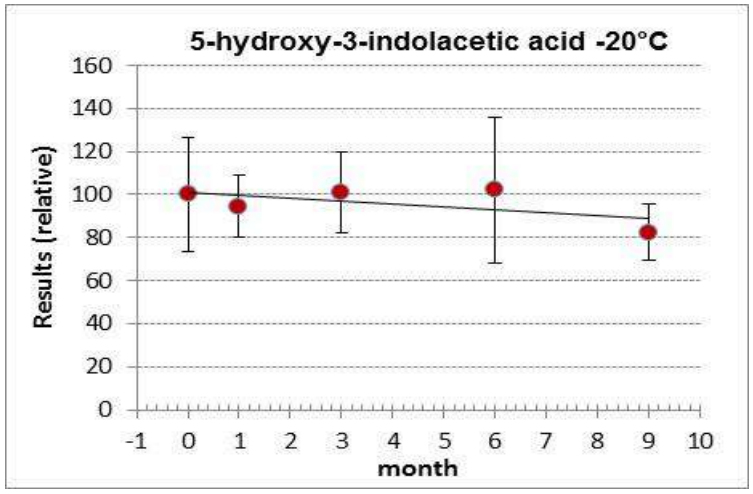

Figure A154. Long Term Stability Plot for 5-hydroxy-3-indolacetic at -20 °C

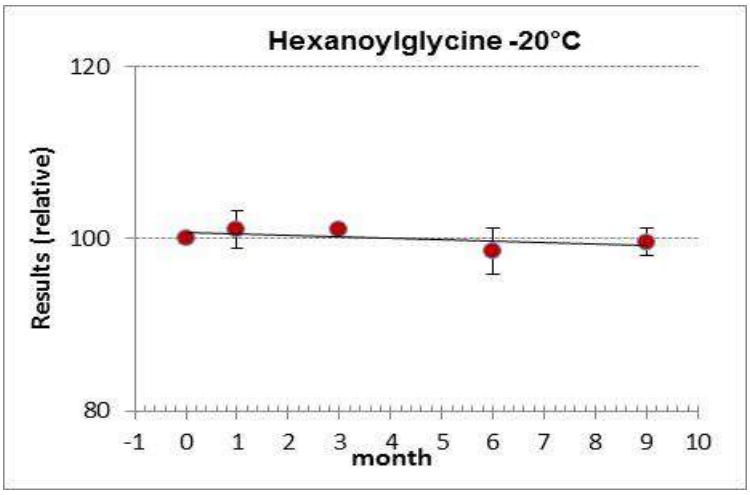

Figure A155. Long Term Stability Plot for Hexanoylglycine at -20 °C

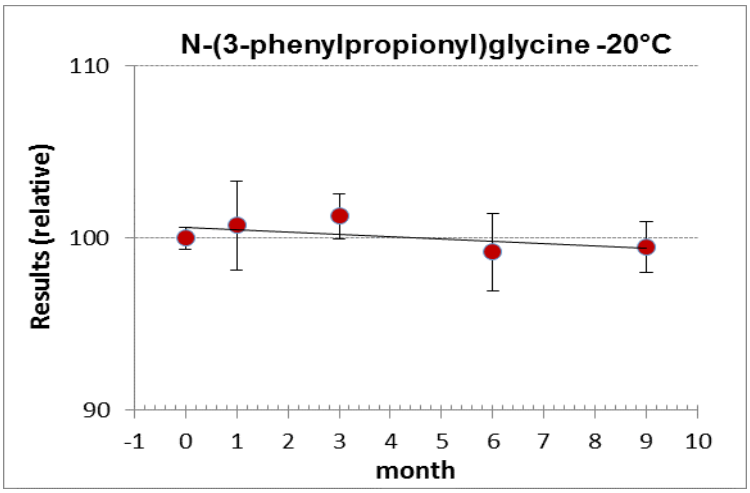

Figure A156. Long Term Stability Plot for N-(3-phenylpropionyl)glycine at -20 °C

