Journal of Visualized Experiments

Quantitative Metabolomics of Saccharomyces Cerevisiae Using Liquid Chromatography Coupled with Tandem Mass Spectrometry

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE62061R2
Full Title:	Quantitative Metabolomics of Saccharomyces Cerevisiae Using Liquid Chromatography Coupled with Tandem Mass Spectrometry
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Additional Information:	
Question	**Response**
Please specify the section of the submitted manuscript.	Biochemistry
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Montreal, Quebec, Canada
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TITLE:

Quantitative Metabolomics of *Saccharomyces Cerevisiae* Using Liquid Chromatography Coupled with Tandem Mass Spectrometry

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KEYWORDS:

water-soluble metabolites, metabolomics profiling, quenching of metabolic activity, propidium iodide staining, fluorescence microscopy, extraction of metabolites, energy carrier molecules, nucleotides, amino acids, monosaccharides, liquid chromatography, mass spectrometry

SUMMARY:

We present a protocol for the identification and quantitation of major classes of water-soluble metabolites in the yeast *Saccharomyces cerevisiae*. The described method is versatile, robust, and sensitive. It allows the separation of structural isomers and stereoisomeric forms of water-soluble metabolites from each other.

ABSTRACT:

Metabolomics is a methodology used for the identification and quantification of many low-molecular-weight intermediates and products of metabolism within a cell, tissue, organ, biological fluid, or organism. Metabolomics traditionally focuses on water-soluble metabolites. The water-soluble metabolome is the final product of a complex cellular network that integrates various genomic, epigenomic, transcriptomic, proteomic, and environmental factors. Hence, the metabolomic analysis directly assesses the outcome of the action for all these factors in a plethora of biological processes within various organisms. One of these organisms is the budding yeast *Saccharomyces cerevisiae*, a unicellular eukaryote with the fully sequenced genome. Because *S. cerevisiae* is amenable to comprehensive molecular analyses, it is used as a model for dissecting mechanisms underlying many biological processes within the eukaryotic cell. A versatile analytical method for the robust, sensitive, and accurate quantitative assessment of the water-soluble metabolome would provide the essential methodology for dissecting these mechanisms. Here we present a protocol for the optimized conditions of metabolic activity quenching in and water-soluble metabolite extraction from *S. cerevisiae* cells. The protocol also

describes the use of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for the quantitative analysis of the extracted water-soluble metabolites. The LC-MS/MS method of non-targeted metabolomics described here is versatile and robust. It enables the identification and quantification of more than 370 water-soluble metabolites with diverse structural, physical, and chemical properties, including different structural isomers and stereoisomeric forms of these metabolites. These metabolites include various energy carrier molecules, nucleotides, amino acids, monosaccharides, intermediates of glycolysis, and tricarboxylic cycle intermediates. The LC-MS/MS method of non-targeted metabolomics is sensitive and allows the identification and quantitation of some water-soluble metabolites at concentrations as low as 0.05 pmol/µL. The method has been successfully used for assessing water-soluble metabolomes of wild-type and mutant yeast cells cultured under different conditions.

INTRODUCTION:

Water-soluble metabolites are low-molecular-weight intermediates and products of metabolism that contribute to essential cellular processes. These evolutionarily conserved processes include the conversion of nutrients into usable energy, synthesis of macromolecules, cellular growth and signaling, cell cycle control, regulation of gene expression, stress response, post-translational regulation of metabolism, maintenance of mitochondrial functionality, vesicular cellular trafficking, autophagy, cellular aging, and regulated cell death[1-3].

Many of these essential roles of water-soluble metabolites have been discovered by studies in the budding yeast *S. cerevisiae*[1,3,4,7,9,14-22]. This unicellular eukaryote is a useful model organism for dissecting mechanisms through which water-soluble metabolites contribute to cellular processes due to its amenability to advanced biochemical, genetic, and molecular biological analyses[23-26]. Although the LC-MS/MS methods of non-targeted metabolomics have been used for studying the roles of water-soluble metabolites in budding yeast[3,18,22,27], this type of analysis requires the improvement of its versatility, robustness, sensitivity, and ability to distinguish between different structural isomers and stereoisomeric forms of these metabolites.

Recent years are marked by significant advances in applying the LC-MS/MS methods of non-targeted metabolomics to the profiling of water-soluble metabolites in vivo. However, many challenges in using this methodology remain[2,28-36]. These challenges include the following. First, the intracellular concentrations of many water-soluble metabolites are below a threshold of sensitivity for the presently used methods. Second, the efficiency of metabolic activity quenching is too low, and the extent of quenching-associated cell leakage of intracellular metabolites is too high for current methods; hence, the presently used methods under-estimate the intracellular concentrations of water-soluble metabolites. Third, the existing methods cannot differentiate the structural isomers (i.e., molecules with the same chemical formula but different atomic connectivity) or stereoisomers (i.e., molecules with the same chemical formula and atomic connectivity, but with the different atomic arrangement in space) of specific metabolites; this prevents the correct annotation of certain metabolites by the presently used methods. Fourth, the existing mass spectral online databases of parent ions (MS1) and secondary ions (MS2) are incomplete; this affects the correct identification and quantitation of specific metabolites using the raw LC-MS/MS data produced with the help of the current methods. Fifth, the existing

methods cannot use a single type of metabolite extraction to recover all or most classes of water-soluble metabolites. Sixth, the existing methods cannot use a single type of the LC column to separate from each other all or most classes of water-soluble metabolites.

Here, we optimized conditions for quenching of metabolic activity within *S. cerevisiae* cells, maintaining most of the water-soluble metabolites within these cells before extraction, and extracting most classes of water-soluble metabolites from yeast cells. We developed a versatile, robust, and sensitive method for the LC-MS/MS-based identification and quantification of more than 370 water-soluble metabolites extracted from *S. cerevisiae* cells. This method of non-targeted metabolomics enables to assess the intracellular concentrations of various energy carrier molecules, nucleotides, amino acids, monosaccharides, intermediates of glycolysis, and tricarboxylic cycle intermediates. The developed LC-MS/MS method permits the identification and quantification of different structural isomers and stereoisomeric forms of water-soluble metabolites with diverse structural, physical, and chemical properties.

PROTOCOL:

1. Making and sterilizing a medium for growing yeast

1.1. Make 180 mL of a complete yeast extract with bactopeptone (YP) medium. The complete YP medium contains 1% (w/v) yeast extract and 2% (w/v) bactopeptone.

1.2. Distribute 180 mL of the YP medium equally into four 250 mL Erlenmeyer flasks. Each of these flasks contains 45 mL of the YP medium.

1.3. Sterilize the flasks with YP medium by autoclaving at 15 psi/121 °C for 45 min.

2. Wild-type yeast strain

2.1. Use the BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) strain.

3. Growing yeast in the YP medium containing 2% glucose

3.1. Sterilize a 20% (w/v) stock solution of glucose by autoclaving at 15 psi/121 °C for 45 min.

3.2. Add 5 mL of the autoclaved 20% (w/v) stock solution of glucose to each of the two Erlenmeyer flasks with 45 mL of the sterilized YP medium. The final concentration glucose in the YP medium is 2% (w/v).

