MetAmino Kit®

Free (Physiological) LC-MS Amino Acid and Metabolites Analysis

User Manual

Ver. EN220914

Important notes: **Read this manual carefully before the product use. This product is intended for research use only. No responsibility will be acceptedfor use in IVD (diagnostic) procedures.**

1. Contents of the manual

2. Introduction

Thank you for purchasing the Chromservis MetAmino® Amino Acid and Metabolite Kit. This user manual provides information regarding the care and use the sample preparation kit including the workflow protocol.

3. Kit contents

3.1. Reagents, liquid media and chemicals

Reagents, liquid media and chemicals The MetAmino® kit includes the following per 100 samples (Start-up Kit). The Basic Kit (400 samples kit) includes two boxes:

- The Basic kit (100 samples)
- Extended box (reagents, liquid media, chemicals and accessories for an additional 300 samples)

Item	Vial type	Volume in vial (mL)	No. of vials (100 samples)	No. of vials (400 samples)
Amino Acid Standards SD1 Solution	2 mL vial	0.25	1	4
Amino Acid Standards SD2 Dried	2 mL vial	n/a	2	8
Solution with Internal Standard (IS)	2 mL vial	1.1	1	4
Amino Acid Standard Diluting Medium (AASDM)	4 mL vial	1.4	1	4
MSPE sorbent activation medium (WES)	40 mL vial	22	1	4
MSPE sorbent equilibration medium (EQS)	40 mL vial	22	1	4
Catalytic Solution (CTS)	4 mL vial	2.2	1	4
Reagent (Derivatization) Solution (RDS)	4 mL vial	1.1	1	4
Diluting and Washing Medium (DWM)	40 mL vial	33	2	8
Eluting Medium (ELM)	40 mL vial	22	1	4
Precipitating Medium (PM)	40 mL vial	11	1	4

3.2. Accessories

Item	Note	Amount (100 samples)	Amount (400 samples)
Reagent tray for up to 80 Centrigufal Tubes	See Fig. 5 in Section 5.1	1 pc	1 pc
Microspin Filters with the MetAmino® sorbent	Inner tube incl. 0.22 µm membrane	100 pcs	400 pcs
Centrifugal Tubes (2 mL)	Outer tube	400 pcs	1,600 pcs
Autosampler Vials (9 mm screw-top)	Including septa and caps	100 pcs	400 pcs
MetAmino® HPLC Column	Proprietary stationary phase	1 pc	1 pc

Note: Microspin filters with the Metamino sorbent are only compatible with the centrifugal tubes included in the kit. The use of other types of centrifugal tubes may cause problems during use. Use of improper consumables may lead to the invalidity of the warranty.

Figure 1 - MetAmino® basic kit for 100 samples

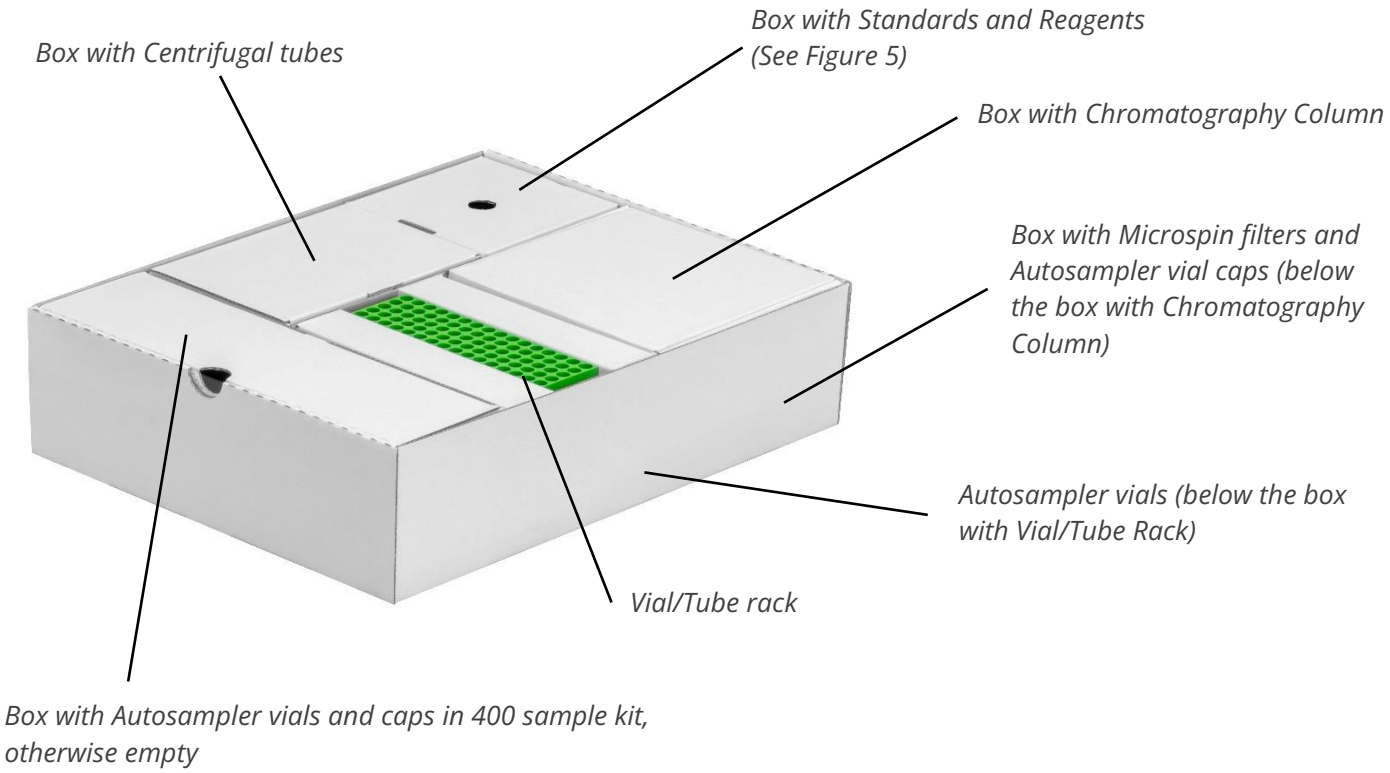


Figure 2 - MetAmino® extended box for a further 300 samples

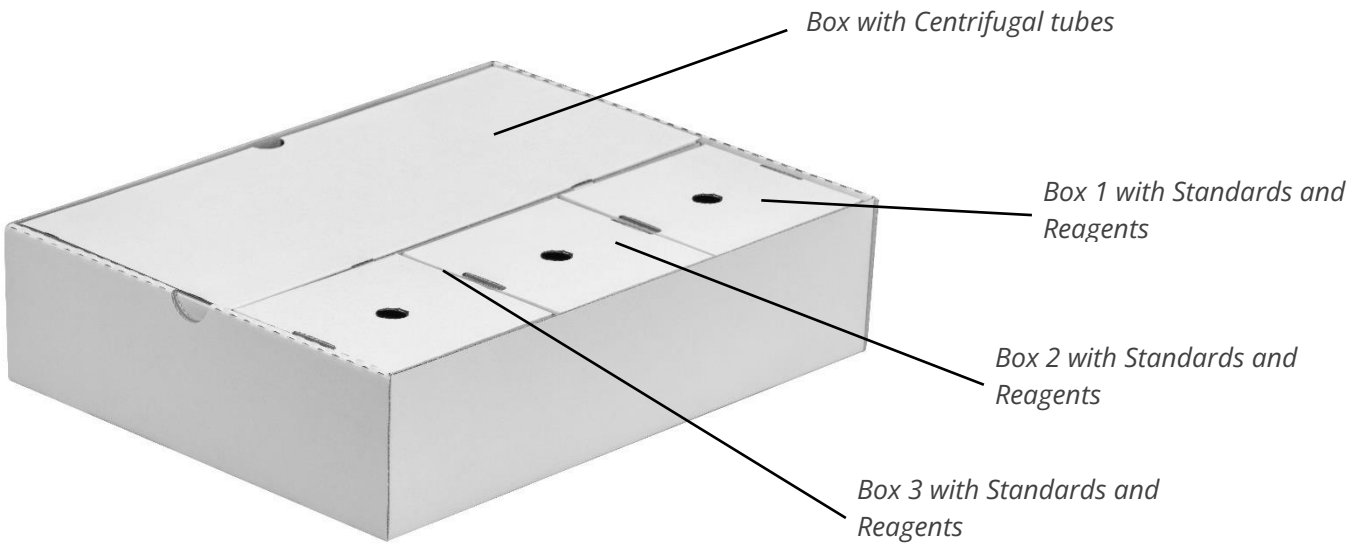
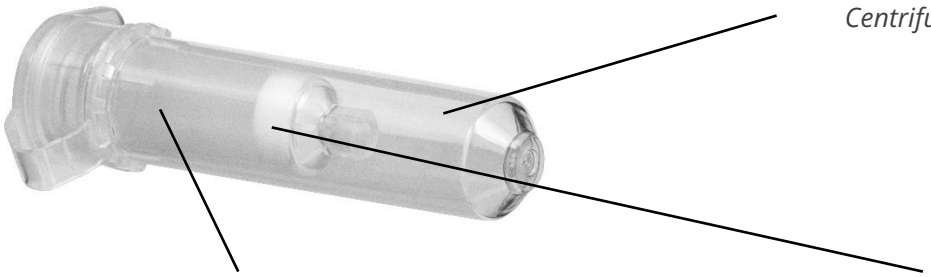


Figure 3 – MetAmino® Centrifugal Tube with Microspin Filters



3.3. Materials and tools

The items listed below are necessary for the workflow. These items are **NOT** included and supplied with the MetAmino® Kit.

No.	Material/Tool
1	Pipette 10 µL to 100 µL
2	Pipette 100 µL to 1000 µL
3	Pipette tips
4	Vortex
5	Bench-top microcentrifuge capable 6000 rpm (min 1500 g)
6	Container for proper waste disposal

4. Overview

4.1. Method description

The MetAmino® kit is designed for the comprehensive analysis of amino acids, biogenic amines and other metabolic analysis by liquid chromatography-mass spectrometry (LC-MS) using various mass spectrometric detectors (ion trap, triple quadrupole, high resolution MS). The method has been validated for the determination of 36 amino acids and related compounds with primary or secondary amino groups in human serum and urine, matrices of plant origin or in other matrices. It includes an efficient derivatization step in which the protic functional groups are converted to nonpolar derivatives, which are subsequently purified on a dedicated Micro-Solid Phase Extraction (**MSPE**) spin-off filter. The sample preparation procedure is simple, inexpensive, and does not require expensive laboratory equipment. The MetAmino® kit is supplied with all materials and information necessary to adapt the kit to any analytical laboratory equipped with the LC-MS technique.

The analytical procedure using the MetAmino® kit consists of the following steps:

- Extraction of the sample (MSPE)
- In-situ derivatization of the amino functional groups

The Micro-Solid-Phase Extraction uses a special MetAmino® Microspin filter, which adsorbs the treated amino acid analytes gaining much less polarity. They are eluted through the integrated 0.22µm membrane and directly available for LC-MS analysis on a dedicated MetAmino® HPLC column. Sample preparation time takes around 8 minutes. LC-MS analysis is accomplished in 12 minutes. Total analysis time is around 20 minutes.

The MetAmino® kit video is provided to facilitate familiarization and to demonstrate the simplicity of the described procedure. Please note that some of the sample preparation steps described in the video may be slightly different than what is described in this User Manual. The MetAmino® kit video can serve as a general User Manual. The whole MetAmino® kit is described in detail in this User Manual.

4.2. Free Amino Acids in Biological Samples

The MetAmino® method has been developed for the LC-MS analysis of more than 70 amino acids and related compounds. For the complete list, please refer to **Table 1**. However, the amino acid set can further be expanded to other compounds containing primary or secondary amino functional groups. If other analytes are a subject of the extended application, please contact your Chromservis technical consultant for assistance.

Table 1 *Comprehensive list of amino acids and related compounds prepared by MetAmino® kit for (+ESI) LC-MS analysis (included internal standards are highlighted in bol).*

Analyte			RT	Mol. Ion	MRM MS/MS transitions		
No.	**Name**	**Synonyms**	**[min]**	**$[M+H]^+$**	**Precursor**	**Quantifier (CE)**	**Qualifier (CE)**
1	Cotinine	Syringe standard	1,15	177,1024	177,1	98 (24)	80 (28)
2	Putrescine	PUT	1.40	189.1599	189.2	72.1 (16)	115.9 (12)
3	Cadaverine	CAD	1.48	203.1755	203.2	86 (20)	69.2 (28)
4	Homoserine	HSER	1.67	202.1074	202.1	146 (4)	102 (8)
5	Pyroglutamic acid	pGLU	1.86	186.1125	186.1	84 (20)	130 (8)
6	Arginine	ARG	3.35	331.2341	331.2	70.1 (48)	115.9 (32)
7	**Homoarginine**	**IS**	**3.63**	**345.2497**	**345.2**	**84 (52)**	**125.9 (24)**
8	Glutamine	GLN	4.12	303.1916	303.2	186 (12)	84 (44)
9	Anserine	ANS	4.20	397.2447	397.2	109.1 (52)	226.2 (28)
10	Citrulline	CIT	4.20	332.2181	332.2	70.2 (36)	215 (16)
11	Methionine sulfoxide	MET-SO	4.29	322.1683	322.2	248 (12)	100 (32)
12	Methionine sulfone	MET-SO_2	4.30	338.1632	338.2	238 (12)	182 (16)
13	5-Hydroxylysine	HLY	4.40	345.2021	345.2	82 (48)	128.1 (28)
14	1-Methylhistidine	1MHIS	4.52	326.2075	326.2	124 (36)	224 (20)
15	3-Methylhistidine	3MHIS	4.55	326.2075	326.2	95.7 (52)	270.1 (24)
16	Prolylhydroxyproline	PHP	4.71	385.2333	385.2	170.1 (16)	114 (36), 70.2 (56)
17	Serine	SER	4.73	262.1615	262.2	106 (16)	60.1 (40)
18	Asparagine	ASN	4.78	271.1649	271.2	240.1 (16)	254.1 (16)
19	4-Hydroxyproline	HYP	5.23	288.1807	288.2	86.1 (32)	188 (12)
20	Glycine	GLY	5.23	232.1544	232.2	76 (12)	132 (4)
21	N-Acetylaspartic acid		5.36	288.1807	288.2	88 (28)	144.1 (20), 158 (8)
22	Glycylproline	GPR	5.39	329.2072	329.2	70.1 (52)	172.1 (16), 115.9 (36)
23	Threonine	THR	5.45	276.1806	276.2	74.2 (28)	176.1 (12)
24	5-Aminolevulinic acid	5-ALA	5.63	288.1807	288.2	158 (8)	86.1 (32)
25	Ethanolamine	EAM	6.09	262.1649	262.2	88 (16)	144.1 (4)
26	*beta*-Alanine	3-ALA	6.22	246.1701	246.2	116 (12)	90 (12)
27	Alanine	ALA	6.25	246.1701	246.2	90 (16)	146 (8)

	Analyte		RT	Mol. Ion		MRM MS/MS transitions	
28	Spermine		6.39	503.3804	503.4	102 (44)	229.2 (32)
29	Histamine	HTA	6.52	312.1919	312.2	212 (12)	95 (40), 112 (20)
30	Indoleacetic acid	IAA	6.65	232.1333	232.1	130 (24)	176 (8)
31	*gamma*-Aminobutyric acid	GABA	6.75	260.1857	260.2	87.1 (24)	86 (24)
32	Sarcosine	SAR	6.75	246.1701	246.2	90 (12)	146.1 (8)
33	4-Aminobenzoic acid	PABA	7.00	294.1701	294.2	134.1 (20)	91 (56)
34	*beta*-aminoisobutyric acid	BAIBA	7.05	260.1857	260.2	130 (12)	112 (20)
35	2-aminobutyric acid	ABA	7.35	260.1857	260.2	160.2 (8)	104 (12)
36	Proline	PRO	7.58	272.1857	272.2	70.1 (36)	172 (12)
37	Methionine	MET	7.65	306.1735	306.2	204 (8)	104 (20)
38	**Methionine-d3**	**IS**	**7.65**	**309.1922**	**309.2**	**207 (8)**	**107 (16)**
39	Thiaproline	TPR	8.03	290.1422	290.1	88 (28)	134 (16), 190.1 (8)
40	Asparatame		8.15	451.2439	451.2	120 (48)	88 (44)
41	Serotonine		8.22	377.2072	377.2	160 (32)	303.1 (8)
42	2,4-diaminobutyric acid	DABA	8.25	375.2490	375.2	201.3 (16)	245 (12)
43	Valine	VAL	8.30	274.2014	274.2	72 (32)	116 (12)
44	Norvaline		8.35	274.2014	274.2	72.1 (24)	174 (8)
45	Alanyl-lysine	ALA-LYS	8.36	474.3174	474.3	84.1 (60)	400.2 (12)
46	Carnosine	CAR	8.36	483.2814	483.3	110.1 (40)	212.1 (24)
47	Ornithine	ORN	8.45	389.2647	389.3	70.1 (60)	315.2 (8)
48	Tryptophan	TRP	8.45	361.2123	361.2	259 (12)	159 (28)
49	Ethionine	ETH	8.60	320.1890	320.2	218.1 (8)	75.1 (44)
50	Histidine	HIS	8.90	412.2443	412.2	110 (44)	312,1 (16)
51	Lysine	LYS	8.92	403.2804	403.3	84.1 (56)	329.1 (8)
52	Phenylalanine	PHE	9.04	322.2014	322.2	120 (36)	164 (16)
53	Leucine	LEU	9.06	288.2170	288.2	86.1 (20)	188.2 (8), 130.1 (12)
54	Aspartic acid	ASP	9.06	346.2225	346.2	88.1 (24)	159.9 (12)
55	Spermidine		9.11	446.3226	446.3	198.1 (28)	298.1 (12)
56	Glutamic acid	GLU	9.17	360.2382	360.2	163 (16)	105 (36)
57	*allo*-isoleucine	aILE	9.22	288.2169	288.2	130.1 (12)	86.1 (24)
58	Isoleucine	ILE	9.24	288.2170	288.2	130.1 (16)	86.1 (24)
59	Norleucine	NLEU	9.40	288.2170	288.2	86.2 (24)	130.1 (12)
60	Pipecolic acid	PIP	9.45	286.2013	286.2	128.1 (12)	84.1 (40)

	Analyte		RT	Mol. Ion		MRM MS/MS transitions	
61	**Homophenylalanine**	**IS**	**9.67**	**336.2170**	**336.2**	**91 (56)**	**117 (32)**
62	2-Aminoadipic acid	AAA	9.67	374.2538	374.3	98 (32)	172 (16)
63	Adrenaline	ADN	10.15	484.2541	484.3	166 (36)	466.2 (4)
64	Cysteine	CYS	10.16	378.1946	378.2	120.1 (32)	278 (12)
65	2-Aminoheptane dioic acid	APA	10.20	388.2695	388.3	112 (36)	186.1 (20)
66	Glutathione	GSH	10.27	620.3213	620.3	186.1 (28)	130.2 (44)
67	Dopamine	DAM	10.27	454.2435	454.2	196.1 (24)	152 (40)
68	Glutamyl-lysine	GLU-LYS	10.44	588.3855	588.4	84.1 (56)	128.1 (52), 157.9 (32)
69	Homocysteine	HCYS	10.48	392.2103	392.2	292.2 (12)	118.1 (16)
70	Diaminopimelic acid		10.54	503.3328	503.3	82.1 (60)	127.9 (48)
71	Tyrosine	TYR	10.61	438.2487	438.2	136 (44)	179.9 (28)
72	Cystathionine	CTH	10.68	535.3049	535.3	201.9 (24)	88.1 (56)
73	Cystine	C-C	10.76	553.2613	553.3	276.1 (16)	219.9 (24)
74	Kynurenic acid		10.78	346.1649	346.2	246 (8)	144 (52), 190 (24)
75	Selenocystine	Se-C-C	11.12	649.1501	649.2	323.9 (20)	575 (12)
76	Kynurenine		11.17	465.2596	465.3	146 (44)	274.2 (12)
77	Homocystine	HC-CH	11.28	581.2926	581.3	290 (16)	190 (20)
78	3,4-dihydroxy-phenylalanine	DOPA	11.55	554.2961	554.3	152.1 (44)	454,1 (12)

Note: CE = Collision energy

Storage and Stability

Amino Acid standards **SD1** are supplied in the solution and Amino Acid standards **SD2** are supplied in dried form. The standard stock solution **SD2** should be used **freshly** prepared on the day of consumption. We recommend storing the complete Box with Standards and Reagents **at 4°C in the dark**. If this is not possible, store only the Amino Acid Standards **SD1** and **SD2** (in dried form), Internal Standard Solution **(ISS**), Catalytic Solution (**CTS**) and Reagent (Derivatization) Solution (**RDS**) **at 4°C in the dark**. All other components can be stored at room temperature.