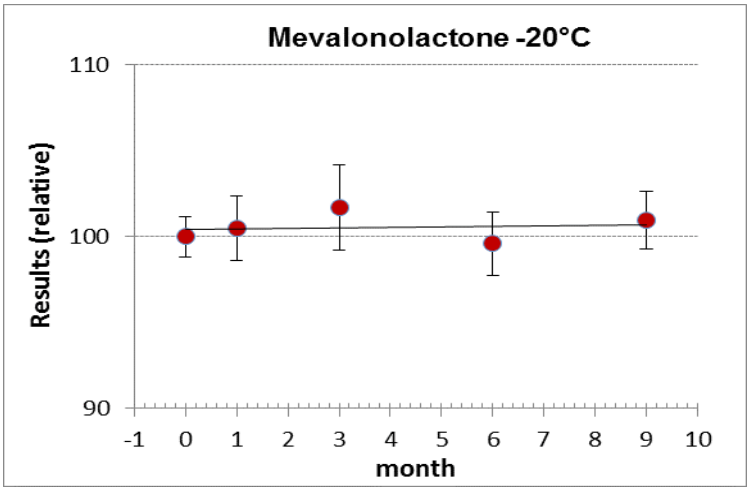

Figure A157. Long Term Stability Plot for Mevalonolactone at -20 °C

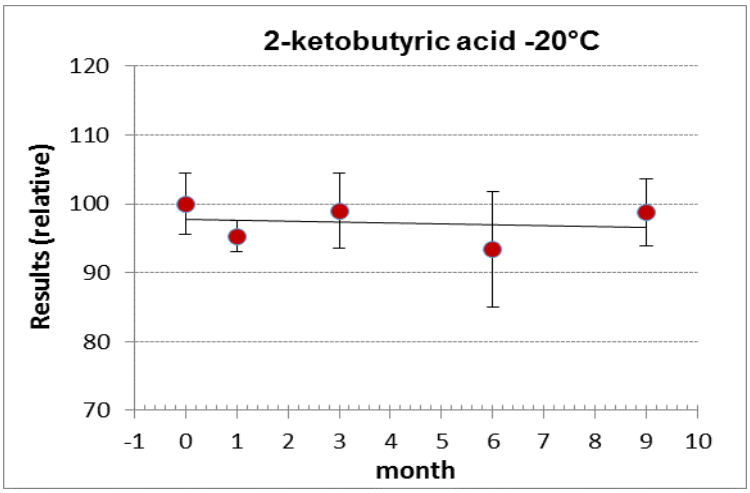

Figure A158. Long Term Stability Plot for 2-ketobutyric acid at -20 °C

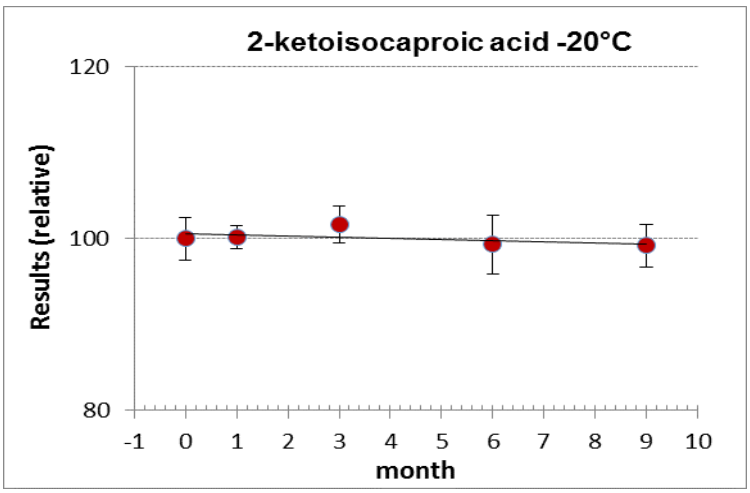

Figure A159. Long Term Stability Plot for 2-ketoisocaproic at -20 °C

