Application Note

Life Science Research

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Fast and Accurate Quantitation of Organic Acids Using an Agilent 6546 LC/Q-TOF

Authors

Dr. Julie Cichelli and
Natalie Rasmussen
Agilent Technologies, Inc.

Abstract

Organic acid analysis is critical for identifying inborn errors of metabolism. Traditional GC/MS workflows require derivatization and long run times, often leading to false positives or negatives. This study demonstrates a high-resolution accurate mass (HRAM) workflow using the Agilent 6546 LC/Q-TOF. The method enables simultaneous qualitative and quantitative analysis of over 60 organic acids in under 20 minutes without derivatization. Enhanced selectivity and resolution allow confident identification of isobaric compounds. The workflow supports life science research applications requiring fast, accurate, and comprehensive organic acid profiling.

Introduction

Organic acids are important biomarkers in life science research, especially for identifying inborn errors of metabolism. Traditionally, gas chromatography/mass spectrometry (GC/MS) has been used for their analysis.[1] However, GC/MS workflows require derivatization and long run times, which can delay results and increase the risk of inaccurate findings.[2]

The Agilent 6546 LC/Q-TOF offers a faster, more selective alternative. It uses high-resolution accurate mass (HRAM) detection, which improves confidence in compound identification. HRAM enables precise measurement of molecular masses, helping distinguish compounds even in complex mixtures. This LC/Q-TOF can detect small differences in mass that lower-resolution instruments may miss, supporting more reliable identification and quantitation.

Chromatographic separation also plays a critical role in accurate analysis. Selecting the right analytical HPLC column is essential for resolving compounds that may have similar chemical structures or retention times. In this workflow, an Agilent InfinityLab Poroshell 120 Phenyl Hexyl column was chosen for its ability to separate a broad range of organic acids, including challenging isobaric pairs. This ensures cleaner separation, better peak shape, and more dependable quantitation.

Together, HRAM detection and optimized chromatography enable a fast, streamlined LC/MS workflow that supports both qualitative and quantitative analysis of over 60 organic acids.

Experimental

This workflow targets over 60 organic acids relevant to life science research (Table 1). A custom personal compound database and library (PCDL) was created using Agilent MassHunter PCDL Manager to support high-resolution identification. Thirteen isobaric sets were resolved using HRAM and optimized chromatography (Table 2).

Table 1. Organic acid analyte panel.

No.	Organic Acid	No.	Organic Acid	No.	Organic Acid	No.	Organic Acid
1	Lactic acid	16	p-OH-Phenylacetic acid	31	Malonic acid	46	N-Acetyl-leucine
2	Pyruvic acid	17	p-OH-Phenyllactic acid	32	3-Hydroxy-3-methylglutaric acid	47	Erythro-2,3 dihydroxy-methylbutyric acid
3	Succinic acid	18	p-OH-Phenylpyruvic acid	33	Isovalerylglycine	48	Uracil
4	Fumaric acid	19	Succinylacetone	34	Hexanoylglycine	49	3-Methylcrotonylglycine
5	2-Ketoglutaric (2-KG)	20	Glutaric acid	35	Suberylglycine	50	Isobutyrylglycine
6	Methylmalonic acid	21	3-Methylglutaconic acid	36	Orotic acid	51	Homovanillic acid
7	3-OH-Butyric acid	22	3-Hydroxyglutaric acid	37	Hydantoin 5-propionate	52	Vanilmandelic acid
8	Acetoacetic acid (AAA)	23	2-Hydroxyglutaric acid	38	4-OH-Butyric acid	53	Vanilactic acid
9	2-Keto-3-methylvaleric acid (KMV)	24	Pyroglutamic acid	39	Homogentisic acid	54	Propionylglycine
10	2-Ketoisocaproic acid (KIC)	25	3-OH-Propionic acid	40	Mevalonic acid	55	2-Methylbutyrylglycine
11	2-Ketoisovaleric acid (KIV)	26	Methylsuccinic acid	41	N-Acetylaspartic acid	56	4-Phenylbutyryic acid
12	Ethylmalonic acid	27	2-OH-Isovaleric acid	42	Glycolic acid	57	Phenylacetic acid
13	Adipic acid	28	3-OH-isovaleric acid	43	Glyceric acid	58	Phenylpropionylglycine
14	Suberic acid	29	3-Methylglutaric acid	44	3-OH-Isobutyric acid	59	Tiglylglycine
15	Sebacic acid	30	2-Methylcitric acid	45	Phenyllactic acid	60	N-Butyrylglycine

Table 2. Isobaric sets of organic acids.