3.3. Use a microbiological loop to inoculate yeast cells into each of the two Erlenmeyer flasks with the YP medium containing 2% glucose.

3.4. Grow the yeast cells overnight at 30 °C in a rotational shaker set at 200 rpm.

3.5. Take an aliquot of yeast culture. Determine the total number of yeast cells per mL of culture. Count cells using a hemocytometer.

4. Cell transfer to and cell grows in the YP medium with 2% glucose

4.1. Add 5 mL of the sterilized 20% (w/v) stock solution of glucose to each of the remaining two Erlenmeyer flasks with the autoclaved YP medium. The final concentration of glucose is 2% (w/v).

4.2. Transfer a volume of the overnight yeast culture in YP medium with 2% glucose that contains the total number of 5.0×10^7 cells into each of the two Erlenmeyer flasks with the YP medium containing 2% glucose. Use a sterile pipette for the cell transfer.

4.3. Grow the yeast cells for at least 24 h (or more, if the experiment requires) at 30 °C in a rotational shaker set at 200 rpm.

5. Making reagents, preparing labware, and setting up equipment for cell quenching

5.1. Prepare the following: 1) a quenching solution (60% high-grade (>99.9%) methanol in 155 mM ammonium bicarbonate (ABC) buffer, pH = 8.0); 2) an ice-cold ABC solution (pH = 8.0); 3) a digital thermometer capable of measuring up to -20 °C; 4) 500 mL large centrifuge bottles; 5) a pre-cooled high-speed centrifuge with a pre-cooled rotor and pre-cooled 500 mL centrifuge bottles for this rotor, all at -5 °C; 6) metabolite extraction tubes (15 mL high-speed glass centrifuge tubes with polytetrafluoroethylene lined caps); and 7) dry ice.

6. Cell quenching

6.1. Use a hemocytometer to determine the number of yeast cells per mL of YP with 2% glucose culture.

6.2. Transfer a volume of the culture in YP medium with 2% glucose that contains the total number of 5.0×10^8 cells into pre-cooled 500 mL centrifuge bottles.

6.3. Quickly fill the centrifuge bottle containing the cells up to the volume of 200 mL with a quenching solution stored at -20 °C.

6.4. Centrifuge the bottles in a high-speed centrifuge at 11,325 x g for 3 min at -5 °C.

6.4. Quickly and tenderly recover the bottle from the centrifuge; gently unscrew the lid and remove the supernatant without disturbing the pellet.

6.5. Quickly resuspend the cell pellet in 10 mL of ice-cold ABC buffer and transfer the suspension into a 15 mL high-speed glass centrifuge tube with a polytetrafluoroethylene-lined cap for metabolite extraction.

6.6. Collect cells by centrifugation in a clinical centrifuge at 3,000 x g for 3 min at 0 °C.

6.7. Quickly remove the supernatant and place the tube on dry ice to begin metabolite extraction or store the tube at -80 °C until extraction.

7. Preparation of reagents, labware and equipment for metabolite extraction

7.1. Prepare the following: 1) LC-MS grade chloroform; 2) LC-MS grade methanol; 3) LC grade nano-pure water; 4) LC-MS grade (ACN); 5) glass beads (acid-washed, 425–600 µm); 6) a vortex with a foam tube holder kit with retainer; 7) 15 mL high-speed glass centrifuge tubes with polytetrafluoroethylene-lined caps; 8) MS vials; 9) dry ice; and 10) 1.5 mL tubes washed once with ethanol, once with ACN and once with nano-pure water.

NOTE: Use only micropipette tips and tubes made of polypropylene that is resistant to organic solvents.

8. Metabolite extraction

8.1. To the metabolites kept on dry ice or stored at -80 °C tube from step 6.7, add the following: 1) 2 mL of chloroform stored at -20 °C; 2) 1 mL of methanol stored at -20 °C; 3) 1 mL of ice-cold nano-pure water; and 4) 200 µL of 425–600 µm acid-washed glass beads.

8.2. Cover and close the mouth of a tube with aluminum foil. Place tubes in a foam tube holder kit with retainer and vortex them for 30 min at medium speed (i.e., at a speed that is set at 6, with 12 being the maximum speed of a vortex) at 4 °C to facilitate metabolite extraction.

8.3. Incubate the tube for 15 min on ice (NOT dry ice!) to promote protein precipitation and the separation of the upper aqueous from the lower organic phase.

8.4. Centrifuge the tube in a clinical centrifuge at 3,000 x g for 10 min at 4 °C. This centrifugation step allows separating the upper aqueous phase (which contains water-soluble metabolites) from the middle layer (which contains cell debris and proteins) and from the lower organic phase (which contains mostly lipids).

8.5. Use a micropipette to transfer the upper aqueous phase (400 µL) to a washed and labeled 1.5 mL tube containing 800 µL of ACN that was stored at -20 °C.

NOTE: There will be white cloud precipitation after adding the upper aqueous phase to ACN kept at -20 °C.

8.6. Centrifuge the tube with the sample in a tabletop centrifuge at 13,400 x g for 10 min at 4 °C.

NOTE: The white cloud precipitation will disappear after centrifugation.

8.7. Transfer 800 µL from the upper portion of a liquid in the tube to a labeled MS vial. Store the sample at 0 °C until it is analyzed by LC-MS/MS.

9. Preparation of reagents, labware, and equipment for LC

9.1. Prepare the following: 1) a vortex; 2) an ultrasonic sonicator; 3) MS glass vials; 4) an LC system equipped with a binary pump, degasser, and autosampler; 5) a zwitterionic-phase chromatography column (5 µm polymer, 150 x 2.1 mm) named in **Table of Materials**; 6) a column heater; and 7) mobile phases, including phase A (5:95 ACN:water (v/v) with 20 mM ammonium acetate, pH = 8.0) and phase B (100% ACN).

10. Separation of extracted metabolites by LC

10.1. Subject the content of the MS vial to ultrasonic sonication for 15 min.

10.2. Vortex the MS vial 3x for 10 sec at room temperature (RT).

10.3. Place the MS vial into the well plate.

10.4. During chromatograph, maintain the column at 45 °C and a flow rate of 0.250 mL/min. Keep the sample in the well plate at 0 °C. Refer to **Table 1** for the LC gradients that need to be used during chromatography.

NOTE: A representative total ion chromatogram of water-soluble metabolites that were extracted from cells of the wild-type strain BY4742 is shown in **Figure 1**. The metabolites separated by LC were identified and quantified by mass spectrometric analysis that was performed in positive ionization [ESI (+)] mode, as described for step 11.

11. Mass spectrometric analysis of metabolites separated by LC

11.1. Use a mass spectrometer equipped with heated electrospray ionization (HESI) for the identification and quantitation of water-soluble metabolites that were separated by LC. Use the mass spectrometer's analyzer for MS1 ions and the mass spectrometer's detector for MS2 ions. Use the settings provided in **Table 2** and **Table 3** for the data-dependent acquisition of MS1 and MS2 ions, respectively.