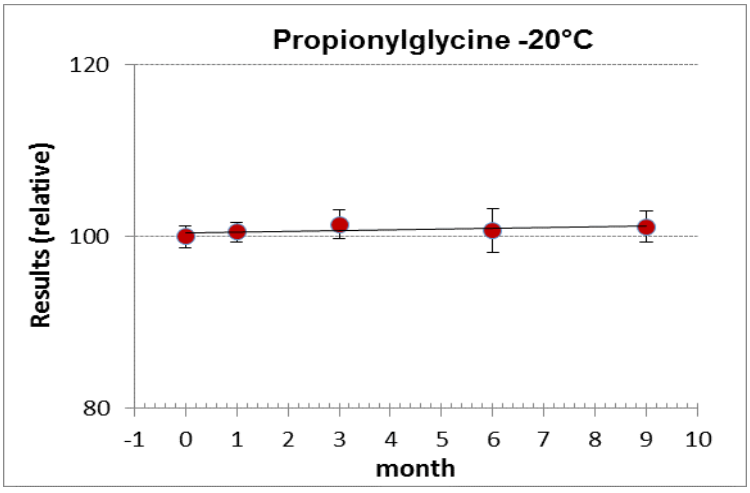

Figure A160. Long Term Stability Plot for Propionylglycine at -20 °C

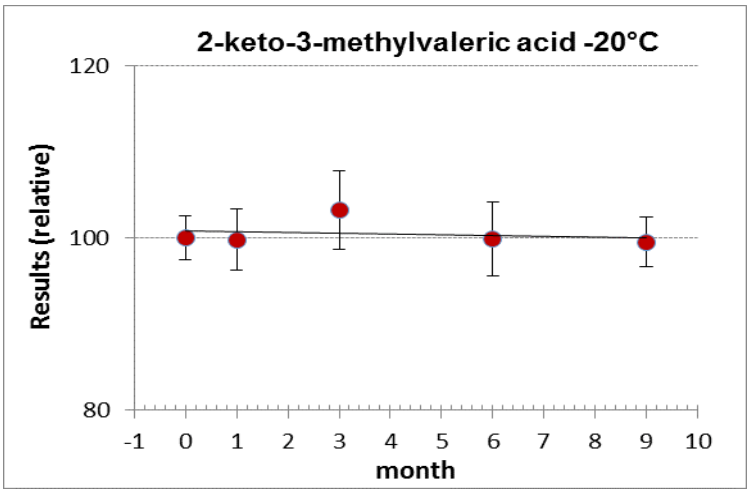

Figure A161. Long Term Stability Plot for 2-keto-3-methylvaleric acid at -20 °C

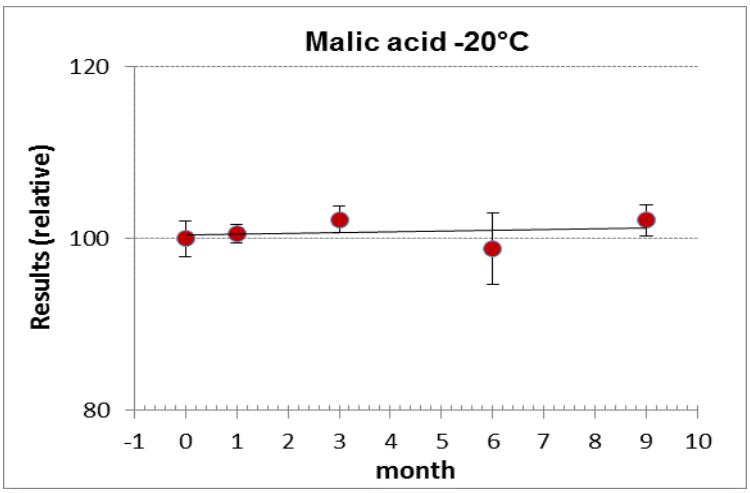

Figure A162. Long Term Stability Plot for Malic acid at -20 °C