Table 2 *Overview of the stability of the standards and reagents contained in the MetAmino® kit*

Item	Storage conditions	Stability
Amino Acid Standards Solution **(SD1)**	4 °C	12 months
Amino Acid Standards Dried **(SD2)**	4 °C	12 months
Solution with Internal Standard **(IS)**	4 °C	12 months
Amino Acid Standard Diluting Medium **(AASDM)**	4 to 25 °C	12 months
MSPE sorbent activation medium **(WES)**	4 to 25 °C	12 months
MSPE sorbent equilibration medium **(EQS)**	4 to 25 °C	12 months
Catalytic Solution **(CTS)**	4 °C	12 months
Reagent (Derivatization) Solution **(RDS)**	4 °C	6 months
Diluting and Washing Medium **(DWM)**	4 to 25 °C	12 months
Eluting Medium **(ELM)**	4 °C	12 months
Precipitating Medium **(PM)**	4 °C	12 months

4.3. Safety

For safety reasons, the sample preparation station should be placed in a fume hood and protective gloves and goggles should be worn. When working with biological fluids, please take any necessary precautions to prevent infection with blood borne pathogens. Appropriate bio-safety precautions and disposal of bio-hazardous wastes should be followed. Follow the national standards and rules in your country.

5. Sample Preparation Procedure

5.1. Setup

The MetAmino® kit package was designed to be an efficient workstation. It contains a reagent tray, vial rack, and vial section. To speed up and simplify the sample preparation, it is recommended to use the Vial/Tube rack (See **Figure 4**). If the kit will not be used for several days, the Reagent tray (See **Figure 5**) can be conveniently removed and placed in the refrigerator at 4 °C.

Figure 4 – Vial/Tube Rack

Figure 5 – Box with Reagents and Standards

5.2. Sample Preparation Protocol

Note: The sample preparation process should be performed in a well-ventilated area (fume hood). The centrifugation steps require the sample to pass the membrane fluently and completely. We recommend 6,000 rpm at 1,500 × g.

5.2.1. Extraction/precipitation step

For each sample line up use one Centrifugal tube (outer tube), one inner microspin filter with sorbent placed in the outer Centrifugal tube and one empty outer Centrifugal tube for the final eluate.

STEP 1: Sample precipitation (serum, plasma): Pipette 100 µL of sample (serum, plasma) and add 100 µL Precipitating Medium (**PM**). Centrifuge for 30 to 60 sec. at 1,500 ×g.

Note: The sample must not contain alcohol. For instance, if a methanolic solution is handled, it must be dried and reconstituted in an aqueous environment not containing any alcohol! If you have not sufficient amount of the sample, the Sample Preparation Protocol can be scaled down as well.

STEP 2: Pipette 25 µL of sample (precipitated serum, plasma, urine or other), and 10 µL of solution with internal standards (**IS**) into each sample preparation vial.

Note: If low concentrations of analytes need to be quantified, the volume of sample to be prepared should be 50 µL or more. The total amount of amino acids in the sample to be loaded onto the MSPE should not exceed 1.2 µmoles!

5.2.2. Derivatization step

STEP 3: Pipette 20 µL of Catalytic Solution (**CTS**) into each sample preparation vial and vortex briefly (5 to 10 sec).

STEP 4: Pipette 10 µL of Reagent (Derivatization) Solution (**RDS**) into each sample preparation vial and vortex briefly (5 to 10 sec) and let the derivatization reaction proceed for at least 2 to 3 min. The workflow step will provide a **derivatized reaction mixture** as referred below.

Note: *If the derivatization takes longer than 3 minutes, it does NOT have an influence on the analytical results. After pipetting the reagent solution, the pipette tip should immediately be removed and disposed of*.

5.2.3. MSPE step

STEP 5: Activate and equilibrate the sorbent in microspin filter (inner tube):

Wet the sorbent in the microspin filter with 200 µL of MSPE sorbent activation medium (**WES**).

Centrifuge for 30 to 60 sec. at 1,500 ×g (6,000 rpm).

Equilibrate the sorbent with 200 µL of MSPE sorbent equilibration medium (**EQS**).

Centrifuge for 30 to 60 sec. 1,500 ×g (6,000 rpm)

STEP 6: Discard the solutions which passed through.

STEP 7: Dilute the derivatized reaction mixture with 400 µL of Diluting and Washing Medium (**DWM**) and vortex briefly (5 to 10 sec).

STEP 8: Load the diluted reaction mixture (typically 450 to 500 µL) to the wetted microspin filter sorbent and let it stand for 1 to 2 minutes. Centrifuge for 30 to 60 sec. at 1,500 ×g (6,000 rpm).

STEP 9: Discard the solutions which passed through.

STEP 10: Wash the sorbent in the microspin filter with 200 µL of Diluting and Washing Medium (**DWM**) and Centrifuge 30 to 60 sec. at 1,500 ×g (6,000 rpm).

STEP 11: Put the microspin filter into a new centrifugal vial, add 200 µL of Eluting Medium (**ELM**) and centrifuge 30 to 60 sec. at 1,500 ×g (6,000 rpm).

Note: In exceptional cases the *solution may pass through the microspin filter before centrifugation begins. This phenomenon does not have a negative effect on sample preparation.*

STEP 12: Transfer the eluate into the autosampler vial. The sample is ready for LC-MS analysis.

5.3. Optimizing Sample Preparation Time

After a short period of practice the complete sample preparation protocol can be completed in 7 to 12 minutes per sample. This process can be further improved if samples are prepared in multiples of three. For example, add Catalytic Solution (**CTS**) (STEP 3) to three vials sequentially using,, the same pipette tip. Similarly, at step 4 dispense the Reagent (Derivatization) Solution (**RDS**) to three vials in succession. Vortex all three vials simultaneously. Continue from STEP 8 for each one of the tree samples prepared

5.4. Column for MetAmino® LC-MS analysis

The HPLC column of the following parameters is included in the kit:

Metamino® LC-MS column 100 x 2.1 mm (flowrate 0.3 mL/min)

The column should be equilibrated by running a blank gradient. The column can be stored in the mobile phase when not in use.

Note: Because of column length, and the use of a sorbent with small particle size and a mobile phase of high viscosity (methanol/water), the expected column backpressure is 300 to 350 bar (4,350 to 5,100 psi). The column supplied with the kit tolerates this backpressure very well.

Instrument settings:

5.4.1. HPLC method

Mobile phase:	A: 5mM Ammonium formate in water	
	B: 5mM Ammonium formate in methanol	
Gradient	**RT (min)**	**Solvent B (%)**
	0:00	55
	10:00	90
	10:50	100
	12:00	100
	12:01	55
	15:00	55
Flow rate	0.30 mL/min	
Column temperature	35 °C	
Injection volume	5 µL	
HPLC column equilibration	For 3 to 6 min before the next injection	

5.4.2. MS method

Ionization	ESI
Mode	Positive Ion
Scan range	100 to 750 m/z
Ion source temperature	300 °C

5.5. Tuning the Mass Spectrometer

Some mass spectrometers require a concentrated calibration solution for tuning the instrument (if not then calibration solution No. I {see Section 5.7} can be used). To prepare the concentrated solution, dispense 40 µL aliquot of Amino Acid Standards **SD1** Solution and 40 µL aliquot of Amino Acid Standards **SD2** Dried (40 nmol) into each of two sample vials (internal standard solutions can be omitted if not relevant). Perform the **MSPE** and derivatization steps for each vial as described by the MetAmino® procedure (Section 5.2). Transfer the eluate from the microspin filter centrifugal vial into one autosampler vial and use to tune the mass spectrometer.

Most mass spectrometers will not allow for concomitant tuning of a large number of ions as required for amino acid profiling. This impediment can easily be overcome by creating time segments (periods) in the run file where a selected group of ions are analysed within each segment. This use of segments allows for optimal tuning of a large number of desired analytes.

A suggested breakdown of the MS analysis into three segments looks as follows:

Time	Suggested Tune AA	AA in Range	AA at End of Range
0-5.5 min	GLN and ASN	ARG, GLN, SER, ASN, GLY, THR	THR
5.5-8.9 min	MET and VAL	ALA, 2-ABA, PRO, MET, VAL, ORN, TRP, HIS	HIS
8.9-13.0 min	LEU and CC	LYS, PHE, ASP, LEU, GLU, ILE, CYS, TYR, CC	CC

A suggested breakdown of the MS analysis into six segments looks as follows:

Time	Suggested Tune AA	AA in Range	AA at End of Range
0-5.5 min	GLN and ASN	ARG, GLN, SER, ASN, GLY, THR	THR
5.5-7.0 min	ALA	ALA	ALA
7.0-8.1 min	PRO and MET	2-ABA, PRO, MET	MET
8.1-8.9 min	VAL and TRP	VAL, ORN, TRP, HIS	HIS
8.9-9.8 min	PHE and LEU	LYS, PHE, ASP, LEU, GLU, ILE	ILE
9.8-13.0 min	CC and TYR	CYS, TYR, CC	CC

The segment time limits and amino acids to be tuned may be different depending on instrument and application. HPLC pumps having larger gradient delay times will produce longer retention times and segments must be adjusted accordingly.

5.6. Calibration Standards

For quantitation purposes, prepare aliquots of the standard mixtures following the Sample Preparation by Derivatization and Microspin solid phase extraction described in this manual in Section 5.2. Two vials with amino acid standards are supplied in the kit **SD1** (33 amino acids in solution) and **SD2** (3 dried amino acids – dry material). The concentrations of standards SD1 and SD2 (after dissolution according to this manual) are 1 mmolL^{-1} of each amino acid. After reconstitution, the standard solution **SD2** should be used on the same day of preparation. Standard mixtures **SD1** should be stored in the refrigerator at 4 °C.

One vial with standard mixture solution **SD1** included in the kit contains 33 amino acids:

AAA, 2-ABA, ALA, APA, ARG, ASP, BAIBA, CC, CIT, CTH, GABA, GLU, GLY, GPR, HIS, HLY, HYP, ILE, LEU, LYS, MET, 1MHIS, 3MHIS, ORN, PHE, PHP, PRO, SAR, SER, THR, TPR, TYR, VAL

The **SD2** vials with lyophilized mixture of 3 amino acids:

ASN, GLN, TRP

To prepare the **SD2** solution, dissolve dry amino acids **SD2** in 0.300 mL **AASDM** Amino Acid Standard Diluting Medium.

*Note: Vials with dry unstable amino acids **SD2** are include in the kit. The standard stock solution **SD2** should be used on the day of preparation, as the stability of GLN, ASN, TRP is limited. The internal standards (IS) are supplied in the solution of concentration 0.3 mmolL^{-1}.*

5.7. Calibration Procedure

Use the following standard amino acid mixtures and make duplicate injections of each to generate the desired calibration:

Calibration solution:

No. I. 20 µL of amino acid standard kit solution **SD1**, plus 20 µL of amino acid standard kit solution **SD2**, plus 10 µL of solution with internal standards (20 nmol of each amino acid and 3 nmol of each **IS**)

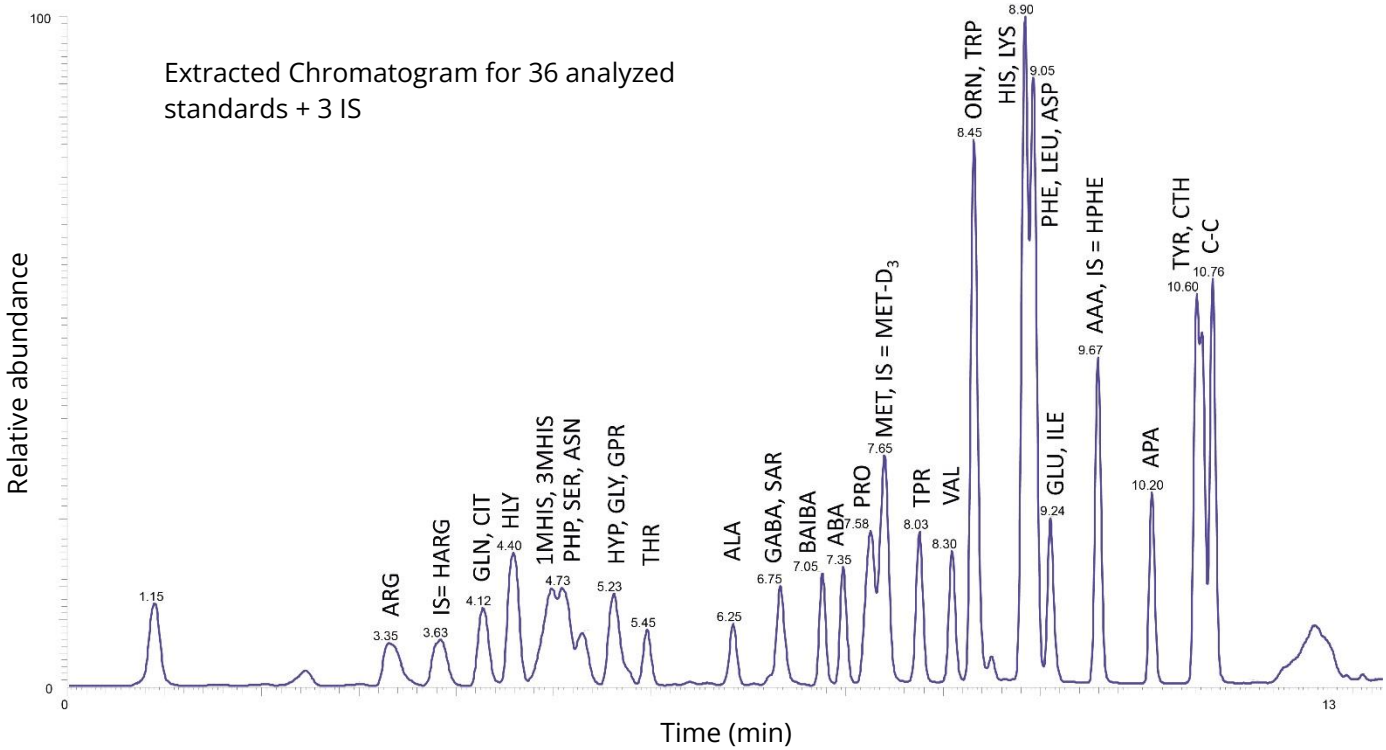
No. II 10 µL of amino acid standard kit solution **SD1**, plus 10 µL of amino acid standard kit solution **SD2**, plus 10 µL of solution with internal standards (10 nmol of each amino acid and 3 nmol of each **IS**)

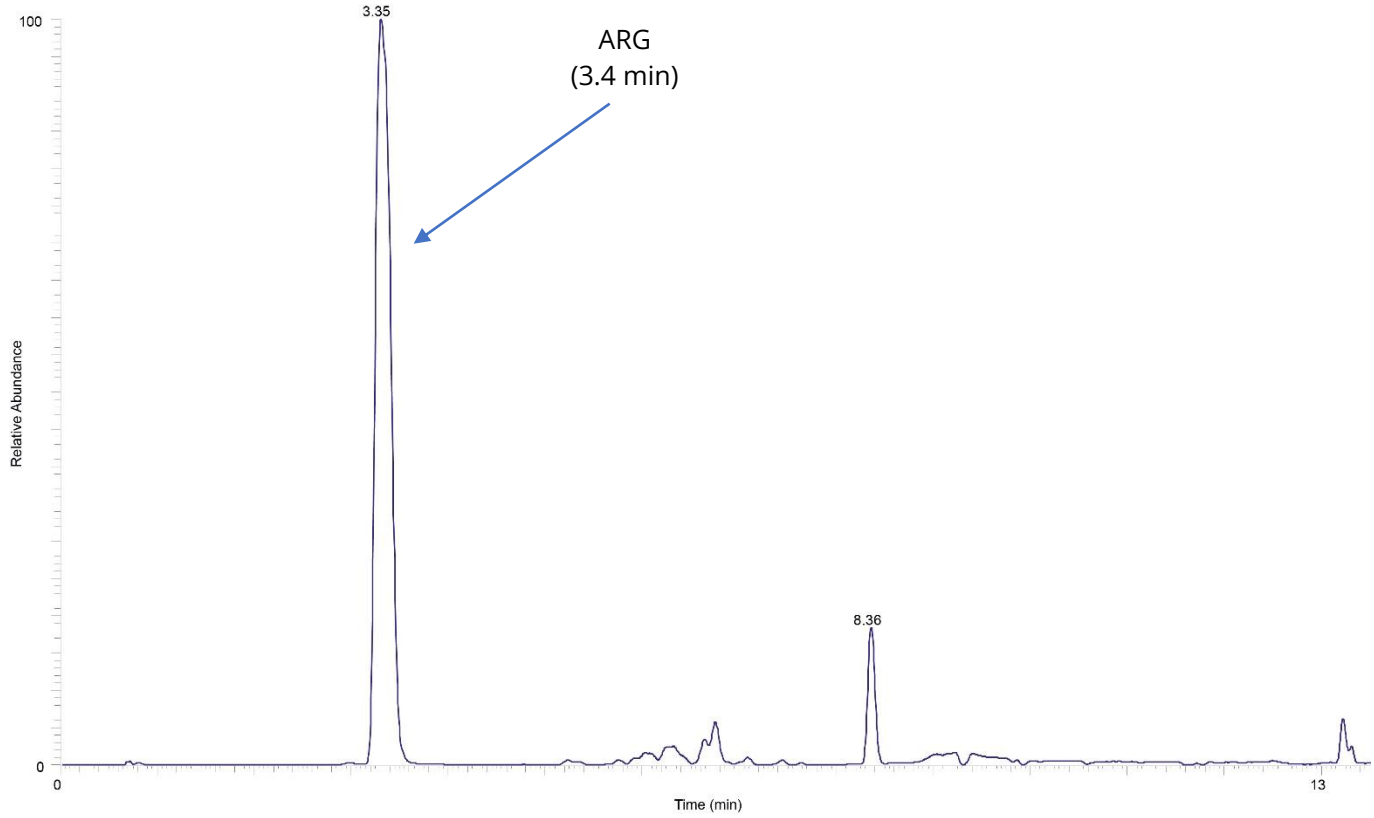
No. III 10 µL of ten times diluted amino acid standard kit solution **SD1**, plus 10 µL of ten times diluted amino acid standard kit solution **SD2**, plus 10 µL of solution with internal standards (1 nmol of each amino acid and 3 nmol of each **IS**)

