MetAmino Kit®

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

User Manual

Ver. EN231122

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

1. Content 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 of the sample preparation kit including the workflow protocol.

3. Kit Contents

3.1. Reagents, Liquid Media and Chemicals

The MetAmino® Start-up kit is designed for 100 samples. Reagents, liquid media and chemicals are included. The Basic kit (400 samples kit) includes two boxes:

- The Start-up 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
Reducing Agent (RA)	4 mL vial	2.75	1	4
Basic Medium (BM)	4 mL vial	2.75	1	4
Catalytic Solution (CTS)	40 mL vial	5.5	1	4
Reagent (Derivatization) Solution (RDS)	40 mL vial	5.5	1	4
Extraction Medium (EM)	40 mL vial	5.5	1	4
Acidic Medium (AM)	4 mL vial	2.75	1	4

3.2. Accessories

Item	Note	Amount (100 samples)	Amount (400 samples)
MetAmino® GC Column	Proprietary stationary phase	1 pc	1 pc
Reaction glass tubes		100 pcs	400 pcs
Tube rack for up to 80 reaction Tubes	See Fig. 3 in Section 5.1	1 pc	1 pc
Autosampler Vials (9 mm screw-top)	Including septa and caps	100 pcs	400 pcs
Inserts for Autosampler Vials		100 pc	400 pc

Figure 1 - MetAmino® start-up kit for 100 samples

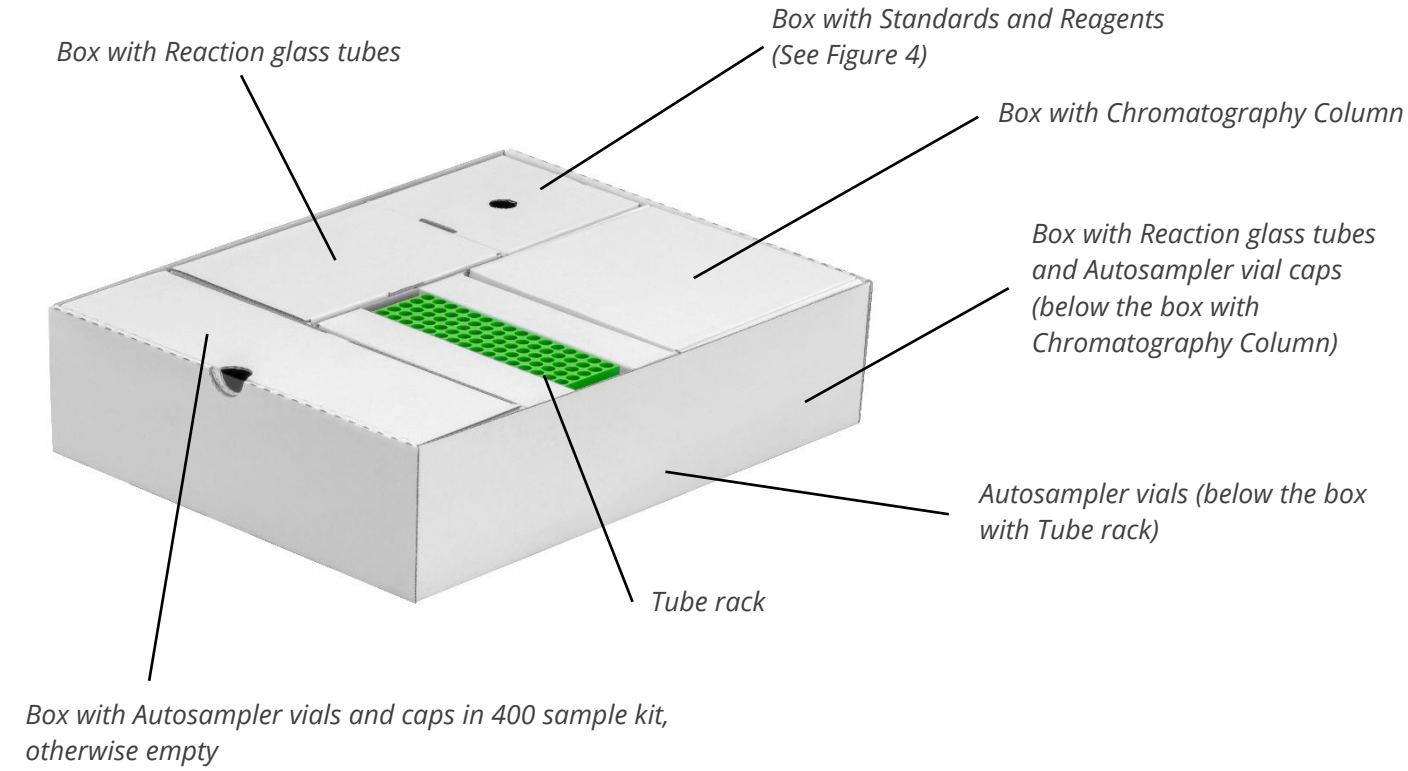
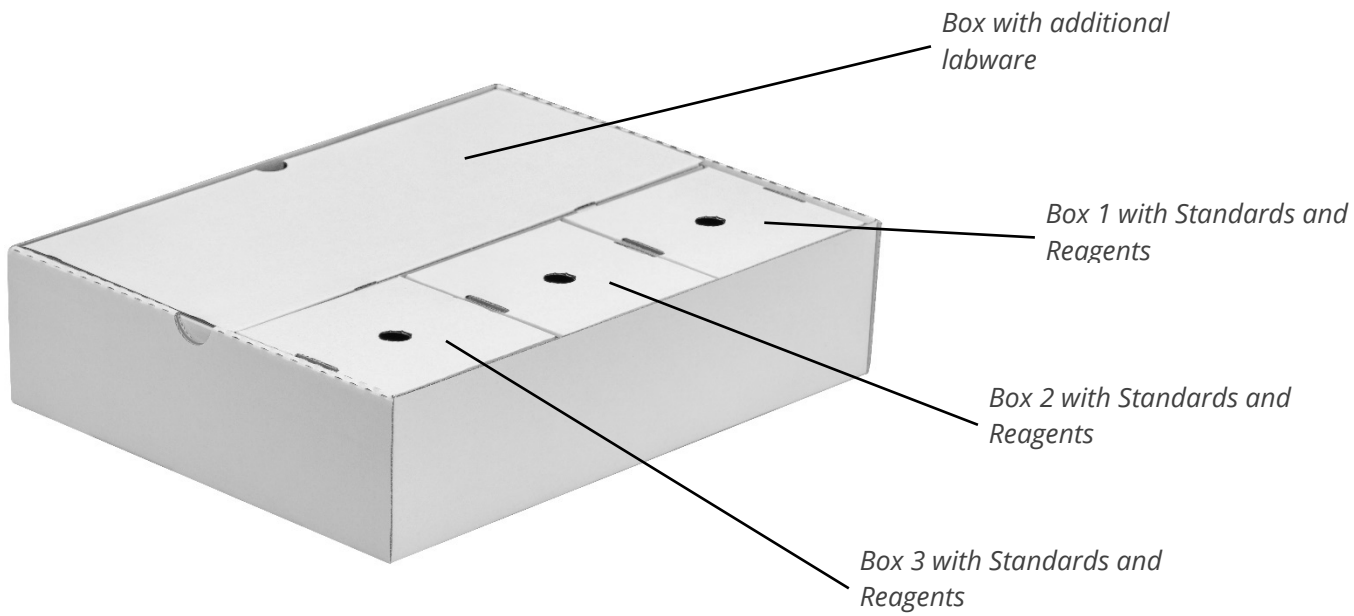


Figure 2 - MetAmino® extended box for a further 300 samples



3.3. Materials and Tools

All 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	Pasteur pipettes for sample transfer (see section 5.2 step 9)
7	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 analytes by gas chromatography-mass spectrometry (GC-MS) using various mass spectrometric detectors (quadrupole, triple quadrupole). The method has been validated for the determination of 21 amino acids and related compounds with primary or secondary amino groups in human serum and urine. The MetAmino® acid analysis procedure consists of a derivatization followed by liquid/liquid microextraction (**LLME**) step; derivatized samples are quickly analyzed by gas chromatography-mass spectrometry. Analytes are quickly derivatized with reagent at room temperature in aqueous solution. Derivatized analytes concomitantly migrate to the organic layer for additional separation from interfering compounds. Organic layer is then directly amenable for GC/MS system.

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

- In-situ derivatization of the amino functional groups
- Liquid/liquid microextraction (LLME)

The GC-MS analysis runs on a MetAmino® GC column. Sample preparation time takes around 5 minutes. GC-MS analysis is accomplished in 12 minutes. Total analysis time is around 17 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 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 GC-MS analysis of more than 70 amino acids and related compounds. For the complete list, please refer to **Table 1a and Table 1b**. 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 1a *Comprehensive list of amino acids and related compounds prepared by MetAmino® kit for GC-MS analysis (included internal standards are highlighted in bold).*

	Analyte		RT	Mmi*	Diagnostic ions		
No.	**Name**	**Synonyms**	**[min]**		**m/z**	**m/z**	**m/z**
1	Sarcosine	SAR	2.36	497.0308	270	226	69
2	Alanine	ALA	2.50	497.0308	270	70	69
3	**3,3,3,D3-DL Alanine**	**IS**	**2.61**	**501.0409**	**273**	**73**	**113**
4	N-Acetylglycine		2.65	299.0392	256	113	212
5	Glycine	GLY	2.68	483.0151	256	212	56
6	3-Methylglutaric acid		2.74	510.0512	311	282	227
7	2-Aminobutyric acid	2-ABA	2.90	511.0465	284	84	113
8	3-Hydroxymethylglutaric acid		2.95	752.0325	285	311	85
9	N-Acetylcysteine		3.00	571.0134	282	309	509
10	*beta*-Alanine	3-ALA	3.03	497.0308	269	256	98
11	Valine	VAL	3.03	525.0621	298	283	98
12	β-Aminoisobutyric acid	BAIBA	3.11	511.0465	256	112	113
13	3-Amino-*n*-butyric acid	3-ABA	3.06	511.0465	270	227	283
14	Norvaline	NVA	3.25	525.0621	298	256	98
15	Leucine	LEU	3.42	539.0778	312	270	256
16	*allo*-Isoleucine	aILE	3.43	539.0778	312	283	256
17	Ethanolamine	ETA	3.47	513.0257	256	269	270
18	Isoleucine	ILE	3.48	539.0778	283	312	256
19	Homoserine	HSER	3.48	753.0278	100	283	128
20	Proline	PRO	3.53	523.0465	296	297	69
21	Threonine	THR	3.67	527.0415	100	283	483
22	4-Aminobutyric acid	GABA	3.67	511.0465	112	256	69
23	Norleucine	NLE	3.70	539.0778	312	256	112
24	Pipecolic acid	PIP	3.72	537.0621	310	518	407
25	N-Acetylaspartic acid		3.75	539.0414	270	312	228

	Analyte		RT	Mmi*	Diagnostic ions		
26	Asparagine	ASN	3.79	522.0261	295	496	113
27	Thioproline	TPR	4.06	541.0029	314	287	86
28	2-Hydroxyglutaric acid		4.07	738.0169	283	239	511
29	Aspartic acid	ASP	4.10	723.0173	254	496	296
30	3-Methylcysteine		4.17	543.0185	61	300	316
31	Serine	SER	4.40	739.0122	268	295	51
32	Acetylserine		4.42	555.0363	268	312	113
33	Pyroglutamic acid		4.61	537.0000	282	310	510
34	N-Acetylglutamic acid		4.69	567.0727	282	310	510
35	Glutamic acid	GLU	4.70	737.0329	282	310	510
36	Methionine	MET	4.64	557.0342	283	483	357
37	4-Hydroxyproline	4-HYP	4.61	539.0414	312	294	68
38	Cysteine	CYS	4.99	754.9895	328	285	113
39	Selenomethionine		4.93	604.9786	282	510	405
40	Ethionine	ETH	4.95	571.0498	282	311	571
41	Phenylalanine	PHE	5.09	573.0621	91	330	92
42	2-Aminoadipic acid	AAA	5.20	751.0486	124	282	324
43	3-Hydroxyproline	3-HYP	4.45	539.0414	312	129	256
44	2,4-Diaminobutyric acid	DABA	5.48	752.0438	282	256	325
45	S-Carboxymethyl-cysteine		5.52	769.0050	213	259	314
46	Homocysteine	HCYS	5.56	769.0045	282	342	82
47	2-Aminopimelic acid	APA	5.61	765.0642	338	138	95
48	Histamine	HTA	5.64	563.0526	308	320	113
49	**Homophenylalanine**	**IS**	**5.69**	**587.0778**	**283**	**117**	**483**
50	Glutamine	GLN	5.73	554.0523	84	282	327
51	4-Aminobenzoic acid	PABA	5.87	545.0308	146	345	346
52	1-Methylhistidine		5.89	577.0682	95	150	350
53	Chloro-phenylalanine		5.91	607.0231	125	364	180
54	Methionine sulfone	MET-SO_2	5.99	589.0240	282	82	189
55	Ornithine	ORN	6.09	766.0595	296	256	69
56	Acetyltyrosine		6.15	871.0489	333	289	188
57	Histidine	HIS	6.28	789.0391	307	362	113
58	Glycylproline	GPR	6.36	580.0679	70	153	296