11.2. Use a sample volume of 10 µL for the injection in both the ESI (+) and ESI (-) modes.

12. Identification and quantitation of different metabolites by the processing of raw data from LC-MS/MS

12.1. Use the software named in **Table of Materials** to conduct the identification and quantitation of different water-soluble metabolites from raw LC-MS/MS files. This software uses MS1 for metabolite quantitation and MS2 for metabolite identification. The software exploits the

most extensively curated mass spectral fragmentation library to annotate the metabolites using the LC-MS/MS raw data by matching MS spectra. This software also uses the exact mass of MS1 and isotope pattern match to annotate metabolites using online databases. See **Figure** 2 for details.

12.2. Use the library of databases and spectra, which is freely available online (https://www.mzcloud.org), to search for MS2 spectra of the raw data.

13. Membrane integrity assay by propidium Iodide (PI) staining and fluorescence microscopy

13.1. After cell quenching performed as described for step 6, wash the quenched cells thoroughly with 15 mL of ABC buffer to remove the quenching solution. Collect cells by centrifugation at 3,000 x g for 5 min at 0 °C.

13.2. Resuspend the cell pellet in 1 mL of ABC buffer and add 0.5 mL of the PI solution (0.5 mg/mL).

13.3. Vortex a tube with the sample 3x for 10 s and incubate it for 10 min in the dark and on ice.

13.4. Centrifuge the tube with the sample in a tabletop centrifuge at 13,400 x g for 10 min at 4 °C.

13.5. Remove the supernatant and resuspend the pellet in 1 mL of ABC buffer.

13.6. Centrifuge the tube at 13,400 x g for 10 min at 4 °C and remove the supernatant. Repeat this step 2 more times to remove the PI bound to the cell surface.

13.7. Resuspend the pellet in 300 µL of ABC buffer. Place 10 µL of the suspension on the surface of a microscope slide.

13.8. Capture the differential interference contrast (DIC) and fluorescence microscopy images with a fluorescence microscope. Use a filter set up at the excitation and emission wavelengths of 593 nm and 636 nm (respectively).

13.9. Use a software to count the total cell number (in the DIC mode) and the number of fluorescently stained cells. Also, use this software to determine the intensity of staining for individual cells.

REPRESENTATIVE RESULTS:

To improve a quantitative assessment of water-soluble metabolites within a yeast cell, we optimized the conditions of cell quenching for metabolite detection. Cell quenching for this purpose involves a rapid arrest of all enzymatic reactions within a cell[31,33,37,38]. Such an arrest of cellular metabolic activity is an essential step of any method for the quantitation of water-soluble metabolites *in vivo* because it prevents the under-estimation of their intracellular

concentrations[31,33,37,38]. Cell quenching for metabolite assessment always impairs the integrity of the plasma membrane (PM) (and of the cell wall (CW), if present); this causes metabolite leakage from the cell[31,33,37,38]. The method employs cell quenching conditions that minimize such impairment, thereby significantly decreasing the quenching-associated cell leakage of intracellular metabolites. Indeed, most current methods for cell quenching involve the use of a certain concentration of methanol (i.e., 40% (v/v), 60% (v/v), 80% (v/v), or 100% (v/v)) at specific temperature (i.e., -20 °C, -40 °C, or -60 °C), with or without a buffer[31,33,37,38]. We compared the efficiency of the PM and CW impairment for one of the currently used cell quenching method (i.e., by cell treatment with 80% (v/v) methanol at -40 °C in the absence of a buffer[38]) to that for the modified cell quenching method (i.e., by cell treatment with 60% (v/v) methanol at -20 °C in the presence of an isotonic buffered solution of ABC at pH = 8.0). PI is a fluorescent dye that is impermeable to intact cells; it can enter the cell only if the integrity of the PM (and of the CW, if present) is impaired[39]. Moreover, the intensity of fluorescence emission by PI rises by 30-fold when it is bound to DNA or RNA[39]. Thus, a PI staining assay can be used for assessing the efficiency of quenching-associated cell leakage of intracellular metabolites because these metabolites can leak into the extracellular space only if the PM and CW of a yeast cell are damaged[39]. We found that the modified cell quenching method causes significantly lower damage to the PM and CW than the quenching method by cell treatment with non-buffered 80% (v/v) methanol at -40 °C (**Figure 3**). Indeed, almost all cells subjected to quenching using the method exhibited red fluorescence emission, which is characteristic of the yeast cells whose PM and CW are not damaged (**Figure 3**). In contrast, almost all cells subjected to quenching using the other method, displayed green fluorescence emission characteristic of the yeast cells whose PM and CW are significantly damaged (**Figure 3**). The most intense red fluorescence was converted to green fluorescence by a software to differentiate between the strong red fluorescence and mild red fluorescence emissions.

The modified cell quenching method caused significantly lower leakage of water-soluble metabolites from yeast cells than the - quenching method by cell treatment with non-buffered 80% (v/v) methanol at -40 °C. We subjected equal numbers of yeast cells to quenching using either the method (i.e., by cell treatment with 60% (v/v) methanol at -20 °C in the presence of an isotonic buffered solution of ABC at pH = 8.0; **Figure 4**) or the other method (i.e., by cell treatment with non-buffered 80% (v/v) methanol at -40 °C; **Figure 5**). The extent of quenching-associated cell leakage into the extracellular solution was assessed for specific water-soluble metabolites with the help of LC-MS/MS. The concentrations of different amino acid classes (i.e., large and small acidic, basic, neutral-nonpolar, and neutral polar amino acids) in the extracellular solution were measured before and after cell quenching. We found that the cell quenching method causes significantly lower leakage of all these amino acid classes into the extracellular solution (**Figure 4**) than the other method (**Figure 5**).

The LC-MS/MS method for a quantitative assessment of water-soluble metabolites within a yeast cell uses a single type of the column for chromatographic separation of all water-soluble metabolite classes. This column is the zwitterionic-phase column named in **Table of Materials**. We found that this column provides a much more efficient separation of different classes of water-soluble metabolites than the reverse-phase column named in **Table of Materials**

(**Supplemental Table 1**). Indeed, the retention time (RT) shift values of water-soluble metabolite standards (i.e., NAD+, AMP, GMP, arginine, and glutamic acid) were significantly lower and the peak shapes were substantially sharper for the zwitterionic-phase column, as compared with the reverse-phase column (**Supplemental Table 1**). LC conditions used for chromatographic separation of all metabolites (i.e., water-soluble and water-insoluble) for the reverse-phase column are provided in **Supplemental Table 2**.

Another advantage of the LC-MS/MS method consists in the ability of chromatographic separation on the above zwitterionic-phase column to efficiently separate from each other different water-soluble metabolites with diverse structural, physical, and chemical properties. These water-soluble metabolites include the following metabolite classes: 1) acidic, basic, neutral polar, and non-neutral polar amino acids, including their different structural isomers (**Figure 6**); 2) stable and unstable nucleotides and their derivates that perform vital functions within a cell (**Figure 7**); and 3) various monosaccharides, including their different stereoisomeric forms (**Figure 8**).