No.	Isobars
1	3-Hydroxypropionic acid, lactic acid
2	3-Hydroxybutyric acid, 4-hydroxybutyric acid, 3-hydroxyisobutyric acid
3	Succinic acid, methylmalonic acid
4	2-Hydroxyvaleric acid, 3-hydroxyvaleric acid
5	2-Keto-3-methylvaleric acid, 2-keto-n-caproic acid
6	Ethylmalonic acid, glutaric acid, methylsuccinic acid
7	Isobutyrylglycine, *n*-butyrylglycine
8	Adipic acid, 3-methylglutaric acid
9	2-Hydroxyglutaric acid, 3-hydroxyglutaric acid
10	3-Methylcrotonylglycine, tiglylglycine
11	Isovalerylglycine, 3-methylbutyrylglycine
12	Hexanoylglycine, acetyl-leucine
13	Hydroxyphenylacetic acid, homovanillic acid

LC separation was performed on an Agilent 1290 Infinity II LC with an Agilent InfinityLab Poroshell 120 Phenyl Hexyl column. Method conditions are summarized in Table 3. Agilent MassHunter Acquisition software was used to control the LC and MS systems.

Mass spectrometry was conducted on an Agilent 6546 LC/Q-TOF, operating in positive ion mode with an Agilent Jet Stream Technology ion source (AJS). Parameters are listed in Table 4. Compound confirmation was performed using MassHunter Qualitative Analysis, and quantitation was completed using MassHunter Quantitative Analysis.

Results and discussion

The 6546 LC/Q-TOF workflow enabled fast, accurate separation and quantitation of over 60 organic acids. Chromatographic separation using the InfinityLab Poroshell 120 Phenyl Hexyl column produced clean peak shapes and consistent retention times across the full panel. An example chromatogram demonstrating separation of all 60 compounds is shown in Figure 1.

Table 3. LC method parameters.

Parameter	Value
LC System	Agilent 1290 Infinity II LC
HPLC Column	Agilent InfinityLab Poroshell 120 Phenyl Hexyl, 2.1 × 150 mm, 2.7 µm (p/n 693775-912)
Column Temperature	30 °C
Mobile Phase	A) 0.01% Formic acid in water B) Methanol
Flow Rate	0.5 mL/min
Injection Volume	1 µL
Gradient	Time (min) B (%) 0.00 0 3.50 0 18.00 60 18.01 95 20.00 95
Post Time	3.0 min
Stop Time	19 min

Table 4. MS method parameters.

Parameter	Value
MS System	Agilent 6546 LC/Q-TOF
Gas Temperature	150 °C
Gas Flow	10 L/min
Nebulizer	35 psig
Sheath Gas Temperature	400 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	2,000
Nozzle Voltage	500
Fragmentor	125