	Analyte		RT	Mmi*	Diagnostic ions		
59	Lysine	LYS	6.46	780.0751	310	256	153
60	Tyramine		6.48	589.0570	346	333	289
61	2,6-Diaminopimelic acid (isomers)		6.93	1006.0616	308	536	
62	Tyrosine	TYR	6.95	815.0435	333	289	113
63	5-Hydroxylysine (isomers)	5-HLY	7.17	1022.0565	269	256	69
64	Cystathionine	CTH	7.57	1038.0337	328	282	69
65	Dopamine	DAM	7.52	831.0000	256	531	113
66	Tryptophan	TRP	7.49	612.0730	130	131	385
67	3,4-Dihydroxyphenylalanine	DOPA	7.73	1057.0249	149	531	575
68	Cystine	C-C	7.78	1055.9901	496	268	113
69	Prolylhydroxyproline	PHP	7.84	862.0806	296	297	294
70	3-Nitrotyrosine		7.95	860.0286	334	113	378

** Mmi = monoisotopic mass of the derivative of the relevant metabolite fragmentation in ion source*

Table 1b *Comprehensive MRM transitions of amino acids and related compounds prepared by MetAmino kit for GC-MS analysis (included **internal standards** listed in bold).*

	Analyte		MRM MS/MS transitions		
No.	**Name**	**Synonyms**	**Quantifier [a] (CE[b])**	**Qualifier[c] (CE)**	**Qualifier (CE)**
2	Alanine	ALA	270 > 113 (20)	270 > 226 (20)	113 > 69 (20)
5	Glycine	GLY	256 > 113 (20)	212 > 73 (20)	212 > 192 (10)
11	Valine	VAL	298 > 55 (20)	283 > 83 (10)	298 > 98 (10)
15	Leucine	LEU	312 > 256 (10)	256 > 113 (20)	312 > 113 (20)
18	Isoleucine	ILE	312 > 256 (10)	283 > 83 (20)	312 > 113 (20)
29	Aspartic acid	ASP	296 > 96 (10)	296 > 254 (10)	496 > 454 (20)
20	Proline	PRO	296 > 113 (30)	296 > 70 (30)	504 > 296 (30)
21	Threonine	THR	100 > 50 (30)	100 > 69 (30)	283 > 83 (10)
26	Asparagine	ASN	295 > 113 (20)	95 > 68 (10)	295 > 95 (20)
31	Serine	SER	295 > 113 (10)	268 > 240 (20)	268 > 113 (20)
35	Glutamic acid	GLU	282 > 82 (10)	282 > 113 (20)	310 > 282 (10)
37	trans-4-L-Hydroxyproline	4-HYP	294 > 94 (20)	294 > 113 (30)	521 > 294 (10)
36	Methionine	MET	283 > 83 (20)	483 > 283 (10)	483 > 83 (20)

	Analyte		MRM MS/MS transitions		
38	Cysteine	CYS	285 > 213 (30)	328 > 101 (20)	328 > 113 (20)
41	Phenylalanine	PHE	91 > 65 (30)	330 > 131 (20)	330 > 103 (30)
57	Histidine	HIS	307 > 113 (20)	362 > 135 (20)	307 > 236 (20)
59	Lysine	LYS	256 > 113 (20)	310 > 113 (30)	256 > 192 (10)
50	Glutamine	GLN	327 > 84 (10)	327 > 282 (10)	282 > 82 (20)
62	Tyrosine	TYR	333 > 289 (20)	333 > 113 (20)	289 > 107 (20)
63	5-Hydroxylysine (isomers)	5-HLY	269 > 86 (10)	269 > 113 (20)	256 > 113 (20)
66	Tryptophan	TRP	130 > 77 (30)	130 > 103 (30)	130 > 51 (30)
3	**3,3,3,D3-DL Alanine**	**IS**	**273 > 113 (20)**	**273 > 229 (20)**	
49	**Homophenylalanine**	**IS**	**91 > 65 (30)**	**283 > 83 (10)**	**283 > 113 (20)**

[a] *Quantifier ion transition (Optimized for Agilent 6495B)*
[b] *Collision energy (Optimized for Agilent 6495B)*
[c] *Qualifier ion transition (Optimized for Agilent 6495B)*

4.3. 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 as **freshly** prepared on the day of consumption. We recommend storing the complete Box with Standards and Reagents **at 4 °C in the dark**.

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

Item	Storage conditions	Stability
Amino Acid Standards SD1 Solution	4 °C	12 months
Amino Acid Standards SD2 Dried	4 °C	12 months
Solution with Internal Standard (IS)	4 °C	12 months
Amino Acid Standard Diluting Medium (AASDM)	4 °C	12 months
Reducing Agent (RA)	4 °C	12 months
Basic Medium (BM)	4 °C	12 months
Catalytic Solution (CTS)	4 °C	12 months
Reagent (Derivatization) Solution (RDS)	4 °C	6 months
Extraction Medium (EM)	4 °C	12 months
Acidic Medium (AM)	4 °C	12 months

4.4. 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.

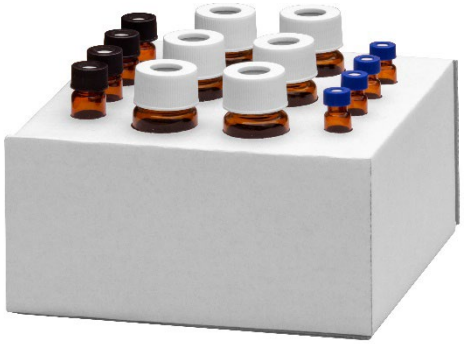
5. Sample Preparation Procedure

5.1. Setup

The MetAmino® kit package was designed to be an efficient workstation. It contains a Reagent tray, Tube rack, and vial section. To speed up and simplify the sample preparation, it is recommended to use the Tube rack (See **Figure 3**). If the kit will not be used for several days, the Reagent tray (See **Figure 4**) can be conveniently removed and placed in the refrigerator at 4 °C.

Figure 3 – Tube rack

Figure 4 – 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 spinning at 6,000 rpm (1,500 ×g).

5.2.1. Sample preparation step

For each sample line up use one reaction glass tube.

STEP 1: Pipette 25 µL of sample (serum, plasma, urine or other), and 10 µL of solution with Internal Standards (**IS**) into each reaction glass tube.

Note: If low concentrations of analytes need to be quantified, the volume of sample to be prepared should be 50 µL or more.

5.2.2. Derivatization step

STEP 2: Pipette 20 µL of Reducing Agent **(RA)**, into each reaction glass tube and vortex briefly (5 to 10 sec) and let it stand for 1 to 2 minutes.

Note: For all subsequent sample preparation steps use a vortex mixer set in the touch (pulse) mode (to about 80 % of max speed) for any mixing operations.

STEP 3: Pipette 25 µL of Basic Medium (**BM**) into each reaction glass tube

STEP 4: Pipette 50 µL of Reagent (Derivatization) Solution (**RDS**) into each reaction glass tube and vortex briefly (5 to 10 sec).

STEP 5: Pipette 25 µL of Catalytic Solution (**CTS**) into each reaction glass tube and vortex briefly (5 to 10 sec).

STEP 6: Pipette again 25 µL of Catalytic Solution (**CTS**) into each reaction glass tube and vortex briefly (5 to 10 sec) and let it stand for 1 to 2 minutes. The emulsion will gradually separate into two layers.

Note: A longer time than 2 minute each at steps 4 and 5, or later, at step 6, it does NOT have an influence on the analytical results.

5.2.3. LLME step

STEP 7: Pipette 50 µL of Extraction Medium (**EM**) into each reaction glass tube and vortex briefly (5 to 10 sec).

STEP 8: Pipette 25 µL of Acidic Medium (**AM**) into each reaction glass tube and vortex briefly (5 to 10 sec) and centrifuge 30 to 60 sec. at 1,500 ×g (6,000 rpm).

STEP 9 : Transfer the organic (upper) layer (50-100 µL) into the autosampler vial with insert. The sample is ready for GC-MS analysis.

5.3. Optimizing Sample Preparation Time

After a short period of practice the complete sample preparation protocol can be completed in 5 to 6 minutes per sample.

This process can be further improved by preparing up to ten samples at a time. For example, at step 2 dispense Reducing Agent (**RA**) (and at later steps all other reagents) in ten vials successively, using the same pipette tip. At step 5, after dispensing Catalytic Medium (**CTS**), vortex 2-3 vials simultaneously. During each one-minute wait at step 2, prepare autosampler vials for sample transfer.

5.4. Column for MetAmino® GC-MS analysis

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

MetAmino® GC column 10 m x 0.25 mm x 0.25 µm

5.4.1. MetAmino® GC column conditioning

After the proper column installation and absence of leaks, the column is ready for conditioning. As first, connect the column to the injector. Do not connect the column to the detector (the other end of the column should be inside of the thermostat).

Set the temperature on your GC thermostat to 40 ˚C and keep it for 15 minutes. Afterwards, set the temperature gradient to 10 ˚C/min reaching the temperature of 330 ˚C and keep it at this temperature for 2 hours. Do not exceed the upper column temperature limit otherwise column damage will occure.

After this heating procedure is completed, connect the column to the detector. For a control, inject the solvent and check the quality of a chromatogram. If the signal to noise ratio is not optimal, inject the solvet couple of times and compare the results. A flat base line should be established.

The column is ready for a use.

Note: Our MetAmino® GC columns are pre-conditionized and therefore there is no need to conditionalize it over night.

Instrument settings:

5.4.2. GC method

Injection	splitless, 280 °C
Flow rate	1.5 mL/min constant flow
Carrier Gas	Helium
Injection volume	1 µL
Oven program	60°C (hold 1 min) at 20°C /min to 280°C

5.4.3. MS method

Source	230 °C
Quad	150 °C
Auxiliary	280 °C
Scan range	65-700 m/z
Scan speed	8 scans/s

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 liquid/liquid microextraction (**LLME**) steps for each vial as described by the MetAmino® procedure (Section 5.2). Transfer the organic (upper) layer for each vial into autosampler vial and use to tune the mass spectrometer.

Most mass spectrometers do not allow 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 is done for an optimal tuning of a large number of desired analytes.

5.6. Calibration Standards

For quantitation purposes, prepare aliquots of the standard mixtures following the Sample Preparation by Derivatization and liquid/liquid microextraction described in this manual in Section 5.2. Two vials with amino acid standards are supplied in the kit - **SD1** (18 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 18 amino acids:
ALA, GLY, VAL, LEU, ILE, PRO, THR, ASP, SER, GLU, MET, 4-HYP, CYS, PHE, HIS, LYS, TYR, 5-HLY

The **SD2** vials contains 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}.*

Hint: If you are not familiar with the mmol/L units, you can simply convert here: 1 mmol/L = 1nmol/µL = 1 µmol/mL. The conversion to mass concentration units (mg/L) can be done by multiplying the molar concentration by the molar mass of the corresponding analyte.

***Example**: Glycine (M = 75.07 g/mol) is supplied in SD1 in a concentration of 75.07 mg/L.*

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. 10 µL of amino acid standard solution **SD1**, plus 10 µL of amino acid standard solution **SD2**, plus 10 µL of solution with internal standards (10 nmol of each amino acid and 2 nmol of each **IS**)

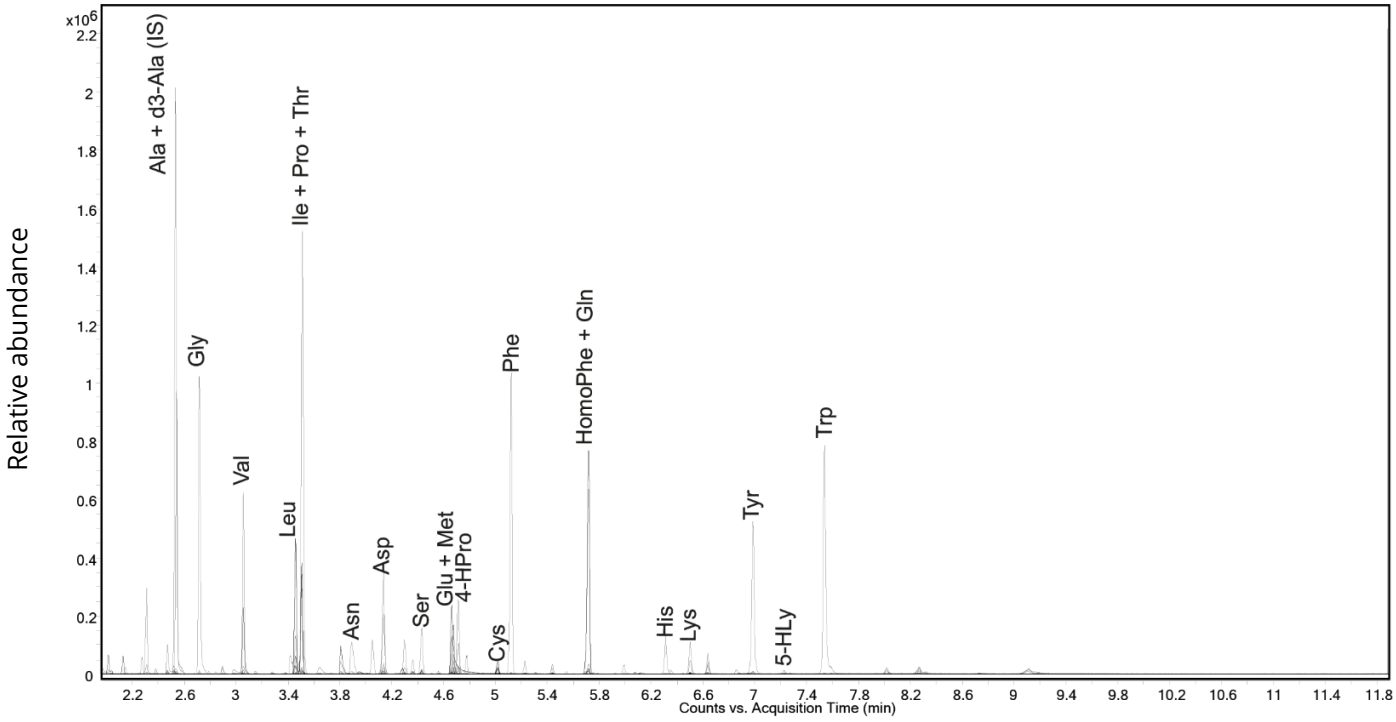
No. II 5 µL of amino acid standard solution **SD1**, plus 5 µL of amino acid standard solution **SD2**, plus 10 µL of solution with internal standards (5 nmol of each amino acid and 2 nmol of each **IS**)

No. III 10 µL of ten times diluted amino acid standard solution **SD1**, plus 10 µL of ten times diluted amino acid standard solution **SD2**, plus 10 µL of solution with internal standards (1 nmol of each amino acid and 2 nmol of each **IS**). For dilution use Amino Acid Standard Diluting Medium (**AASDM**).