Importantly, the LC-MS/MS method for a quantitative assessment of water-soluble metabolites within a yeast cell was versatile and robust. It allowed us to identify and quantify 374 water-soluble metabolites with diverse structural, physical, and chemical properties in *S. cerevisiae* cells that were cultured in the complete YP medium initially containing 2% glucose (**Supplemental Table 3**). 240 metabolites were detected in the positive ionization mode and 134 metabolites were detected in the negative ionization mode. The identities of all these water-soluble metabolites were confirmed by matching their data-dependent acquisition (DDA) MS2 fragments acquired both in the positive and negative ionization modes to the MS2 mzCloud spectral library. This online library includes the spectra of metabolite standards that differ in their MS1 and DDA MS2 parameters. To maximize the extent of matching the MS2 spectra of the sample with the online spectral library, we used different DDA MS2 parameters. These parameters are provided in **Supplemental Table 4**. Almost 6,000 features (putative metabolites) for the same sample were acquired with the help of the high-energy-induced-collision-dissociation (HCD) or collision-induced dissociation (CID) fragmentation method, using top 5 MS2 events, 35 collision energy values, and 10 ms activation times. After filtering the resulting files with > 95% MS2 matching and > 90% MS1 isotopic pattern matching criteria, only 162 metabolites under HCD fragmented condition and 142 metabolites under CID fragmented condition were identified. 81 out of 162 metabolites were unique to the HCD fragmentation method, whereas 42 out of 142 were unique to the CID fragmentation method (see a sheet named "T5_35E__10 ms_HCD vs CID" in **Supplemental Table 4**). Therefore, we concluded that the correct annotation of water-soluble metabolites with the help of the LC-MS/MS method requires the use of many different DDA MS2 parameters.

The LC-MS/MS method is also highly sensitive. It allows to identify and quantitate some water-soluble metabolites at concentrations as low as 0.05 pmol/µL (see data for phenylalanine in **Table 4**). This limit of quantitation varies within a wide range of concentrations for different metabolite classes (**Table 4**).

The MS system used here has a wide (at least two orders of magnitude) linear dynamic range for measuring the concentrations of various metabolites (**Supplemental Table 5**).

FIGURE AND TABLE LEGENDS:

Figure 1: The total ion chromatogram (TIC) from liquid chromatography/mass spectrometry (LC-MS) data of water-soluble metabolites that were extracted from cells of the wild-type strain BY4742. The metabolites were separated by LC on the zwitterionic-phase chromatography column named in **Table of Materials**. The metabolites were detected by MS of parent ions (MS1) that were created using the positive electrospray ionization mode.

Figure 2: A workflow used for the analysis of water-soluble metabolites with the help of the software named in Table of Materials. All the parameters were autocorrected by the software based on the MS raw data, except the followings: 1) "Detect Compounds" tab: set min, peak intensity 10,000; and 2) "Search ChemSpider" tab: 4 online databases were selected for the identification of metabolites, including the BioCyc, Human Metabolome Database, KEGG, and Yeast Metabolome Database.

Figure 3: Differential interference contrast (DIC; top row) and fluorescence (bottom row) microscopic images of yeast cells quenched either with ammonium bicarbonate (ABC) buffer (pH = 8.0; control) or with a different quenching solution. After cell quenching, equal numbers of cells were incubated with the PI solution for 10 min in the dark and on ice. The most intense red fluorescence was converted to green fluorescence by software to differentiate between the strong red fluorescence and mild red fluorescence emissions. The efficiency of damage to the PM and CW was compared for the method of cell quenching (i.e., by cell treatment with 60% (v/v) methanol at -20 °C in the presence of an isotonic buffered solution of ABC at pH = 8.0) and one of the currently used methods (i.e., by cell treatment with 80% (v/v) methanol at -40 °C in the absence of a buffer). A scale bar is shown.

Figure 4: The leakage percentage of different amino acid classes for the method of cell quenching. 5.0 x 10^8 of yeast cells were subjected to quenching by the treatment with 60% (v/v) methanol at -20 °C in the presence of an isotonic buffered solution of ABC at pH = 8.0. The leakage percentage of different amino acid classes was assessed using LC-MS/MS to measure their concentrations in the extracellular solution before and after quenching. The mean values ± SD and individual data points are shown (n = 3). * $p < 0.05$.

Figure 5: The leakage percentage of different amino acid classes for one of the currently used cell quenching methods. 5.0 x 10^8 of yeast cells were subjected to quenching by the treatment with 80% (v/v) methanol at -40 °C in the absence of a buffer. The leakage percentage of different amino acid classes was assessed using LC-MS/MS to measure their concentrations in the extracellular solution before and after quenching. The mean values ± SD and individual data points are shown (n = 3). * $p < 0.05$.

Figure 6: Efficient chromatographic separation of acidic, basic, neutral polar and non-neutral polar amino acid classes, including two structural isomers (i.e., leucine and isoleucine), on the

zwitterionic-phase column named in Table of Materials. All these amino acids were detected by MS/MS in the positive ionization [ESI (+)] mode. Conditions for LC on the zwitterionic-phase chromatography column are described in **Table 1**. Conditions for MS/MS are described in **Table 2** and **Table 3**. All amino acid standards are bought commercially (e.g., Sigma). The retention time shifts between 3 independent chromatography runs are less than ± 10 seconds.

Figure 7: Efficient chromatographic separation of different classes of nucleotides, including energetically unstable nucleotides (nucleoside monophosphates, nucleoside diphosphates, and nucleoside triphosphates) and electron carrier molecules (NADH and NAD+), on the zwitterionic-phase column named in Table of Materials. All these nucleotides were detected by MS/MS in the positive ionization [ESI (+)] mode. Conditions for LC on the zwitterionic-phase chromatography column are described in **Table 1**. Conditions for MS/MS are described in **Table 2** and **Table 3**. All nucleotide standards are bought commercially (e.g., Sigma). The retention time shifts between 3 independent chromatography runs are less than ± 10 seconds.

Figure 8: Efficient chromatographic separation of different classes of monosaccharides, including structural isomers and stereoisomeric forms of aldo- and ketohexoses (fructose, mannose, and galactose), and aldopentoses (ribose and arabinose), on the zwitterionic-phase column named in Table of Materials. All these monosaccharides were detected by MS/MS in the positive ionization [ESI (+)] mode. Conditions for LC on the zwitterionic-phase chromatography column are described in **Table 1**. Conditions for MS/MS are described in **Table 2** and **Table 3**. All monosaccharide standards are bought commercially (e.g., Sigma). The retention time shifts between 3 independent chromatography runs are less than ± 10 seconds.

Table 1: LC condition used for the separation of water-soluble metabolites with the help of the zwitterionic-phase column named in Table of Materials. These conditions were used in all experiments described here.

Table 2: The mass spectrometer settings used to analyze metabolites that were separated by LC. These conditions were used for the analysis of metabolites in all experiments described here. Abbreviations: FTMS = Fourier transform (FTMS); HCD = high-energy-induced-collision-dissociation; LTQ = linear trap quadrupole; AGC = automatic gain control, an ion population value for MS and MS/MS.