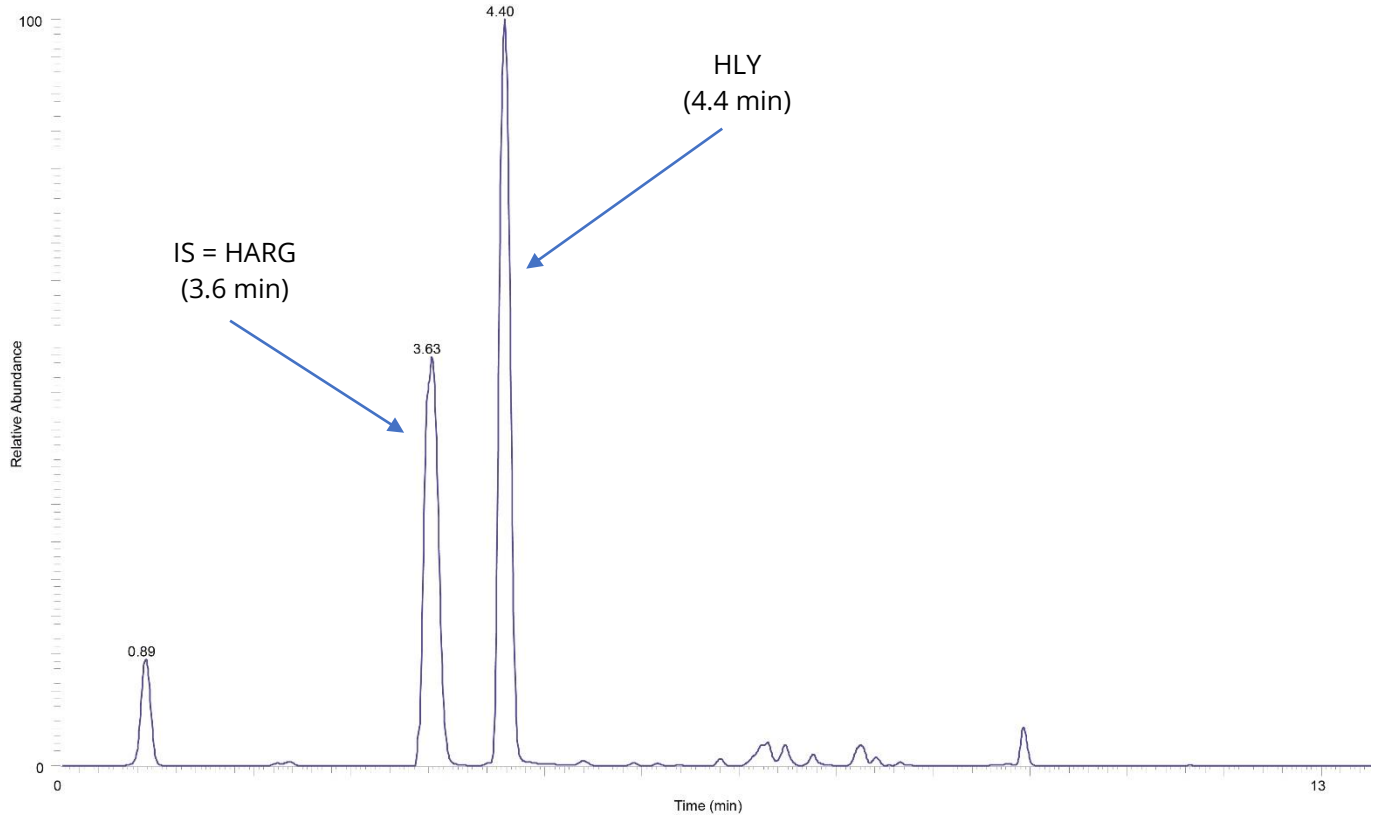
No. IV 10 µL of hundred times diluted amino acid standard kit solution **SD1**, plus 10 µL of hundred times diluted amino acid standard kit solution **SD2**, plus 10 µL of solution with internal standards (0.1 nmol of each amino acid and 3 nmol of each **IS**)

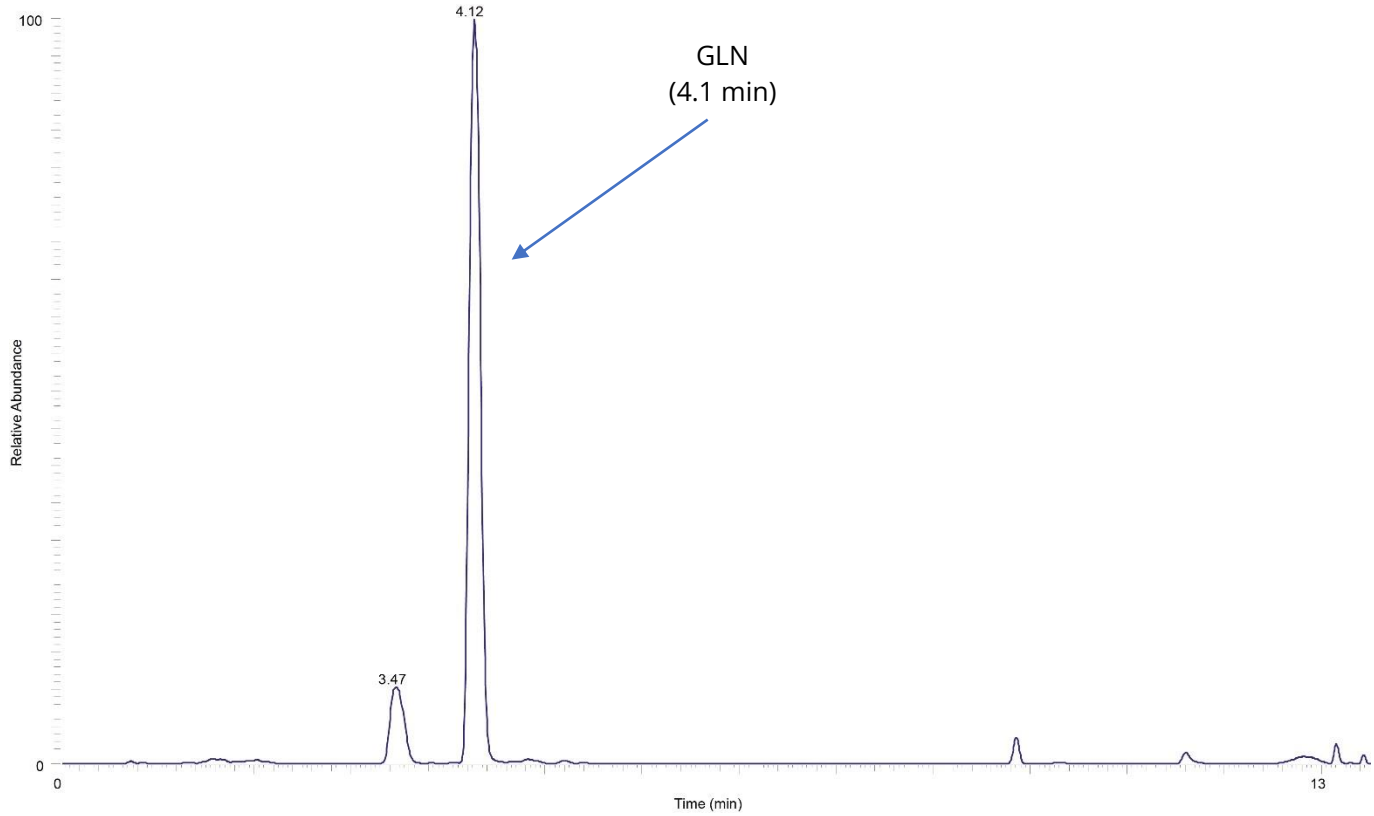
The concentration of each internal standard (**IS**) - HARG, MET-d3 and HPHE - in calibrators and samples prepared for chromatographic analysis is 15 µmolL^{-1}. While the use of the ideal internal standard will vary based on instrument and application, we recommend using HARG as the internal standard for ARG, GLN, CIT, HLY, 1MHIS, 3MHIS, PHP, SER, and THR; MET-d3 as the internal standard for ASN, , GLY, GPR, , ALA, GABA, SAR, BAIBA, 2-ABA, PRO, MET, TPR, LYS;; and HPHE for HYP, VAL, ORN, TRP, HIS, PHE, ASP, LEU, GLU, ILE, AAA, APA, TYR, CTH and CC. Other amino acids can be added and used as internal standards based on the application.

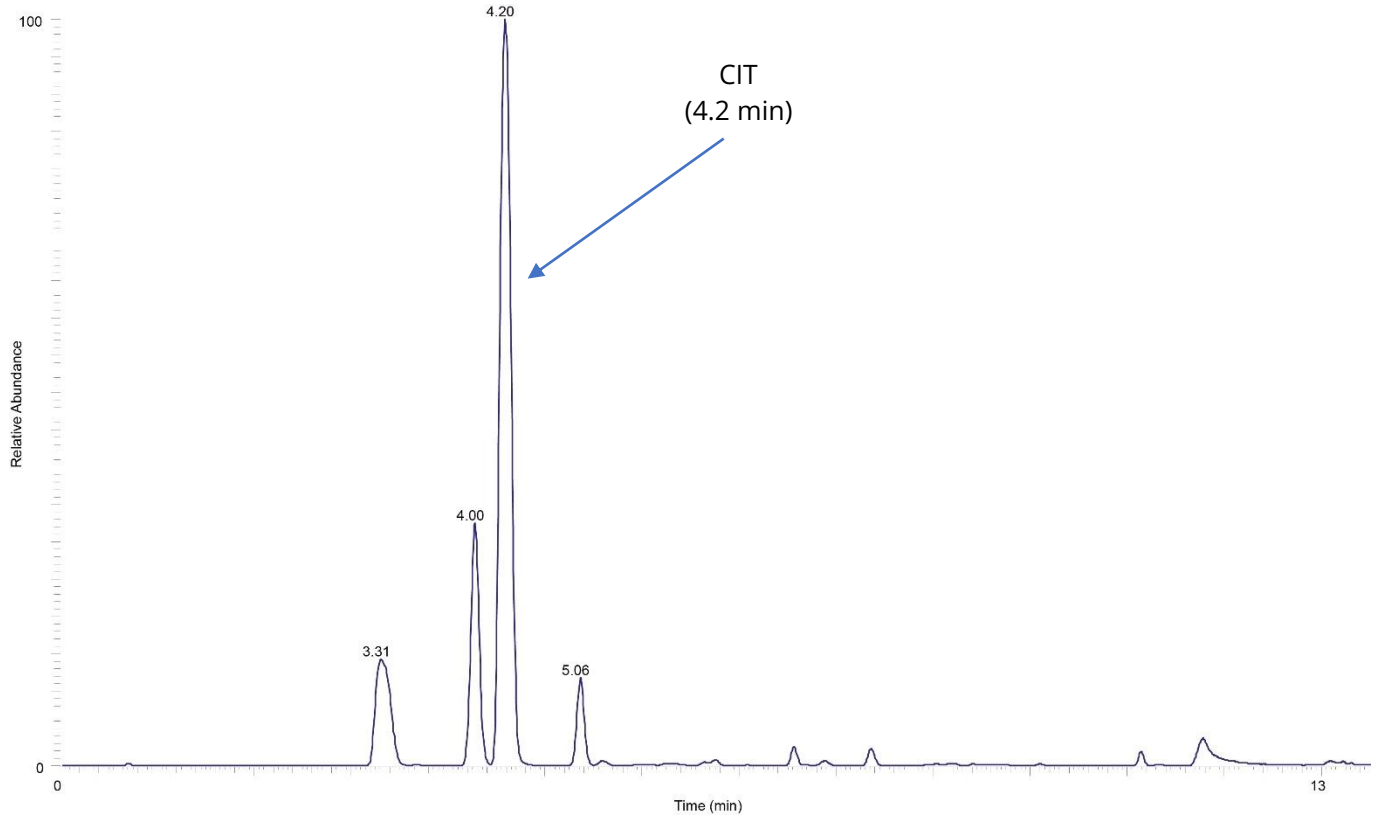
Remember: the standard solution vials should be placed in the Extracted Ion Chromatograms for the amino acids included in standard mixtures provided with the kit (in order of elution):

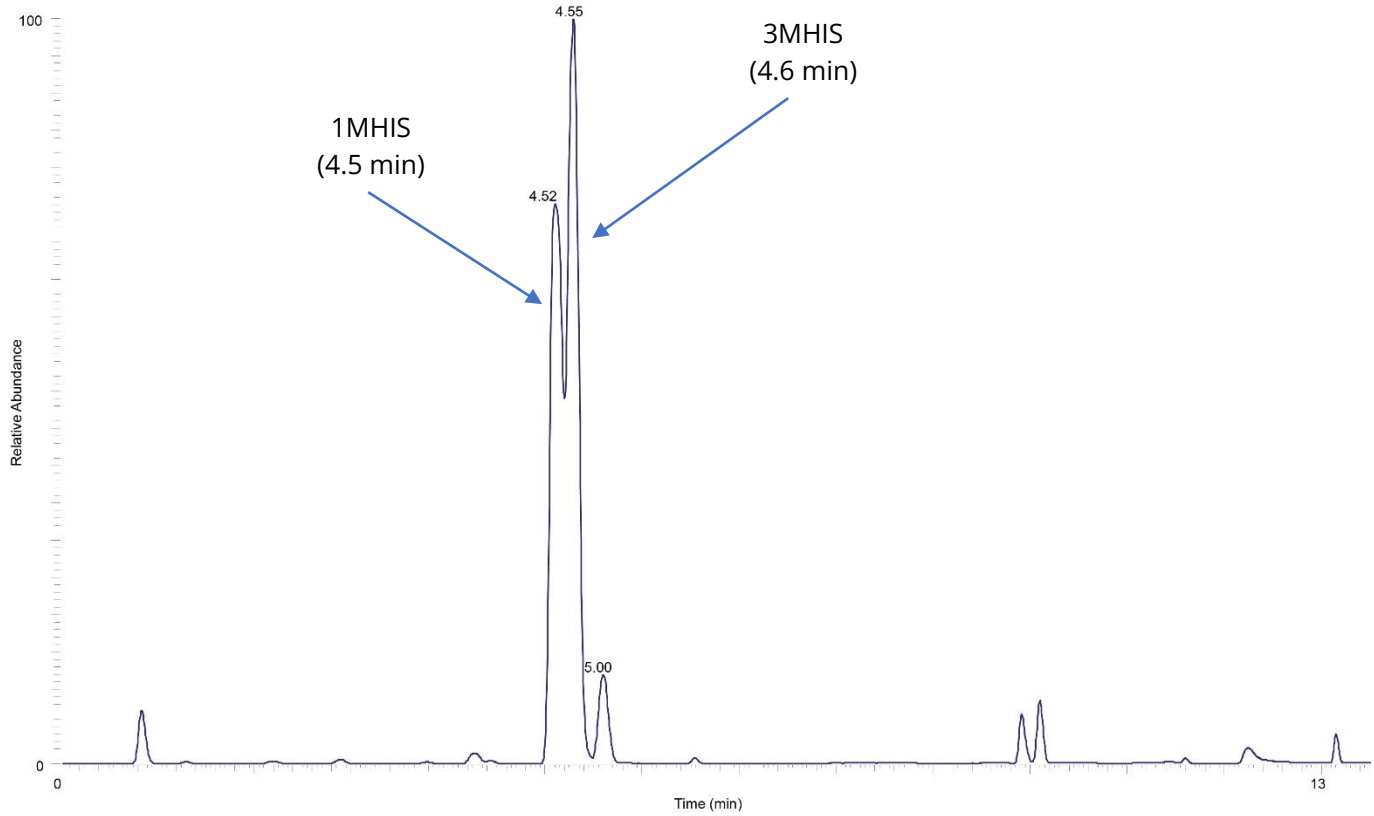


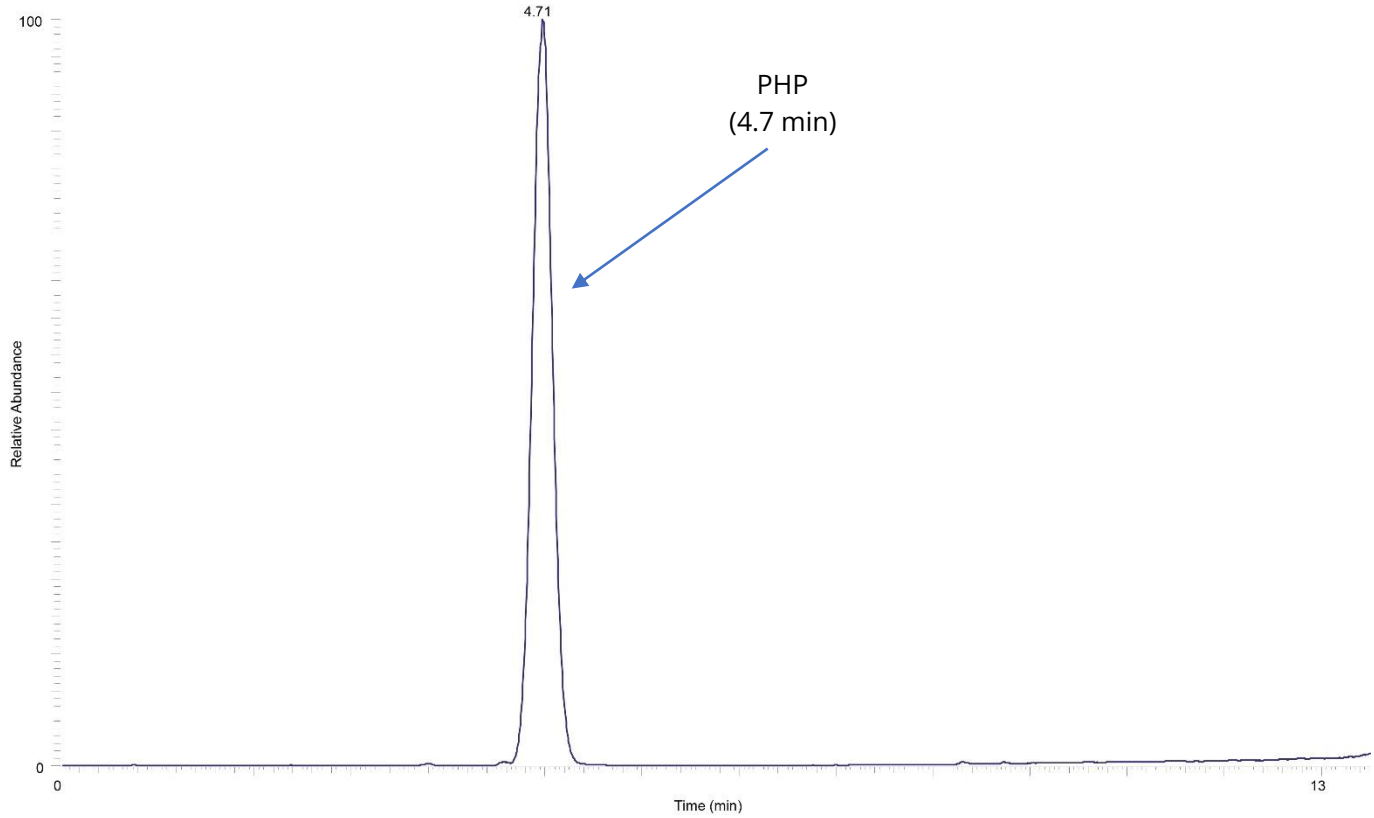

100
3.35
ARG
(3.4 min)
Relative Abundance
8.36
0
0
13
Time (min)