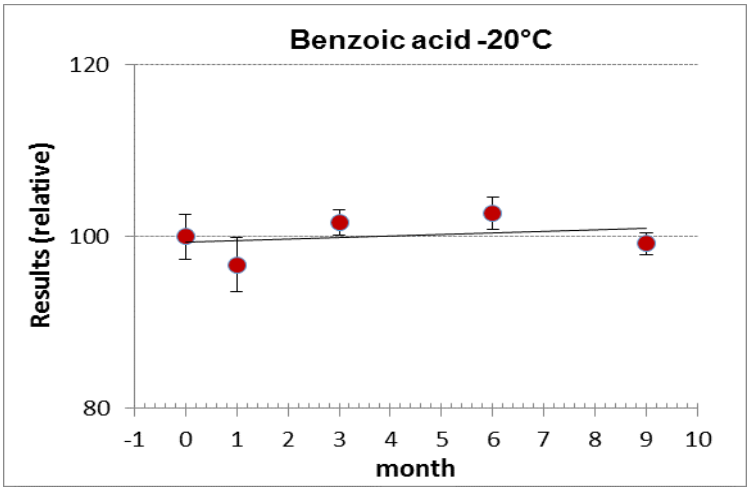

Figure A163. Long Term Stability Plot for Benzoic acid at -20 °C

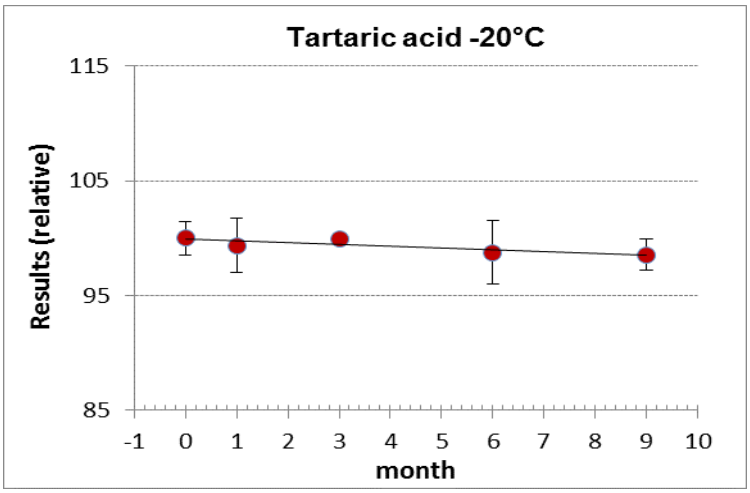

Figure A164. Long Term Stability Plot for Tartaric acid at -20 °C

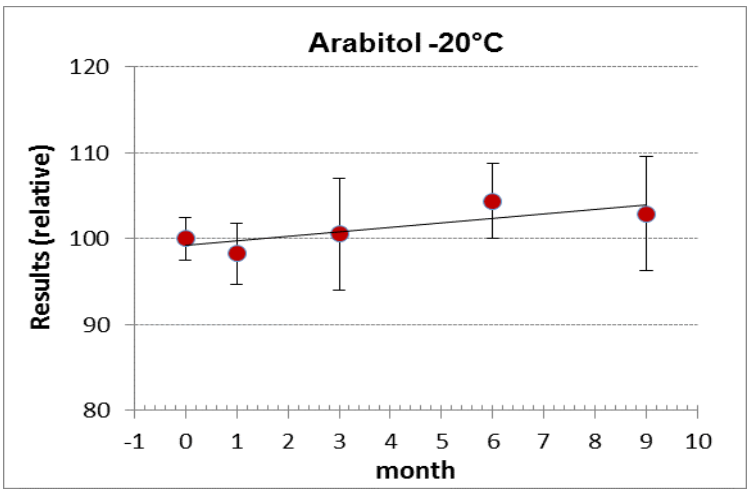

Figure A165. Long Term Stability Plot for Arabitol at -20 °C