Table 3: The mass spectrometer settings used to detect secondary ions (MS2). Abbreviations: HCD = high-energy-induced-collision-dissociation; CID = collision induced dissociation; ms = milliseconds.

Table 4: The lowest concentrations of different water-soluble metabolite standards that the LC-MS/MS method can identify and quantitate. The MS1 peak area of each metabolite standard was used to estimate the lowest quantifiable concentration for this metabolite. Mean values of two independent experiments are shown. Three technical replicates were performed for each of the two independent experiments. NOTE: Threonine* can be detected but cannot be quantified due to its co-elution with homoserine, a chemical isomer of threonine. Glucose** cannot be

identified because it creates multiple chromatography peaks. Metabolite standards*** can be identified and quantitated only in individual samples, but not in a mixture of metabolite standards or a biological mixture of metabolites.

Supplemental Table 1: Retention Time (RT) shift values of the same set of metabolites for the zwitterionic-phase and reverse-phase columns (see Table of Materials) after column equilibration. The table compares the retention reproducibility of the zwitterionic-phase and reverse-phase columns for a distinct set of metabolites the differ from each other in their hydrophilicity and hydrophobicity. These metabolites were identified with the help of LC-MS/MS. Note that the RT shift values of hydrophilic metabolites (i.e., NAD+, AMP, GMP, arginine, and glutamic acid) are significantly lower and the peak shapes are substantially sharper for the zwitterionic-phase column, as compared to the reverse-phase column. In contrast, the RT shift values of hydrophobic metabolites (i.e., stearic acid, lauric acid, and decanoic acid) are significantly lower and the peak shapes are substantially sharper for the reverse-phase column, as compared to the zwitterionic-phase column. Conditions for the chromatographic separation of metabolites with the help of the zwitterionic-phase and reverse-phase columns are detailed in **Table 1** and **Supplemental Table 2**, respectively. The reported here RT shift values are based on the measurement of 20 different samples taken from different vials. The samples were analyzed after column equilibration. The metabolites in each sample were extracted from 5.0×10^8 yeast cells. RT shift values are the means of 20 different samples (n = 20). The p values derived from the unpaired t test were used to compare the two columns with the equal variance between both sample types.

Supplemental Table 2: LC conditions for the reverse-phase 8 column named in Table of Materials and used to separate different metabolites in this study. Column properties were as follows: 150 x 2.1 mm, 5 µm polymer.

Supplemental Table 3: A list of all 374 water-soluble metabolites recovered and annotated using the LC-MS/MS method. All metabolites were recovered from the same LC gradient run, subjected to a data-dependent acquisition (DDA) fragmentation algorithm described in **Supplemental Table 4**, and annotated using the software named in **Table of Materials**. The MS1 peak shapes of 211 metabolites (whose status in the table is indicated as a blank) were appropriate to be used for quantification, and their respective MS2 spectra had full matches with the mzCloud spectral library. MS2 spectra of 38 metabolites (whose status in the table is indicated as a d) had full matches with the online spectral library. Still, their MS1 peak shapes were not appropriate to be used for quantification. MS2 spectra of 125 metabolites (whose status in the table is indicated as an n) did not have full matches with the online spectral library. These metabolites were annotated as follows: 1) using the "Predict composition node" (which annotates metabolites based on the exact match of MS1 values, number of matched and missed isotopes, and isotope % intensity matched with that of the theoretical reference standards); 2) using the "ChemSpider node" (which annotates metabolites based on the exact match of MS1 values and similarity match score of MS2 spectra with the online spectral library); and 3) using the retention time (RT) shift values of metabolites (these values depend on the physical structure and chemical properties of metabolites). The metabolites listed in the table were found in all 3

biological replicates performed, with the RT shift values of < 0.2 min.

Supplemental Table 4: A data-dependent acquisition (DDA) fragmentation algorithm that was used here for the annotation of water-soluble metabolites with the help of the software named in Table of Materials.

Supplemental Table 5: A typical linear dynamic range that we observed when we measured the concentrations of different amino acids with the help of the MS system named in Table of Materials. The MS system used here has a wide (at least two orders of magnitude) linear dynamic range for measuring various metabolites' concentrations.

Table of Materials.

DISCUSSION:

To successfully use the protocol described here, follow the preventive measures described below. Chloroform and methanol extract various substances from laboratory plasticware. Therefore, handle them with caution. Avoid the use of plastics in steps that involve contact with any of these two organic solvents. Use borosilicate glass pipettes for these steps. Rise these pipettes with chloroform and methanol before use. Use only micropipette tips and tubes made of polypropylene that is resistant to organic solvents. During sample preparation for LC-MS/MS, eliminate all air bubbles in the glass vials before inserting them into a wellplate.

The zwitterionic-phase column used here requires extensive re-conditioning after each run to minimize the RT shift. For column re-conditioning, we recommend using a volume of the re-conditioning solution that is about 20 volumes of the column. The column needs to be re-conditioned with the initial mobile phase for 15 min at an increased flow rate of 0.4 mL/min. To avoid any damage to the column during its re-conditioning, keep the column pressure below the upper limit of pressure recommended by the manufacturer.

Of note, some mixed-separation chromatography columns that operate in the reverse-phase mode allow resolution of charged metabolites[40,41]. These mixed-separation columns are based on the reverse-phase column used here (see **Table of Materials**) and contain polar embedded groups that can separate metabolites based on charge[40,41].

Here, we described an LC-MS/MS-based method of non-targeted metabolomics for the quantitative analysis of many water-soluble metabolites extracted from yeast cells. The method provides several advantages over the LC-MS/MS methods of non-targeted metabolomics currently used for this purpose. These advantages include the following. First, the method is sensitive and allows the identification and quantitation of some water-soluble metabolites at concentrations as low as 0.05 pmol/µL. The reported sensitivity of the existing LC-MS/MS methods is lower[3,22,27,42]. Second, the method uses a cell quenching procedure that elicits a significantly lower leakage of intracellular metabolites from the cell than that reported for the presently used procedures[31,33,38]. Thus, the procedure that we developed for cell quenching lowers the extent to which the currently used procedures under-estimate the intracellular concentrations of water-soluble metabolites. Third, unlike the existing LC-MS/MS methods[2,31,33],

the method distinguishes between different structural isomers and stereoisomeric forms of many metabolites. These metabolites include various energy carrier molecules, nucleotides, amino acids, monosaccharides, intermediates of glycolysis, and tricarboxylic cycle intermediates. Fourth, we used the method to create an extensive mass spectral database of MS1 and MS2 for the correct identification and quantitation of a wide range of specific metabolites using the raw LC-MS/MS data. In contrast, the existing mass spectral online databases of MS1 and MS2 are incomplete[43-45]. Fifth, the method uses a single type of metabolite extraction to recover water-soluble metabolites with diverse structural, physical, and chemical properties. These metabolites' diversity significantly exceeds that of alternative methods for a single-step extraction of water-soluble metabolites from yeast cells[3,22,27,46]. Six, unlike the existing LC-MS/MS methods[3,22,47,48], the method uses a single type of the LC column to separate from each other various structural and functional classes of water-soluble metabolites. Seven, the method enables the identification and quantification of more than 370 water-soluble metabolites extracted from yeast cells. This number of identifiable and quantifiable metabolites exceeds the numbers of metabolites reported for other methods of non-targeted metabolomics in yeast[3,22,27,49,50].