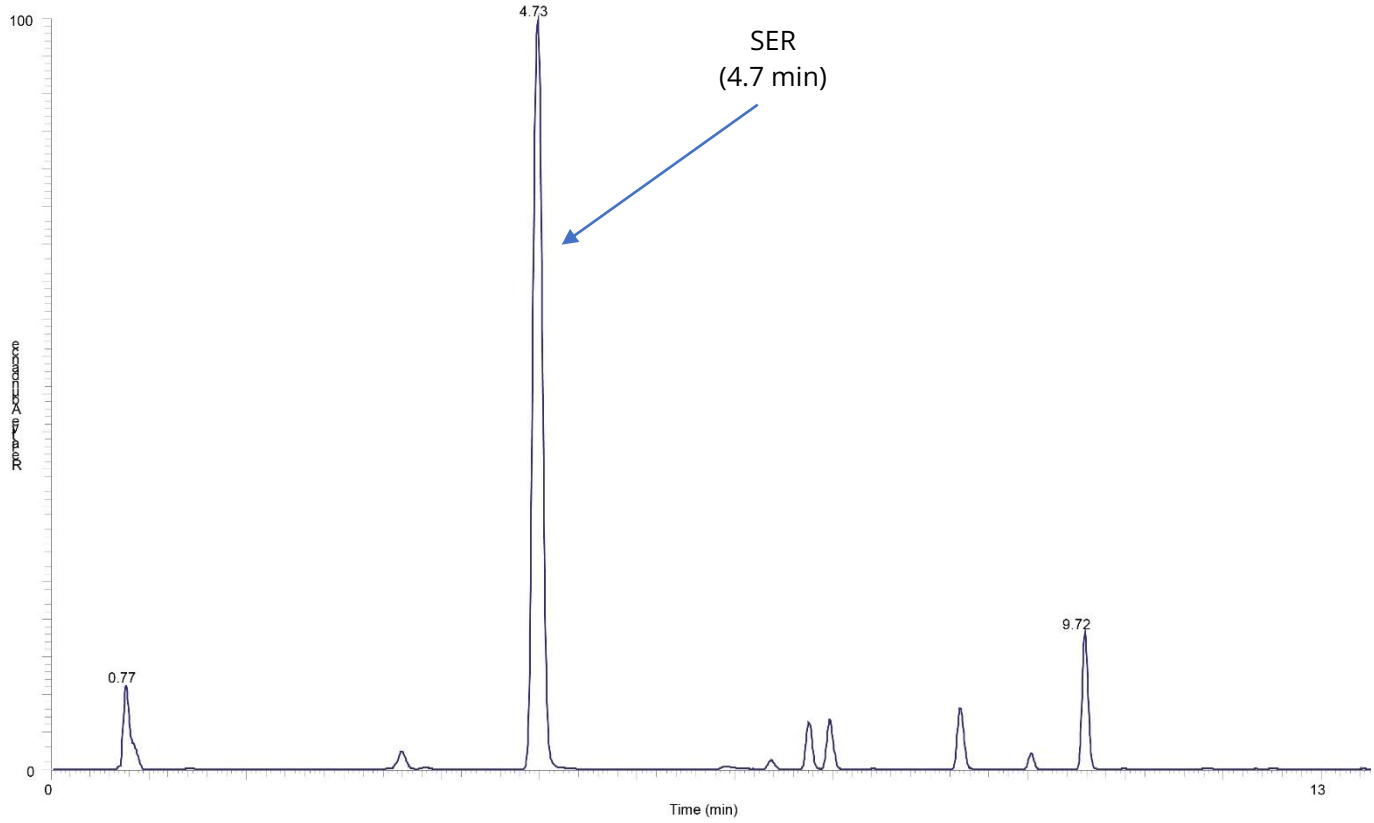

100
4.40
HLY
(4.4 min)
IS = HARG
(3.6 min)
Relative Abundance
3.63
0.89
0
0
13
Time (min)

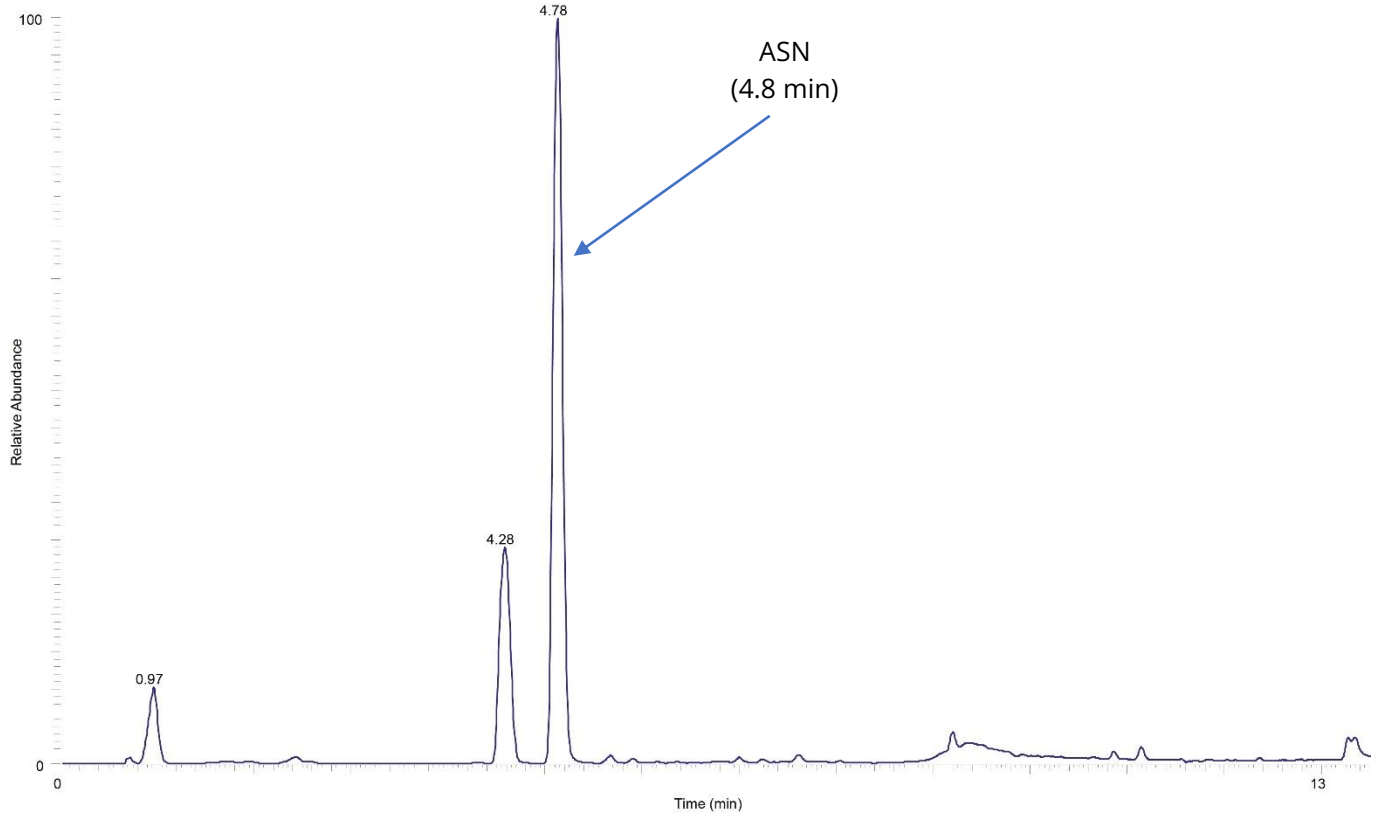

GLN
(4.1 min)
4.12
3.47
100
0
0
13
Relative Abundance
Time (min)

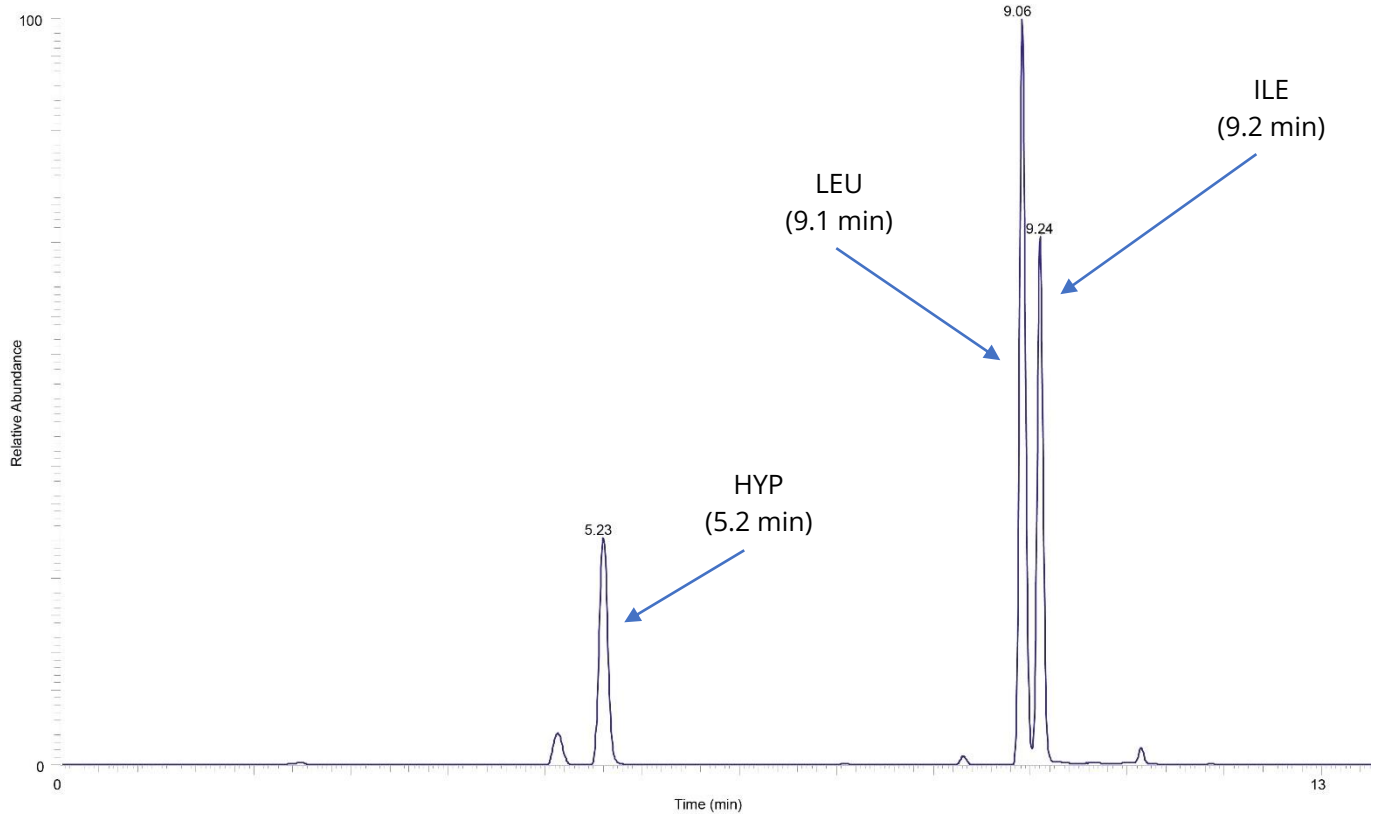

CIT
(4.2 min)
4.20
4.00
3.31
5.06
100
0
0
13
Relative Abundance
Time (min)

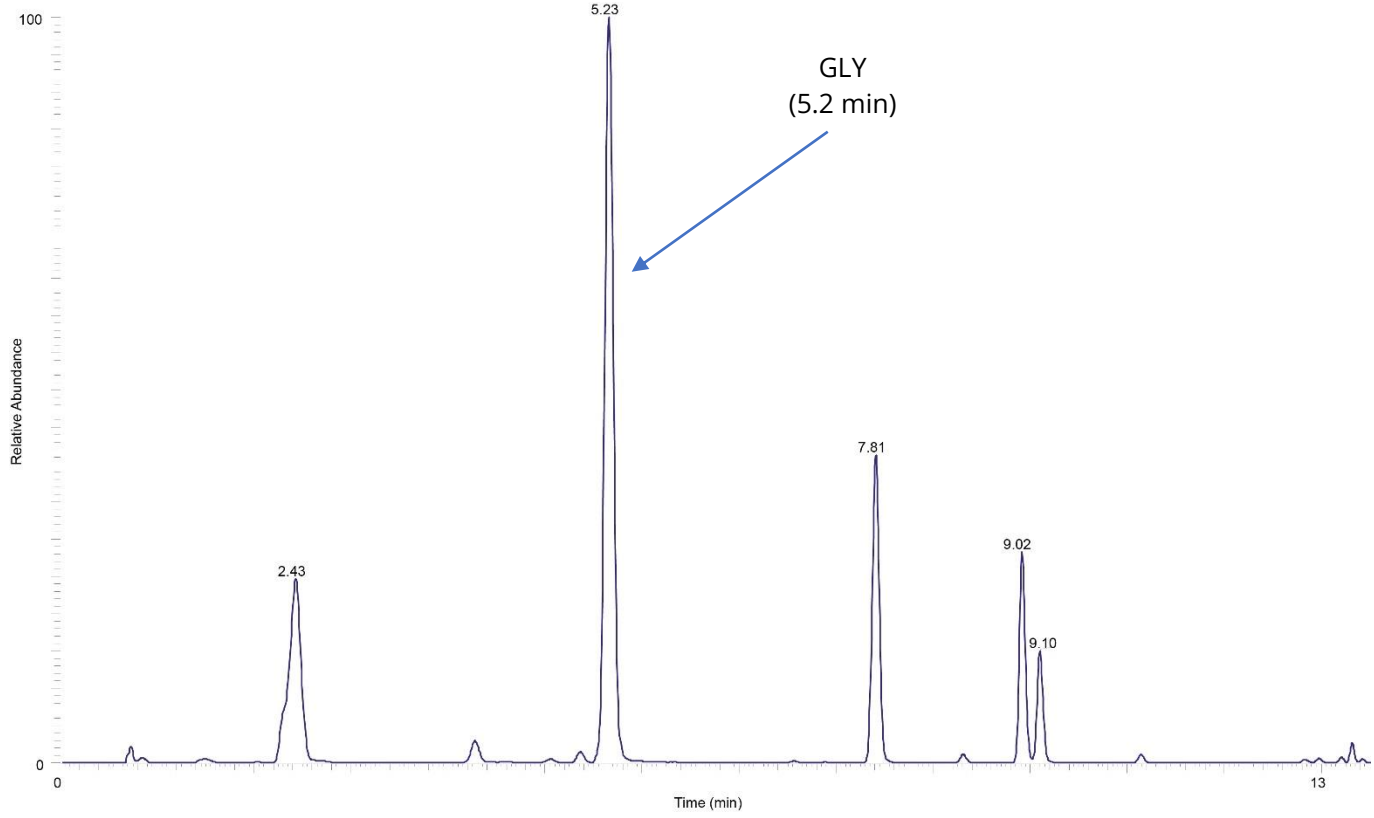

100
4.55
3MHIS
(4.6 min)
1MHIS
(4.5 min)
4.52
5.00
Relative Abundance
0
0
13
Time (min)

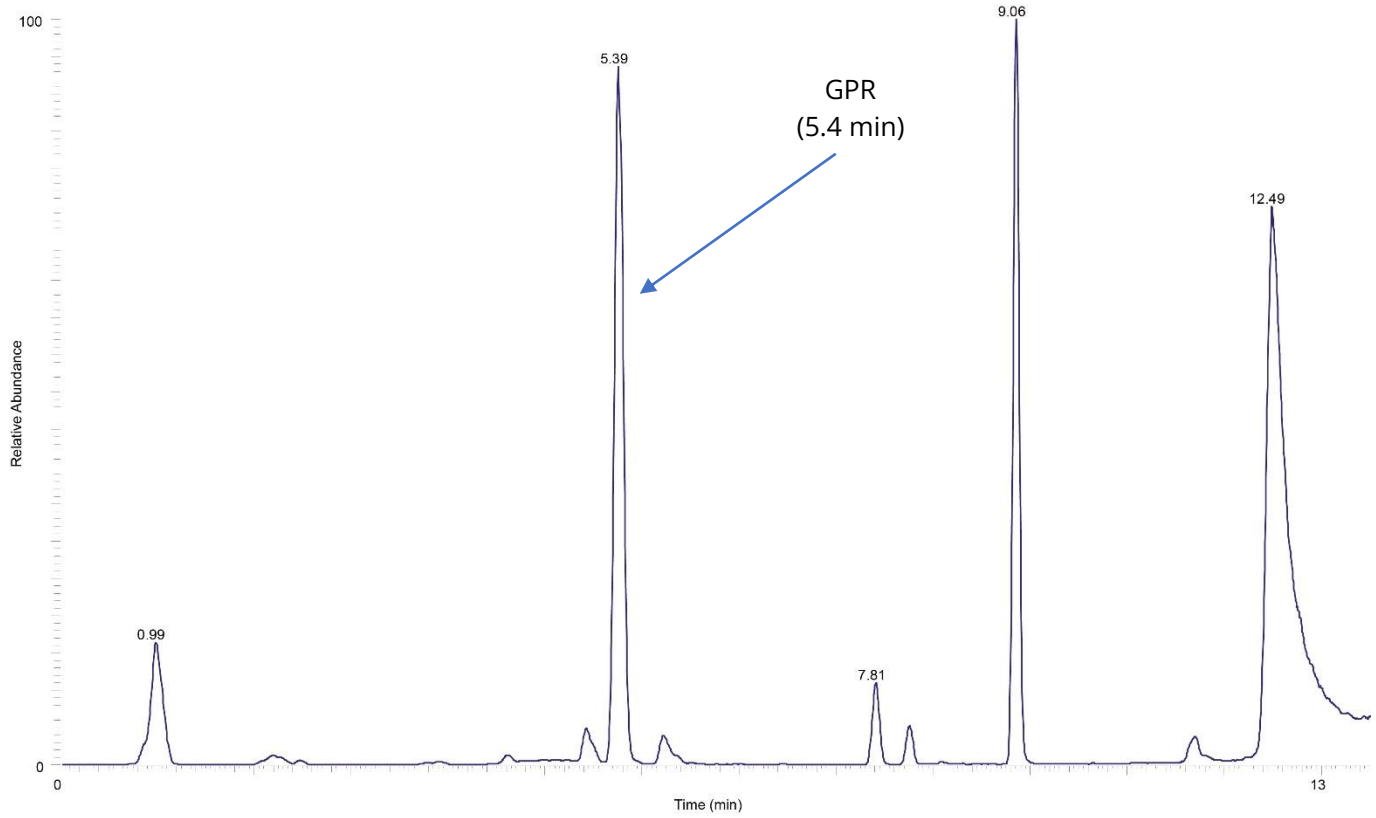

100
4.71
PHP
(4.7 min)
Relative Abundance
0
0
13
Time (min)

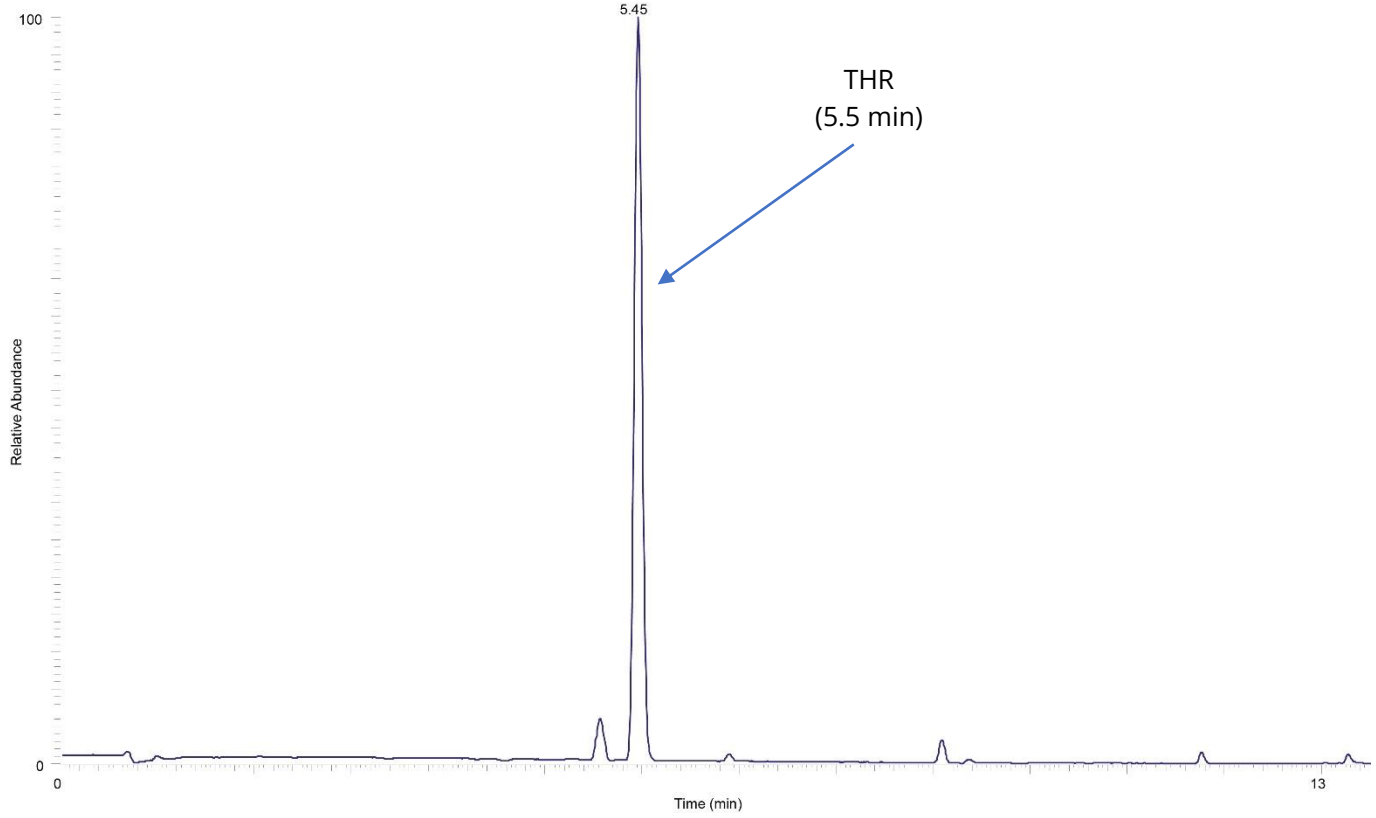

100
4.73
SER
(4.7 min)
0.77
9.72
0
0
13
Time (min)