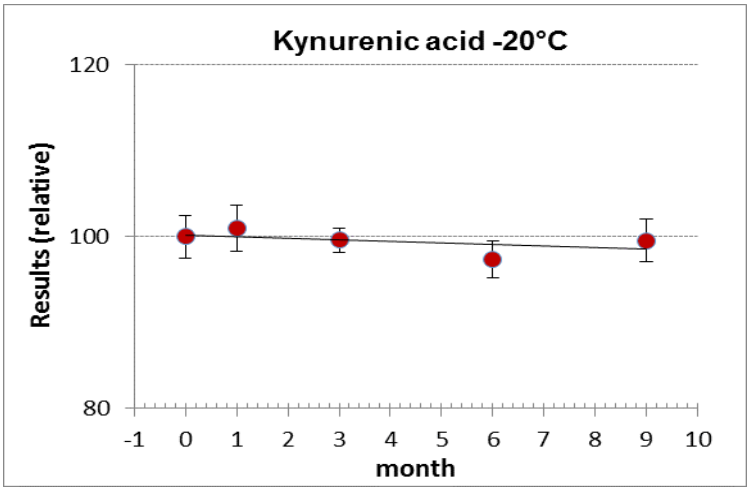

Figure A166. Long Term Stability Plot for Kynurenic acid at -20 °C

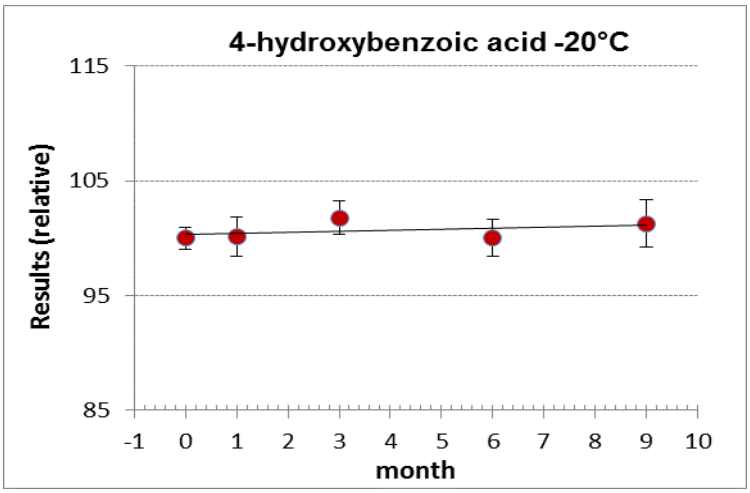

Figure A167. Long Term Stability Plot for 4-hydroxybenzoic acid at -20 °C

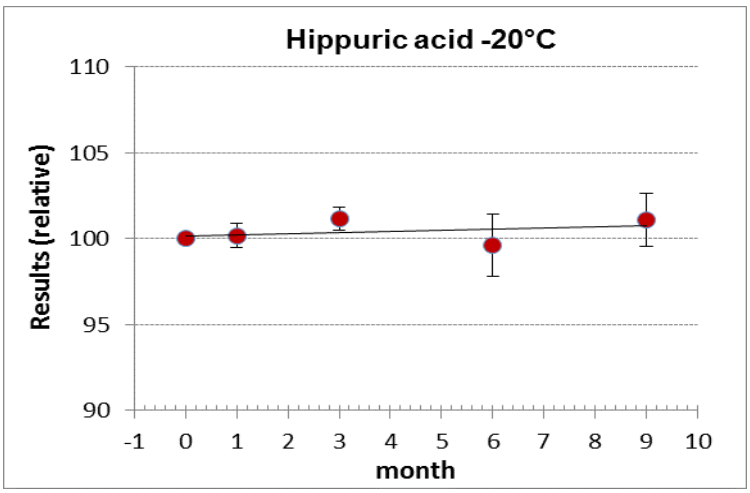

Figure A168. Long Term Stability Plot for Hippuric acid at -20 °C