The method has several limitations. These limitations are as follows. The zwitterionic-phase column used for metabolite separation by LC requires extensive re-conditioning after each run. Furthermore, the method is efficient only for the non-targeted metabolomics of water-soluble, hydrophilic metabolites. Moreover, different isomeric forms of carbohydrates (including isomers of fructose, glucose, and galactose) cannot be quantified with the help of the method. This is because the zwitterionic-phase column used in the method cannot separate these carbohydrate isomers from each other when present in a mixture with other metabolites. Besides, the method cannot be exploited for identifying glucose because this carbohydrate creates multiple peaks during chromatography on the zwitterionic-phase column used in the method. Finally, threonine cannot be quantified with the help of the method due to the co-elution of this amino acid with its isomer homoserine during metabolite separation by chromatography.

We use this LC-MS/MS method to study aging-associated changes in the water-soluble metabolome of the budding yeast *S. cerevisiae*. We also employ this method to investigate how many aging-delaying genetic, dietary, and pharmacological interventions affect the water-soluble metabolome of yeast cells during their chronological aging. Because of its versatility, robustness, and sensitivity, the LC-MS/MS method can be successfully used for the quantitative assessment of the water-soluble metabolomes in evolutionarily distant eukaryotic organisms.

ACKNOWLEDGMENTS:

We are grateful to current and former members of the Titorenko laboratory for discussions. We acknowledge the Centre for Biological Applications of Mass Spectrometry, the Centre for Structural and Functional Genomics, and the Centre for Microscopy and Cellular Imaging (all at Concordia University) for outstanding services. This study was supported by grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada (RGPIN 2014-04482 and CRDPJ 515900 - 17). K.M. was supported by the Concordia University Armand C. Archambault Fellowship and the Concordia University Dean of Arts and Sciences Award of Excellence.

DISCLOSURES:

The authors declare that they have no competing financial interests.

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Figure 1

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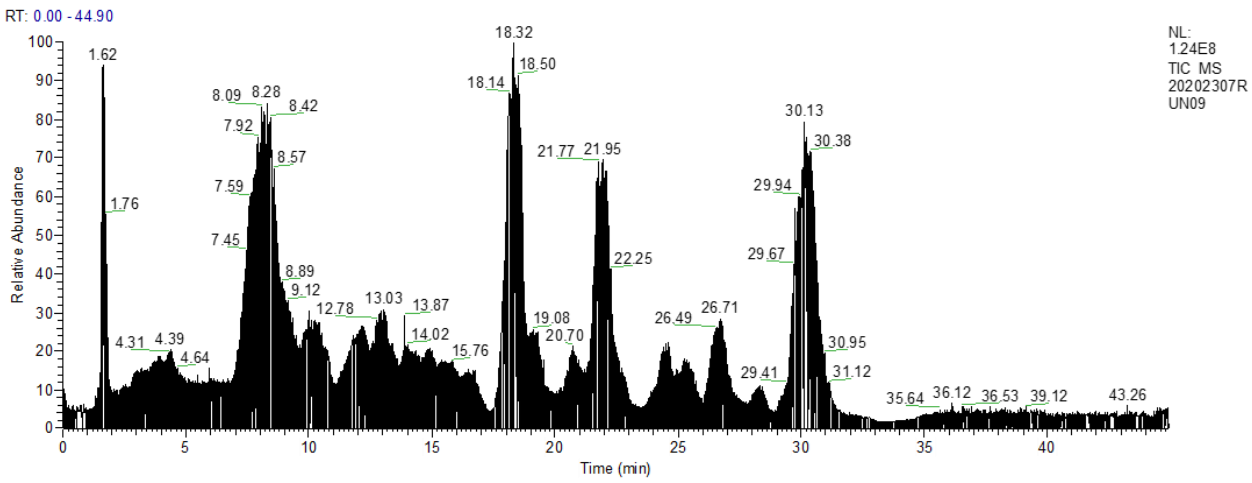

Figure 2

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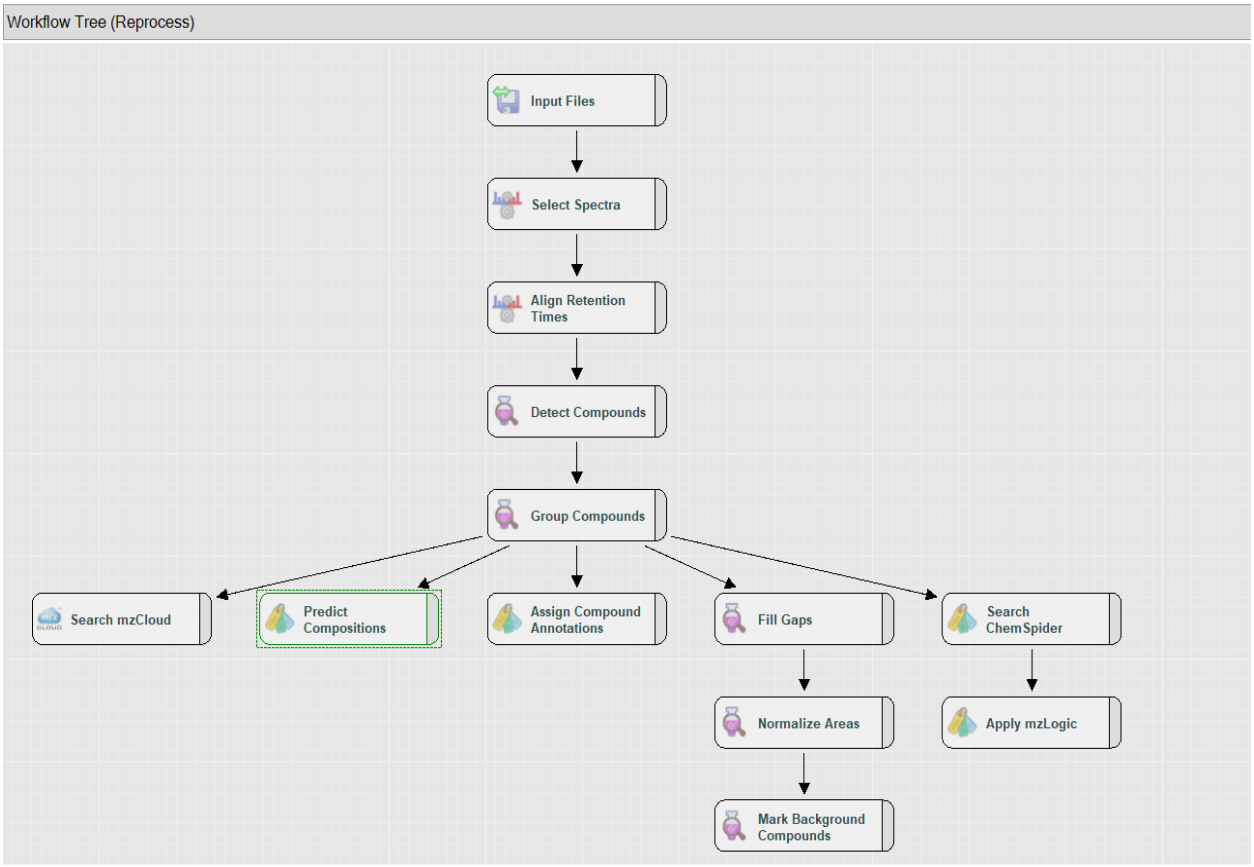