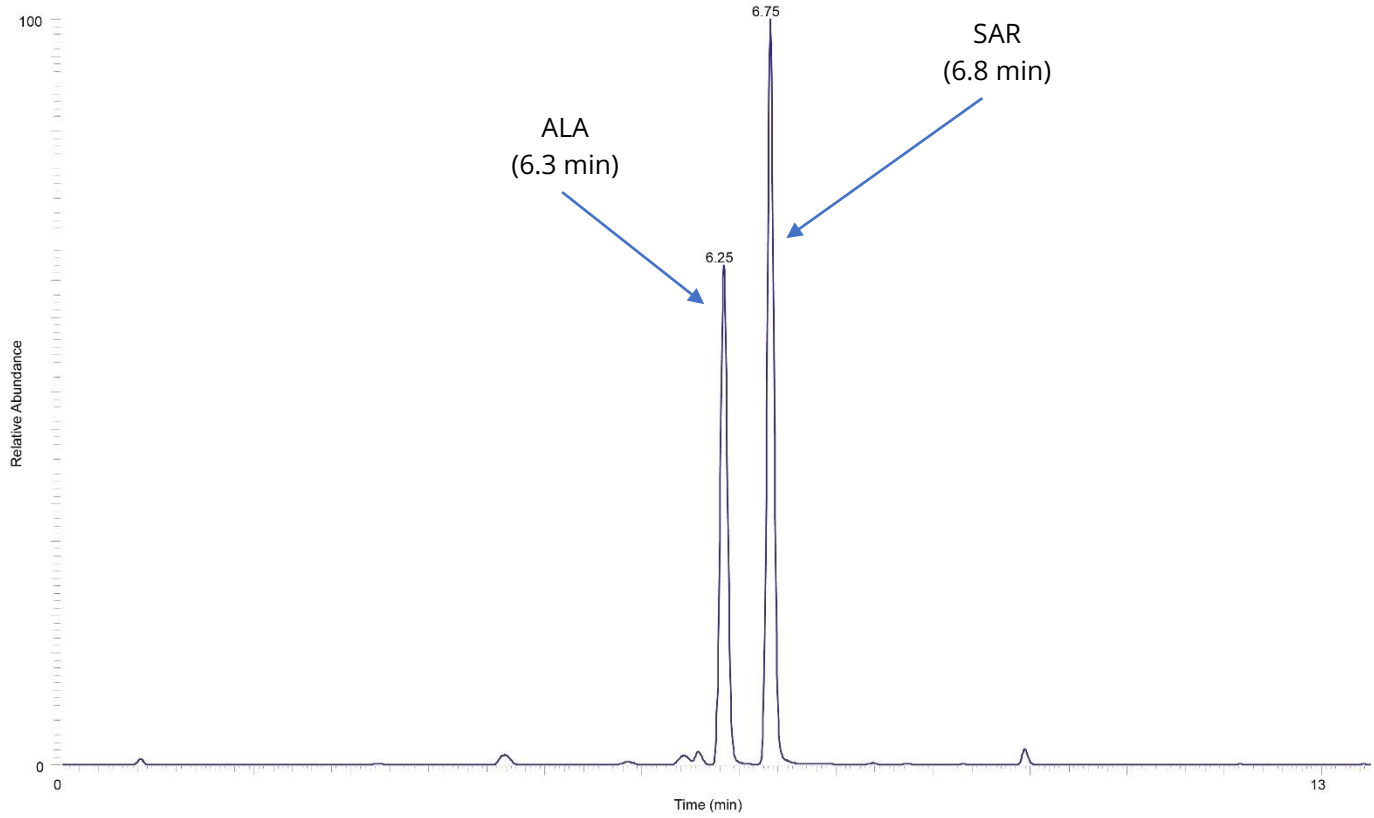

100
4.78
ASN
(4.8 min)
Relative Abundance
4.28
0.97
0
0
13
Time (min)

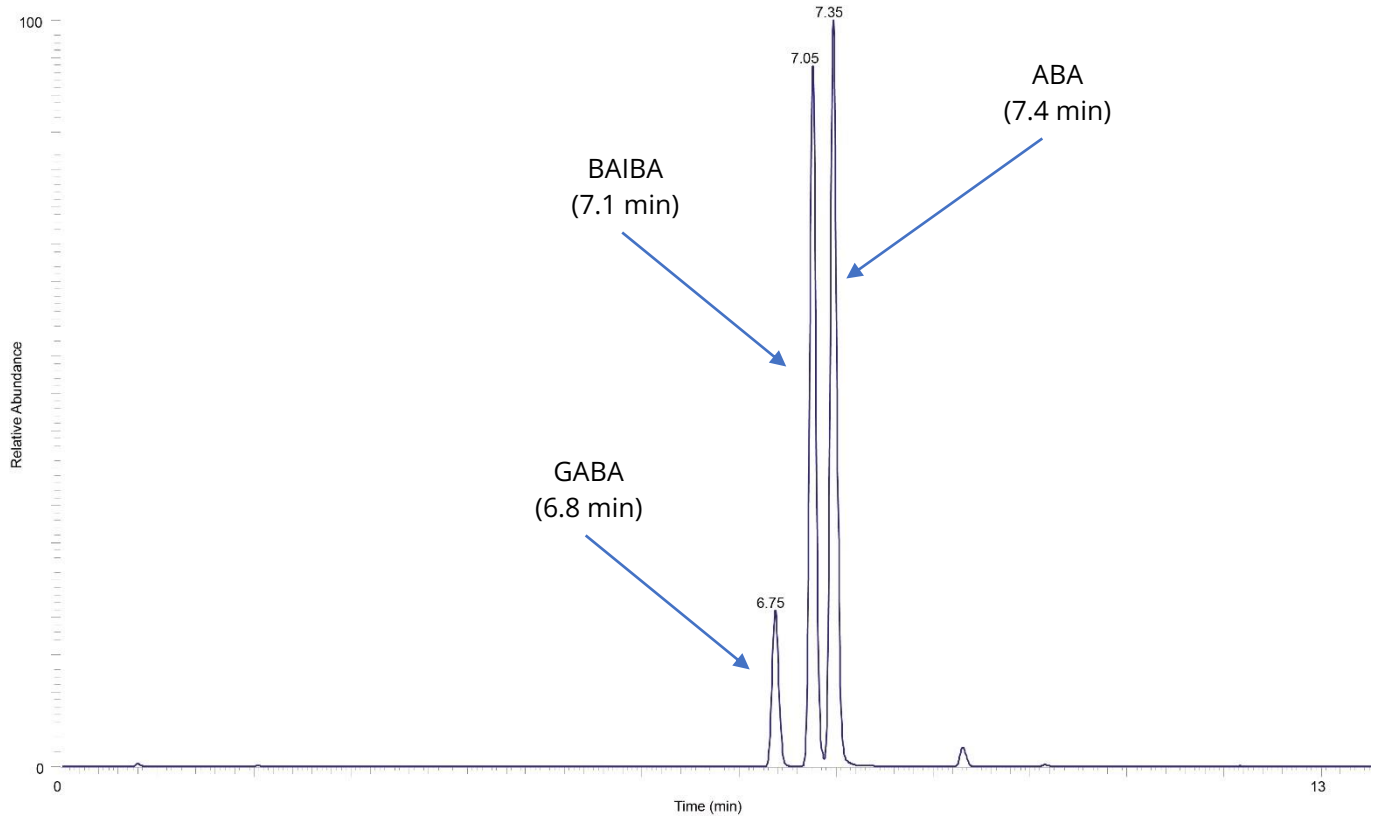

100
Relative Abundance
0
0
13
Time (min)
9.06
9.24
5.23
ILE
(9.2 min)
LEU
(9.1 min)
HYP
(5.2 min)

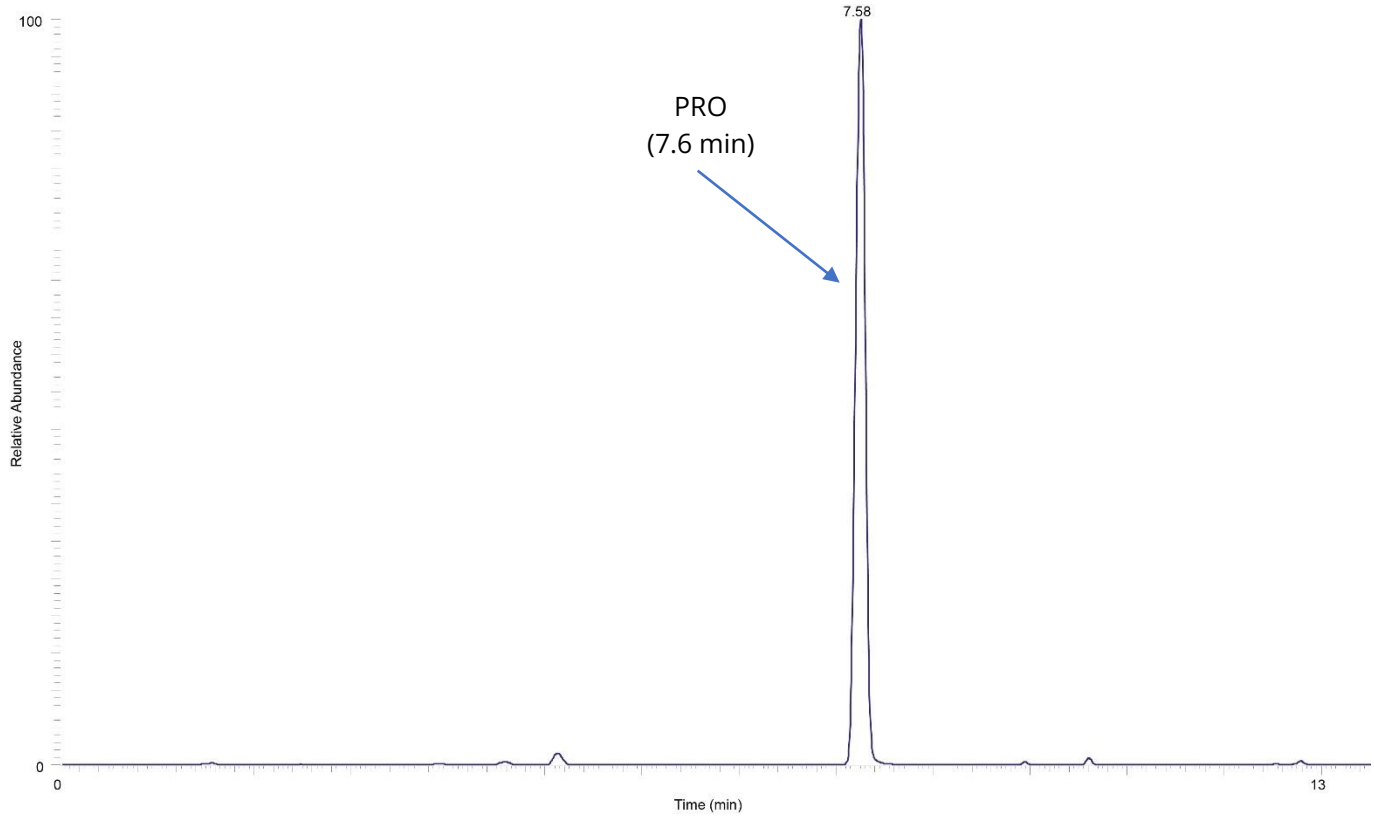

100
Relative Abundance
0
0
13
Time (min)
5.23
GLY
(5.2 min)
7.81
9.02
9.10
2.43

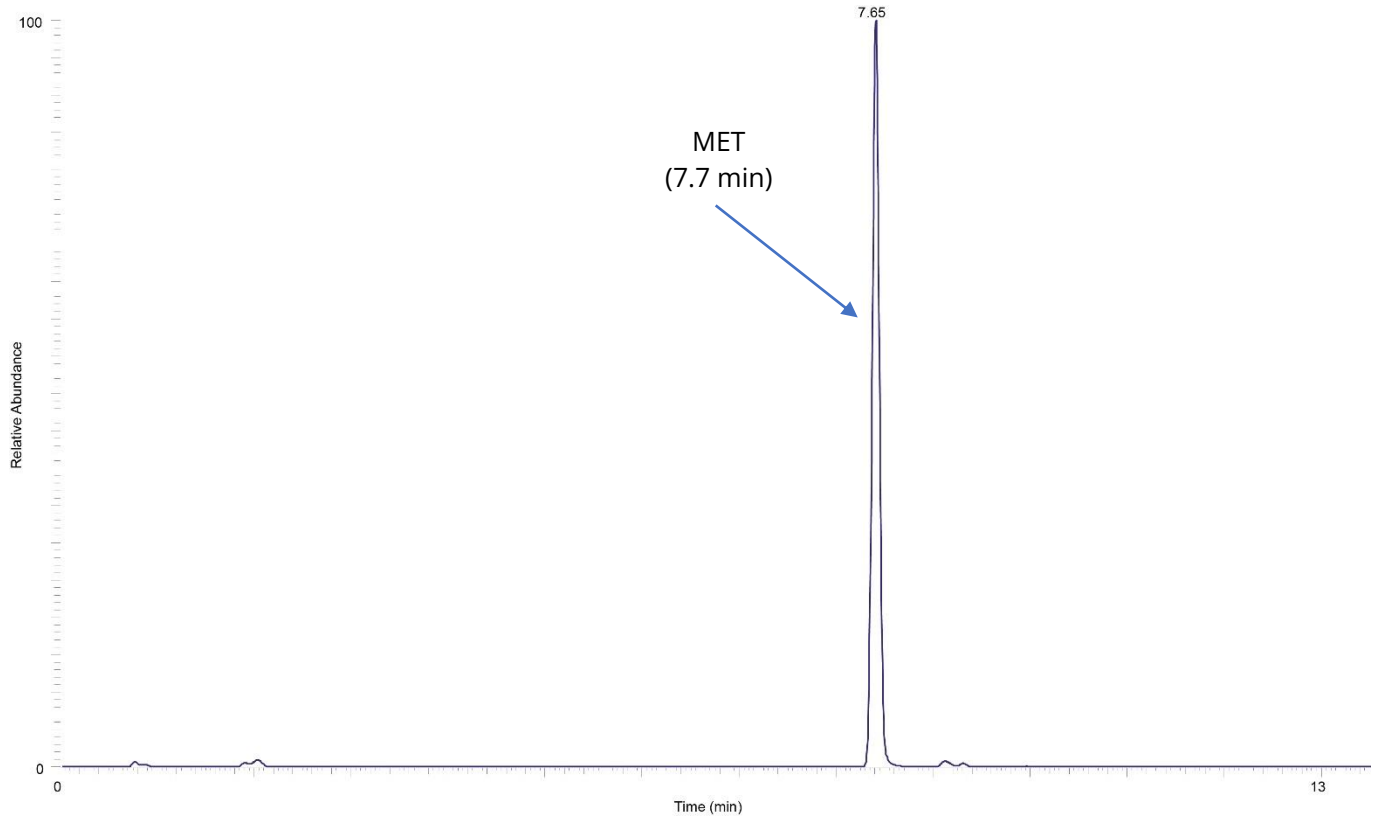

100
5.39
9.06
GPR
(5.4 min)
12.49
Relative Abundance
0.99
7.81
0
0
13
Time (min)

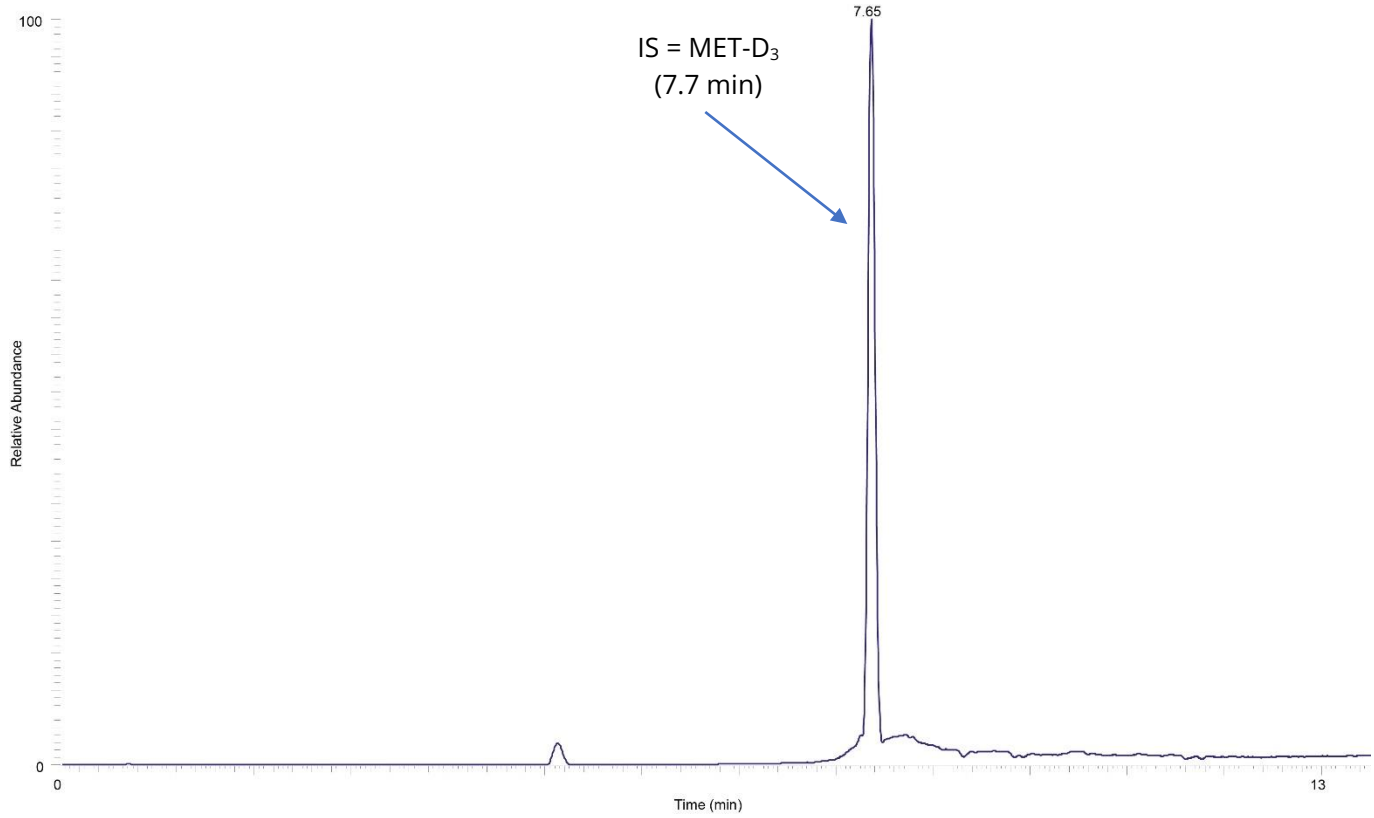

100
5.45
THR
(5.5 min)
Relative Abundance
0
0
13
Time (min)