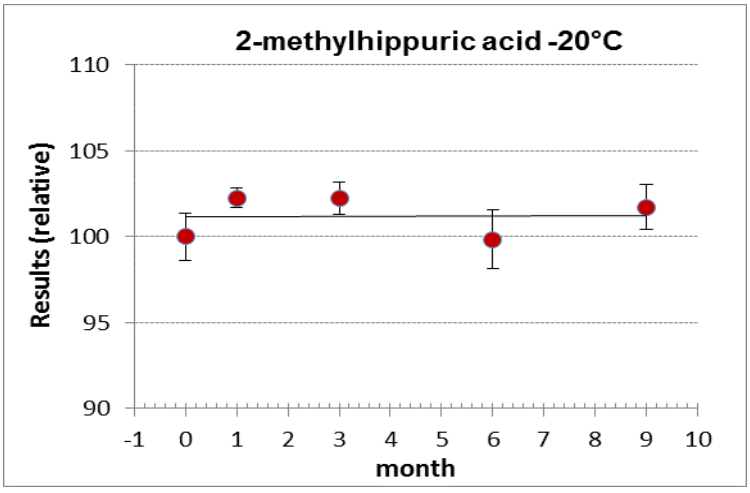

Figure A169. Long Term Stability Plot for 2-methylhippuric acid at -20 °C

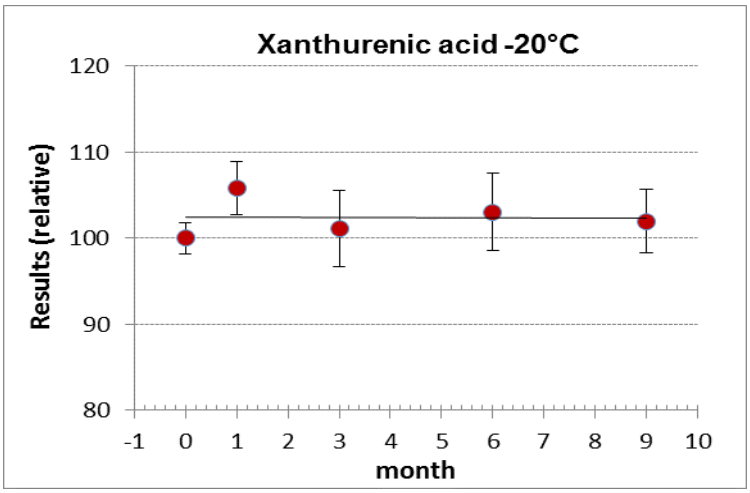

Figure A170. Long Term Stability Plot for Xanthurenic acid at -20 °C

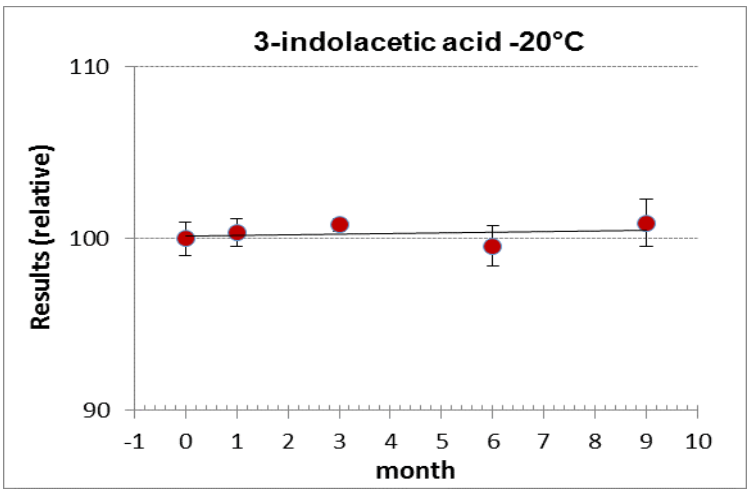

Figure A171. Long Term Stability Plot for 3-indolacetic acid at -20 °C