Figure 3

Control	Our method of cell quenching: 60% (v/v) methanol at -20°C with an isotonic buffered solution of ABC at pH = 8.0	Control	Currently used method of cell quenching: 80% (v/v) methanol at -40°C without a buffer

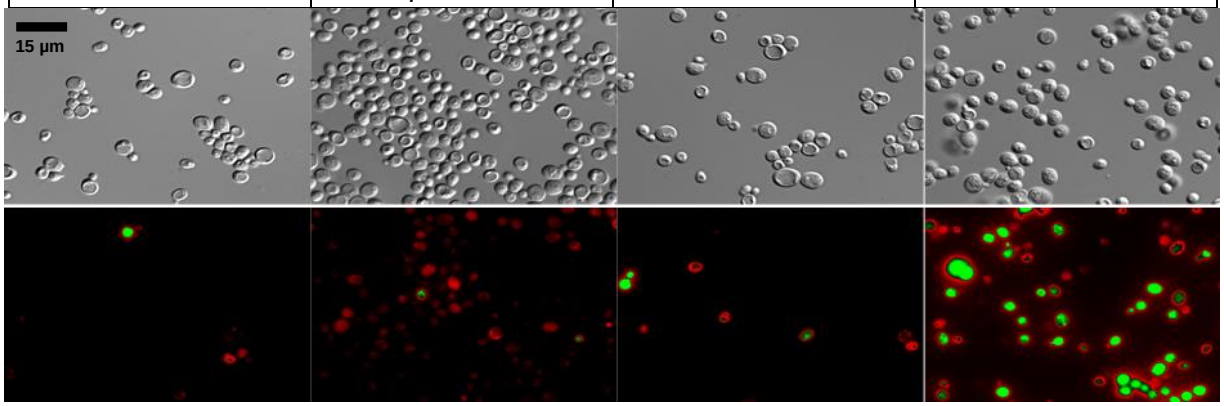

Figure 4

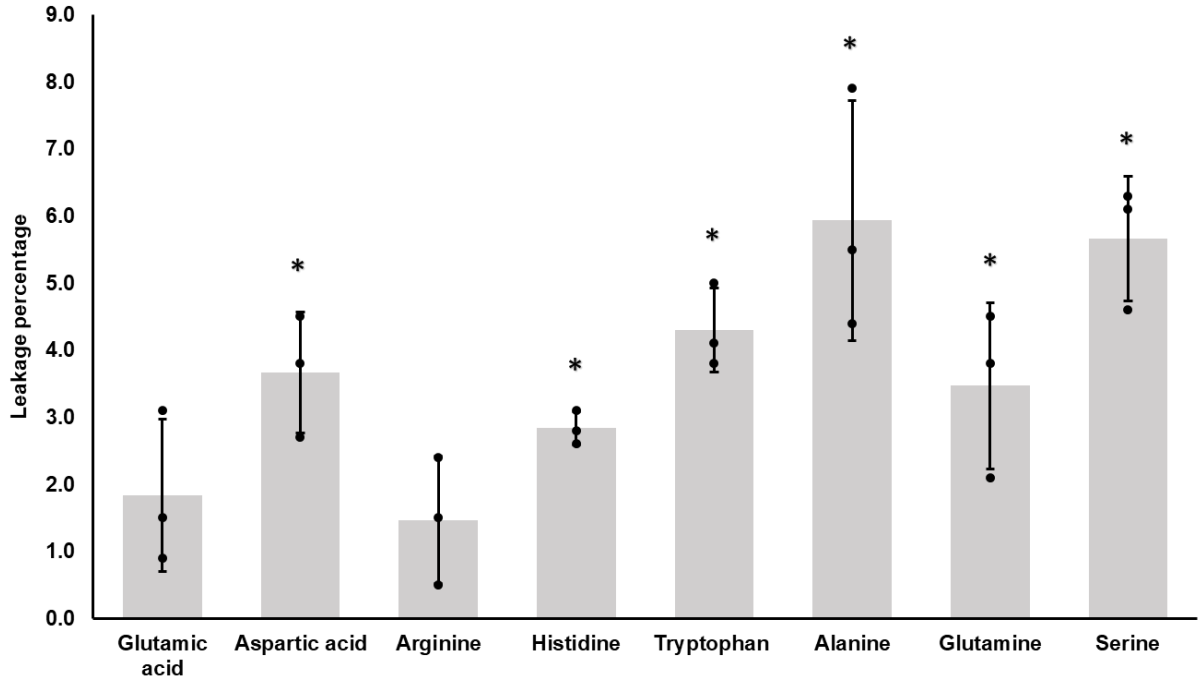