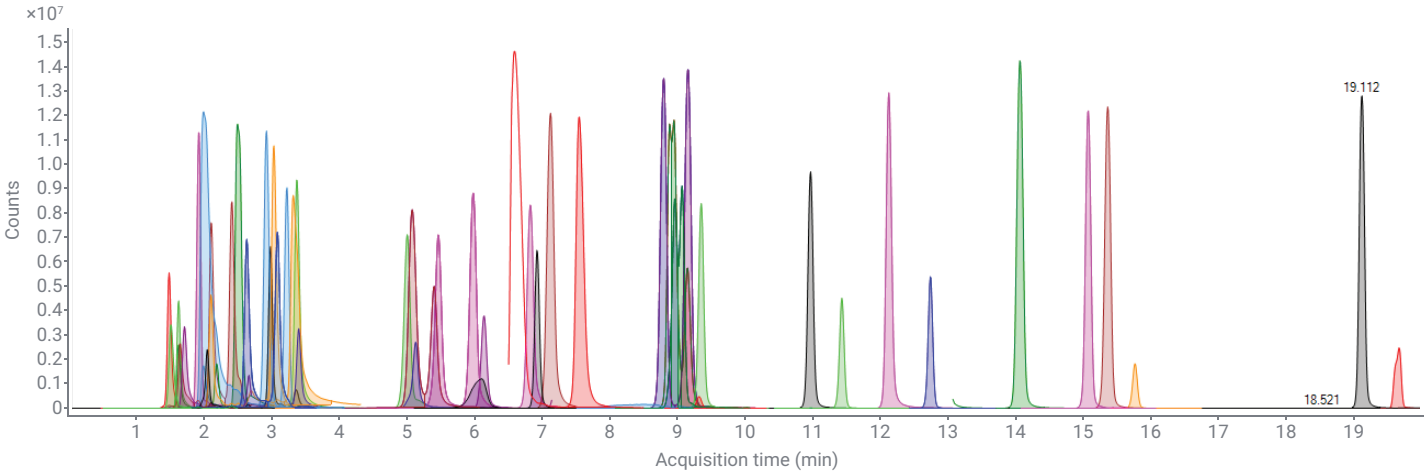


Figure 1. Example chromatogram showing the separation of 60 organic acids.

A key advantage of this method is its ability to resolve isobaric compounds—molecules with identical masses but different structures. Thirteen isobaric sets were successfully separated. Selected examples of these separations are shown in Figure 2.

Quantitative performance was evaluated using four-point calibration curves. Figure 3 shows calibration data for phenyllactic acid, and Figure 4 shows calibration for sebacic acid. Both compounds were chosen for their analytical difficulty and metabolic significance. The method demonstrated consistent linearity and reproducibility across the calibration range.