No. IV 10 µL of hundred times diluted amino acid standard solution **SD1**, plus 10 µL of hundred times diluted amino acid standard solution **SD2**, plus 10 µL of solution with internal standards (0.1 nmol of each amino acid and 2 nmol of each **IS**). For dilution use Amino Acid Standard Diluting Medium (**AASDM**).

The concentration of each internal standard (IS) – d3-Ala, HPHE – in calibrators and samples prepared for chromatographic analysis is 2 nmoles per sample. While the use of the ideal internal standard will vary based on instrument and application, we recommend using d3-Ala as the internal standard for ALA, GLY, VAL, LEU, ILE, PRO, THR, ASP, SER and HPHE as the internal standard for GLU, MET, 4-HYP, CYS, PHE, GLN, HIS, LYS, TYR, 5-HLY and TRP. 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):*

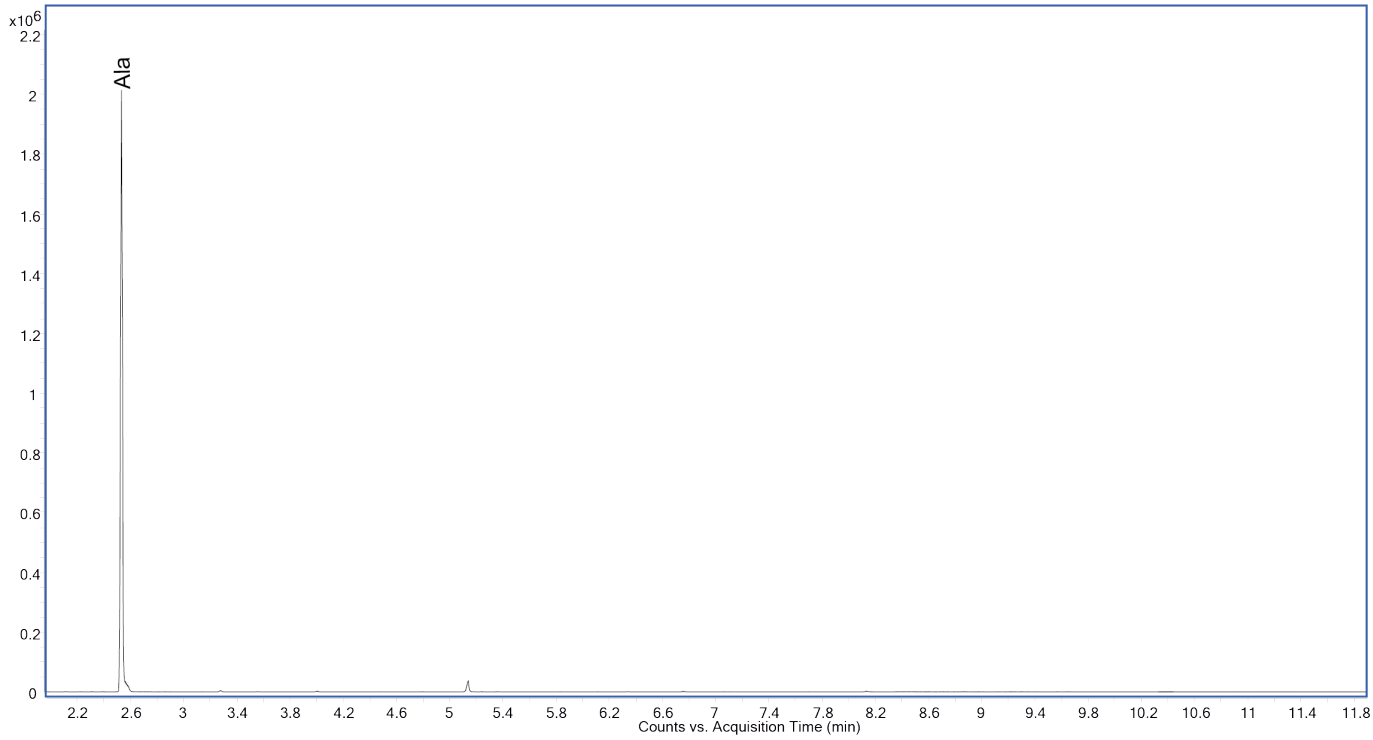


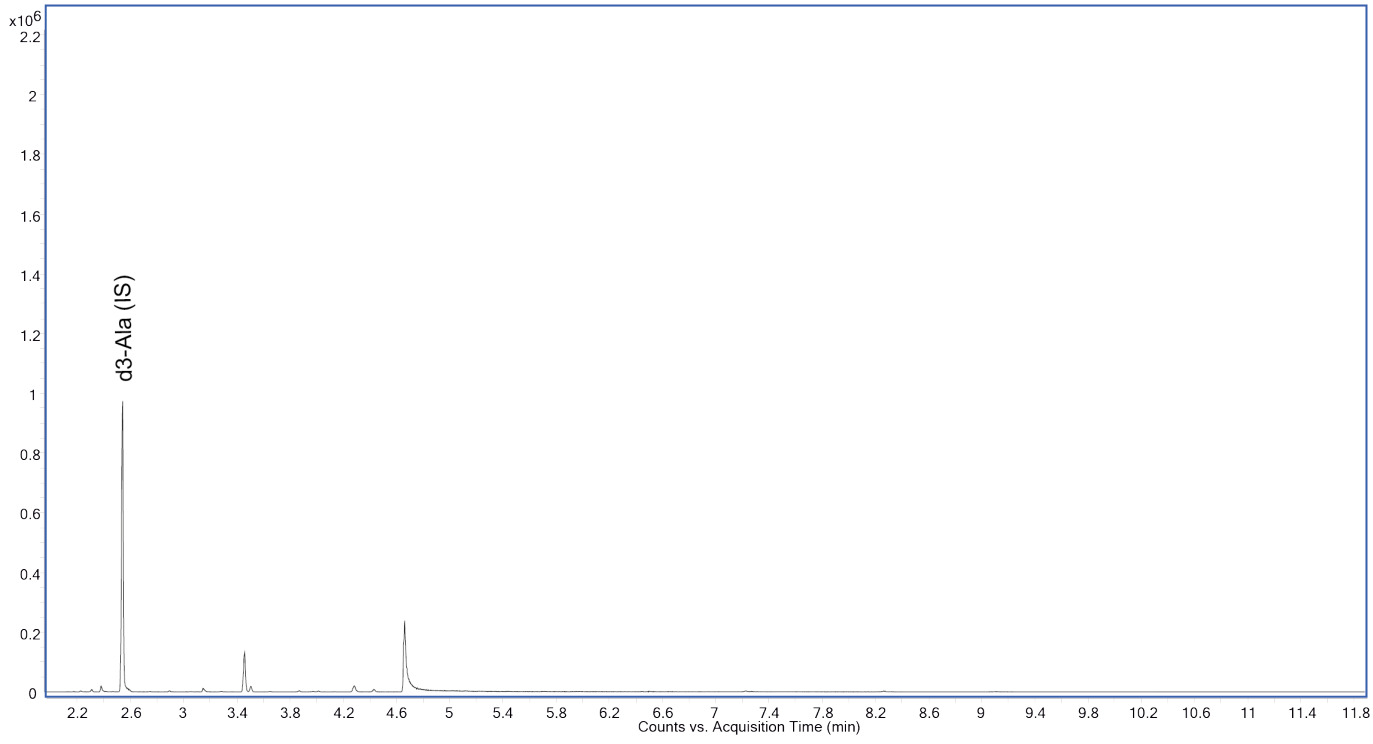
Hint: ***Preparation of the calibration diagram and the units***

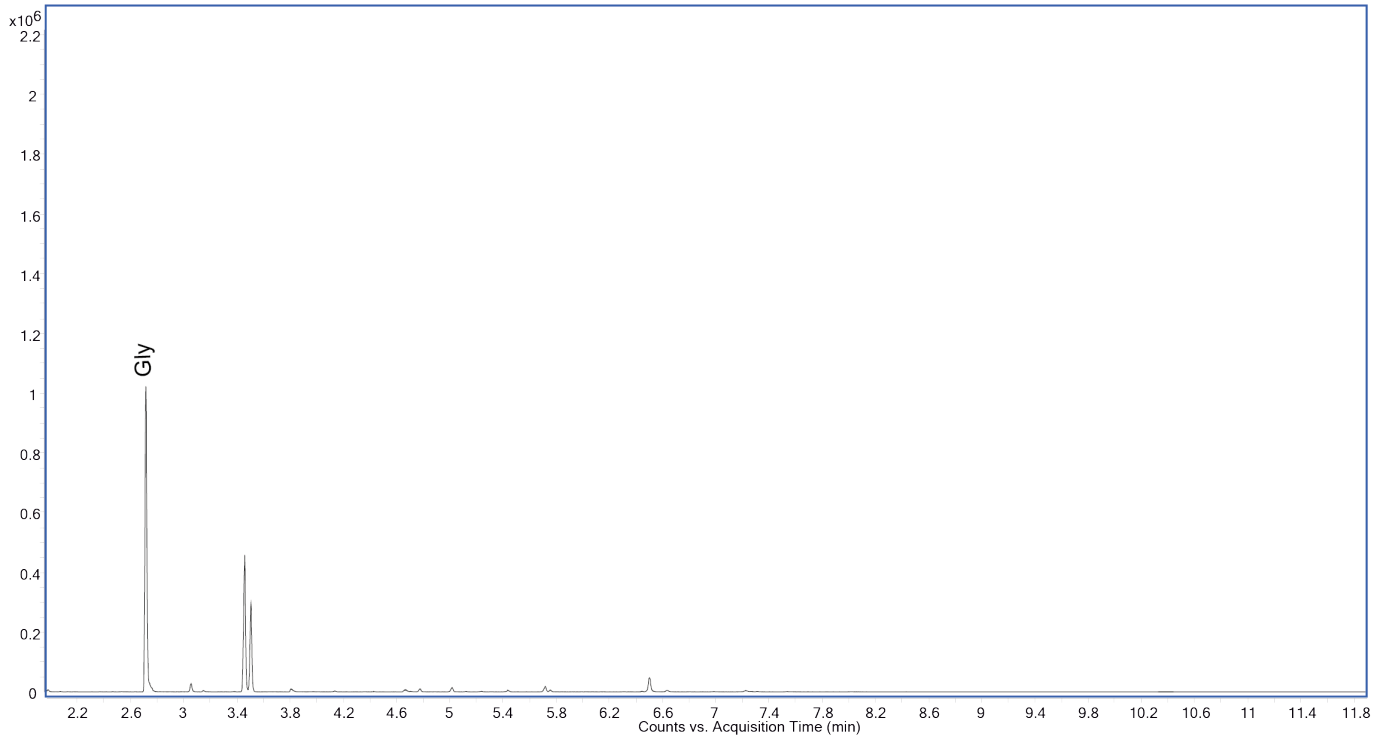
A very convenient method of constructing the calibration plot is to express the units in absolute numbers (nmol/sample) rather than in units of molar concentration or mass concentration.