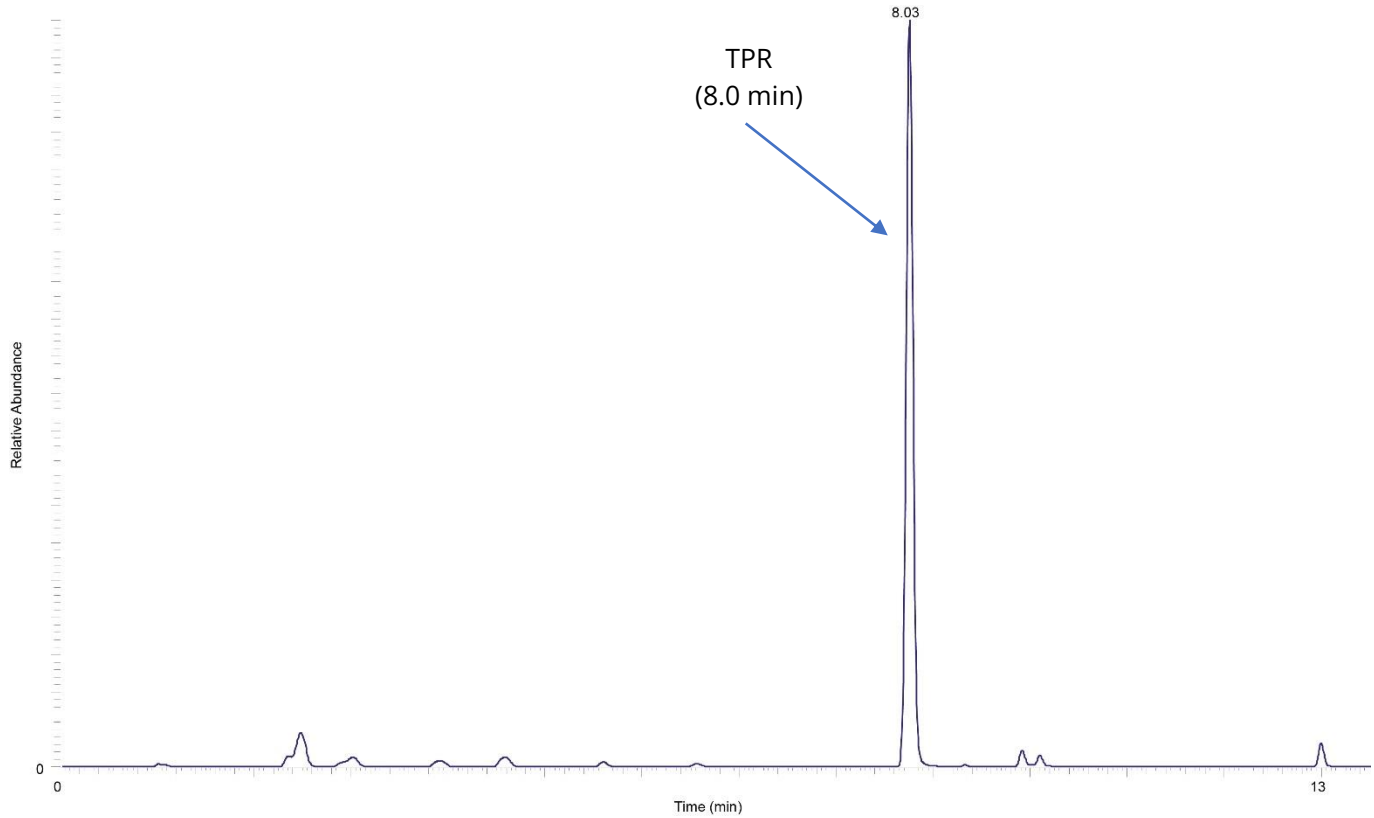

100
6.75
SAR
(6.8 min)
ALA
(6.3 min)
6.25
Relative Abundance
0
0
13
Time (min)

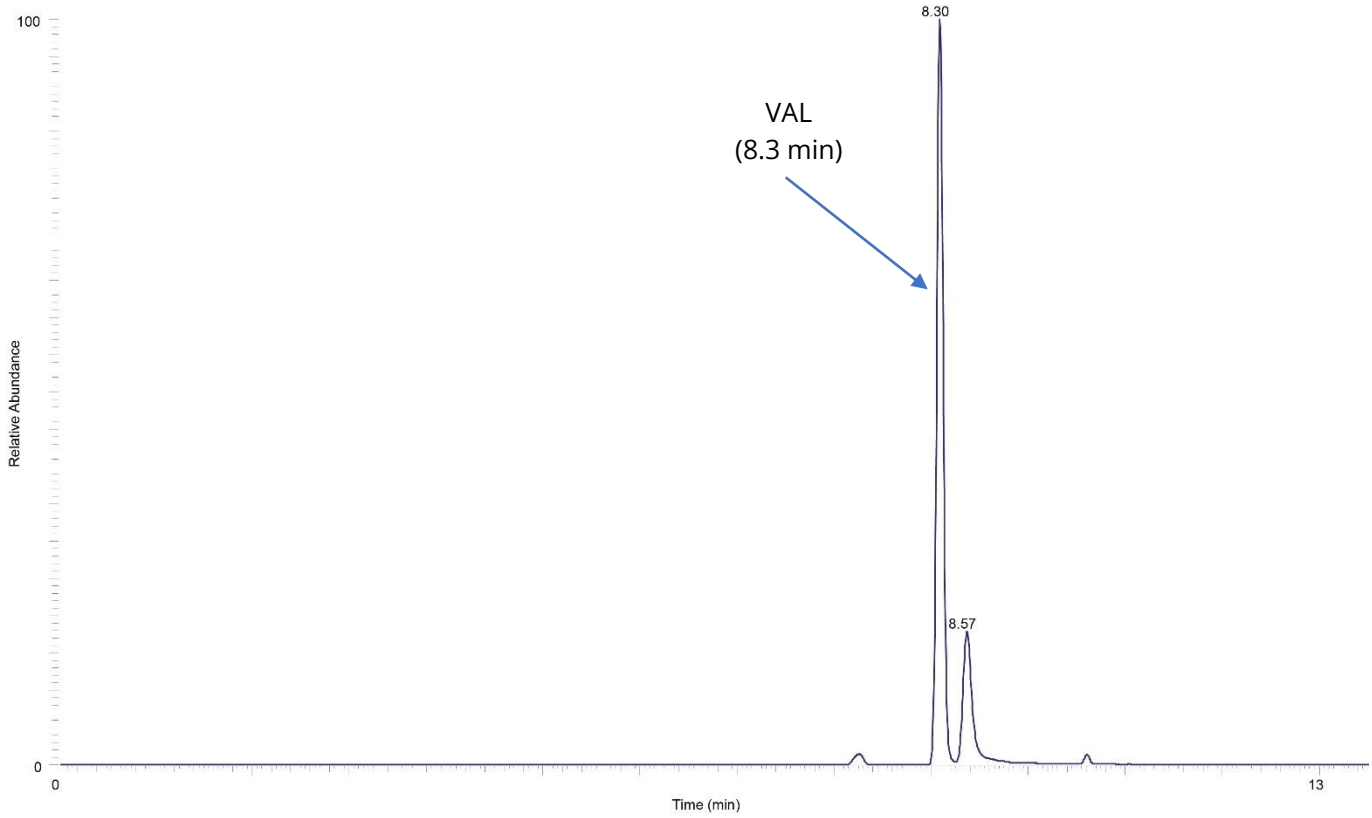

100
7.35
7.05
ABA
(7.4 min)
BAIBA
(7.1 min)
Relative Abundance
GABA
(6.8 min)
6.75
0
0
13
Time (min)

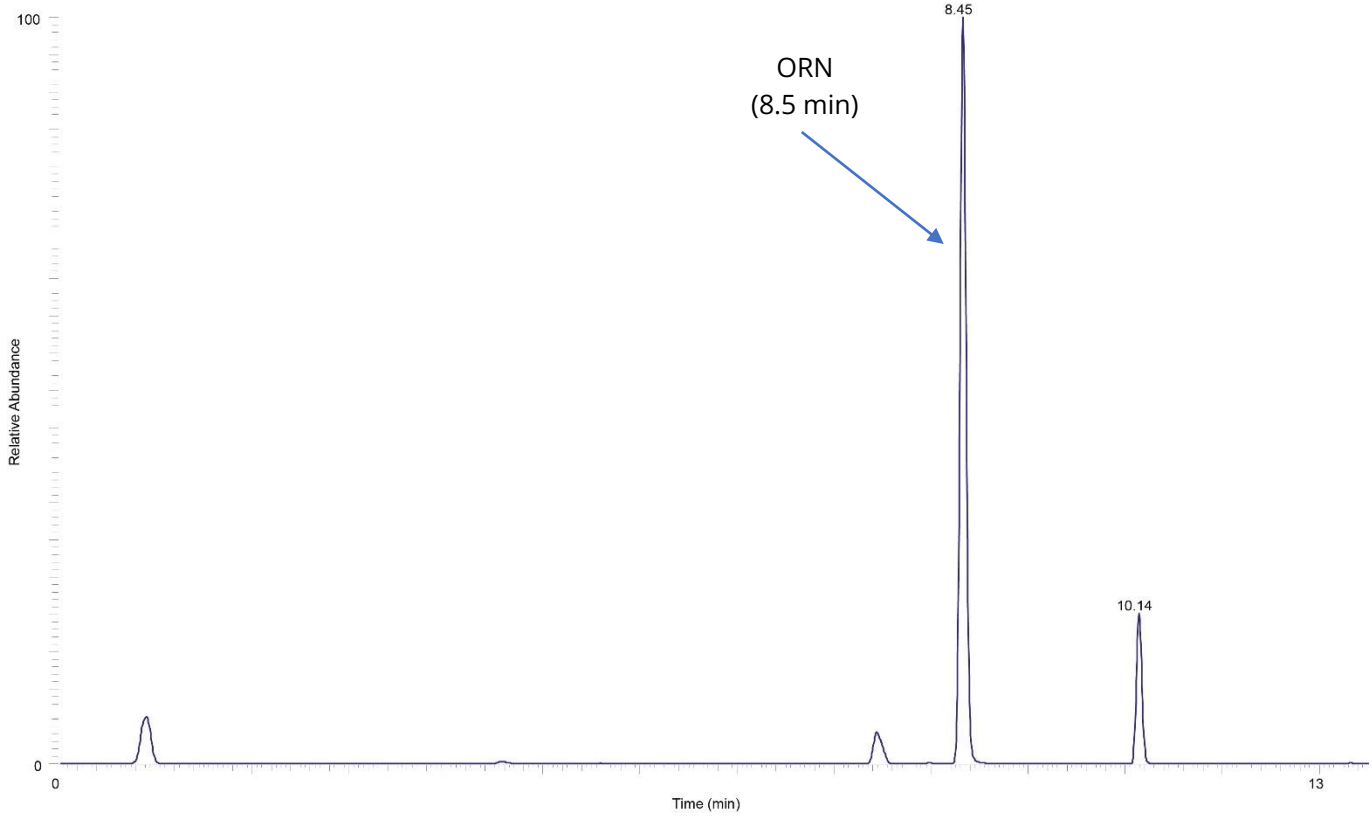

100
7.58
PRO
(7.6 min)
Relative Abundance
0
0
13
Time (min)

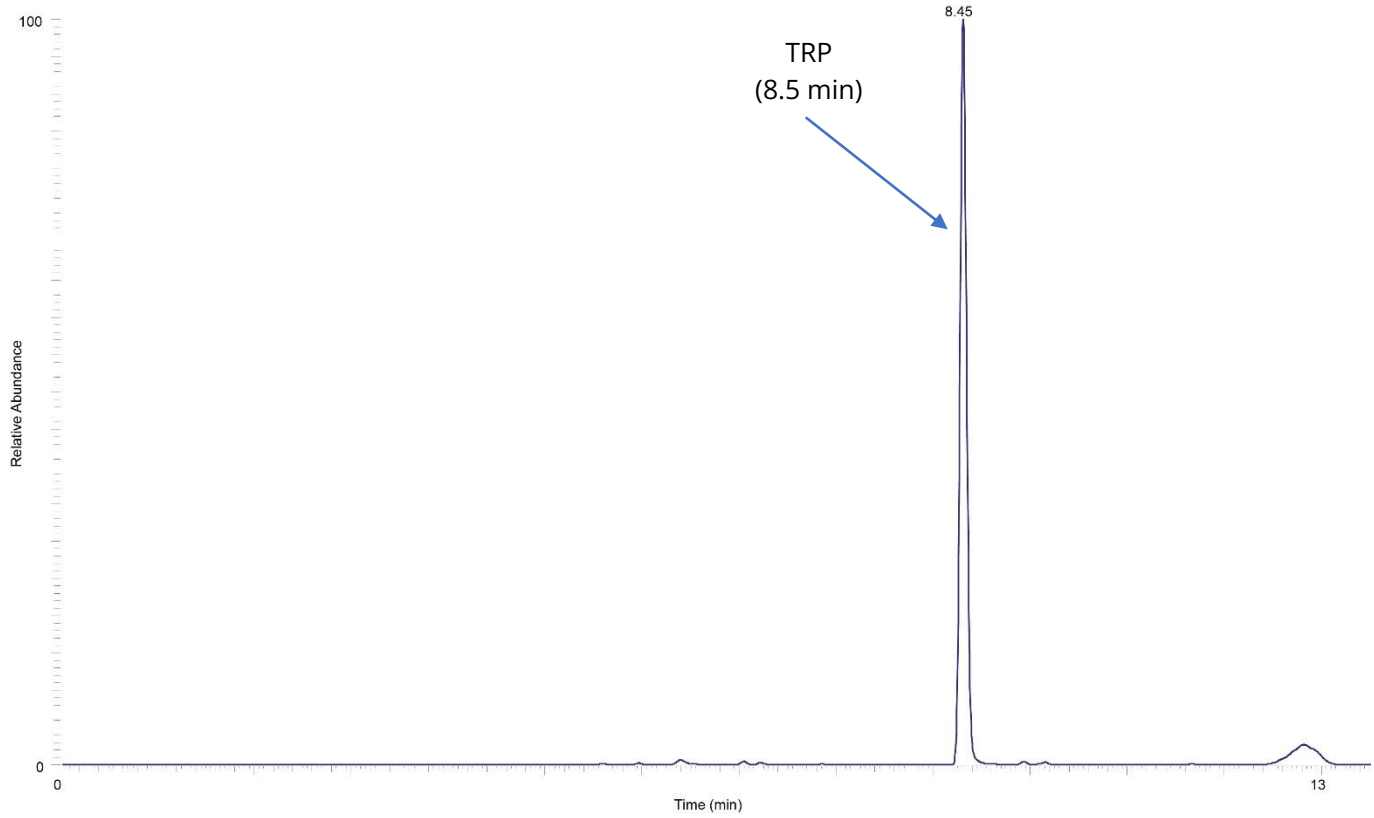

100
7.65
MET
(7.7 min)
Relative Abundance
0
0
13
Time (min)

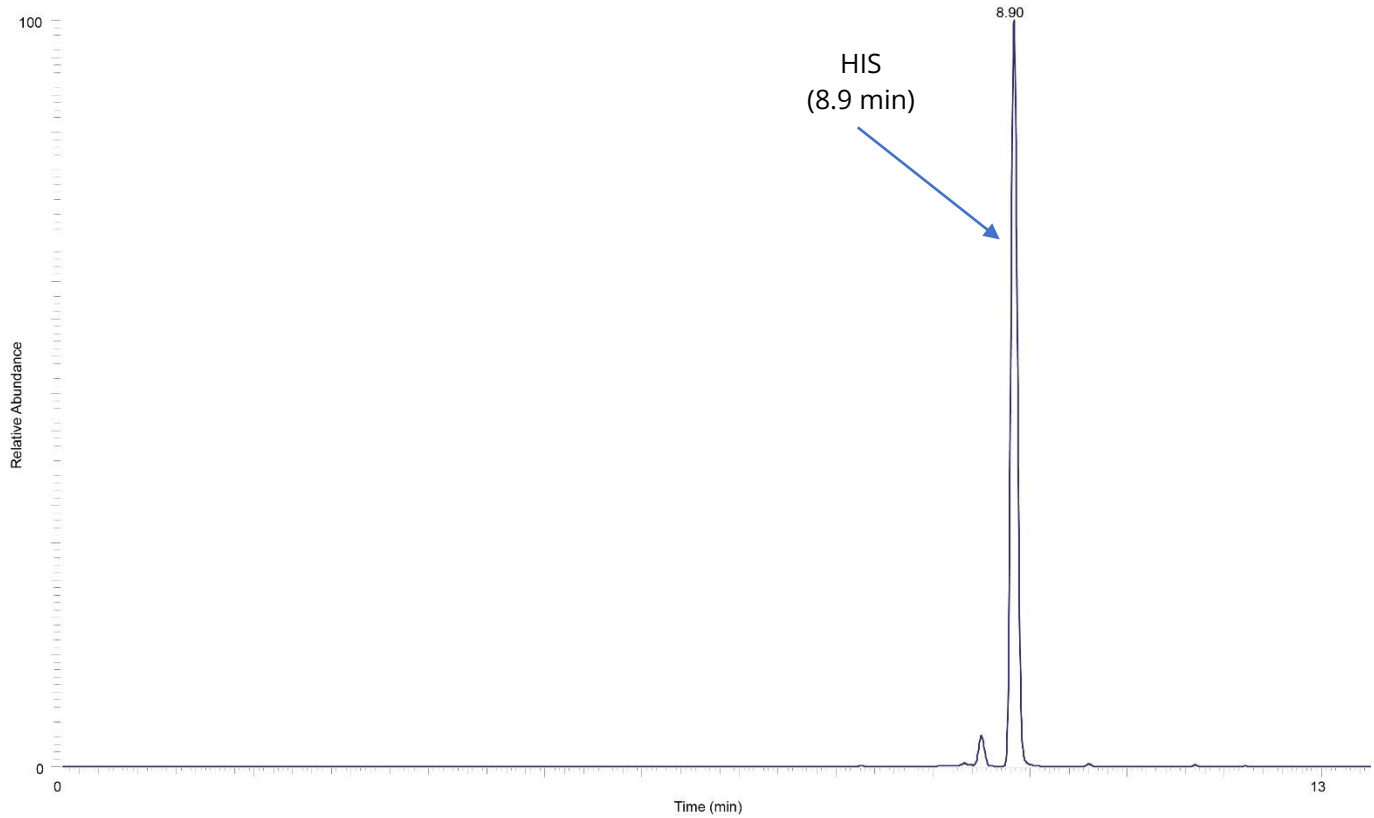

100
7.65
IS = MET-D3
(7.7 min)
Relative Abundance
0
0
13
Time (min)