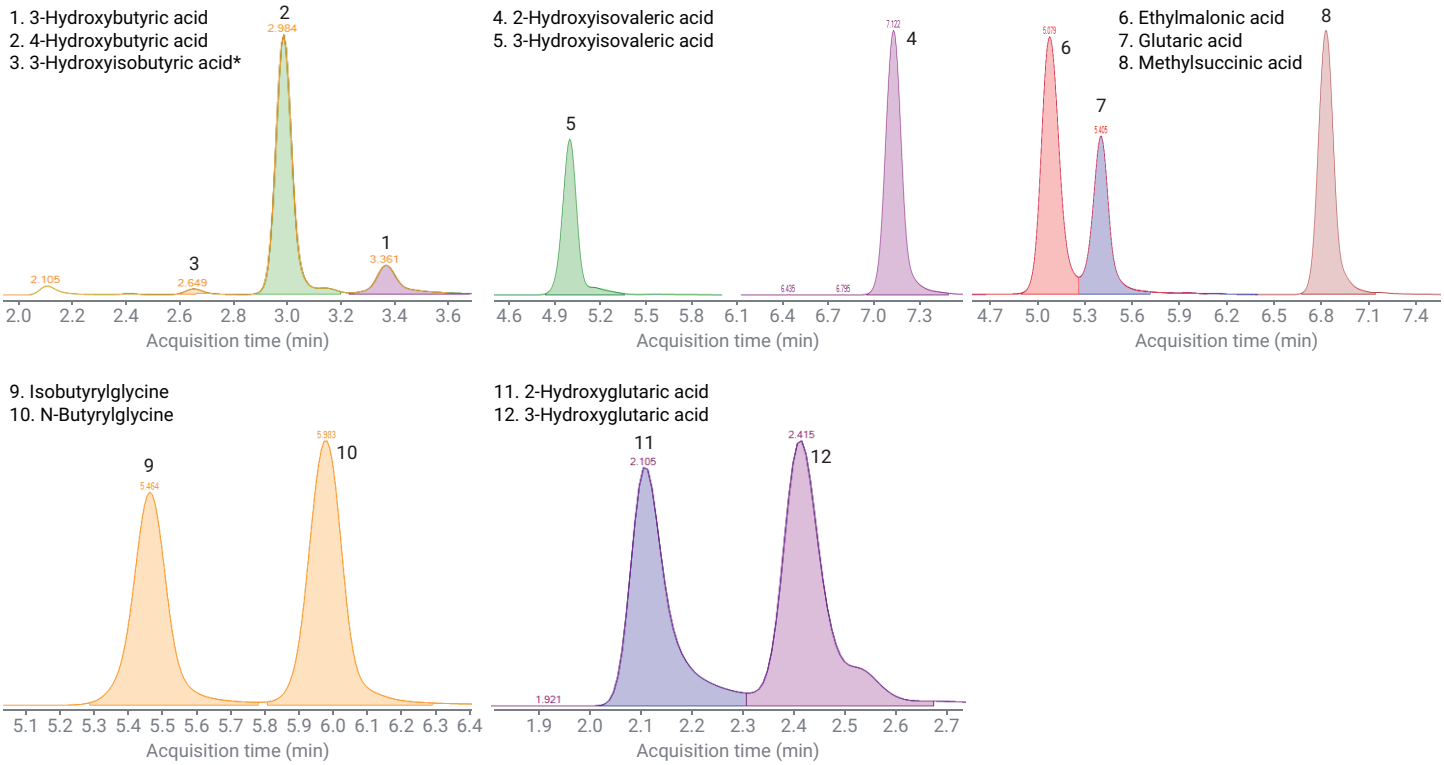


Figure 2. Select separations of isobaric sets of organic acids.