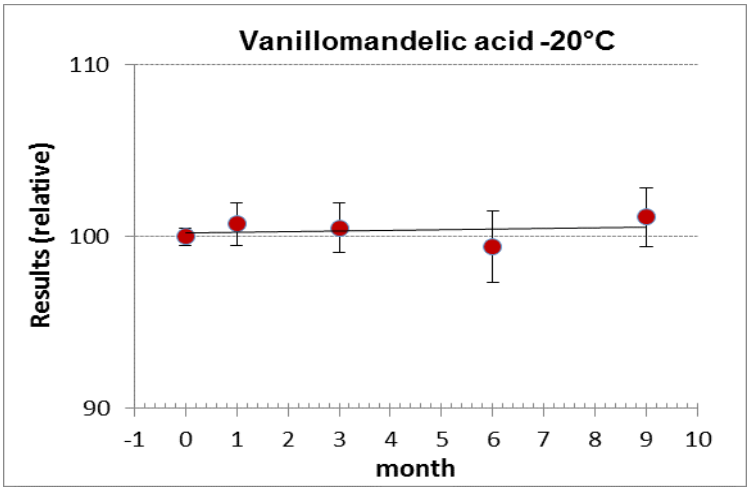

Figure A172. Long Term Stability Plot for Vanillomandelic acid at -20 °C

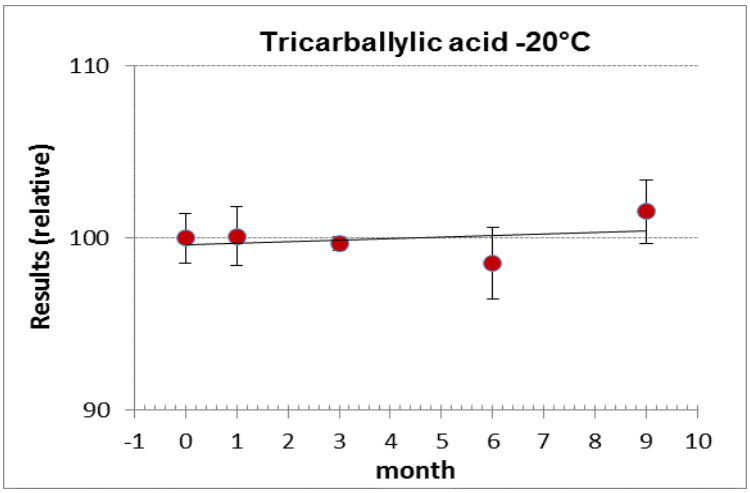

Figure A173. Long Term Stability Plot for Tricarballylic acid at -20 °C

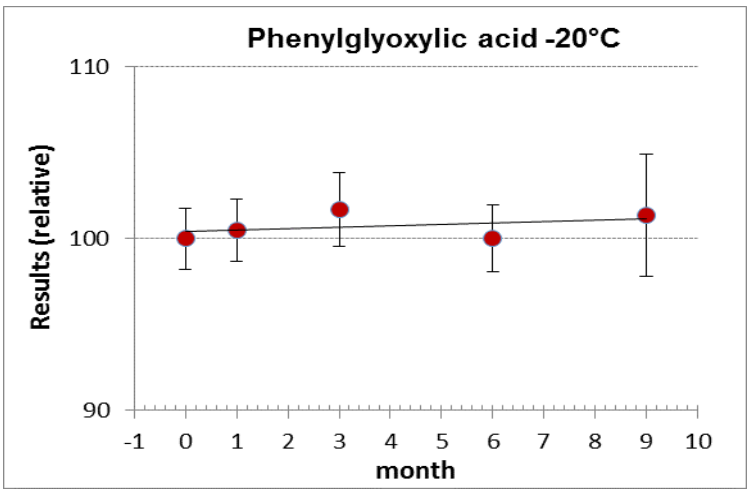

Figure A174. Long Term Stability Plot for Phenylglyoxylic acid at -20 °C