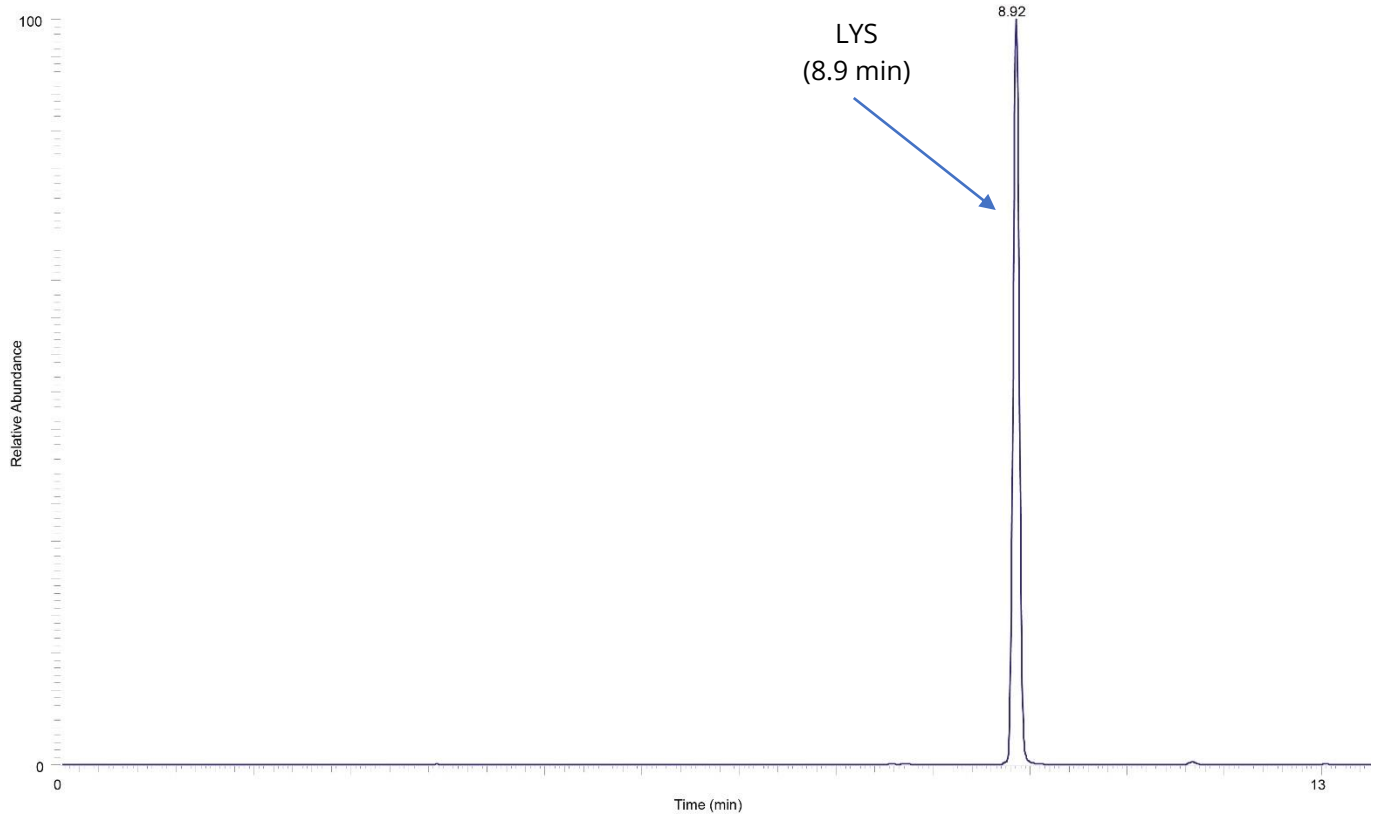

8.03
TPR
(8.0 min)
Relative Abundance
0
0
13
Time (min)

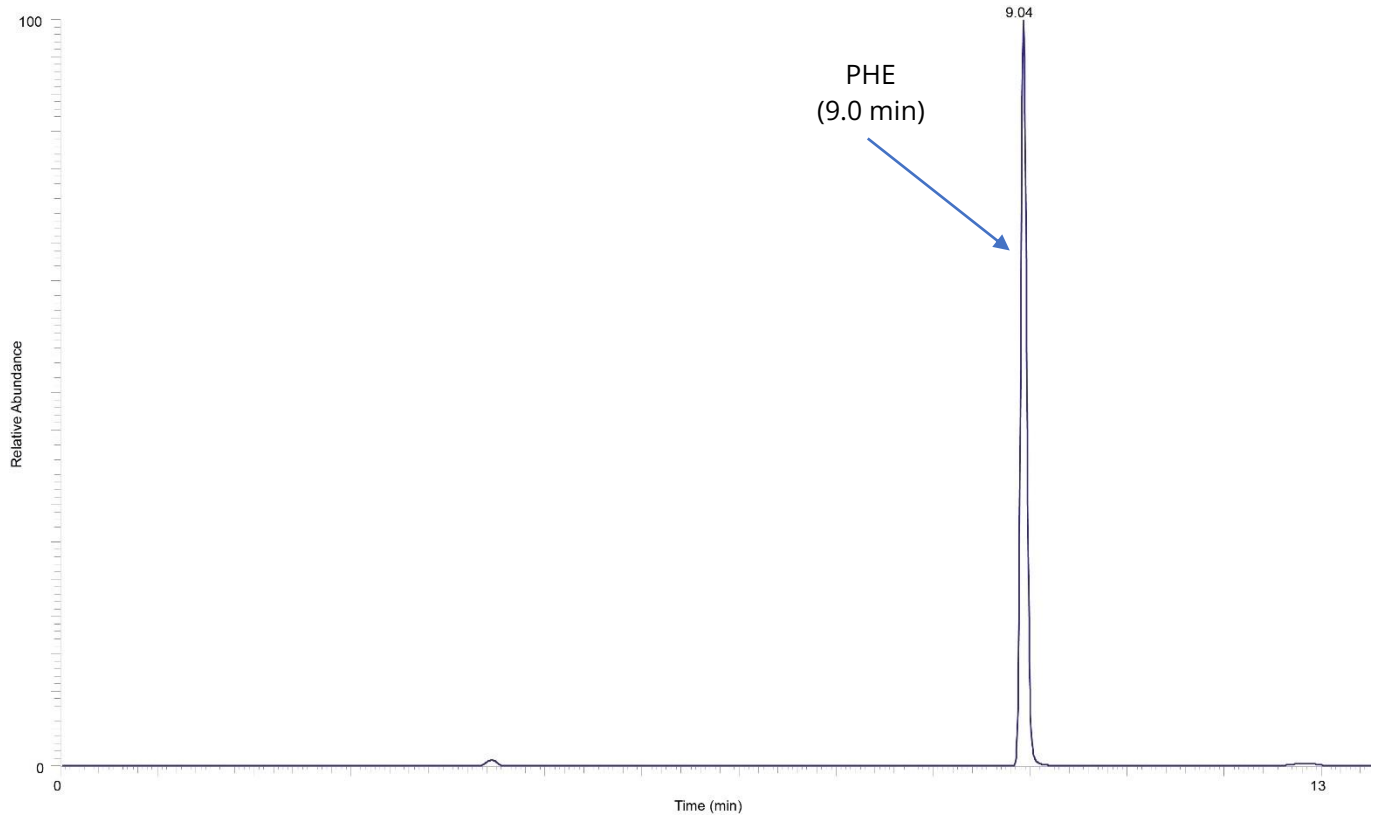

100
8.30
VAL
(8.3 min)
Relative Abundance
8.57
0
0
13
Time (min)

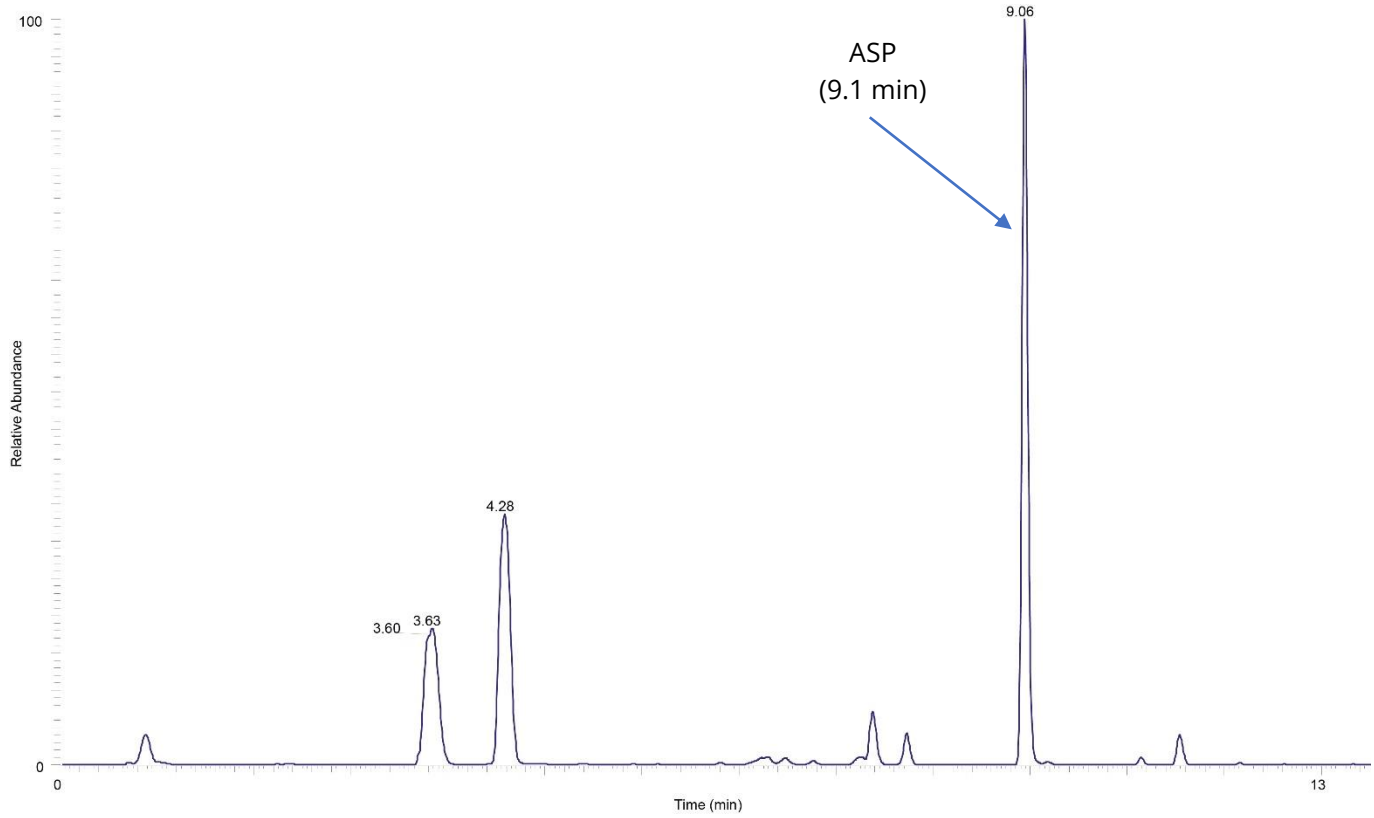

100
8.45
ORN
(8.5 min)
Relative Abundance
10.14
0
0
13
Time (min)

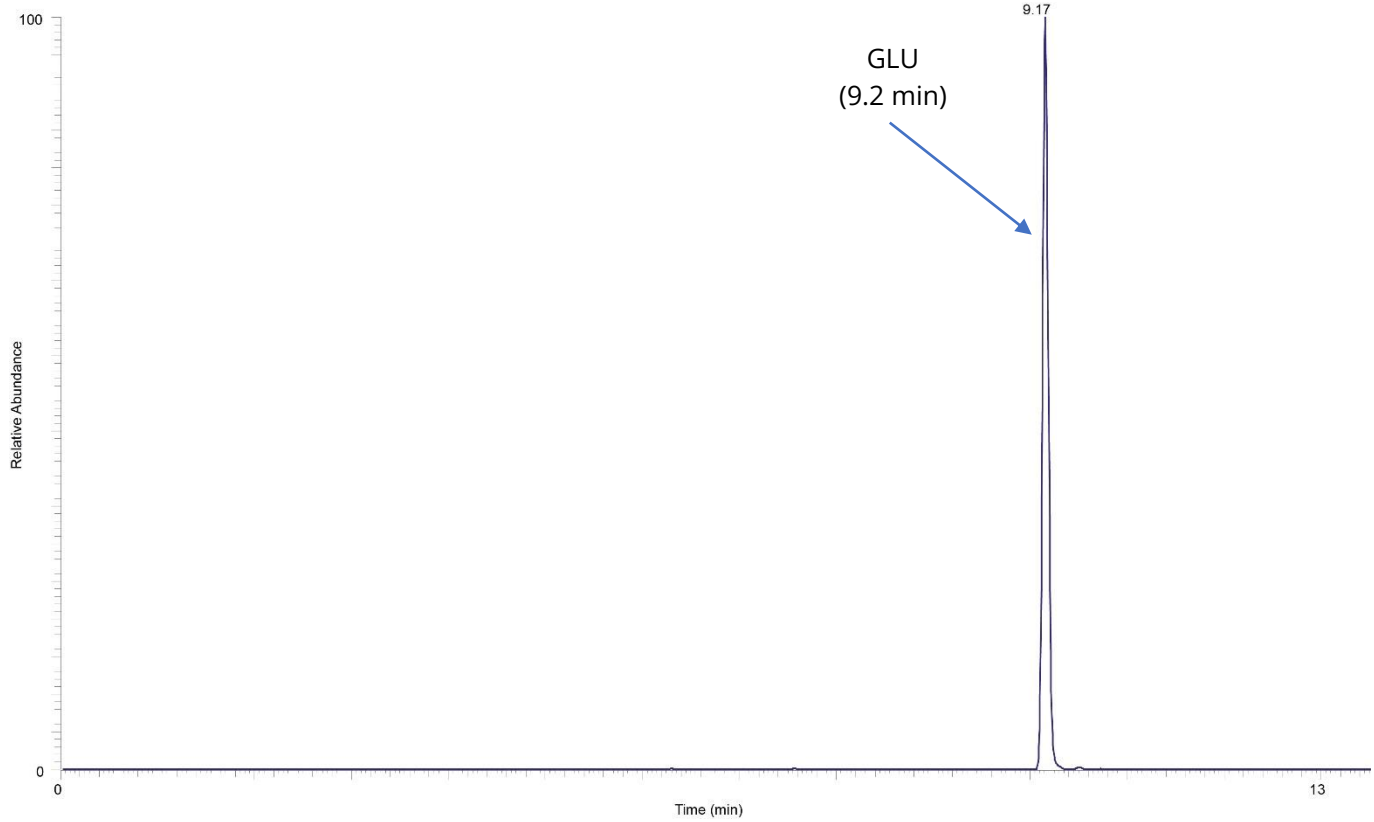

100
8.45
TRP
(8.5 min)
Relative Abundance
0
0
13
Time (min)

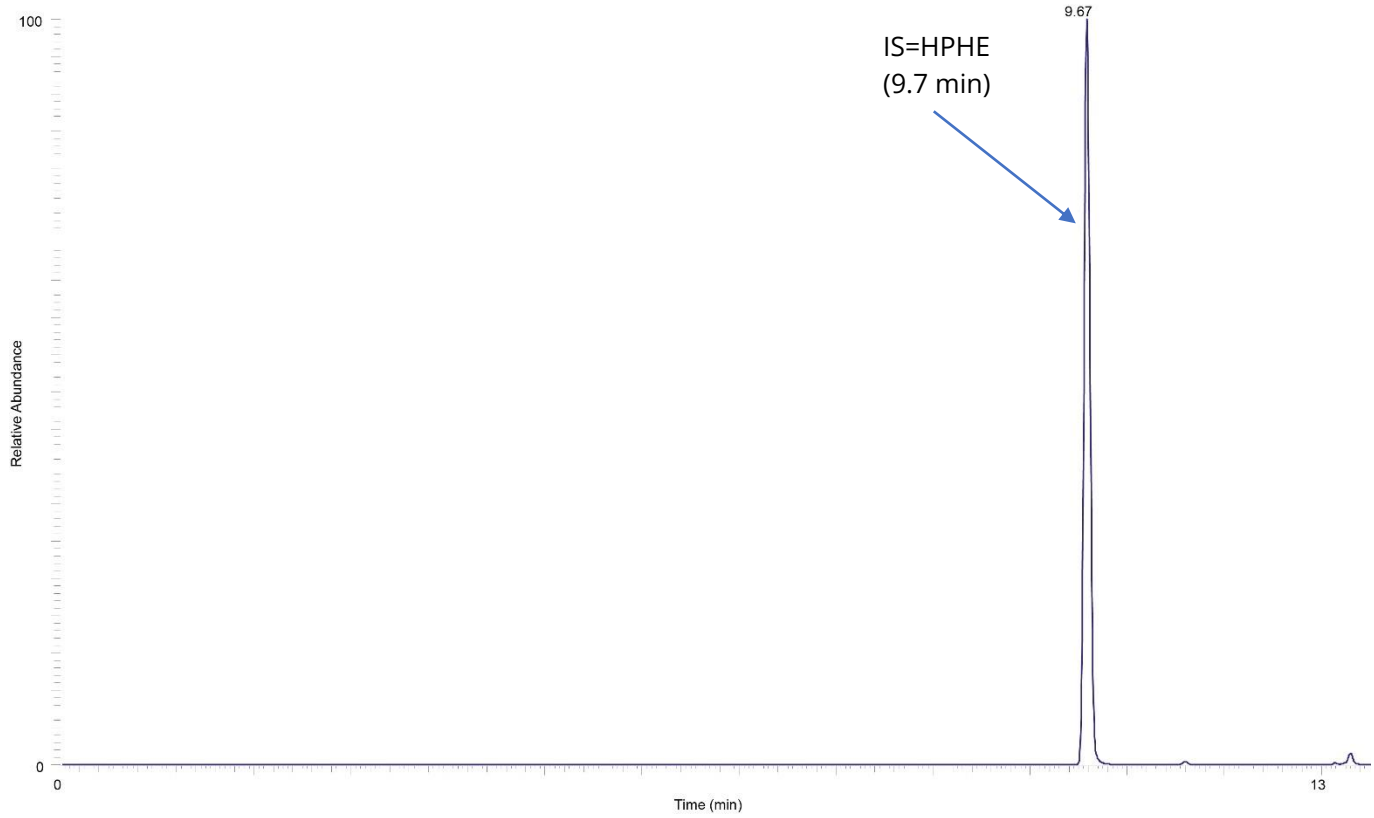

100
8.90
HIS
(8.9 min)
Relative Abundance
0
0
13
Time (min)

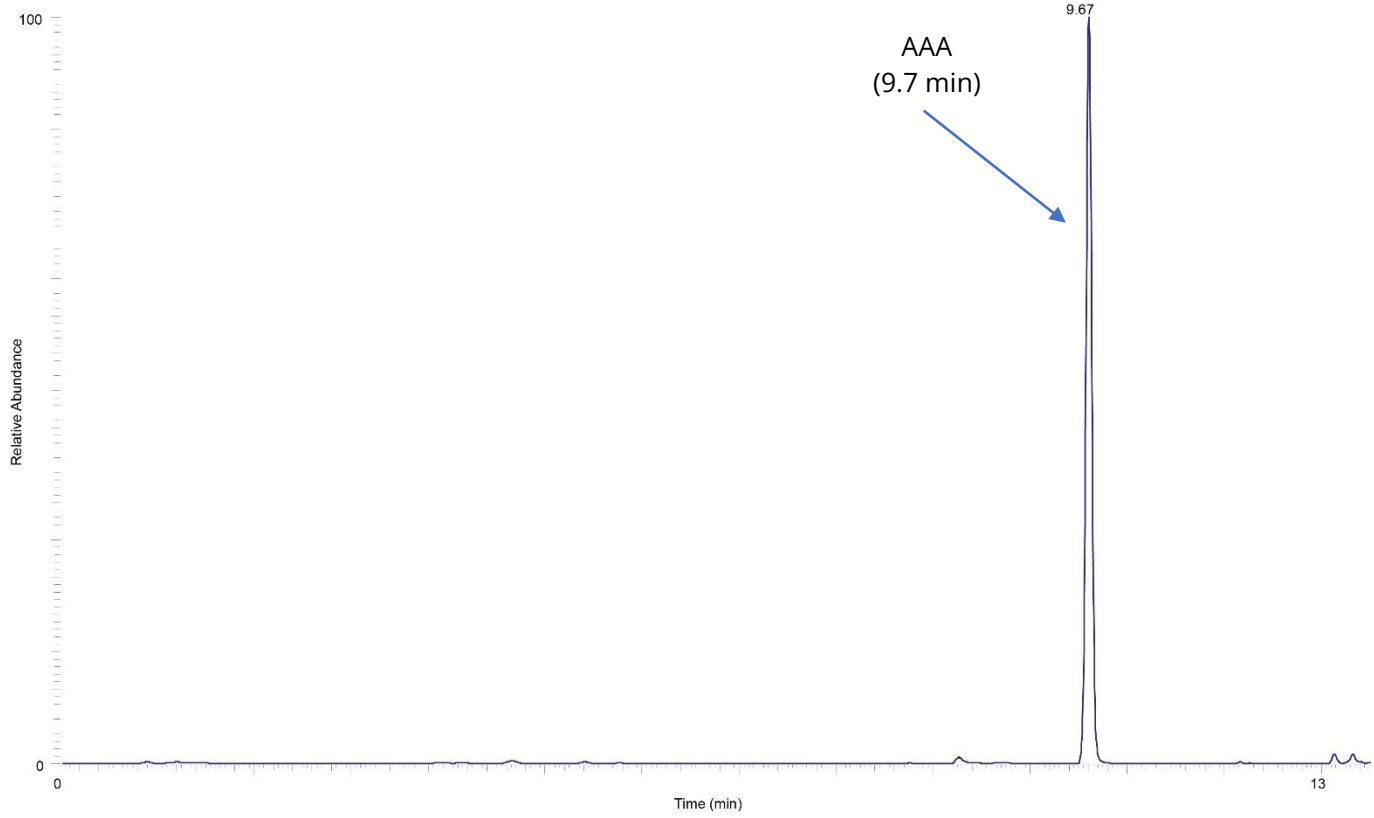

100
8.92
LYS
(8.9 min)
Relative Abundance
0
0
13
Time (min)