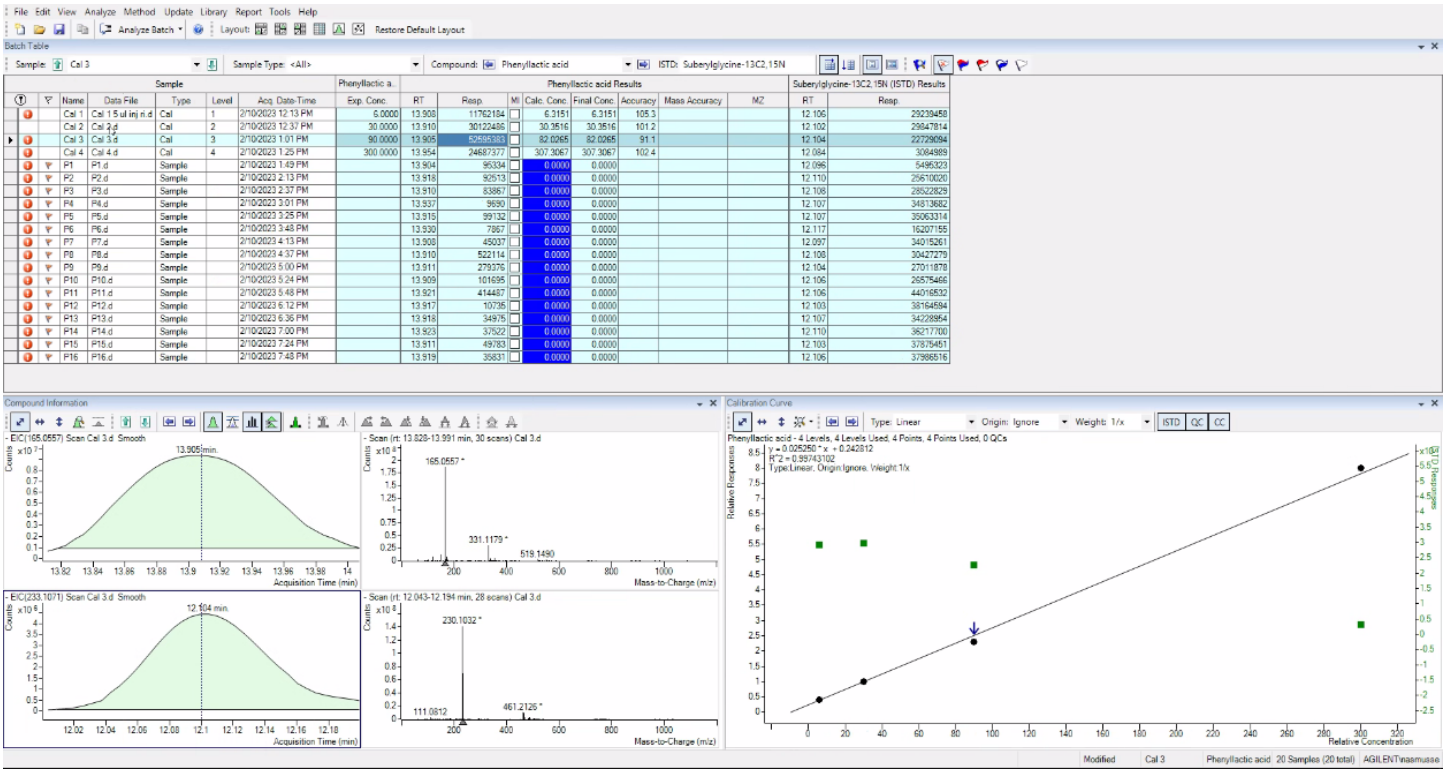


Figure 3. Four-point calibration data for phenyllactic acid.

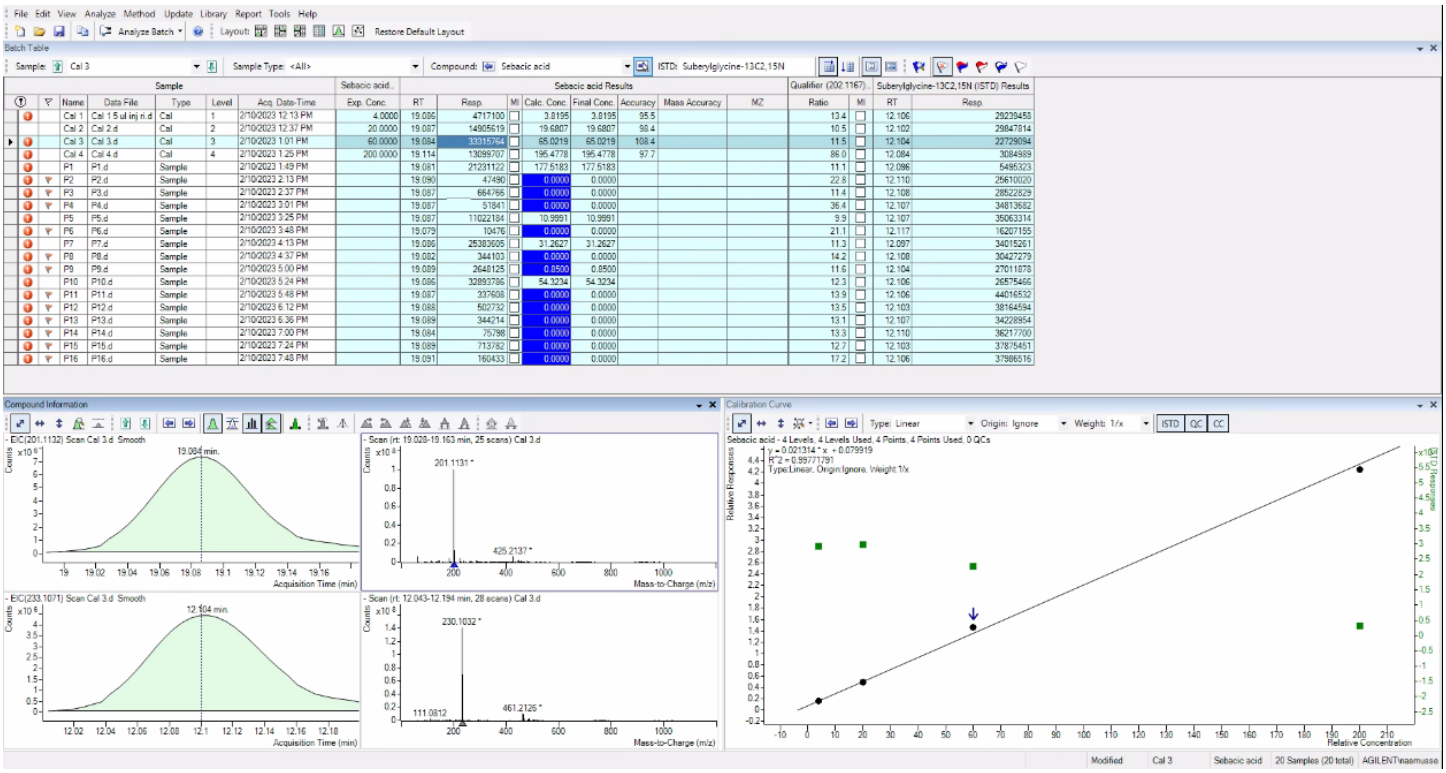
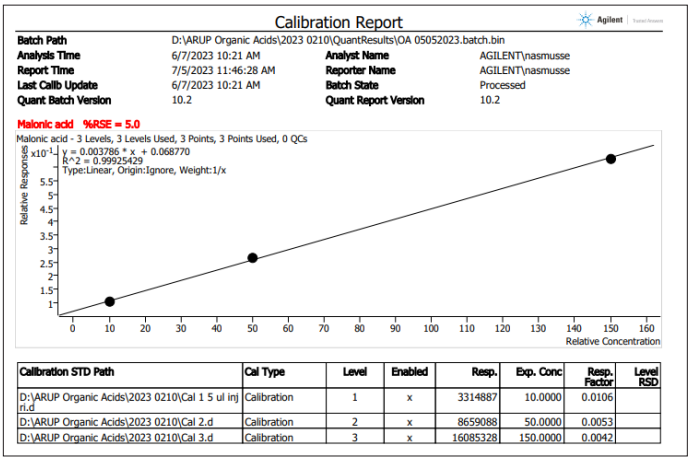