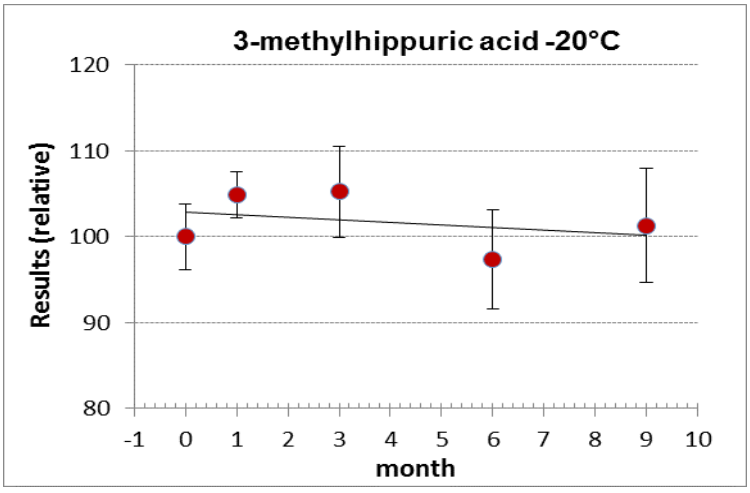

Figure A175. Long Term Stability Plot for 3-methylhippuric acid at -20 °C

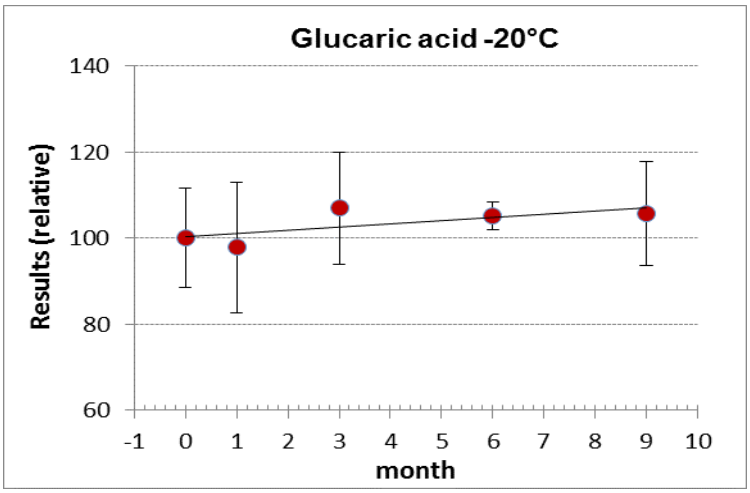

Figure A176. Long Term Stability Plot for Glucaric acid at -20 °C

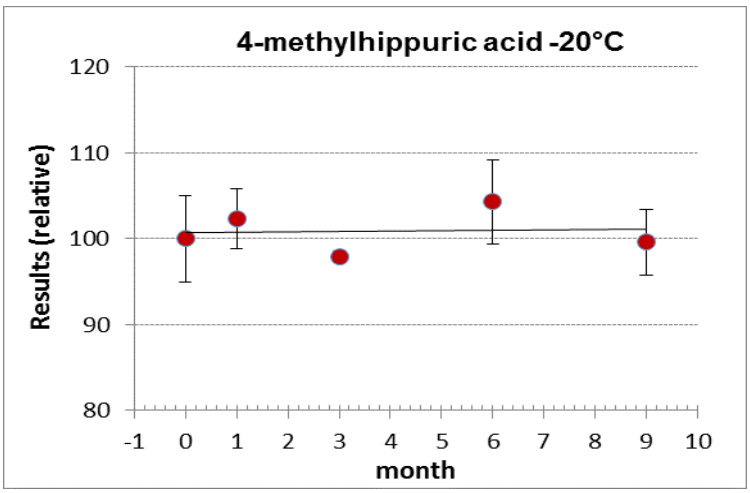

Figure A177. Long Term Stability Plot for 4-methylhippuric at -20 °C