ZİVAK®

IngenieríaAnalítica
Chromatography & Spectrometry

IVD CE ISO 13485 ISO 9001

ZİVAK®
TECHNOLOGIES

Organic Acid Urine
LC-MS/MS Analysis Kit

ZV-3004-0200-20

200

2-8 °C

User Manual

Distribuido en España por:
INGENIERIA ANALITICA S.L.
Tel: (+34) 902.45.6677
Fax: (+34) 902.46.6677
www.ingenieria-analitica.com
inf@ingenieria-analitica.com

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1. INTENDED USE:

Quantitative LC-MS/MS analysis kit for Organic Acids in human urine samples.

2. SUMMARY AND EXPLANATION:

Organic acid disorders (organic acidemia) are a group of inherited metabolic conditions. Most of the organic acidemias caused by defective autosomal genes for various enzymes are for amino acid metabolism. Neurological and physiological harms are caused by this impaired ability to synthesise a key enzyme required to break down a specific amino acid, or a group of amino acids, resulting in acidemia and toxicity to specific organs systems. Most are inherited as autosomal recessive diseases. Organic acid related disorders may affect many metabolic pathways including amino acid, lipid metabolism, fatty acid oxidation and the Krebs cycle.

Organic acidemias are usually diagnosed in infancy, characterized by urinary excretion of abnormal amounts or types of organic acids. The diagnosis is usually made by detecting an abnormal pattern of organic acids in a urine sample. In some conditions, the urine is always abnormal; in the others the characteristic substances are only present intermittently. Many of the organic acidemias are detectable by newborn screening with Tandem Mass spectrometry. These disorders vary in their prognosis, from manageable to fatal, and usually affect more than one organ system, especially the central nervous system.

Quantitative analysis of the organic acids in urine samples has clinical importance for determining organic acidemias and monitoring the organic acid concentrations in human urine. ZIVAK Quantitative Organic Acid LC-MS/MS kit was developed for quantitative detection of the 73 major organic acids in human urine samples. LC-MS/MS is an influential device for the study of metabolic disorders and LC-MS/MS analysis gives results in 20 minutes with minimal sample preparation. Main methods and procedures that have been selected are based on EN ISO 13485 and 98/79/EC.

3. TEST PRINCIPLE:

A dilute and shoot method used for organic acids analysis in urine samples using an acidic dilution solution. After the dilution, organic acids are separated on a reversed phase HPLC column with a gradient elution and analysed by Tandem Mass spectrometry.

4. WARNING AND PRECAUTIONS:

- For in-vitro diagnostic use only.
- For professional use only.
- Read the instructions carefully, before you start.
- In case of damage of the kit package, please contact Zivak or your supplier.
- Do not use expired kits and components.
- Please check the batch no and expiry date before start.
- Although the concentration of all toxic components is low, for safety reasons the sample preparation station should be placed in an exhaust hood.
- Protective gloves and goggles should be worn.
- Please take any necessary precautions to prevent infection with pathogens while working with biological fluids. Appropriate bio-safety precautions and disposal of bio-hazardous wastes should be followed
- Please check the labels on reagent bottles. Reagents of this kit contain hazardous material may cause eye and skin irritations.

5. STORAGE AND STABILITY:

- This analysis kit can be shipped at room temperature.
- After arrival, reagent 1,2,3 , calibrators and controls should be stored at 2-8 °C.
- All other components of the kit should be stored at room temperature.
- All components are guaranteed until expiry date when stored at recommended temperatures and used as described in these instructions.

6. MATERIALS SUPPLIED:

Order No.	Volume	Symbol	Component
ZV-3004-02R1-20	2 x 6 ml	R1	Reagent 1, Contains internal standards
ZV-3004-02R2-20	1 x 6 ml	R2	Reagent 2, Contains acid
ZV-3004-02R3-20	1 x 6 ml	R3	Reagent 3, Contains stabiliser
ZV-3004-02MA-20	2 x 0,5 L	MA	Mobile Phase A, Contains organic solvent
ZV-3004-02MB-20	1 x 0,5 L	MB	Mobile Phase B, Contains organic solvent
ZV-3004-02WB-10	2 x 0,5 L	WB	Washing solution, Contains organic solvent
ZS-2ML-8-1001	2x100pcs		2 ml glass autosampler vial
ZS-9004-0100-00	2x100pcs		2 ml glass autosampler vial caps pre-slit
ZV-3004-KK-20	1 x 1 pc	KK	User Guide

7. MATERIALS REQUIRED BUT NOT SUPPLIED:

ZV-3004-02S1-20	1 x 3 ml	Calibrator Level 1	Lyophilized Urine Calibrator Level 1
ZV-3004-02S2-20	1 x 3 ml	Calibrator Level 2	Lyophilized Urine Calibrator Level 2
ZV-3004-02S3-20	1 x 3 ml	Calibrator Level 3	Lyophilized Urine Calibrator Level 3
ZV-3004-02K1-20	1 x 3 ml	Control Level 1	Lyophilized Urine Control Level 1
ZV-3004-02K2-20	1 x 3 ml	Control Level 2	Lyophilized Urine Control Level 2

- Zivak Organic Acid Urine LC-MSMS Analytical Column
- 20-200 µL pipette
- 200-1000 µL pipette
- Pipette tips
- Vortex mixer
- 1.5 mL capped preparation tube
- Centrifuge
- Bidistilled or deionised water

8. PROCEDURE NOTES:

Any inappropriate handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pre-treatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Leave aside all reagents and specimens to reach room

temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange the vial caps. Always keep vials closed when not been used. Do not re-use wells/tubes or reagents.

Incubation time affects results. All tubes or wells should be handled in the same order and time sequences.

9. LIMITATIONS OF THE PROCEDURE:

Specimen collection and storage have a significant effect on the test results. Urine samples must be kept frozen or acidified with appropriate acid solution before the analysis. (6N HCl,%50 Acetic acid, Na_2CO_3 (crystals), Boric acid (crystals). **If the urine sample is not clear, it must be centrifuged before sample preparation.**

10.PRE-TEST SET-UP INSTRUCTIONS:

Set-up the Instrument:

- Purge the HPLC pumps with a high flow rate of mobile phase(s). This should be done by pumping the mobile phase(s) through the system for 2 minutes at a flow rate of 4.0 ml/min.
- Switch off the pump and connect the column in flow direction.
  Activate the method and allow mobile phase(s) to flow through the column for 15 minutes.
- Make sure the bottle of mobile phase bottle is closed well, otherwise components of the mobile phase could evaporate; this alters the retention times.

Preparation of Zivak Calibrators:

Add **2 ml** deionised water to urine **calibrator** and dissolve.
After preparation, **calibrators** must be aliquoted into **350 µl** volumes and stored at **-20°C.**

Preparation of Zivak Controls

Add **2 ml** deionised water to urine **control level I** and dissolve
After preparation, **control levels** must be aliquoted into **350 µl** volumes and stored at **-20 °C.**

The R2-R3 mixture must be prepared daily before starting sample preparation.

Sample Number	R2 (ml)	R3 (ml)
10	0.25	0.25
20	0.50	0.50
50	1.25	1.25

11.TEST PROCEDURE:

Sample Pre-treatment (Automated):

Take **300 µL** of patient sample (or **calibrators** and **controls**) into the Zivak Multitasker Sample Preparation vials

↓

Make sure that reagents and wash solution are connected to the reagent pump and purged properly.

↓

Place the vials on the **MULTITASKER** system tray.

Note: The prepared sample is stable at **2-8°C** for **24 hours.**

Sample Pre-treatment (Manual)

Take **300 µL** of patient sample (or **calibrators** and **controls**) into the autosampler vial

↓

Add **50 µL** of **Reagent 1** and vortex for **5 seconds.**

↓

Add **50 µL** of **Reagent 2-3 mixture** (described above) and vortex for 5 seconds

↓

Inject **20 µL** to the **LC-MS/MS** system.

Note: The prepared sample is stable at **2-8 °C** for **24 hours**

12.QUALITY CONTROL:

The test results are only valid if the test has been performed by following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards and kit controls must be found within the acceptable ranges as stated on the QC (Quality Control) Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation, the following technical issues should be proven: Expiry dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods. It is recommended to participate at appropriate quality assessment trials.

13.CALCULATION OF RESULTS:

The obtained area of the standard is plotted against their concentration. The standard curve is calculated by a linear regression or a weighted linear regression function. Using computer programs, the curve is best described by a 2-point linear regression fit with linear axes. For the calculation of the regression curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used). The concentration of the samples can be read directly from the regression function. Samples showing signals above the highest standard have to be confirmed by a reference method.

$$\frac{\text{(Peak Area of Sample) x (Peak area of IS in Calibrator)}}{\text{(Peak area of Calibrator) x (Peak area of IS in the Sample)}} \times \text{Concentration of Calibrator} = \textbf{Concentration of the Sample}$$

**IS: Internal Standard*

Calculation for 24 hours urine:

Concentration of the Sample(mg/L) **x** Volume of 24h Urine (L) = mg analyte / 24 hours

Calculation for spot urine:

$$\frac{\text{[(Concentration of the Sample (mg/L)]}}{\text{[(Concentration of Creatinine g/L)]}} = \frac{\textbf{mg} \text{ analyte}}{\textbf{creatinine}}\textbf{g}$$

14.INTERPRETATION OF RESULTS:

Various societies recommend different Cut-Off values for repetition of the measurement and the application of confirmatory assays. Depending on the application of patient samples from different populations, it is **highly recommended that each laboratory establishes its own range of normal values** and that this distribution of values is co-ordinated with the recommendations of the responsible society of this geographic region.

The results themselves should not be the only reason for any therapeutic consequences. They have to be correlated to other clinical observations and diagnostic tests.

15.EXPECTED VALUES:

It is recommended that each laboratory establishes its own range of normal values.