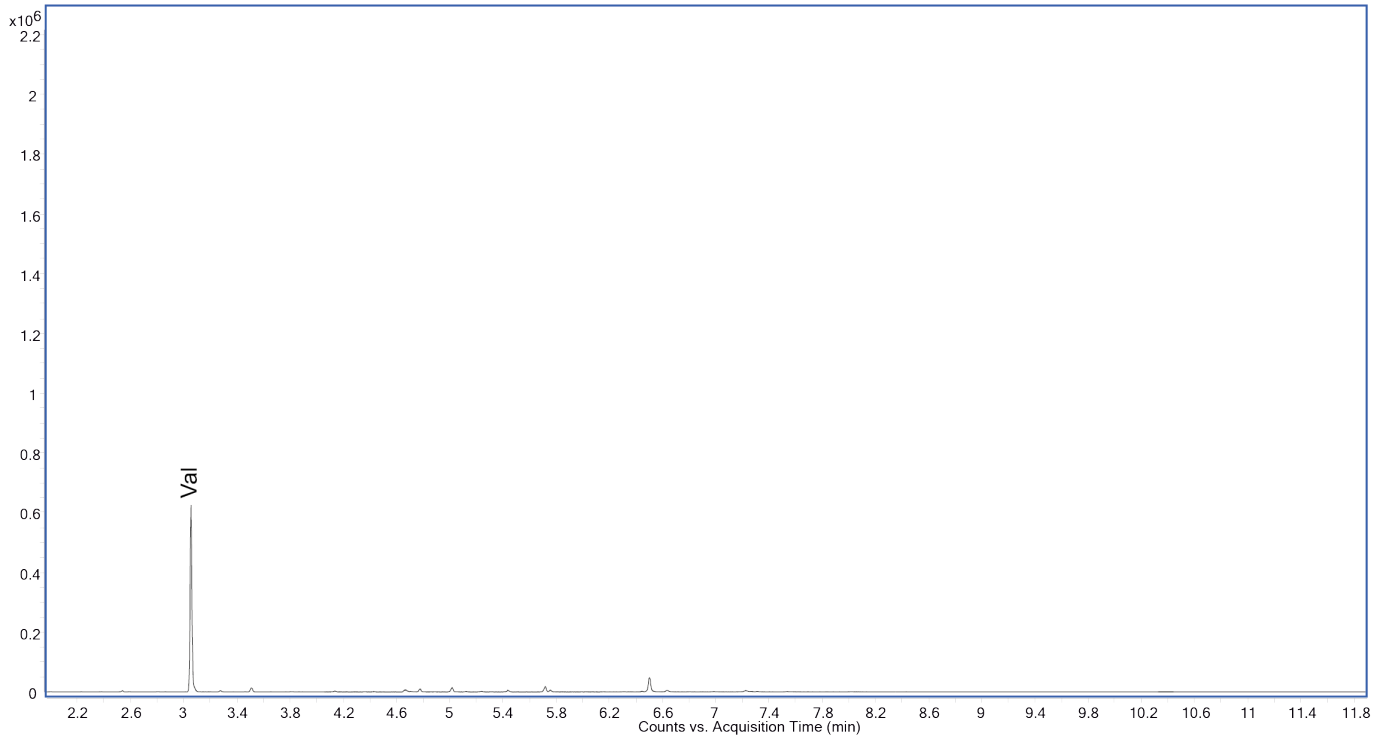
***Example**: The supplied SD1 solution is diluted 10 times and 10 µL of this diluted solution is taken for the Metamino reaction. This calibration point can be labeled as 1 nmol/sample.*

5.7.1. Extracted ion chromatograms






x10^6
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Gly
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)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1.6
1.4
1.2
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0.8
0.6
0.4
0.2
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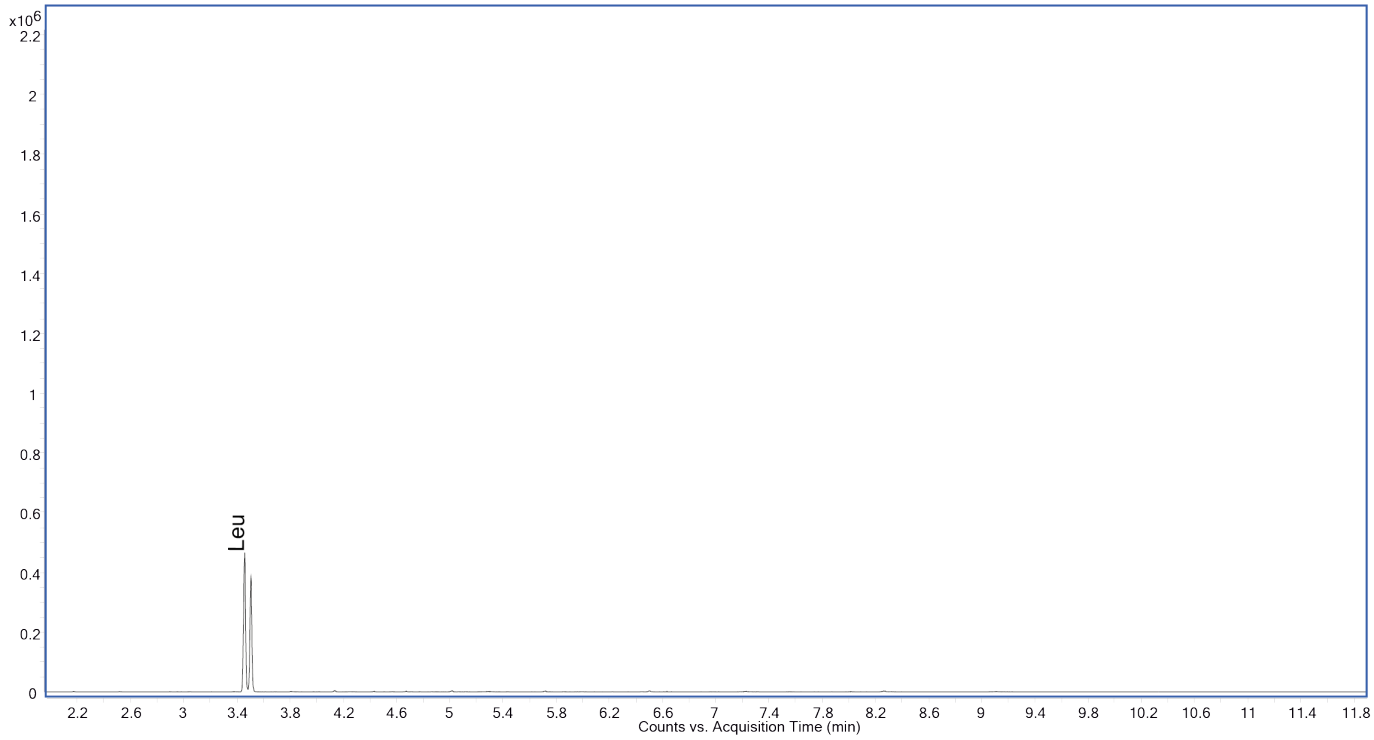
Val
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)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1.8
1.6
1.4
1.2
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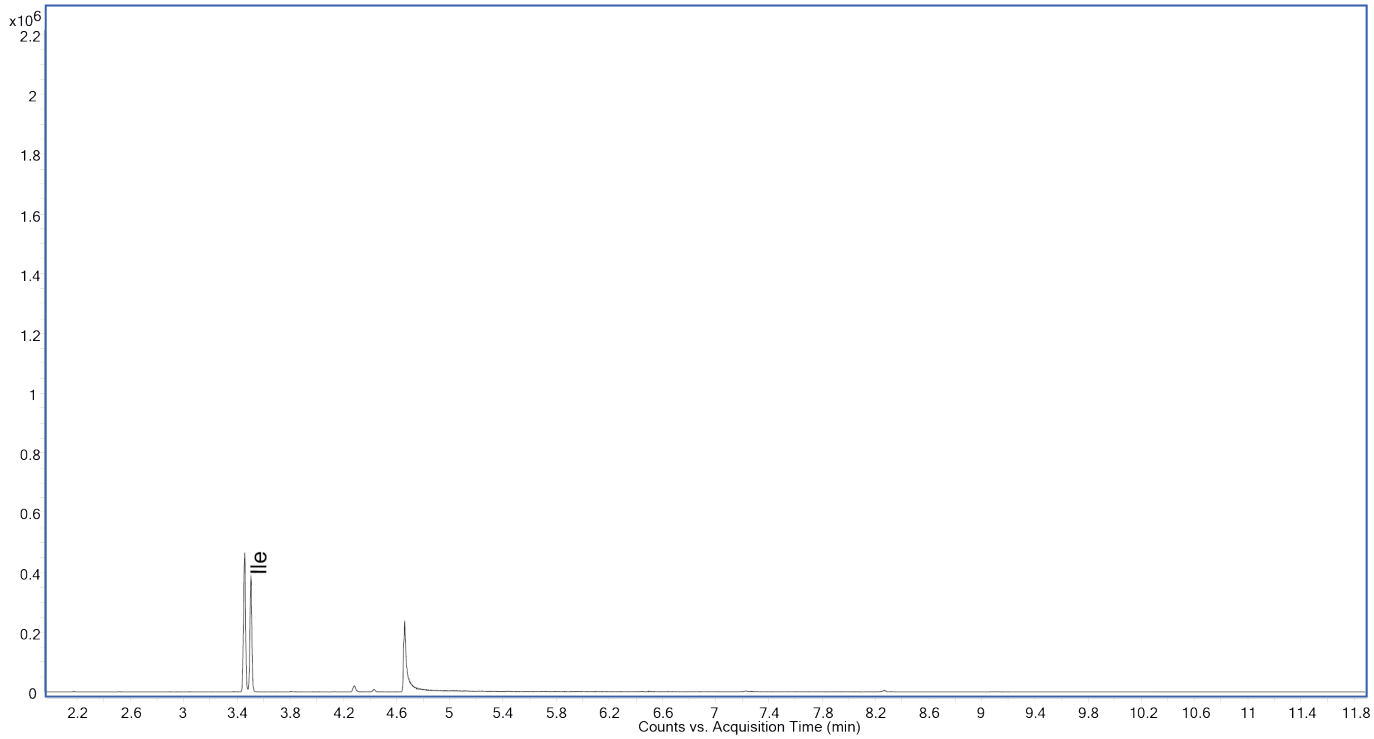
0.8
0.6
0.4
0.2
0
Leu
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)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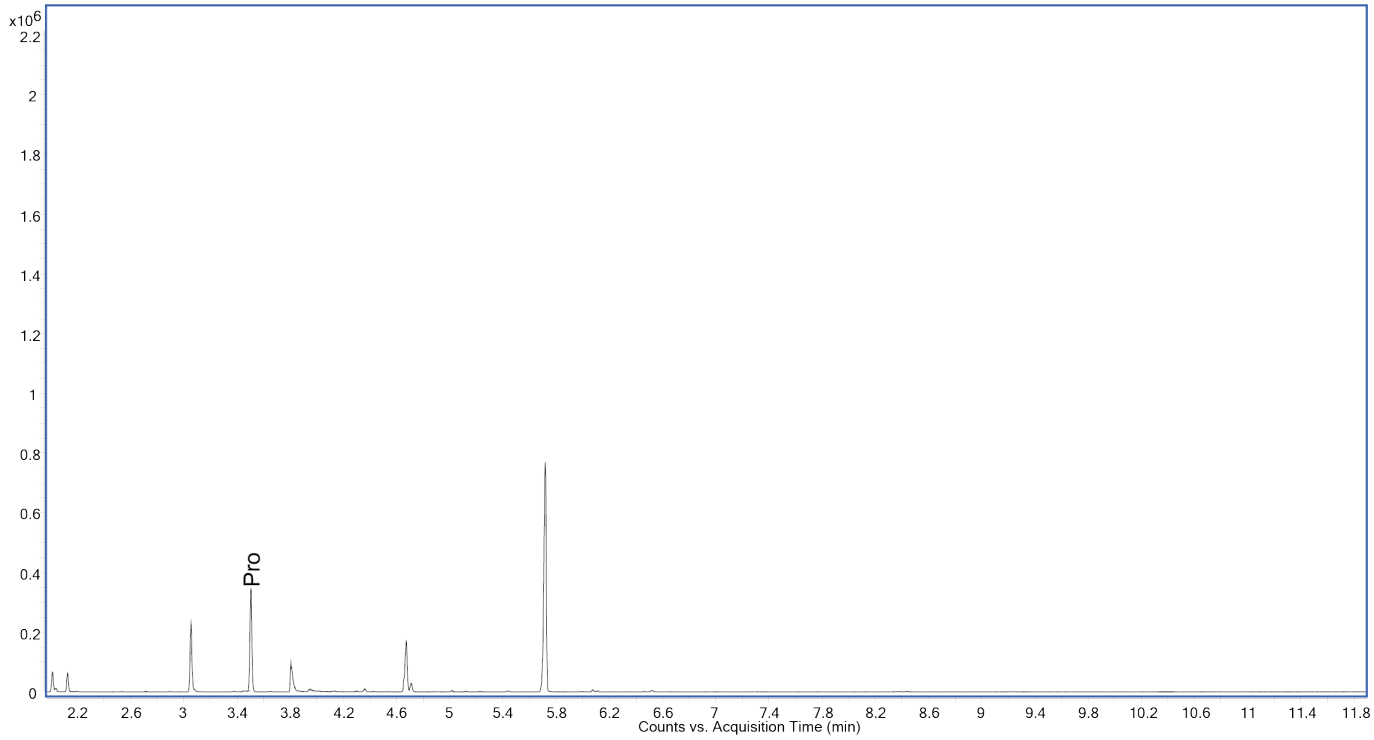
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Ile
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)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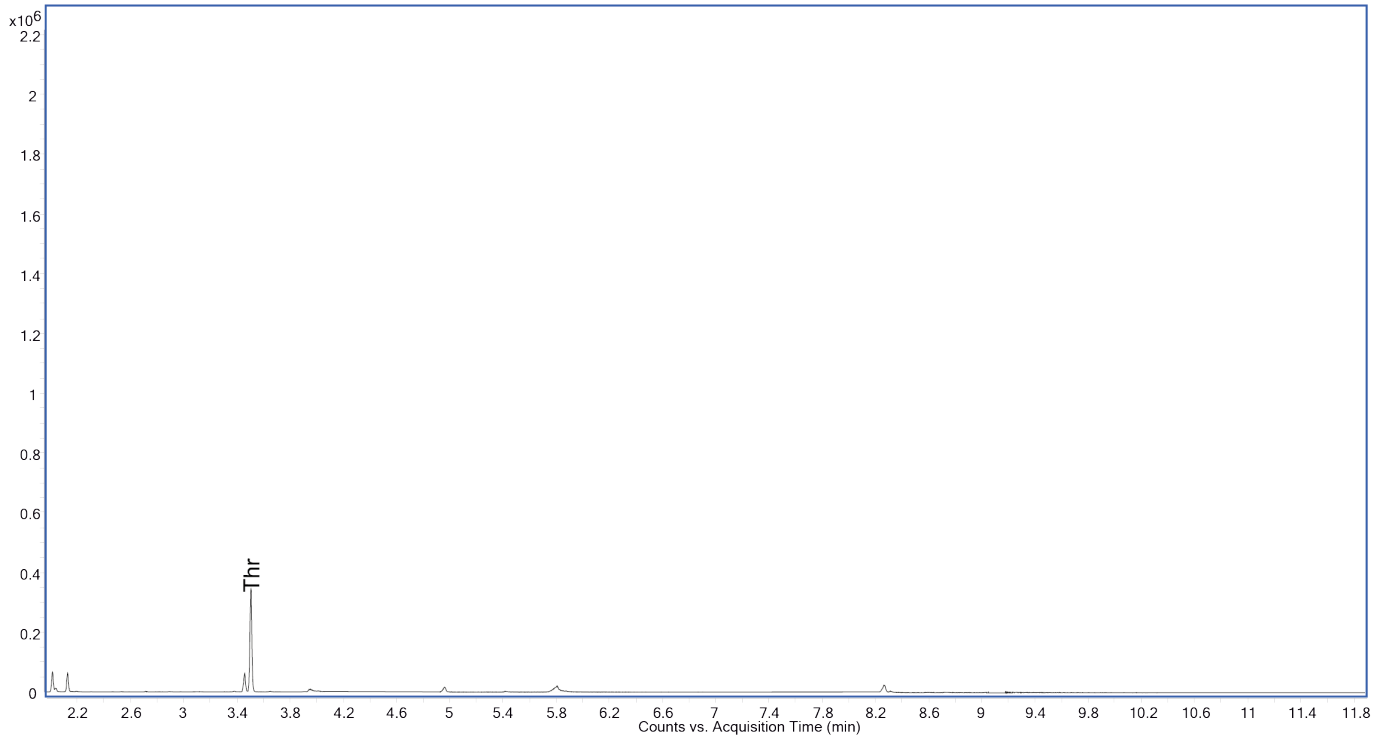
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Pro
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)