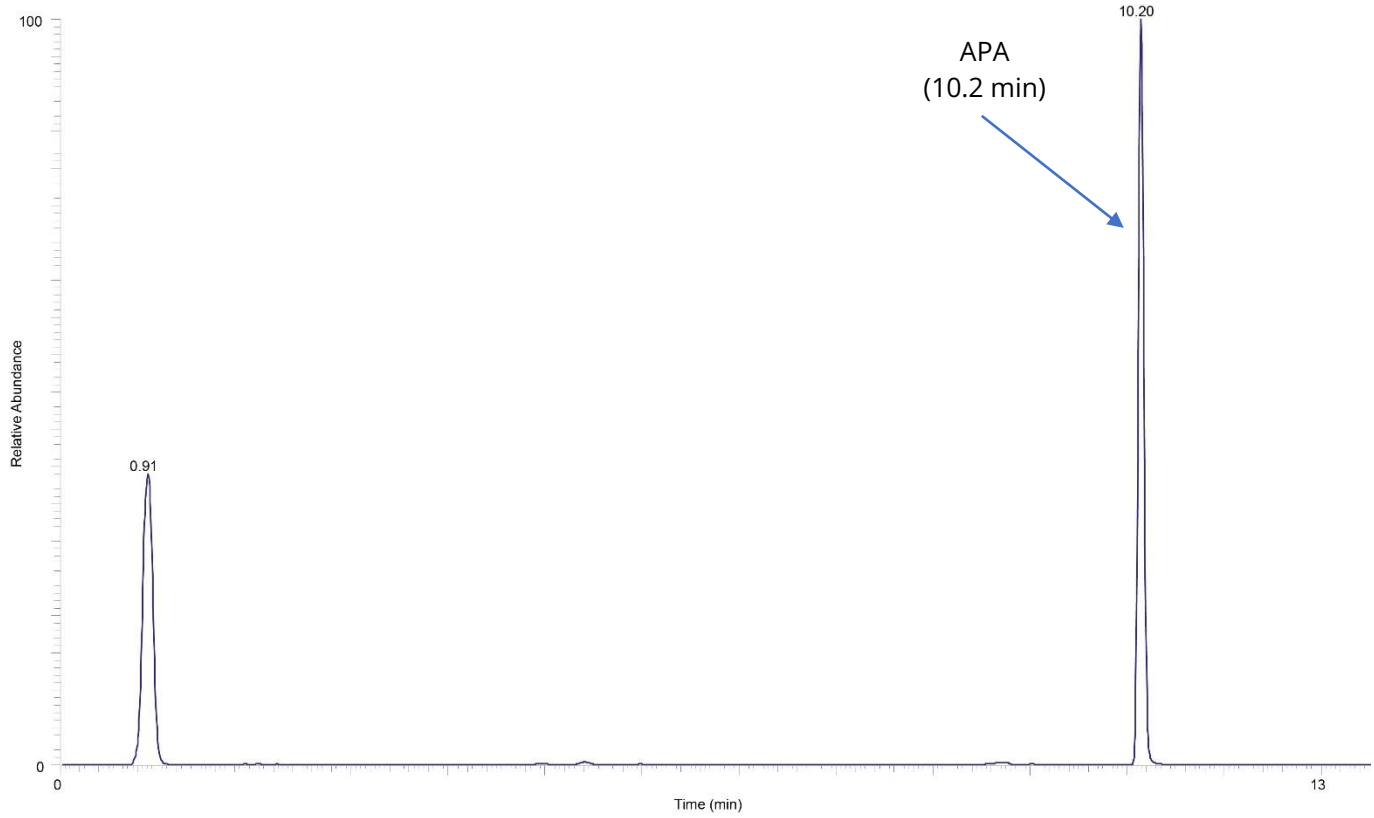

100
9.04
PHE
(9.0 min)
Relative Abundance
0
0
13
Time (min)

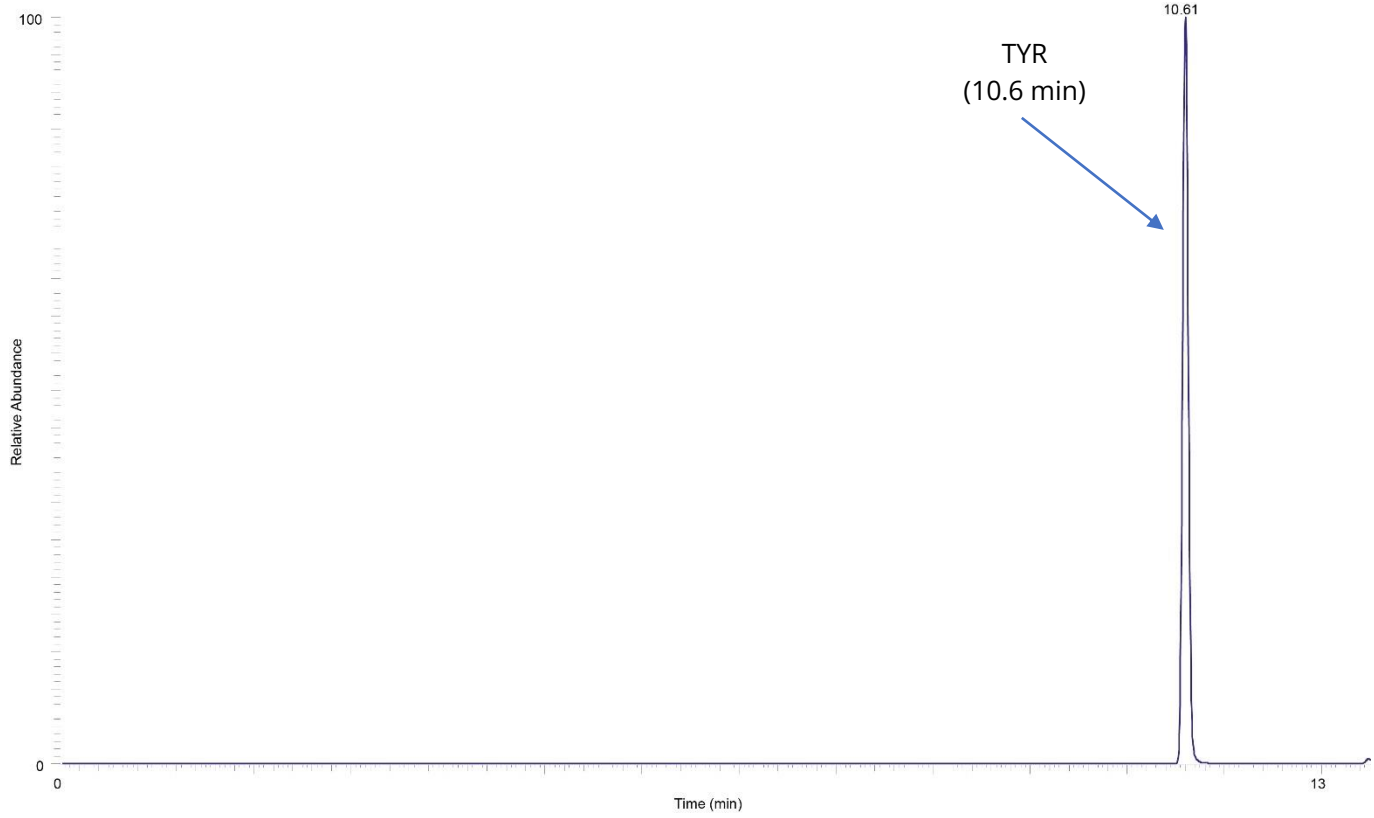

100
Relative Abundance
0
0
13
Time (min)
9.06
ASP
(9.1 min)
4.28
3.60
3.63

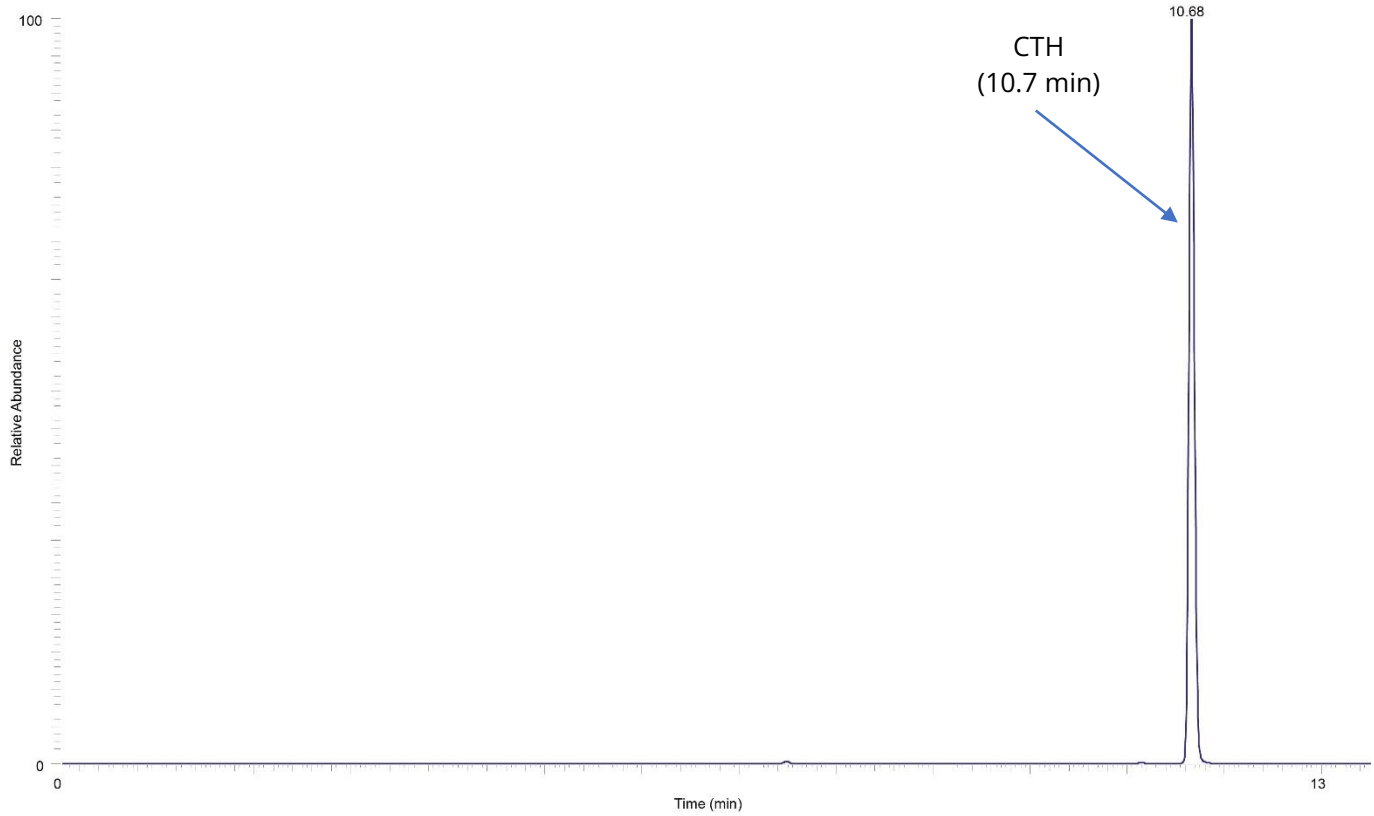

100
Relative Abundance
0
0
13
Time (min)
9.17
GLU
(9.2 min)

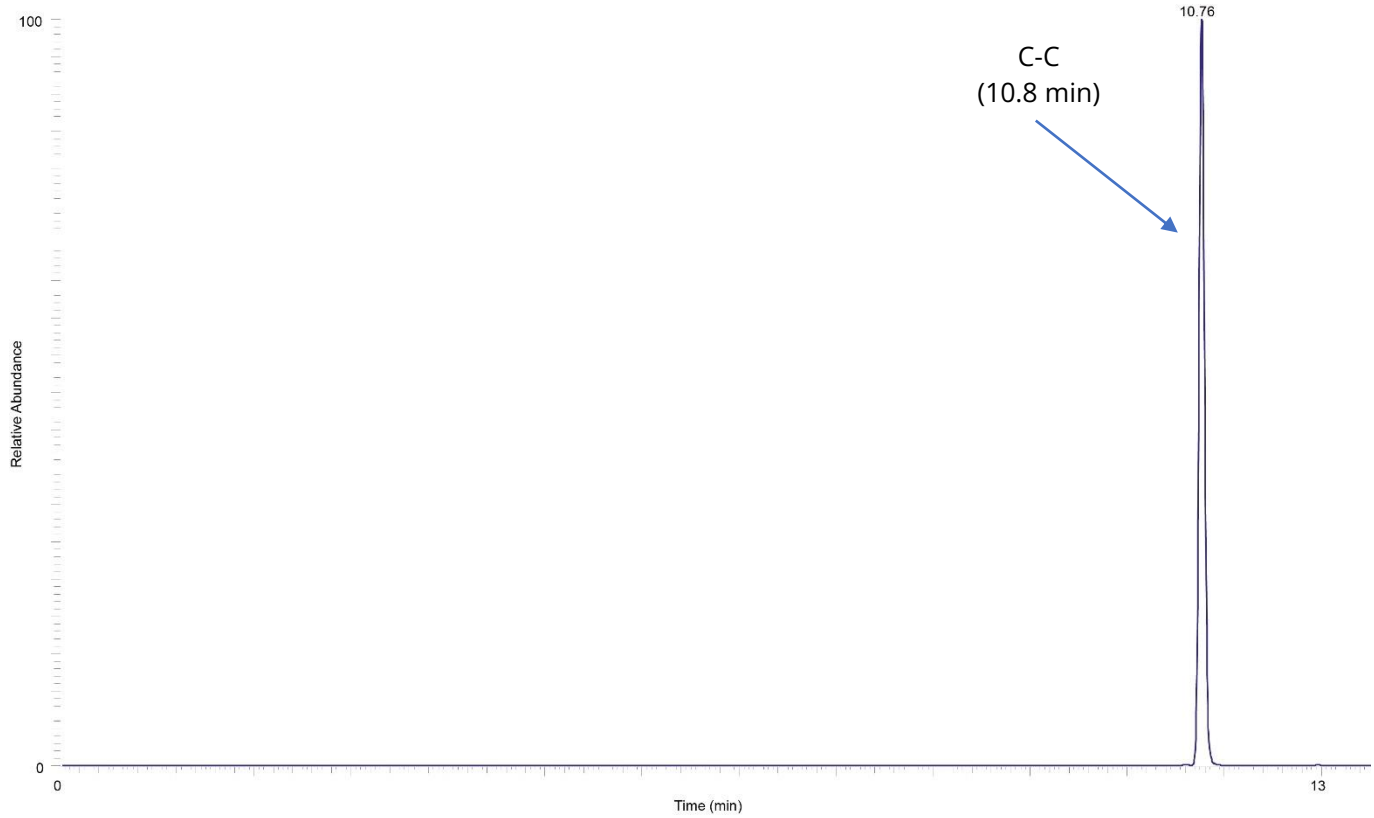

100
9.67
IS=HPHE
(9.7 min)
Relative Abundance
0
0
13
Time (min)


100
9.67
AAA
(9.7 min)
Relative Abundance
0
0
13
Time (min)


100
Relative Abundance
0
0
13
Time (min)
0.91
10.20
APA
(10.2 min)


100
Relative Abundance
0
0
13
Time (min)
10.61
TYR
(10.6 min)


100
0
Relative Abundance
10.68
CTH
(10.7 min)
0
13
Time (min)


100
0
Relative Abundance
10.76
C-C
(10.8 min)
0
13
Time (min)

Note: The extracted ion chromatograms were recorded using a low-resolution mass spectrometer. When using a more selective mass spectrometer (high resolution, QQQ), no contamination peaks are visible in the reconstructed chromatograms. Only in the case of isobaric amino acids (LEU/ILE; 1MHIS/3MHIS) do double peaks remain in the chromatogram.

6. Sample Storage and Stability

Some amino acids are chemically unstable in physiological fluids (e.g., progressive decline of plasma glutamine and cystine over time) as well as in standard mixtures. Keep samples and standard mixtures in the fridge. Old amino acid standard mixtures and mixtures which have not been properly stored should not be used for instrument calibration. Order fresh mixtures from Chromservis (see ordering information in *Section 9*).

Samples prepared for by the procedure describedin this manual may be stored for several days in a freezer prior to LC-MS analysis. Because sample preparation is rapid with this procedure, we recommend analysing freshly prepared samples .

7. Cleaning and Care of Supplies

Always tightly cap all vials containing standards and reagents, especially the Reagent (Derivatization) Solution (RDS) vial, when not in use to avoid evaporation of the solvent and alteration of the reagent composition.

After pipetting the reagent medium, the pipette tip should be immediately removed and disposed of to prevent pipette damage.

8. Quality Assurance

All components of the MetAmino® analysis kit are subject to rigorous quality control testing. These measures help to ensure the best results. If poor results occur, please contact your Chromservis technical consultant or your local distributor.

9. Ordering information

Item	p/n	Amount
MetAmino® sample preparation LC/MS **start-up** kit, CF (for 100 samples)	MAK-5857-AA01	1 kit
MetAmino® sample preparation LC/MS **basic-up** kit, CF (for 400 samples)	MAK-5857-CA04	1 kit
MetAmino® sample preparation reagents kit (for 100 samples)	MAK-5857-L002	1 set

10. Safety data sheets

The MetAmino® kit includes reagents classified according to regulation (EC) 1907/2006 (REACH). All Safety Data Sheets (SDS) are available to download using following QR code.

https://www.chromservis.eu/en/safety-data-sheets

11. Warranty

MetAmino® kit is for research use only, not for use in diagnostic procedures (IVD).

Chromservis s.r.o. warrants that this kit shall perform in accordance with the specification set forth in the labelling and in this User manual. The MetAmino® kit is designed and intended for research purposes only, not for any clinical diagnostic purposes. This kit is for use only by properly qualified personnel. This limited warranty is subject to the conditions mentioned below.

The limited warranty does not apply to any material deviation from the specifications, which result from:

- Improper use or from any purpose other than set forth in this User manual
- Faults and defects in any third-party component
- Modification of the MetAmino® kit components
- Any incorrect use when handling and storing the components of the kit

12. Manufacturer contact

Chromsevis s.r.o.
Jakobiho 327
109 00 Praha 10 – Petrovice
The Czech Republic
www.chromservis.eu