16.LC-MS/MS PARAMETERS:

Device	Zivak Tandem Gold LC-MS/MS System
Column	Organic Acid Urine LC-MS/MS Analytical Column
Injection Volume	20 µL
Pump Program	00:00 min 0 % B 14:00 min 100 % B 16:00 min 100 % B 16:01 min 0 % B 20:00 min 0 % B
Flow	0.25 mL/min
Ionization Mode	ESI positive + ESI negative
CID Gas	2.4 mTorr
API Nebulizing Gas	55 psi
Scan Time	2.1 sec
SIM Width	1.5 amu
Drying Gas	35 psi
Drying Gas Temp.	350 °C
Needle	5000V
Shield	600V
Capillary	40V
Detector	1600 V

17.MS SCAN PARAMETERS:

No	Analyte	MH+ (m/z)	MS/MS	Capillary (eV)	CE (eV)	IS to Use
1	2-3-pyridine carboxylic	(-)165.8	121.8	40	8	IS-1
2	2-Hydroxyphenylacetic acid (o-Hydroxyphenylacetic acid)	(-)150,7	106,8	40	10	IS-2
3	2-me-hippuric	(-)191.7	90.9	40	14	IS-2
4	2-Methylcitric acid	(-)204,8	124,8	40	12	IS-1
5	2-Methylglutaric acid	(-)144,7	100,7	40	8	IS-2
6	3,4-dihydroxy-hidrocinnamic	(-)180.7	136.5	40	10	IS-2
7	3-Hydroxypropanoic acid	(-)88,7	59,0	40	8	IS-1
8	3-indoleacetic	(+)176.1	130.0	40	12	IS-2
9	3-me-hippuric acid	(-)191.7	90.9	40	14	IS-2
10	3-Methylcrotonyl Gylcine	(-)155,7	73,9	40	8	IS-2
11	3-Methylglutaric acid	(-)144,7	100,7	40	8	IS-2
12	3-Hydroxy-3-methylglutaric acid	(-)160.8	98.9	40	10	IS-1
13	3-Phenyllactic acid	(-)164,8	146,9	40	10	IS-2
14	4-Methylhippuric acid	(-)191.7	90.9	40	14	IS-2
15	4-Hydroxybenzoic acid	(-)136.8	92.8	40	8	IS-2
16	4-Hydroxyphenylacetic acid (p-Hydroxyphenylacetic acid)	(-)150.7	106.8	40	10	IS-2
17	5-Hydroxyindole-3-acetic acid	(+)192.1	146.1	40	14	IS-1
18	Adipic	(-)144.7	100.7	40	8	IS-1
19	3-Methyl-2-oxovaleric acid (AKBM)	(-)128.8	84.8	40	8	IS-2
20	Alpha-Ketoisocaproic acid (4-Methyl-2-oxovaleric acid) (AKIC)	(-)128.8	84.8	40	8	IS-2
21	3-methyl-2-oxobutanoic acid sodium salt (alpha-Ketoisovaleric acid)(AKIV)	(-)114.8	71.0	40	8	IS-1
22	Alpha-Hydroxyglutaric acid	(-)146,7	128,9	40	10	IS-1
23	Alpha-Ketobutyric acid	(-)100,8	57,0	40	8	IS-1
24	2-Ketoglutaric acid (Alpha-ketoglutaric acid)	(-)144.7	100.7	40	8	IS-1
25	Alpha-OH-butyric acid	(-)102.8	57.0	40	10	IS-2
26	Benzoic	(-)120.8	77.0	40	10	IS-2
27	3-Hydroxybutyric acid (β-Hydroxybutyric acid)	(-)102.8	59.0	40	8	IS-1
28	Beta-OH-isovaleric (β-Hydroxyisovaleric acid)	(-)116.8	59.0	40	10	IS-2
29	Cis-aconitic	(-)172.8	84.8	40	10	IS-1
30	Citramalic	(-)146.8	87.0	40	12	IS-1
31	Citric	(-)190.8	110.9	40	10	IS-1
32	Ethylmalonic	(-)130.8	87.0	40	12	IS-2
33	Formiminoglutamic	(-)172.8	127.6	40	8	IS-1
34	Fumaric	(-)114.8	71.0	40	8	IS-1
35	D-Saccharic acid potassium salt (Glucaric Acid)	(-)208.9	85.0	40	12	IS-1
36	Glutaric Acid	(-)130.8	87.0	40	12	IS-2
37	Glyceric Acid	(-)104.8	74.7	40	10	IS-1

38	Glycolic Acid	(-)74.8	74.8	40	6	IS-1
39	Hexanoyl glycine	(-)171,8	74.0	40	12	IS-2
40	Hippuric	(-)177.9	133.9	40	10	IS-2
41	Homovanillic	(-)180.9	136.8	40	6	IS-1
42	Isocitric	(-)190.8	110.9	40	10	IS-1
43	Iso-Valeryl Glycine (N-Isovalerylglycine)	(-)157.7	73.9	40	10	IS-2
44	Kynurenic	(-)187.9	144.0	40	12	IS-2
45	Lactic	(-)88.7	43.0	40	10	IS-1
46	Malic	(-)132.9	114.8	40	10	IS-1
47	Malonic acid	(-)102,8	59.0	40	8	IS-1
48	Mandelic	(-)150.7	106.8	40	10	IS-2
49	Methylmalonic	(-)116.8	73.0	40	8	IS-1
50	Methylsuccinic acid (2-Methylsuccinic Acid)	(-)130,8	87.0	40	12	IS-1
51	Mevalonolactone	(+)131,0	69.0	40	10	IS-1
52	N-(3-Phenylpropionyl)glycine	(-)205,8	73,9	40	12	IS-2
53	N-Acetyl-L-aspartic acid	(-)173,8	88,0	40	14	IS-1
54	Orotic	(-)154.8	110.8	40	12	IS-1
55	Phenylacetic	(-)134.7	90.8	40	6	IS-2
56	Phenylglyoxylic acid (Benzoylformic acid) (Benzoylform)	(-)148.8	77.0	40	10	IS-2
57	P-Hydroxyphenyllactic acid	(-)180,8	118,8	40	16	IS-2
58	Picolinic	(+)124.0	78.0	40	16	IS-1
59	Pimelic acid	(-)158,8	96,8	40	12	IS-2
60	Propionyl Glycine (2-propanamidoacetic acid)	(-)129.8	73.9	40	10	IS-1
61	Pyroglutamic	(+)129.9	84.2	40	18	IS-1
62	Pyruvic	(-)88.7	43.0	40	10	IS-1
63	Sebacic Acid	(-)200.8	138.8	40	16	IS-2
64	Suberic	(-)172.8	111.0	40	14	IS-2
65	Suberyl glycine	(-)229,8	73,9	40	18	IS-2
66	Succinic acid	(-)116.8	73.0	40	8	IS-1
67	Succinylacetone (4,6-Dioxoheptanoic acid)	(+)159.0	99.0	40	12	IS-2
68	Tartaric	(-)148.8	87.0	40	12	IS-1
69	Tiglyl glycine	(+)158,0	83.0	40	8	IS-2
70	Tricarballyl	(-)175.0	156.9	40	10	IS-1
71	Vanilmandellic Acid (4-Hydroxy-3-methoxymandelic Acid)	(-)196.8	137.8	40	12	IS-2
72	Xanthurenic acid (4,8-Dihydroxyquinoline-2-carboxylic acid)	(-)203.8	159.7	40	14	IS-2
73	α-Hydroxyisovaleric acid	(-)116,8	71	40	15	IS-1
	IS-1	(-)118.6	74.0	40	10	
	IS-2	(-)150.6	105.8	40	12	

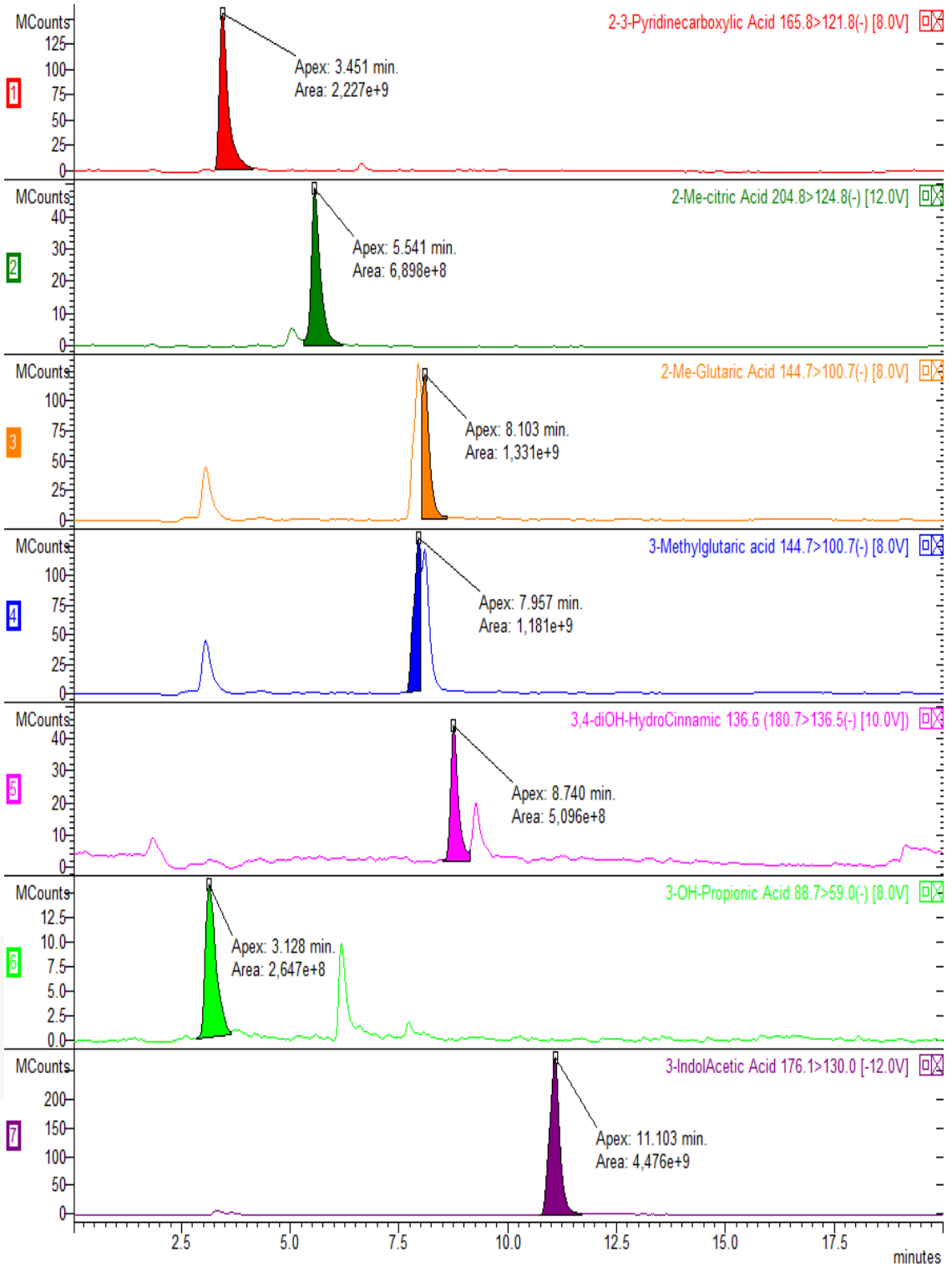
18.ANALYTICAL PERFORMANCE:

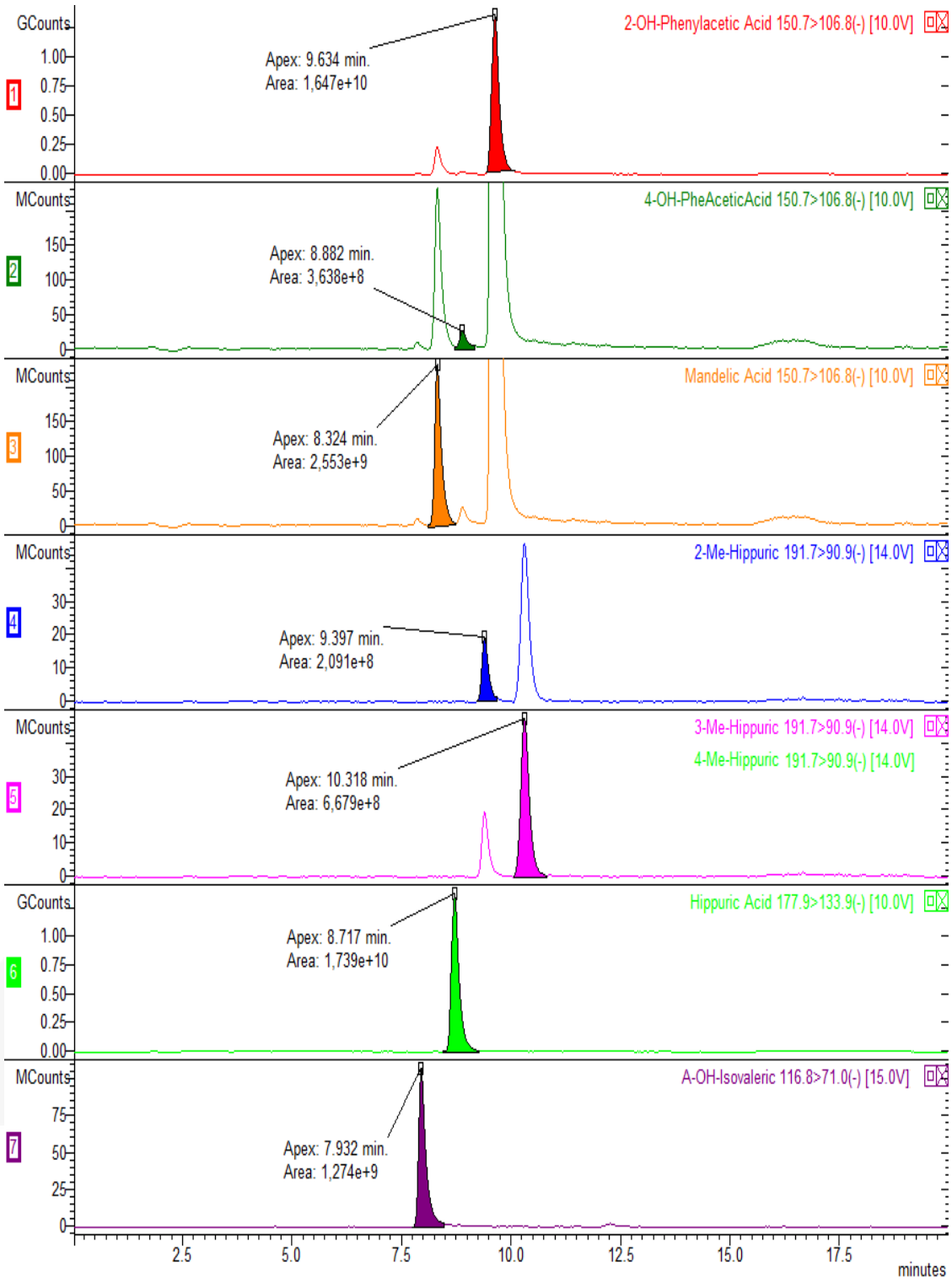
					Inter-Assay Precision (%CV)			
No	**Analyte**	**LOD (mg/L)**	**LOQ (mg/L)**	**Linearity (r^2)**	**1.Day %RSD**	**2.Day %RSD**	**3.Day %RSD**	**% Recovery**
1	2,3-pyridinedicarboxylic acid	0,0070	0,0240	0,9939	6,53	2,45	4,08	105,0
2	2-Hydroxyphenylacetic acid (o-Hydroxyphenylacetic acid)	0,0350	0,1150	0,9984	5,97	6,90	3,60	94,4
3	2-Ketoglutaric acid (Alpha-ketoglutaric acid)	0,0300	0,1000	0,9996	5,88	6,08	5,56	96,0
4	2-Methylcitric acid	0,0170	0,0570	0,9966	6,22	6,58	5,83	95,6
5	2-Methylglutaric acid (Methylglutaric acid)	0,0040	0,0130	0,9995	5,62	7,02	2,94	93,3
6	2-Methylhippuric acid	0,0010	0,0030	0,9994	2,45	5,12	1,43	100,1
7	3,4-Dihydroxyhydrocinnamic acid	0,0100	0,0330	0,9983	3,30	1,57	2,14	94,8
8	3-Hydroxy-3-methylglutaric acid	0,0070	0,0220	0,9980	3,62	3,55	5,48	99,6
9	3-Hydroxybutyric acid (β-Hydroxybutyric acid)	0,0020	0,0060	0,9995	6,11	5,10	5,66	96,4
10	3-Hydroxypropanoic acid	0,0100	0,0330	0,9963	2,57	5,25	5,04	93,8
11	3-İndoleacetic acid	0,0000	0,0010	0,9982	2,82	8,74	6,85	94,0
12	3-methyl-2-oxobutanoic acid sodium salt (alpha-Ketoisovaleric acid)	0,0020	0,0060	0,9952	2,33	2,84	1,25	99,7
13	3-Methyl-2-oxovaleric acid	0,0260	0,0850	0,9964	4,01	1,47	2,56	92,5
14	3-Methylcrotonyl Gylcine	0,0100	0,0340	0,9950	6,34	9,95	7,01	100,2
15	3-Methylglutaric acid	0,0080	0,0260	0,9996	7,04	7,01	6,01	93,3
16	3-methylhippuric acid	0,0010	0,0020	0,9984	6,27	4,04	8,25	104,1
17	3-Phenyllactic acid	0,0030	0,0090	0,9996	3,05	9,73	7,07	96,1
18	4,6-Dioxoheptanoic acid (Succinylacetone)	0,0120	0,0400	0,9948	3,01	2,55	6,35	98,6
19	4-Hydroxy-3-methoxymandelic Acid (Vanilmandellic Acid)	0,0000	0,0010	0,9968	8,21	7,56	2,97	103,0
20	4-Hydroxybenzoic acid	0,0040	0,0140	0,9993	2,08	4,31	4,23	90,4
21	4-Hydroxyphenylacetic acid (p-Hydroxyphenylacetic acid)	0,0020	0,0050	0,9992	4,60	5,43	8,66	93,2
22	4-Methylhippuric acid	0,0010	0,0020	0,9909	8,00	5,63	7,33	98,1
23	5-Hydroxyindole-3-acetic acid	0,0010	0,0020	0,9988	4,59	5,55	5,46	93,2
24	Adipic acid	0,0120	0,0410	0,9996	6,97	4,52	8,90	101,2
25	Alpha-Ketobutyric acid	0,0020	0,0070	0,9913	2,28	2,15	2,36	104,7
26	Alpha-Ketoisocaproic acid (4-Methyl-2-oxovaleric acid)	0,0040	0,0140	0,9992	2,69	2,58	4,12	101,1
27	Alpha-OH-butyric acid	0,0120	0,0400	0,9953	2,36	6,25	2,36	89,6
28	α-Hydroxyglutaric acid	0,0050	0,0160	0,9992	7,64	3,74	7,44	96,6
29	α-Hydroxyisovaleric acid	0,0050	0,0170	0,9977	6,14	8,81	9,42	97,5
30	β-Hydroxyisovaleric acid	0,0010	0,0040	0,9981	6,18	8,45	8,51	99,6
31	Benzoic acid	0,0120	0,0400	0,9995	2,30	7,84	5,16	97,6
32	Cis-aconitic	0,0030	0,0100	0,9948	3,47	4,39	5,48	101,2
33	Citramalic	0,0020	0,0070	0,9910	4,18	3,26	3,47	87,8
34	Citric acid	0,0810	0,2690	0,9973	3,20	1,68	1,31	101,1
35	D-Saccharic acid potassium salt (Glucaric Acid)	0,0010	0,0040	0,9952	8,97	8,91	6,77	98,5
36	Ethylmalonic acid	0,0040	0,0130	0,9985	3,83	2,17	8,19	93,8