x10^6
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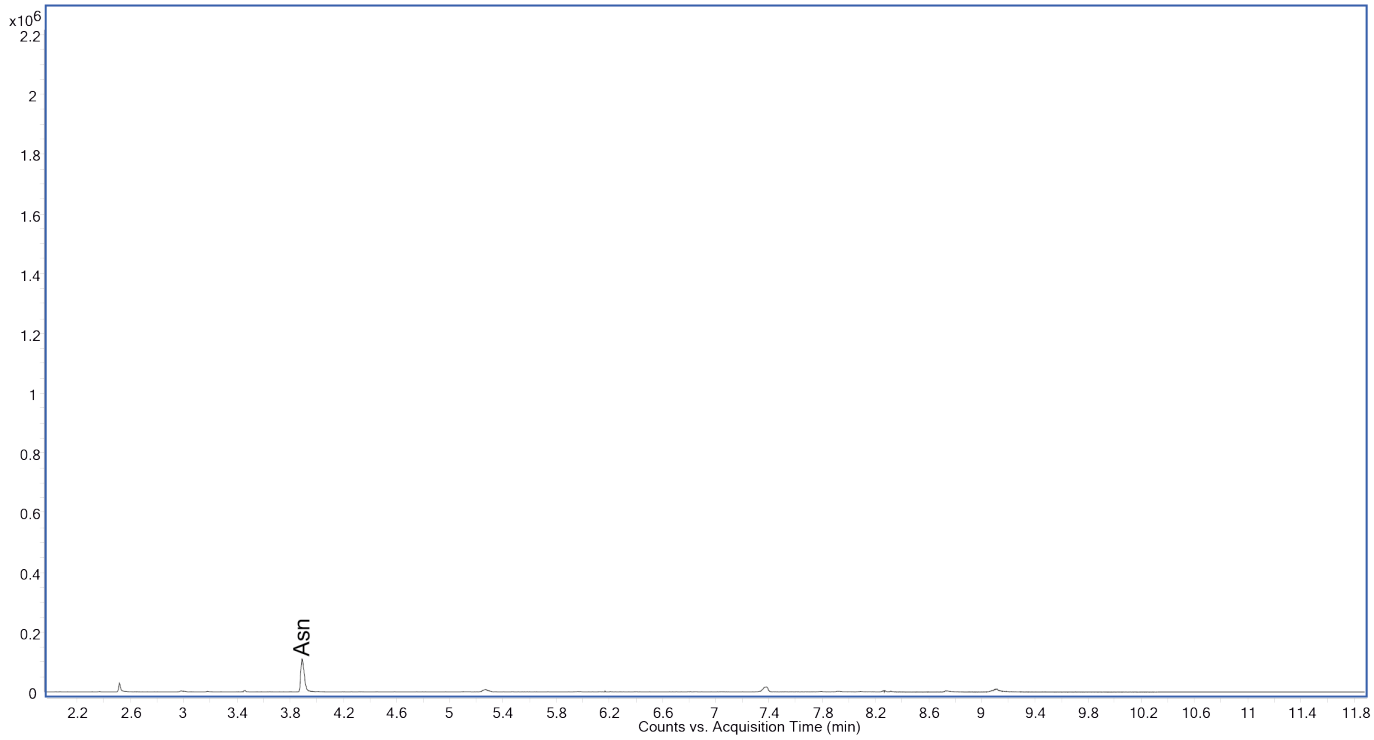
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Thr
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)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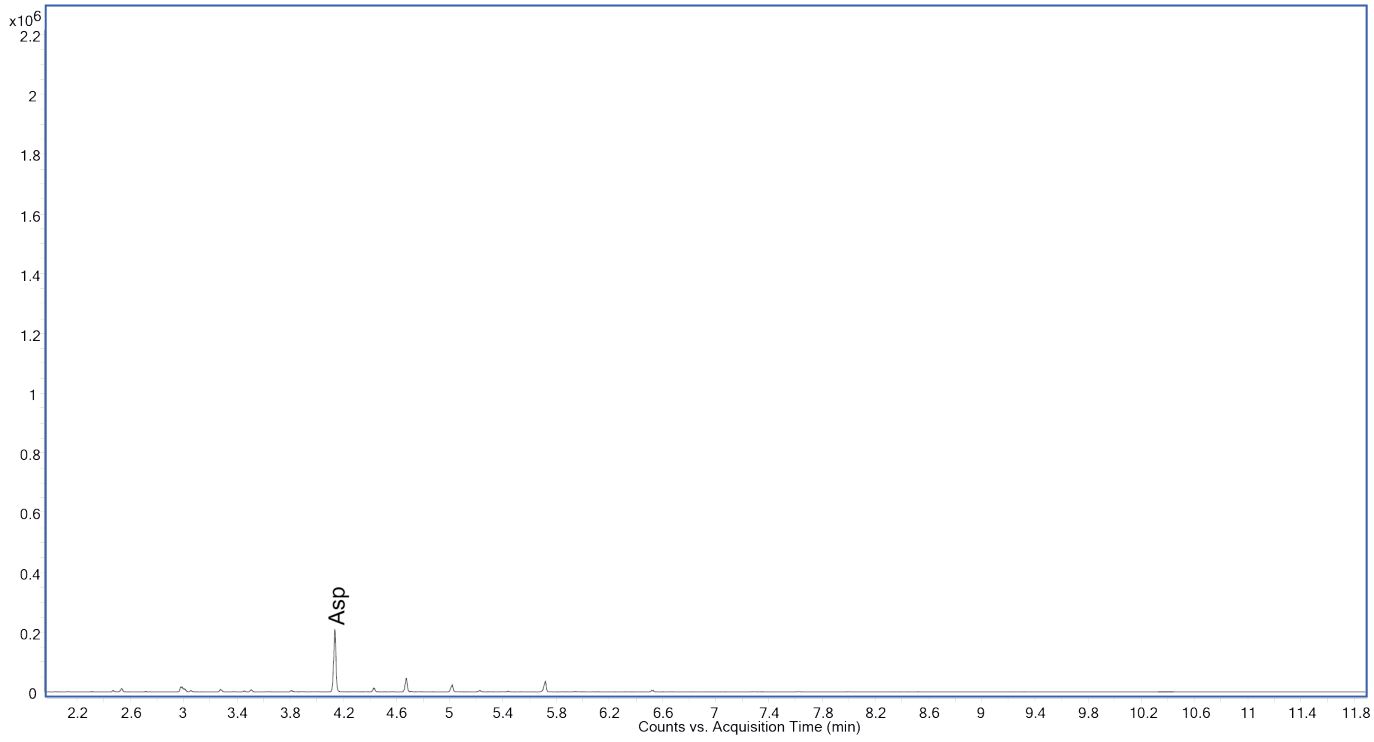
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Asn
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)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1.6
1.4
1.2
1
0.8
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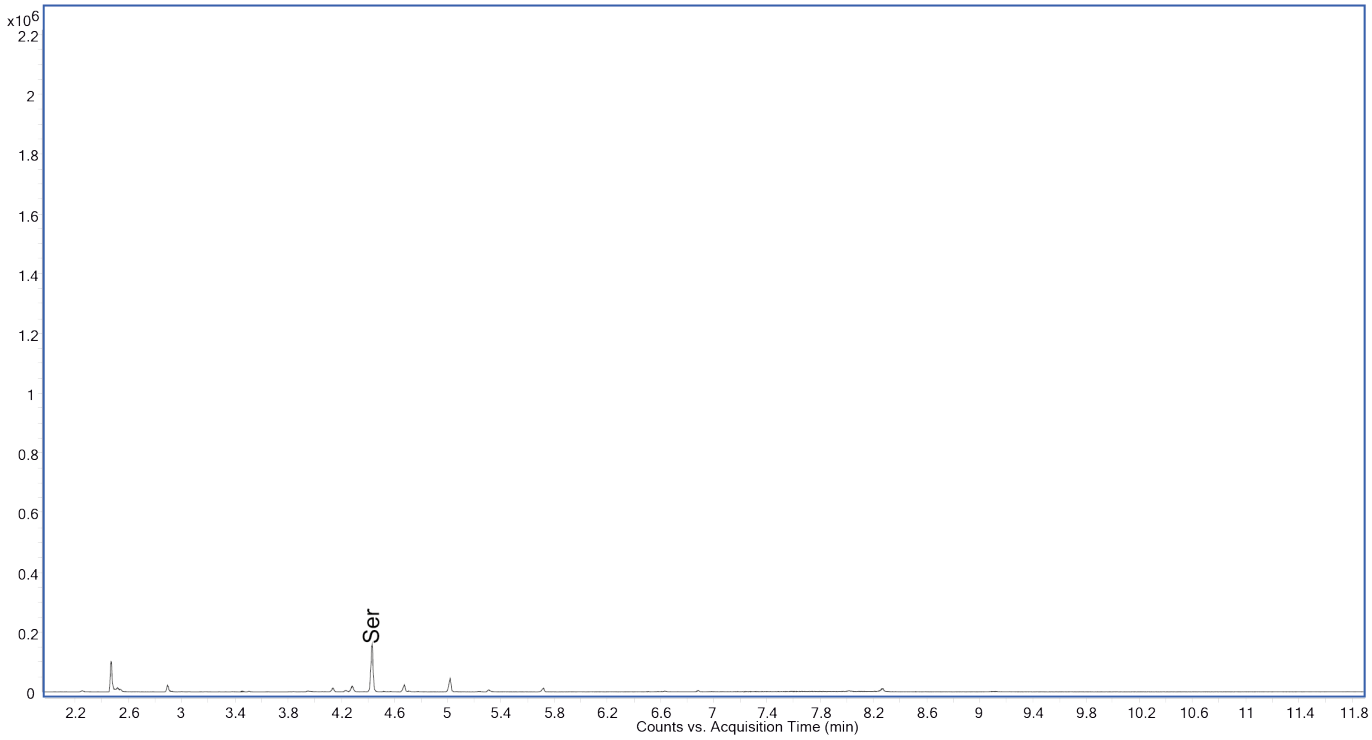
0.4
0.2
0
Asp
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)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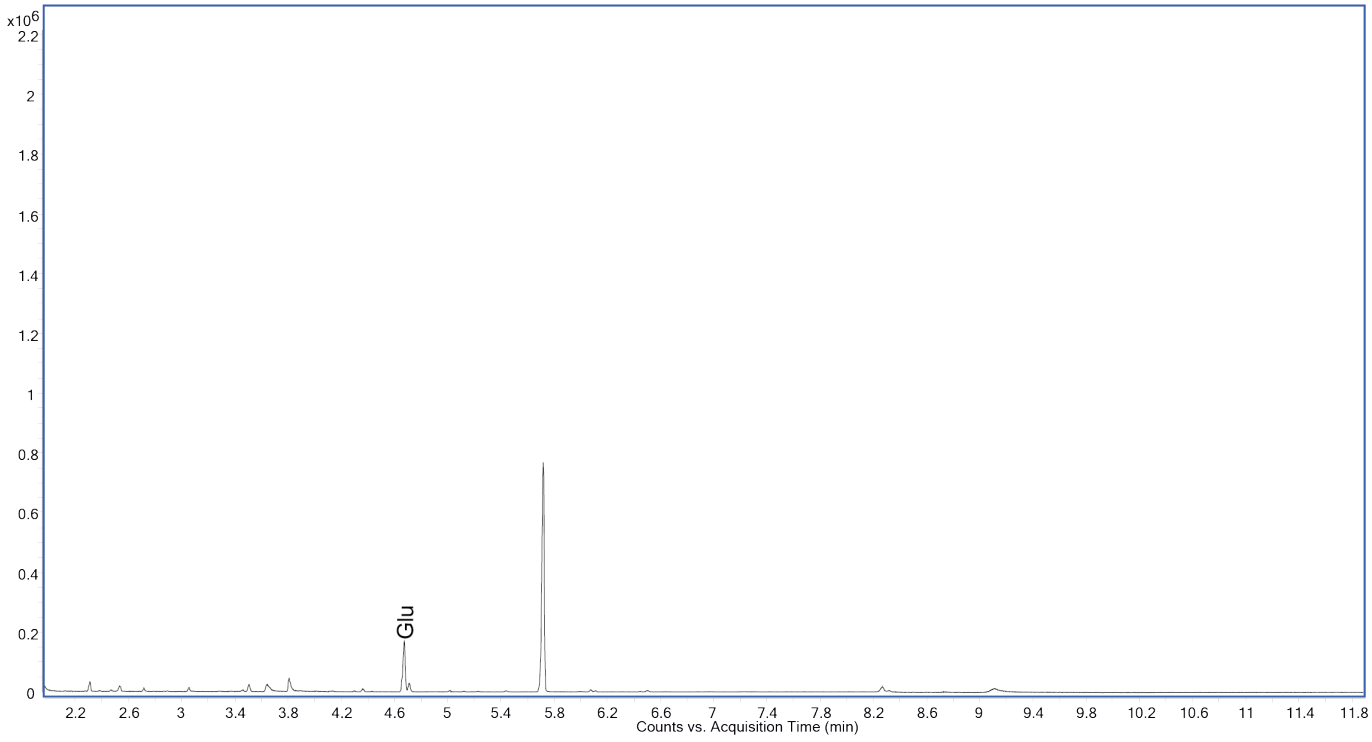
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Ser
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)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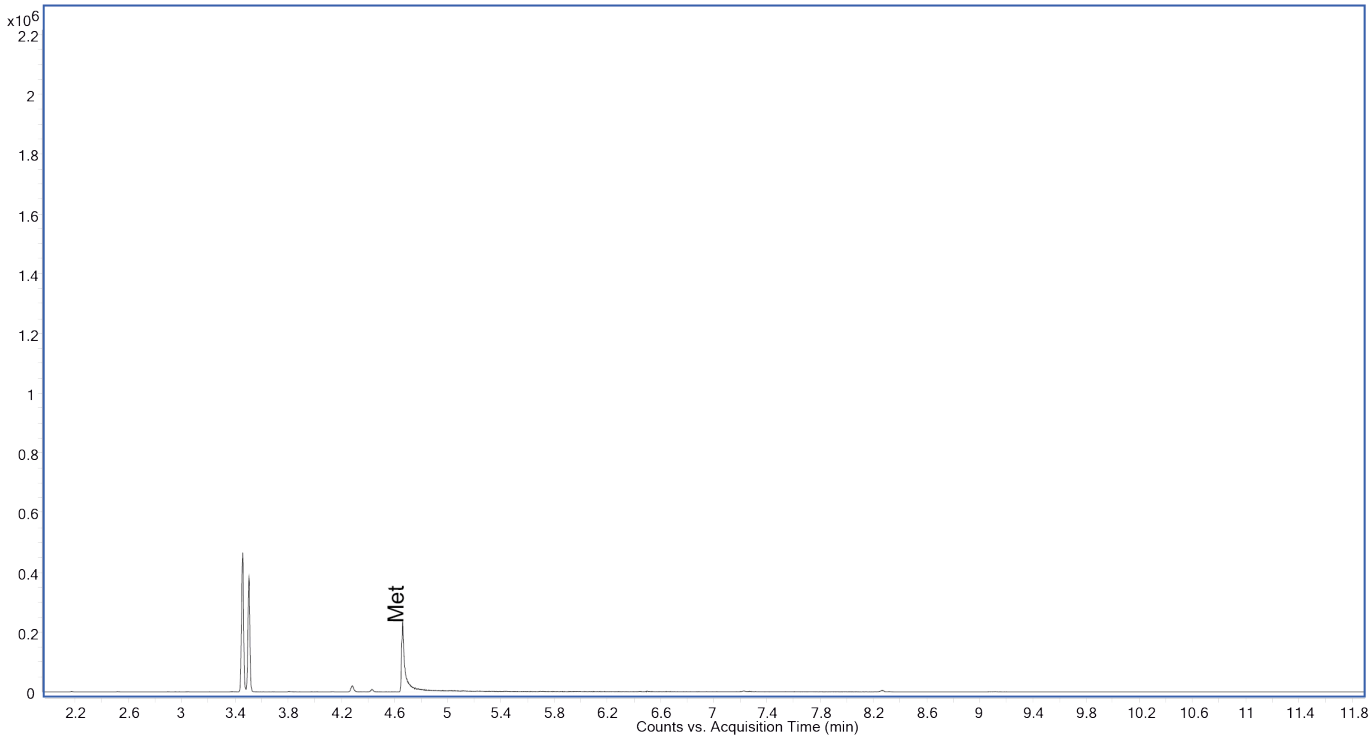
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Glu
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)