Figure 4. Four-point calibration data for sebacic acid.

Data acquisition was performed using MassHunter Acquisition software, with compound confirmation completed using MassHunter Qualitative Analysis and the custom PCDL. Quantitative results were processed using MassHunter Quantitative Analysis, which also supported streamlined reporting. An example of reporting output is shown in Figure 5, illustrating calibration and sample data in a clear, review-ready format.



Calibration Report

Batch Path	D:\ARUP Organic Acids\2023 0210\QuantResults\OA 05052023.batch.bin		
Analysis Time	6/7/2023 10:21 AM	**Analyst Name**	AGILENT\nasmusse
Report Time	7/5/2023 11:46:28 AM	**Reporter Name**	AGILENT\nasmusse
Last Calib Update	6/7/2023 10:21 AM	**Batch State**	Processed
Quant Batch Version	10.2	**Quant Report Version**	10.2

Malonic acid %RSE = 5.0

Malonic acid - 3 Levels, 3 Levels Used, 3 Points, 3 Points Used, 0 QCs
y = 0.003786 * x + 0.068770
R^2 = 0.99925429
Type:Linear, Origin:Ignore, Weight:1/x

Relative Responses (x10^-1)
Relative Concentration

Calibration STD Path	Cal Type	Level	Enabled	Resp.	Exp. Conc	Resp. Factor	Level RSD
D:\ARUP Organic Acids\2023 0210\Cal 1 5 ul inj ri.d	Calibration	1	x	3314887	10.0000	0.0106	
D:\ARUP Organic Acids\2023 0210\Cal 2.d	Calibration	2	x	8659088	50.0000	0.0053	
D:\ARUP Organic Acids\2023 0210\Cal 3.d	Calibration	3	x	16085328	150.0000	0.0042	

By Sample Quant Report

Batch Path	D:\ARUP Organic Acids\2023 0210\QuantResults\OA 05052023.batch.bin		
Analysis Time	6/7/2023 10:21 AM	**Analyst Name**	AGILENT\nasmusse
Report Time	7/5/2023 11:07:54 AM	**Reporter Name**	AGILENT\nasmusse
Last Calib Update	6/7/2023 10:21 AM	**Batch State**	Processed
Quant Batch Version	10.2	**Quant Report Version**	10.2

Analysis Info

Instrument	Instrument 1	**Operator**	
Data File	Cal 1 5 ul inj ri.d	**Sample Name**	Cal 1
Sample Type	Cal	**Dilution**	1
Acq. Method	OA TOF PH 3x150 Final.m	**Acq. Date**	2/10/2023 12:13 PM
Position	P3-A2	**Injection Volume**	5