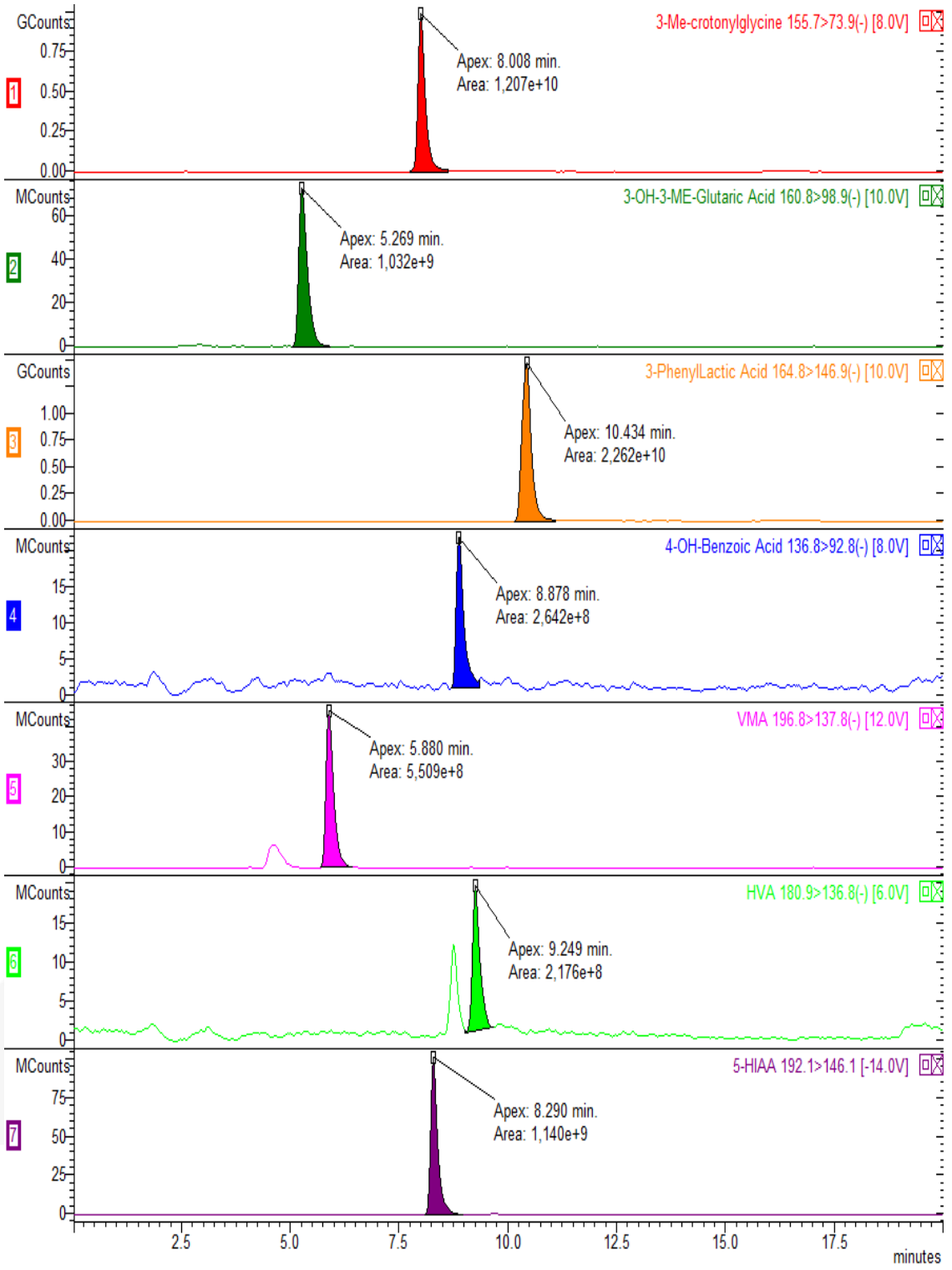
37	Formiminoglutamic	0,0040	0,0140	0,9914	1,88	4,66	4,01	101,4
38	Fumaric acid	0,0380	0,1260	0,9984	3,59	5,79	1,50	99,3
39	Glutaric acid	0,0000	0,0010	0,9899	5,50	7,25	6,61	95,0
40	Glyceric acid hemicalciyum salt monohydrate	0,0100	0,0330	0,9960	2,04	4,54	6,62	93,1
41	Glycolic acid	0,0050	0,0170	0,9961	1,95	3,56	2,54	94,3
42	Hexanoyl glycine	0,0000	0,0020	0,9899	5,27	7,40	6,46	91,2
43	Hippuric acid	0,0260	0,0850	0,9988	2,26	6,68	2,58	95,4
44	Homovanillic acid	0,0030	0,0100	0,9901	2,54	3,65	4,25	94,2
45	Isocitric acid	0,0360	0,1190	0,9982	3,08	6,38	6,87	93,6
46	Kynurenic acid	0,0020	0,0070	0,9976	4,47	7,36	8,60	90,5
47	Lactic acid	0,0650	0,2150	0,9994	8,87	5,88	7,48	105,8
48	Malic acid	0,0020	0,0060	0,9991	8,50	7,77	7,19	99,3
49	Malonic acid	0,0080	0,0260	0,9982	5,49	8,95	6,72	95,9
50	Mandelic acid	0,0690	0,2270	0,9963	3,02	8,46	9,06	100,1
51	Methylmalonic acid	0,0110	0,0370	0,9938	5,06	5,99	4,67	105,2
52	Methylsuccinic acid (2-Methylsuccinic Acid)	0,0020	0,0060	0,9998	3,67	4,52	7,49	87,5
53	Mevalonolactone	0,0010	0,0050	0,9933	7,53	7,88	10,23	89,7
54	N-(3-Phenylpropionyl)glycine	0,0010	0,0040	0,9939	4,52	6,01	7,29	85,4
55	N-Acetyl-L-aspartic acid	0,0050	0,0170	0,9987	2,98	4,23	4,31	96,6
56	N-Isovalerylglycine	0,0010	0,0040	0,9988	5,19	3,19	5,99	89,2
57	Orotic acid	0,0190	0,0630	0,9979	7,68	8,79	8,31	103,1
58	Phenylacetic	0,0050	0,0170	0,9960	1,25	1,36	1,67	85,6
59	Phenylglyoxylic acid (Benzoylformic acid) (Benzoylform)	0,0030	0,0100	0,9887	7,15	7,01	9,05	102,9
60	P-Hydroxyphenyllactic acid	0,0040	0,0120	0,9977	5,97	3,39	5,16	98,9
61	Picolinic acid	0,0010	0,0030	0,9927	2,32	5,36	3,99	90,3
62	Pimelic acid	0,0010	0,0030	0,9987	4,69	4,07	8,71	81,7
63	Propionyl Glycine (2-propanamidoacetic acid)	0,0050	0,0170	0,9878	3,44	7,61	4,61	90,8
64	Pyroglutamic	0,0020	0,0060	0,9967	2,68	2,36	2,54	93,6
65	Pyruvic acid	0,0480	0,1580	0,9961	2,30	3,85	8,13	94,8
66	Sebacic acid	0,0000	0,0010	0,9956	3,10	6,34	9,52	106,2
67	Suberic acid	0,0020	0,0070	0,9981	5,13	7,03	6,66	99,6
68	Suberyl glycine	0,0010	0,0020	0,9936	4,66	8,85	6,44	87,1
69	Succinic acid	0,0720	0,2370	0,9968	7,95	4,46	3,36	105,4
70	Tartaric acid	0,0030	0,0100	0,9991	4,88	3,76	6,49	97,8
71	Tiglyl glycine	0,0000	0,0010	0,9982	4,42	3,65	4,72	100,8
72	Tricarballylic acid	0,0010	0,0020	0,9990	4,91	4,95	2,80	83,0
73	4,8-Dihydroxyquinoline-2-carboxylic acid (Xanthurenic acid)	0,0020	0,0060	0,9992	6,89	4,42	6,64	83,7