x10^6
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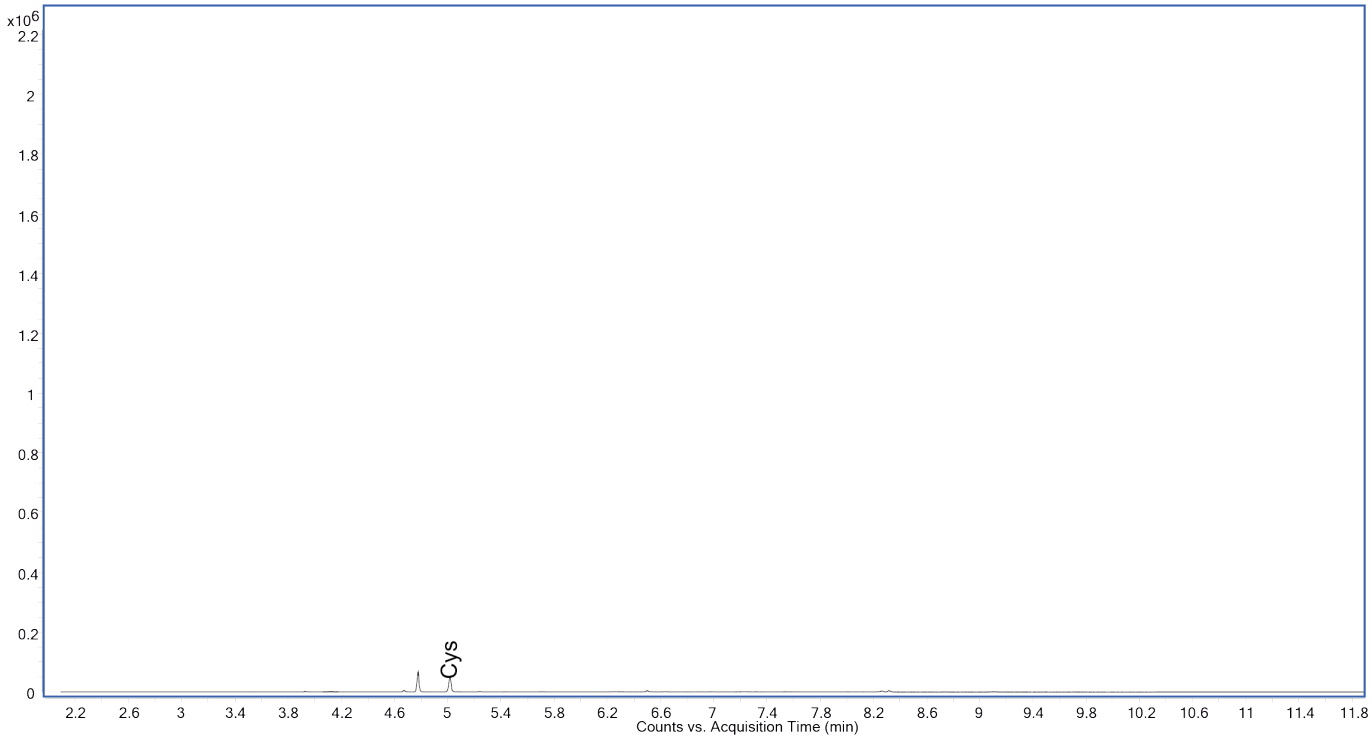
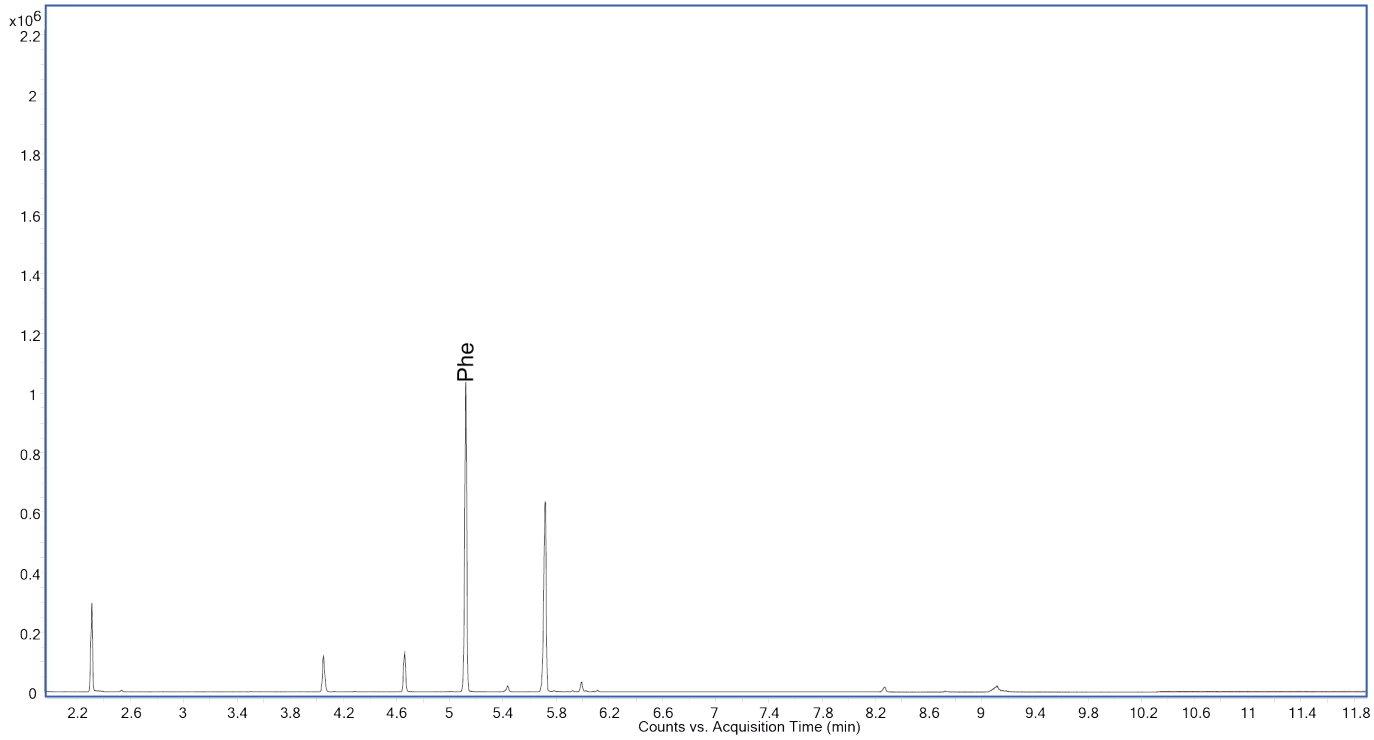
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Met
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)


x10^6
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Cys
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)