- TIC Scan Cal 1 5 ul inj ri.d (Cal 1)

Counts (x10^8)
Acquisition Time (min)

ISTD Compounds	Compound Type	RT	Transition(T)	ISTD Resp.
Suberylglycine-13C2,15N	ISTD	12.106	233.1071	31401202

Quantitation Results

Compound	RT	Ref RT	Transition(T)	Transition(Q)	T-Resp	Q-Resp	QRatio	Ref QRatio	ISTD Compounds	IS Ratio	Final Conc.	Units
Glyceric acid	1.477	1.471	105.0193	No Q.	1871325	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.0596	11.2125	ng/ml
Glycolic acid	1.510	1.505	75.0088	No Q.	25648	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.0008	11.7576	ng/ml
Pyruvic acid	1.505	1.505	87.0088	No Q.	2020614	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.0643	9.2871	ng/ml
Uracil	1.555	1.550	111.0200	No Q.	1164290	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.0371	4.5349	ng/ml
Malonic acid	1.566	1.561	103.0037	No Q.	3314887	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.1056	9.7197	ng/ml
Orotic acid	1.628	1.650	155.0098	No Q.	897657	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.0286	1.8857	ng/ml
2-Ketoglutaric Acid	1.717	1.723	145.0142	No Q.	3160891	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.1007	10.0976	ng/ml
Acetoacetic acid	1.712	1.723	101.0244	No Q.	829428	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.0264	39.2280	ng/ml
Lactic acid	1.885	1.891	89.0244	No Q.	9778690	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.3114	12.2263	ng/ml
N-Acetylaspartic Acid	1.958	1.964	174.0408	No Q.	4985306 2	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	1.5876	75.6519	ng/ml
Fumaric acid	2.014	2.014	115.0037	No Q.	1511734	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.0481	0.0000	ng/ml
3-Hydroxyisobutyric acid	2.070	2.065	103.0401	No Q.	213322	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.0068	18.8878	ng/ml
2,3-Dihydroxy-3-methylbutyric acid	2.076	2.070	133.0506	No Q.	3485386	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.1110	3.0893	ng/ml
2-Hydroxyglutaric Acid	2.070	2.076	147.0299	No Q.	6969897	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.2220	9.8398	ng/ml
3-Hydroxyglutaric acid	2.372	2.372	147.0299	No Q.	9385916	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.2989	10.0996	ng/ml
Pyroglutamic acid	2.456	2.456	128.0353	No Q.	2141754 5	No Q.	No Q.	No Q.	Suberylglycine-13C2,15N	0.6821	36.6320	ng/ml

Figure 5. Example reporting results.

Together, the combination of HRAM detection, optimized chromatography, and integrated software tools supports confident, high-throughput organic acid profiling in under 20 minutes.

Conclusion

This study demonstrates a streamlined LC/MS workflow for the analysis of over 60 organic acids using an Agilent 6546 LC/Q-TOF. By eliminating derivatization and reducing analysis time, the method offers a faster, more efficient alternative to traditional GC/MS approaches. High-resolution accurate mass detection with an optimized chromatographic separation enables confident separation of isobaric compounds with reliable peak shape and retention. Integrated software tools—including Agilent MassHunter Acquisition, Qualitative Analysis, PCDL Manager, and Quantitative Analysis—support a complete workflow from data collection to reporting. Together, these technologies deliver accurate, high-throughput organic acid profiling for life science research applications.

References

1. Lindeque, J. Targeted Analysis of Organic Acids with GC-MS/MS: Challenges and Prospects. *Anal. Biochem.* **2024**, Nov, *694*, 115620. DOI: 10.1016/j.ab.2024.115620.
2. Körver-Keularts, I.; Wang, P.; Waterval, H.; Kluijtmans, L.; Wevers, R.; Langhans, C.; Scott, C.; Habets, D.; Bierau, J. Fast and Accurate Quantitative Organic Acid Analysis with LC-QTOF/MS Facilitates Screening of Patients for Inborn Errors of Metabolism. *J. Inherit. Metab. Dis.* **2018**, May, *41(3)*, 415–424. DOI: 10.1007/s10545-017-0129-0.

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