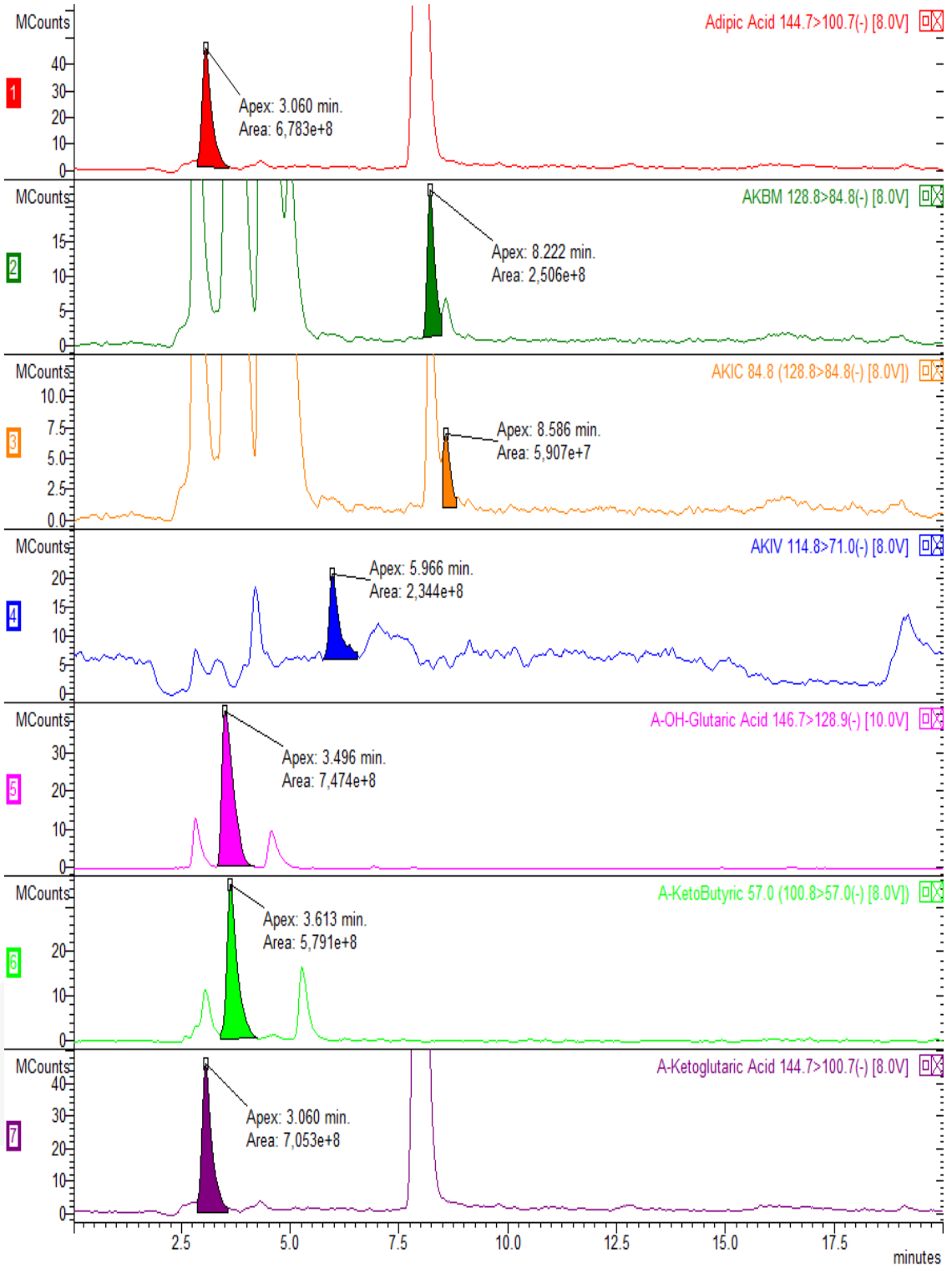
Analytical Specificity (Cross Reactivity): No cross-reactivities were found with the typical substances tested.

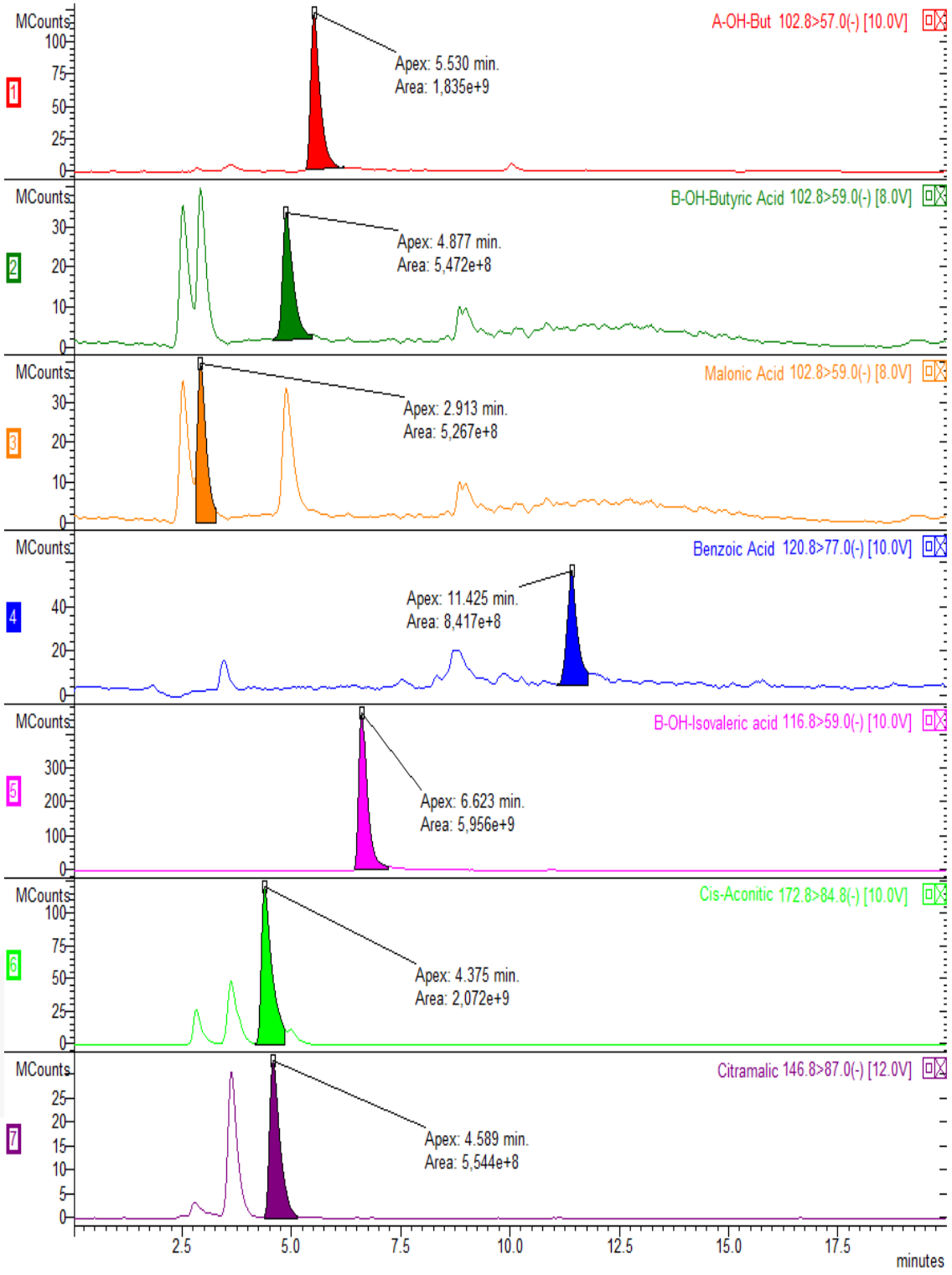
19.SAMPLE CHROMATOGRAM:

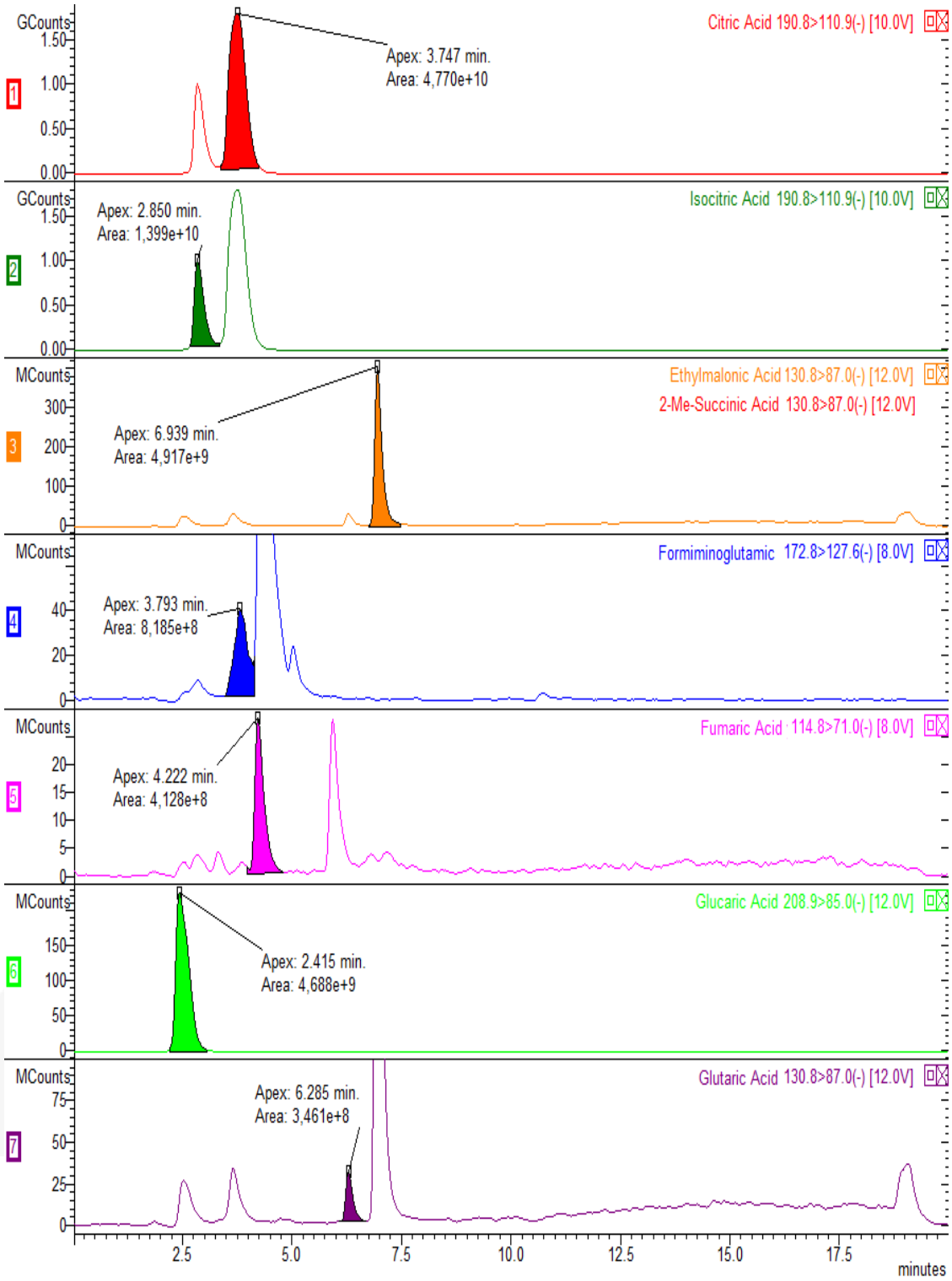


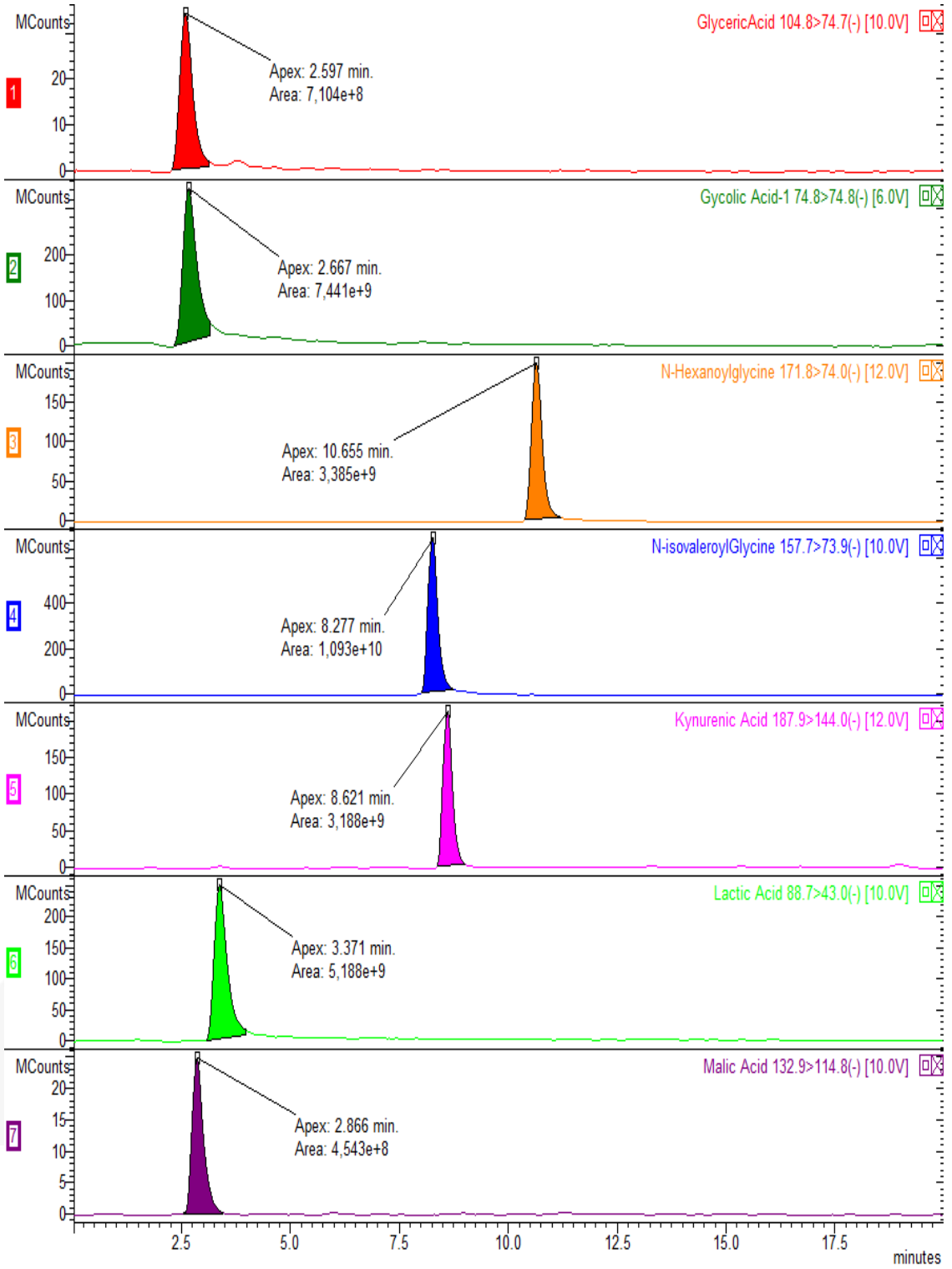

GCounts
2-OH-Phenylacetic Acid 150.7>106.8(-) [10.0V]
Apex: 9.634 min.
Area: 1,647e+10
MCounts
4-OH-PheAceticAcid 150.7>106.8(-) [10.0V]
Apex: 8.882 min.
Area: 3,638e+8
MCounts
Mandelic Acid 150.7>106.8(-) [10.0V]
Apex: 8.324 min.
Area: 2,553e+9
MCounts
2-Me-Hippuric 191.7>90.9(-) [14.0V]
Apex: 9.397 min.
Area: 2,091e+8
MCounts
3-Me-Hippuric 191.7>90.9(-) [14.0V]
4-Me-Hippuric 191.7>90.9(-) [14.0V]
Apex: 10.318 min.
Area: 6,679e+8
GCounts
Hippuric Acid 177.9>133.9(-) [10.0V]
Apex: 8.717 min.
Area: 1,739e+10
MCounts
A-OH-Isovaleric 116.8>71.0(-) [15.0V]
Apex: 7.932 min.
Area: 1,274e+9
minutes

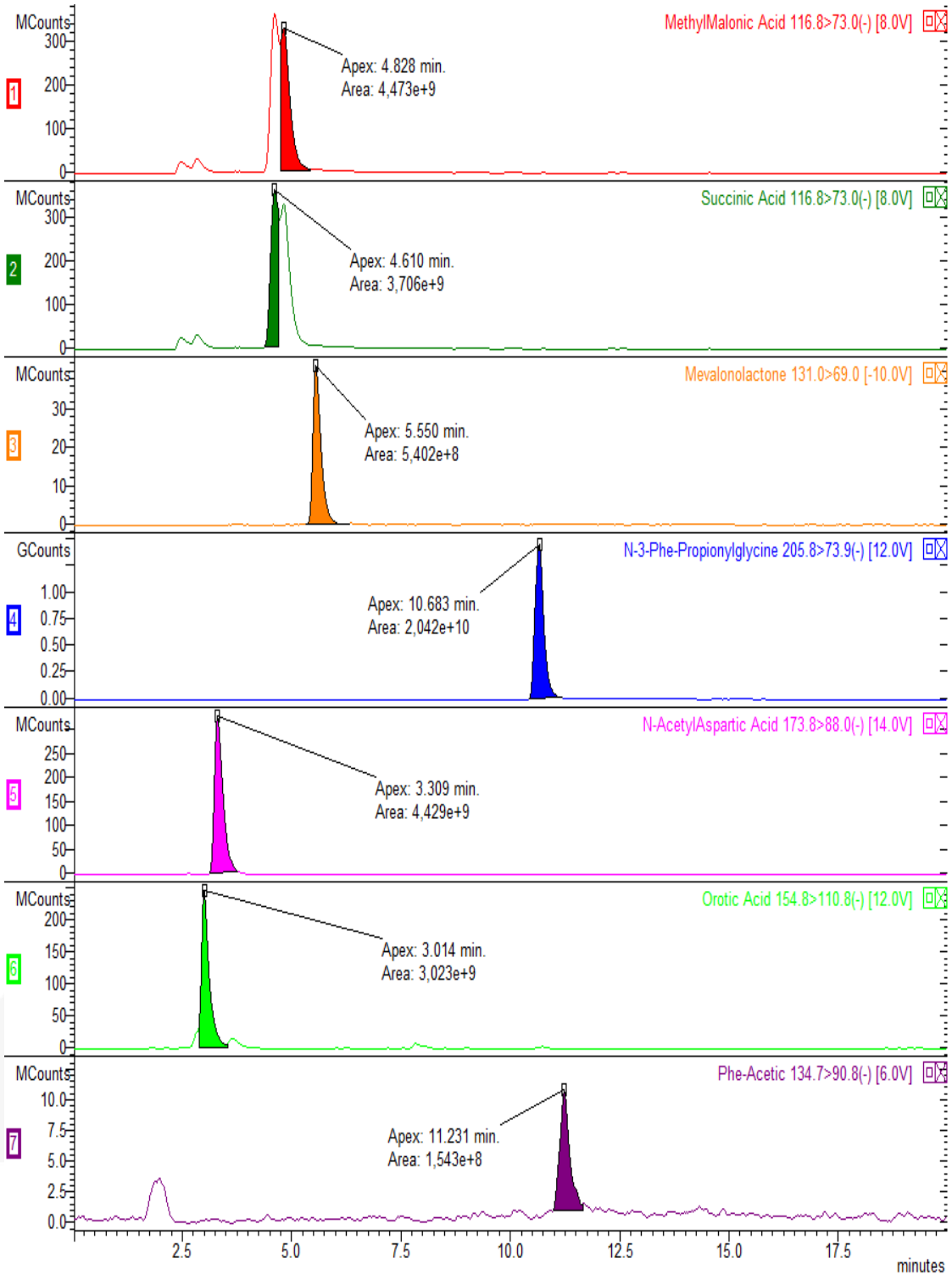

GCounts
3-Me-crotonylglycine 155.7>73.9(-) [8.0V]
Apex: 8.008 min.
Area: 1,207e+10
MCounts
3-OH-3-ME-Glutaric Acid 160.8>98.9(-) [10.0V]
Apex: 5.269 min.
Area: 1,032e+9
GCounts
3-PhenylLactic Acid 164.8>146.9(-) [10.0V]
Apex: 10.434 min.
Area: 2,262e+10
MCounts
4-OH-Benzoic Acid 136.8>92.8(-) [8.0V]
Apex: 8.878 min.
Area: 2,642e+8
MCounts
VMA 196.8>137.8(-) [12.0V]
Apex: 5.880 min.
Area: 5,509e+8
MCounts
HVA 180.9>136.8(-) [6.0V]
Apex: 9.249 min.
Area: 2,176e+8
MCounts
5-HIAA 192.1>146.1 [-14.0V]
Apex: 8.290 min.
Area: 1,140e+9
2.5
5.0
7.5
10.0
12.5
15.0
17.5
minutes