x10^6
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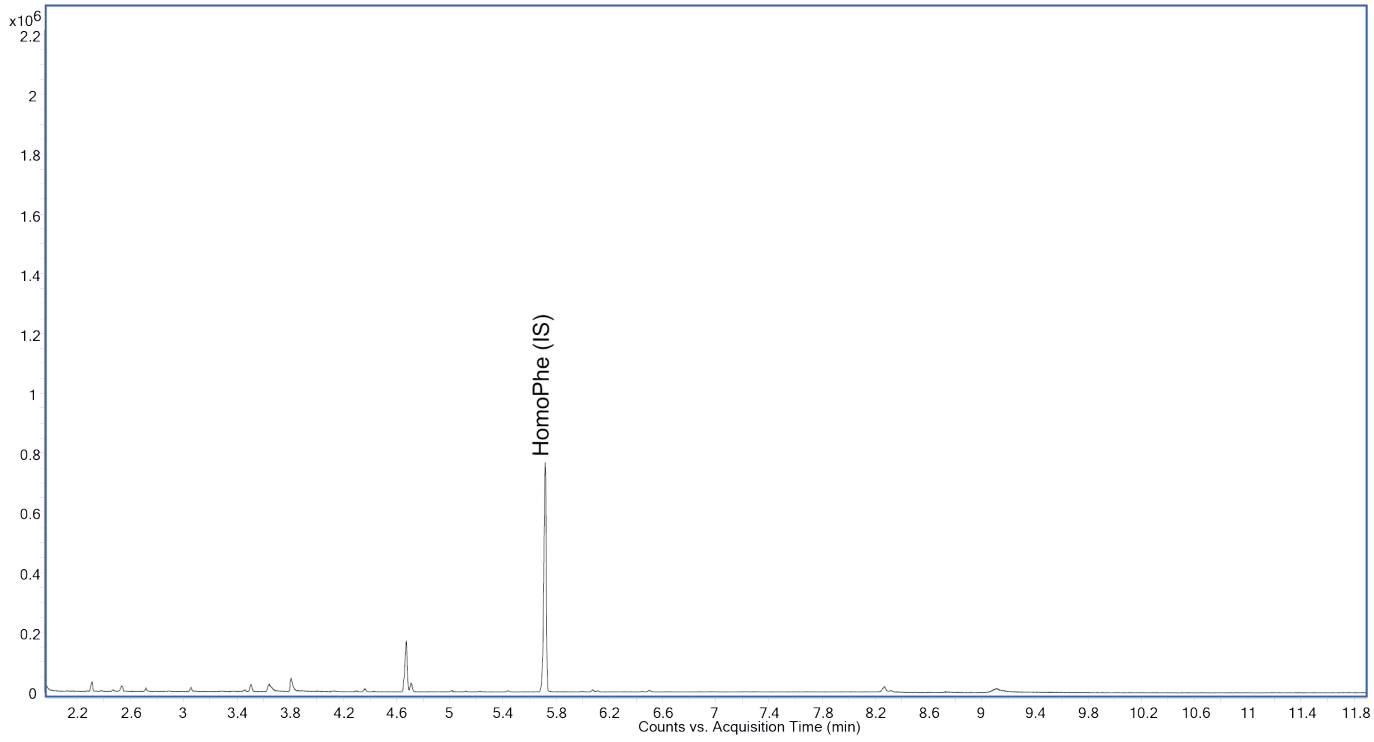
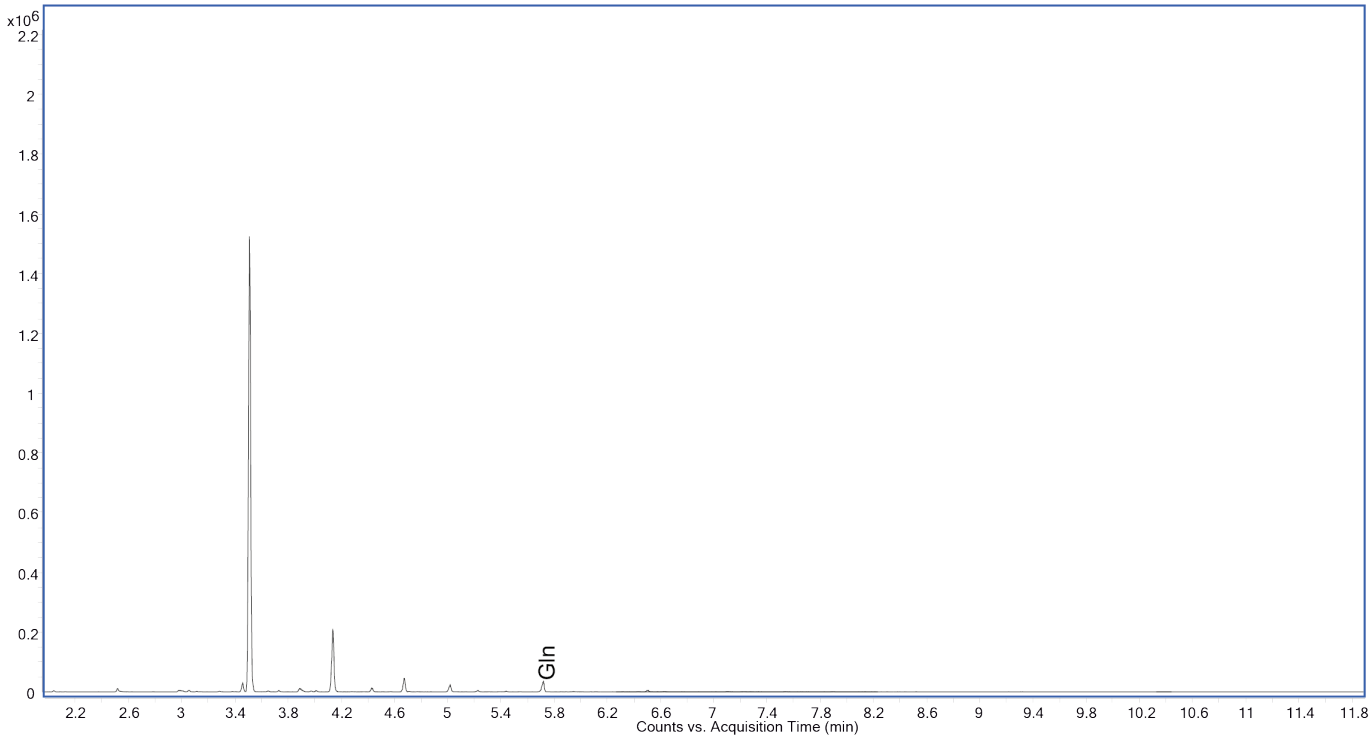
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Phe
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)


x10^6
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
HomoPhe (IS)
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)


x10^6
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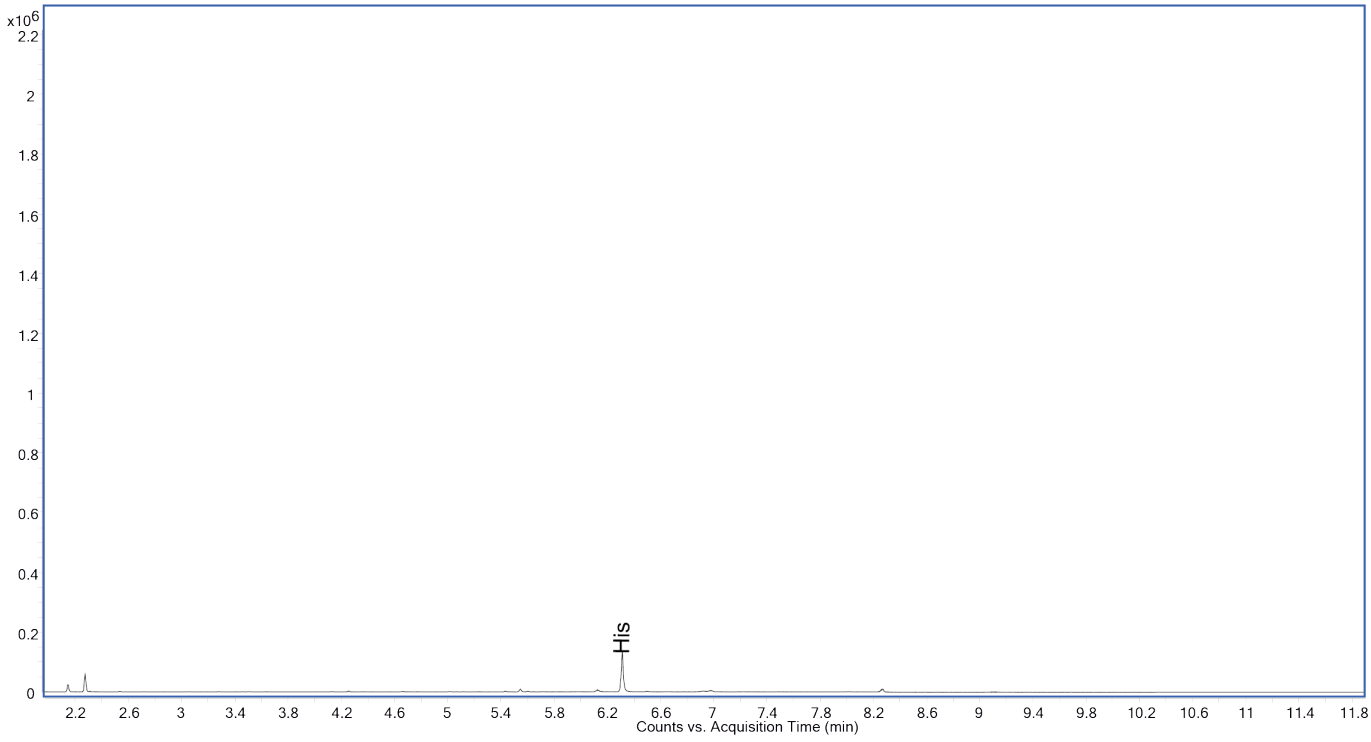
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Gln
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)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2
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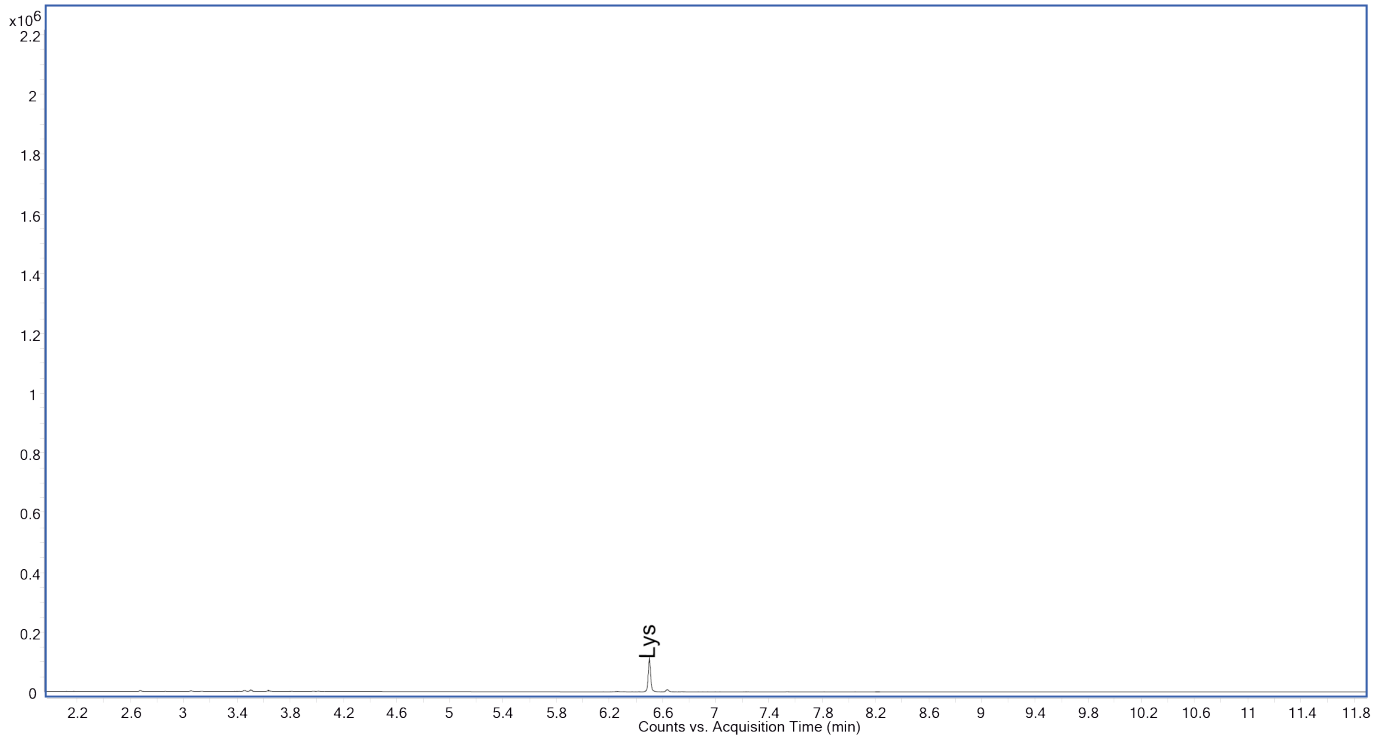
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
His
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)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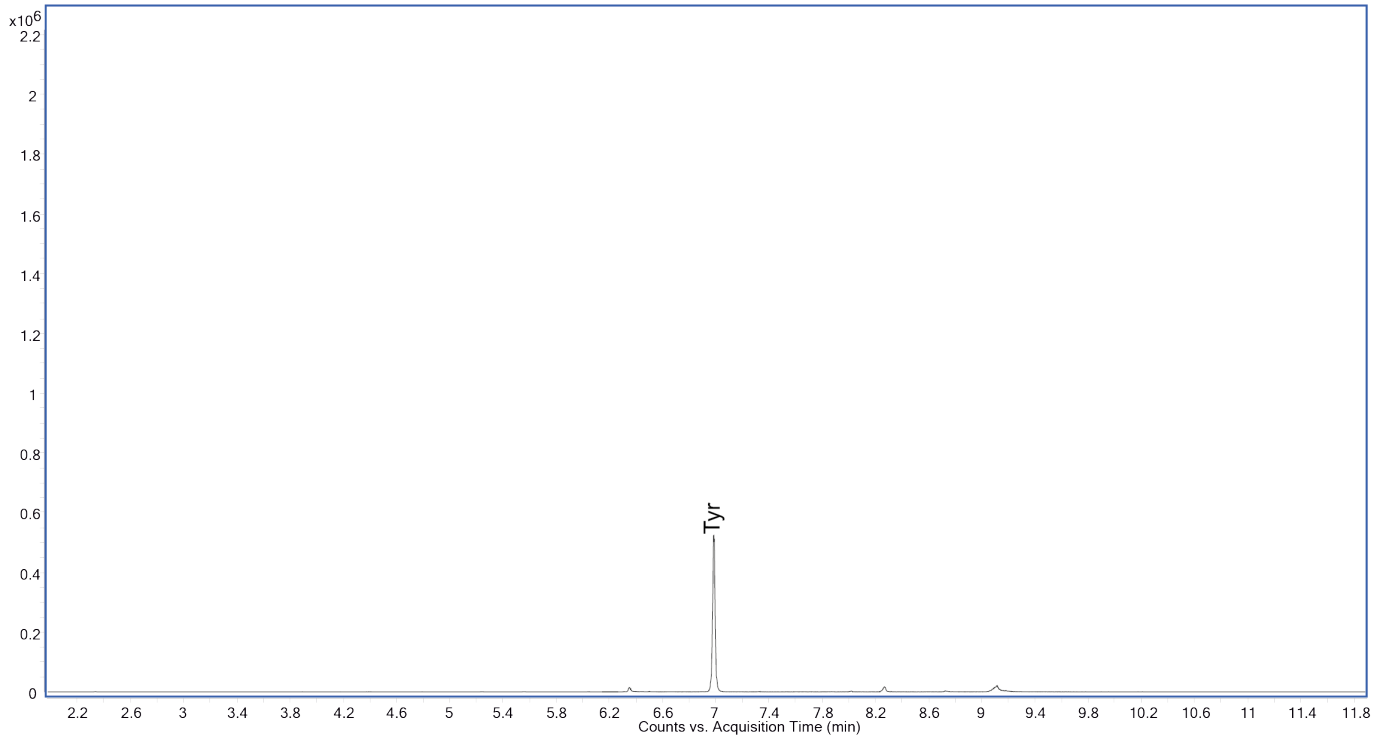
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Lys
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)