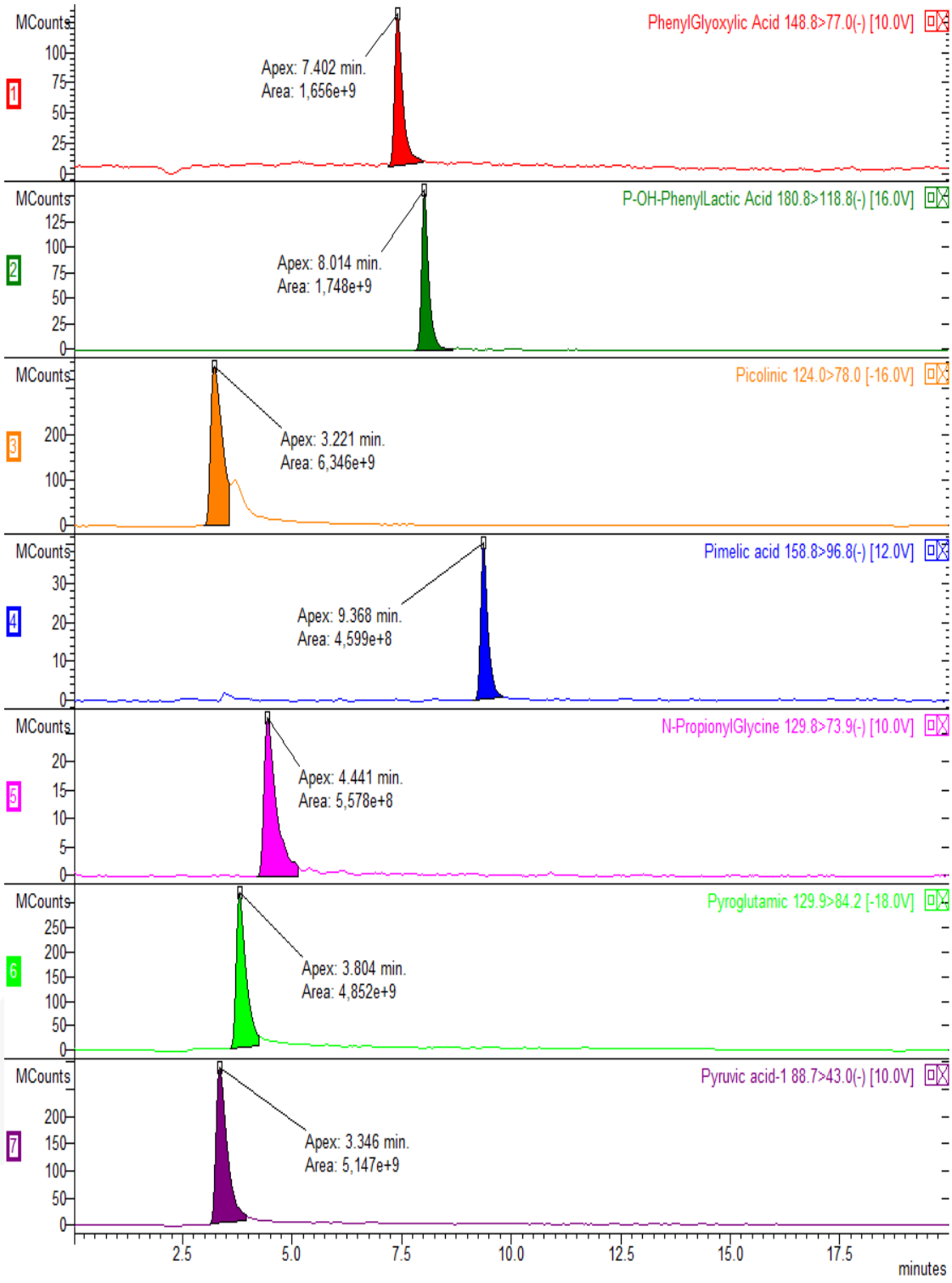

MCounts
Adipic Acid 144.7>100.7(-) [8.0V]
Apex: 3.060 min.
Area: 6,783e+8
AKBM 128.8>84.8(-) [8.0V]
Apex: 8.222 min.
Area: 2,506e+8
AKIC 84.8 (128.8>84.8(-) [8.0V])
Apex: 8.586 min.
Area: 5,907e+7
AKIV 114.8>71.0(-) [8.0V]
Apex: 5.966 min.
Area: 2,344e+8
A-OH-Glutaric Acid 146.7>128.9(-) [10.0V]
Apex: 3.496 min.
Area: 7,474e+8
A-KetoButyric 57.0 (100.8>57.0(-) [8.0V])
Apex: 3.613 min.
Area: 5,791e+8
A-Ketoglutaric Acid 144.7>100.7(-) [8.0V]
Apex: 3.060 min.
Area: 7,053e+8
minutes

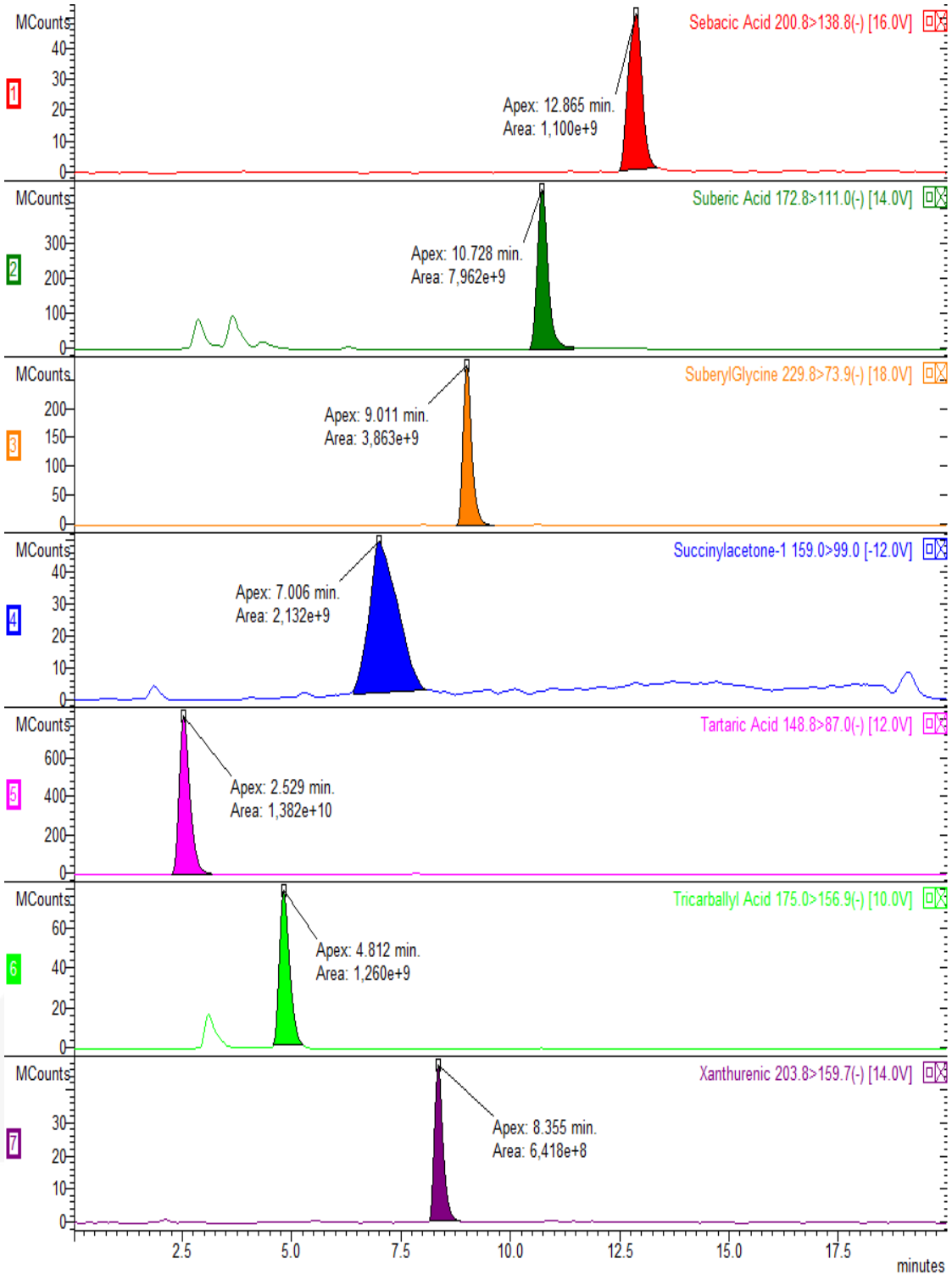

A-OH-But 102.8>57.0(-) [10.0V]
Apex: 5.530 min.
Area: 1,835e+9
B-OH-Butyric Acid 102.8>59.0(-) [8.0V]
Apex: 4.877 min.
Area: 5,472e+8
Malonic Acid 102.8>59.0(-) [8.0V]
Apex: 2.913 min.
Area: 5,267e+8
Benzoic Acid 120.8>77.0(-) [10.0V]
Apex: 11.425 min.
Area: 8,417e+8
B-OH-Isovaleric acid 116.8>59.0(-) [10.0V]
Apex: 6.623 min.
Area: 5,956e+9
Cis-Aconitic 172.8>84.8(-) [10.0V]
Apex: 4.375 min.
Area: 2,072e+9
Citramalic 146.8>87.0(-) [12.0V]
Apex: 4.589 min.
Area: 5,544e+8
MCounts
minutes
2.5
5.0
7.5
10.0
12.5
15.0
17.5

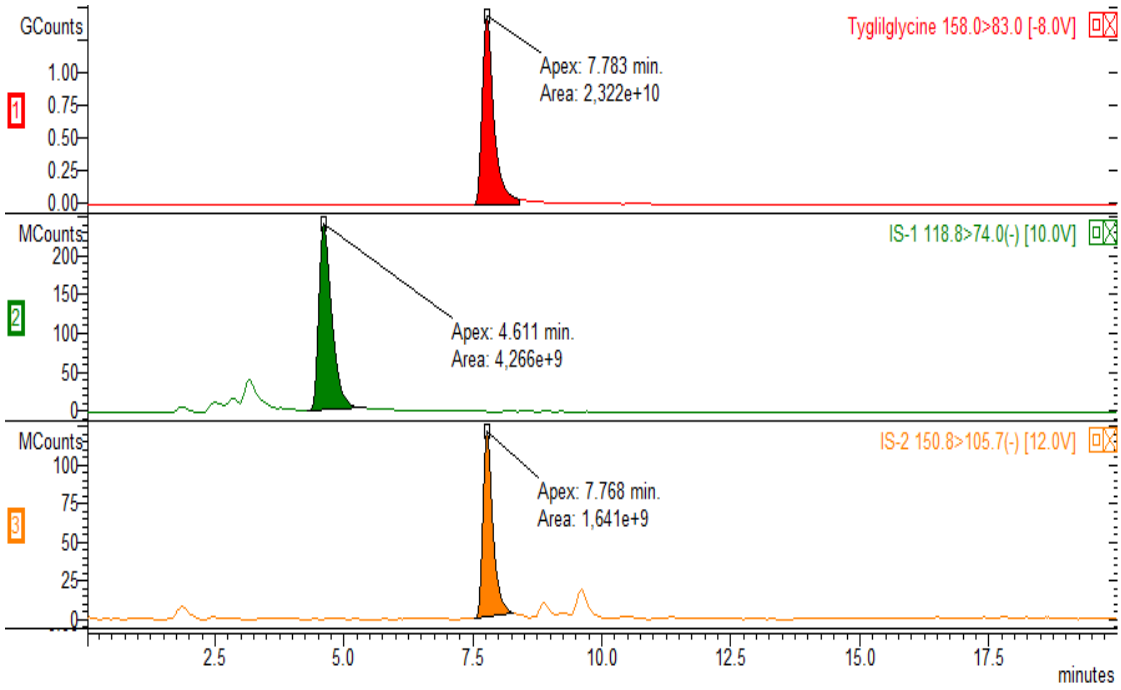

GCounts
Citric Acid 190.8>110.9(-) [10.0V]
Apex: 3.747 min.
Area: 4,770e+10
Isocitric Acid 190.8>110.9(-) [10.0V]
Apex: 2.850 min.
Area: 1,399e+10
MCounts
Ethylmalonic Acid 130.8>87.0(-) [12.0V]
2-Me-Succinic Acid 130.8>87.0(-) [12.0V]
Apex: 6.939 min.
Area: 4,917e+9
Formiminoglutamic 172.8>127.6(-) [8.0V]
Apex: 3.793 min.
Area: 8,185e+8
Fumaric Acid 114.8>71.0(-) [8.0V]
Apex: 4.222 min.
Area: 4,128e+8
Glucaric Acid 208.9>85.0(-) [12.0V]
Apex: 2.415 min.
Area: 4,688e+9
Glutaric Acid 130.8>87.0(-) [12.0V]
Apex: 6.285 min.
Area: 3,461e+8
minutes


MCounts
GlycericAcid 104.8>74.7(-) [10.0V]
Apex: 2.597 min.
Area: 7,104e+8
Gycolic Acid-1 74.8>74.8(-) [6.0V]
Apex: 2.667 min.
Area: 7,441e+9
N-Hexanoylglycine 171.8>74.0(-) [12.0V]
Apex: 10.655 min.
Area: 3,385e+9
N-isovaleroylGlycine 157.7>73.9(-) [10.0V]
Apex: 8.277 min.
Area: 1,093e+10
Kynurenic Acid 187.9>144.0(-) [12.0V]
Apex: 8.621 min.
Area: 3,188e+9
Lactic Acid 88.7>43.0(-) [10.0V]
Apex: 3.371 min.
Area: 5,188e+9
Malic Acid 132.9>114.8(-) [10.0V]
Apex: 2.866 min.
Area: 4,543e+8
2.5
5.0
7.5
10.0
12.5
15.0
17.5
minutes


MethylMalonic Acid 116.8>73.0(-) [8.0V]
Apex: 4.828 min.
Area: 4,473e+9
Succinic Acid 116.8>73.0(-) [8.0V]
Apex: 4.610 min.
Area: 3,706e+9
Mevalonolactone 131.0>69.0 [-10.0V]
Apex: 5.550 min.
Area: 5,402e+8
N-3-Phe-Propionylglycine 205.8>73.9(-) [12.0V]
Apex: 10.683 min.
Area: 2,042e+10
N-AcetylAspartic Acid 173.8>88.0(-) [14.0V]
Apex: 3.309 min.
Area: 4,429e+9
Orotic Acid 154.8>110.8(-) [12.0V]
Apex: 3.014 min.
Area: 3,023e+9
Phe-Acetic 134.7>90.8(-) [6.0V]
Apex: 11.231 min.
Area: 1,543e+8
MCounts
GCounts
minutes


PhenylGlyoxylic Acid 148.8>77.0(-) [10.0V]
Apex: 7.402 min.
Area: 1,656e+9
P-OH-PhenylLactic Acid 180.8>118.8(-) [16.0V]
Apex: 8.014 min.
Area: 1,748e+9
Picolinic 124.0>78.0 [-16.0V]
Apex: 3.221 min.
Area: 6,346e+9
Pimelic acid 158.8>96.8(-) [12.0V]
Apex: 9.368 min.
Area: 4,599e+8
N-PropionylGlycine 129.8>73.9(-) [10.0V]
Apex: 4.441 min.
Area: 5,578e+8
Pyroglutamic 129.9>84.2 [-18.0V]
Apex: 3.804 min.
Area: 4,852e+9
Pyruvic acid-1 88.7>43.0(-) [10.0V]
Apex: 3.346 min.
Area: 5,147e+9
MCounts
minutes
2.5
5.0
7.5
10.0
12.5
15.0
17.5


MCounts
Sebacic Acid 200.8>138.8(-) [16.0V]
Apex: 12.865 min.
Area: 1,100e+9
Suberic Acid 172.8>111.0(-) [14.0V]
Apex: 10.728 min.
Area: 7,962e+9
SuberylGlycine 229.8>73.9(-) [18.0V]
Apex: 9.011 min.
Area: 3,863e+9
Succinylacetone-1 159.0>99.0 [-12.0V]
Apex: 7.006 min.
Area: 2,132e+9
Tartaric Acid 148.8>87.0(-) [12.0V]
Apex: 2.529 min.
Area: 1,382e+10
Tricarballyl Acid 175.0>156.9(-) [10.0V]
Apex: 4.812 min.
Area: 1,260e+9
Xanthurenic 203.8>159.7(-) [14.0V]
Apex: 8.355 min.
Area: 6,418e+8
2.5
5.0
7.5
10.0
12.5
15.0
17.5
minutes


GCounts
Tyglilglycine 158.0>83.0 [-8.0V]
Apex: 7.783 min.
Area: 2,322e+10
MCounts
IS-1 118.8>74.0(-) [10.0V]
Apex: 4.611 min.
Area: 4,266e+9
MCounts
IS-2 150.8>105.7(-) [12.0V]
Apex: 7.768 min.
Area: 1,641e+9
minutes

20. Automated Sample Preparation Method Parameters for Zivak ASP-200

	1 - Reagent addition (Parallel)		2 - Reagent addition (Parallel)		3 - Vortex	4 - Injection (Serial)		
From	**Reagent tray**		**Reagent tray**		**Analysis tray**	**Analysis tray**		
	1 (Vial Position) (SR1)		1 (Vial Position) (SR2)		1 (Vial Position)	1 (Vial Position)		
	0 mm (Needle Height)		0 mm (Needle Height)		0 mm (Needle Height)	0 mm (Needle Height)		
	100 µl		100 µl		0,30 min. / 1000 rpm	200 µl		
	No liquid detection		No liquid detection		-	No liquid detection		
	Parallel		Parallel		Parallel	Serial		
To	**Analysis tray**		**Analysis tray**					
	1 (Vial Position)		1 (Vial Position)					
	15 mm (Needle Height)		18 mm (Needle Height)					
	50 µl		50 µl					
Washing	**Wash Station C**	**Wash Station A**	**Wash Station C**	**Wash Station A**		**Wash Station C**	**Wash Station A**	**Inj.Valve**
	Pump XLP (I6-O4)	Pump XLP (I6-O1)	Pump XLP (I6-O4)	Pump XLP (I6-O1)		Pump XLP (I6-O4)	Pump XLP (I6-O1)	Pump XLP (I6-O1)
	2000 µl	2000 µl	2000 µl	2000 µl		1000 µl	1000 µl	500 µl
	1 repeat	1 repeat	1 repeat	1 repeat		1 repeat	1 repeat	1 repeat
Syringes	**Pump XLP**		**Pump XLP**			**Pump Xcalibur**		
	20 - 1000 ms (speed-wait)		20 - 1000 ms (speed-wait)			16 - 1500 (speed - wait)		
	16 - 1000 ms (speed-wait)		16 - 1000 ms (speed-wait)			16 - 1500 (speed- wait)		
Air segment	First-Last		First-Last			First-Last		
	20 µl		20 µl			20 µl		

Purge Before			
Syringe Pump	**Wash Place**	**Flush Volume**	**Repeat Time**
Pump XLP (10 000 ul)	Wash station A	2000 uL	1

Batch	108