x10^6
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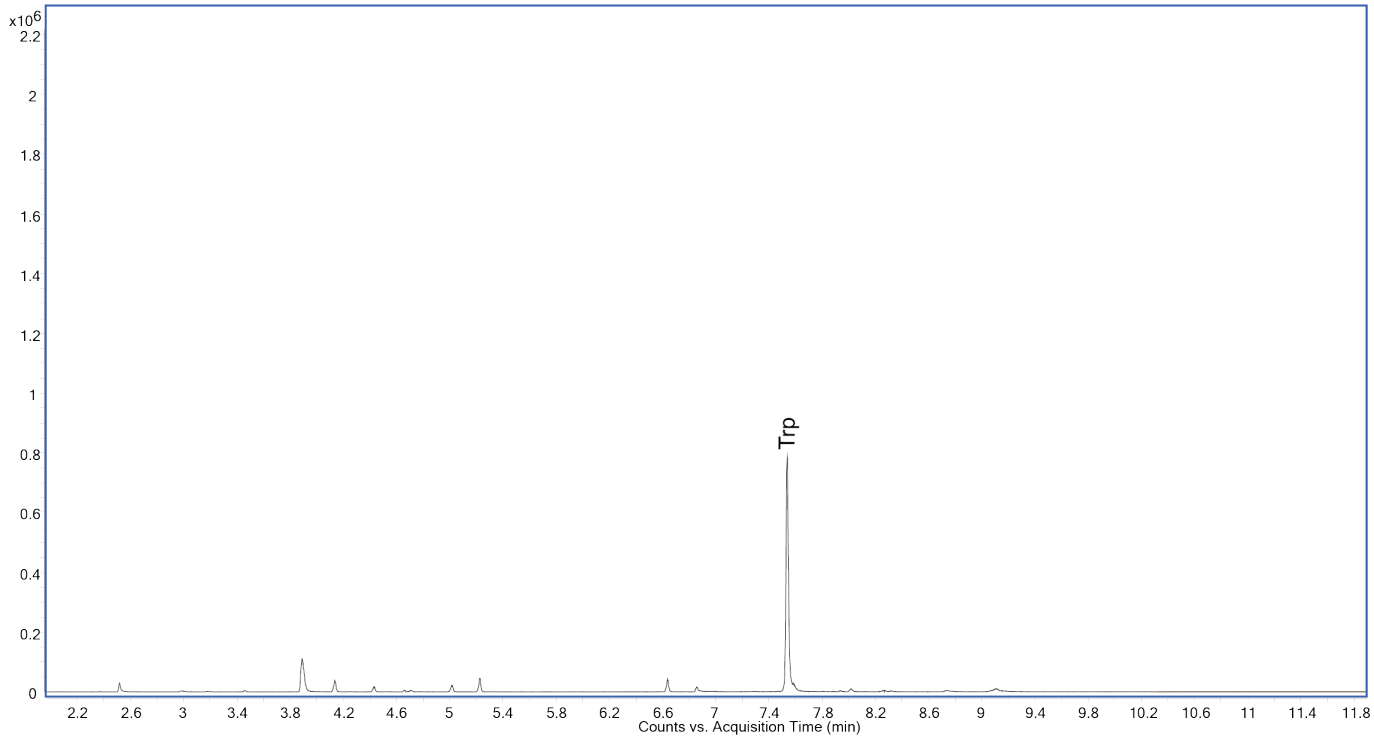
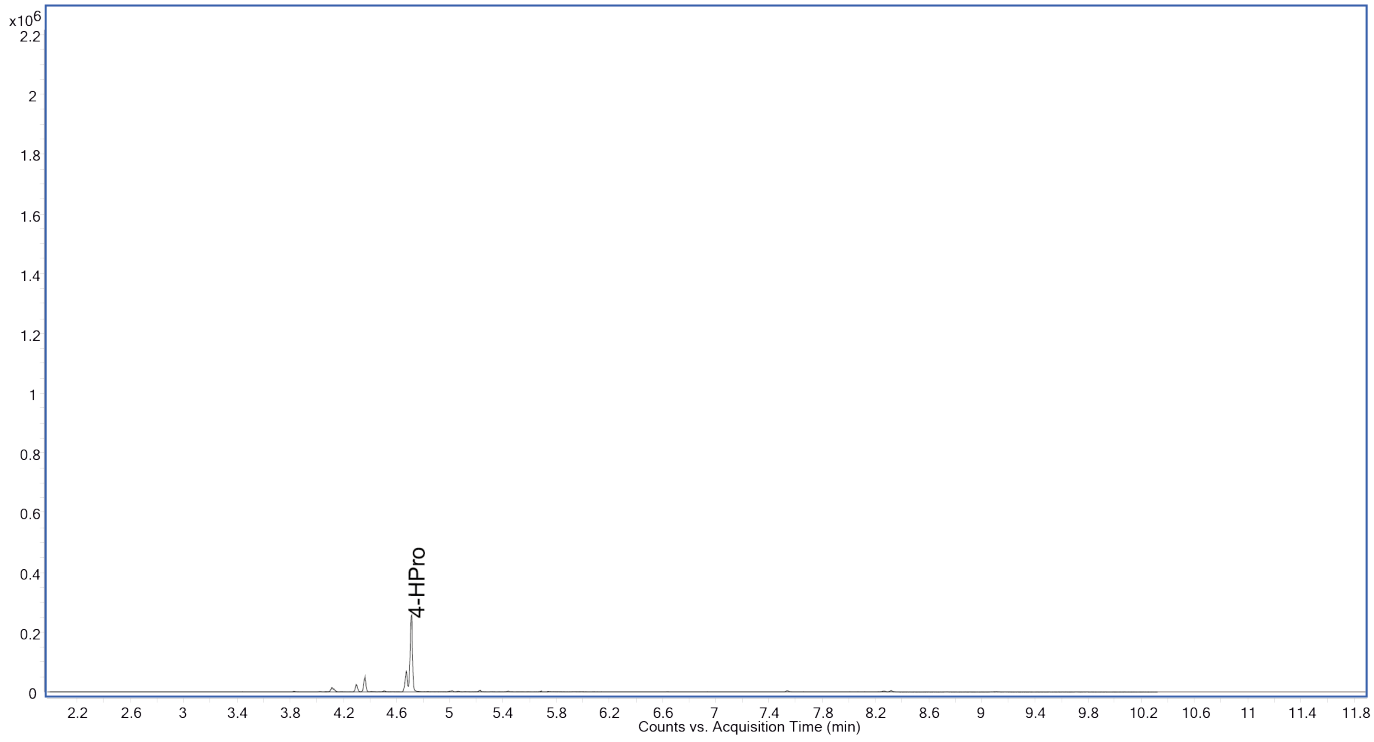
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Tyr
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)


x10^6
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Trp
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)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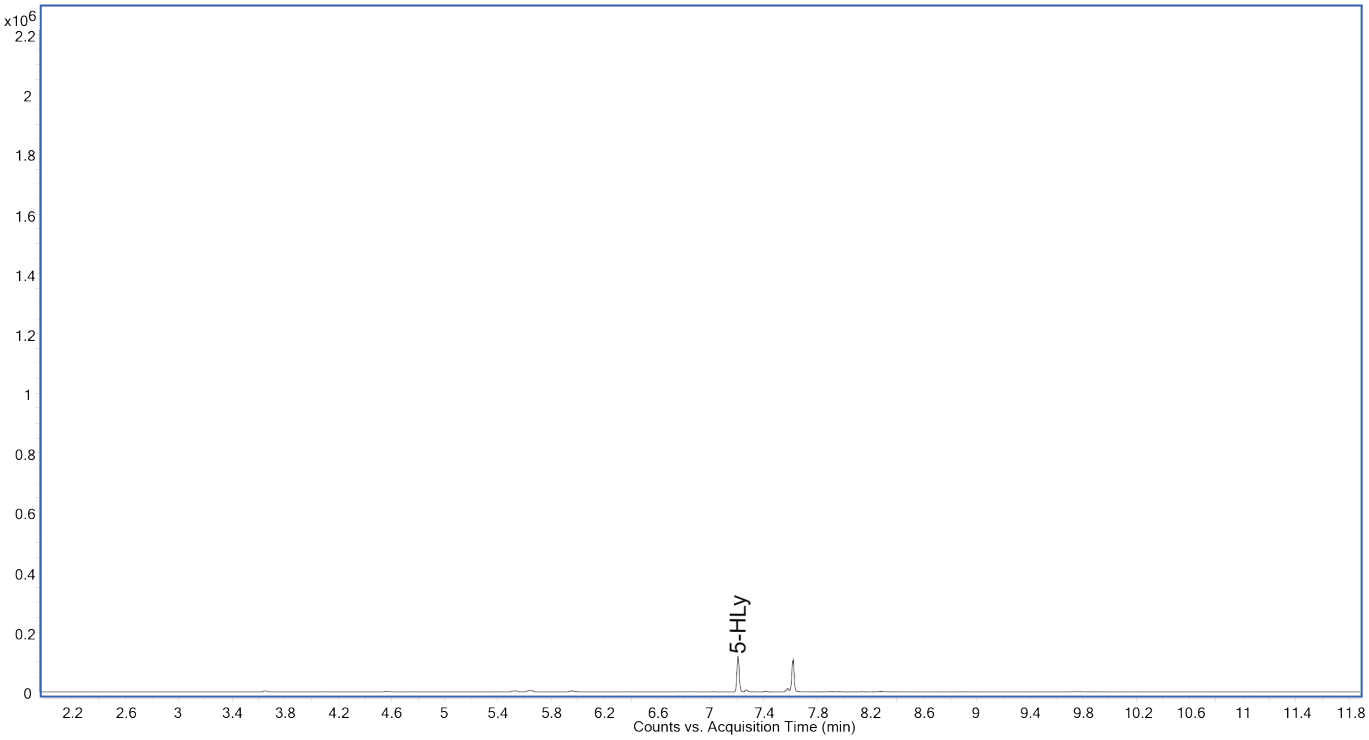
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
4-HPro
2.2 2.6 3 3.4 3.8 4.2 4.6 5 5.4 5.8 6.2 6.6 7 7.4 7.8 8.2 8.6 9 9.4 9.8 10.2 10.6 11 11.4 11.8
Counts vs. Acquisition Time (min)



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 by the procedure described in this manual may be stored for several days in a freezer prior to GC-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 GC/MS **start-up** kit, CF (for 100 samples)	MAK-5857-BA01	1 kit
MetAmino® sample preparation GC/MS **basic** kit, CF (for 400 samples) – NOT AVAILABLE YET	MAK-5857-DA04	1 kit
MetAmino® sample preparation reagents kit (for 100 samples)	MAK-5857-M002	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 a 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 dedicated to a use by properly qualified person only. 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