Figure 5

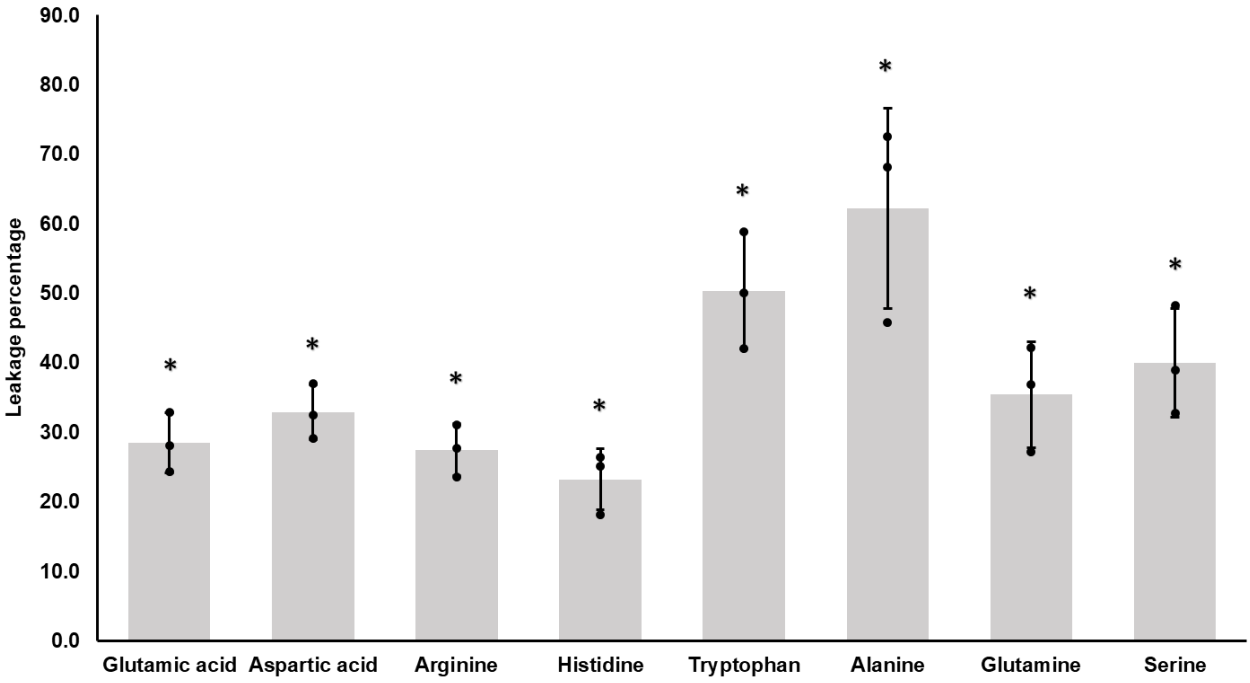

Figure 6

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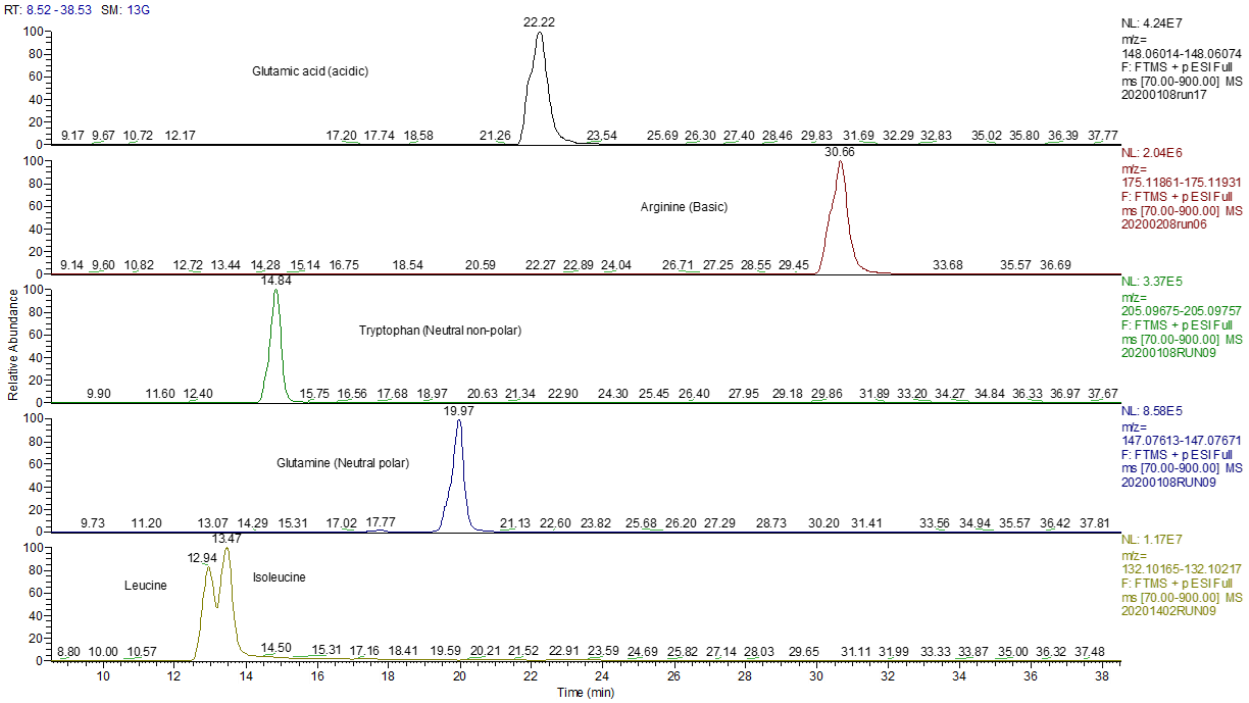

Figure 7

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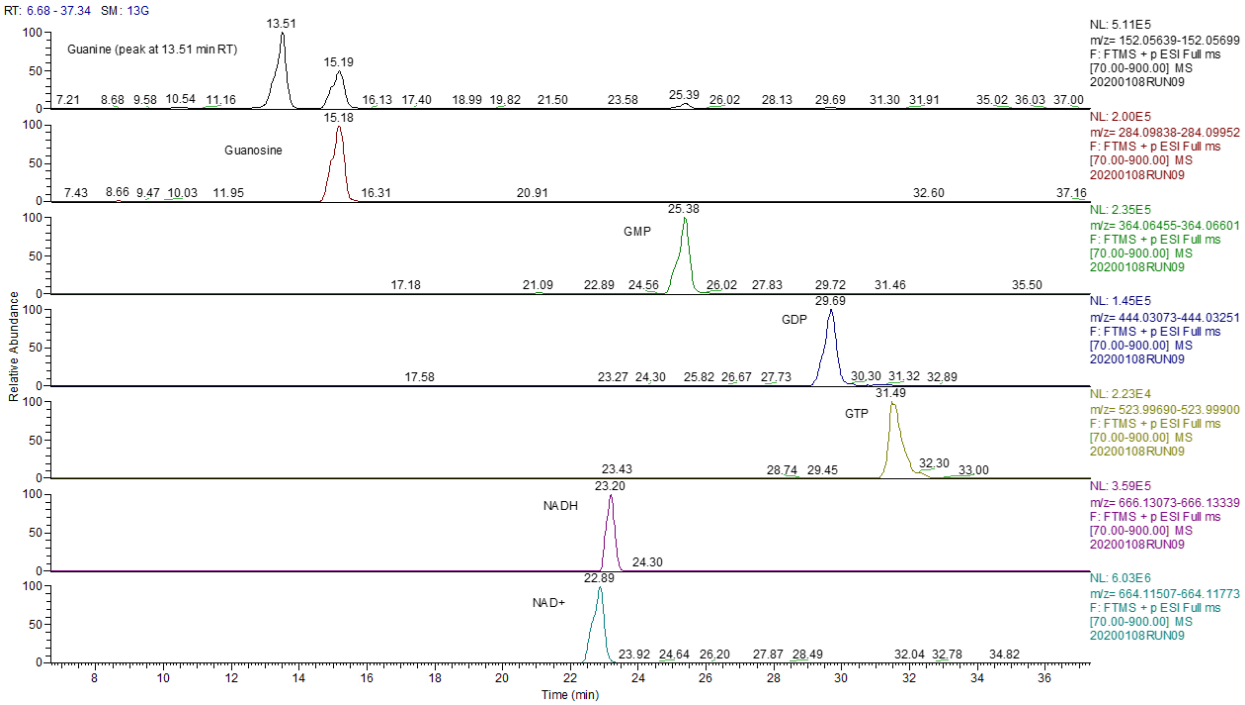

Figure 8

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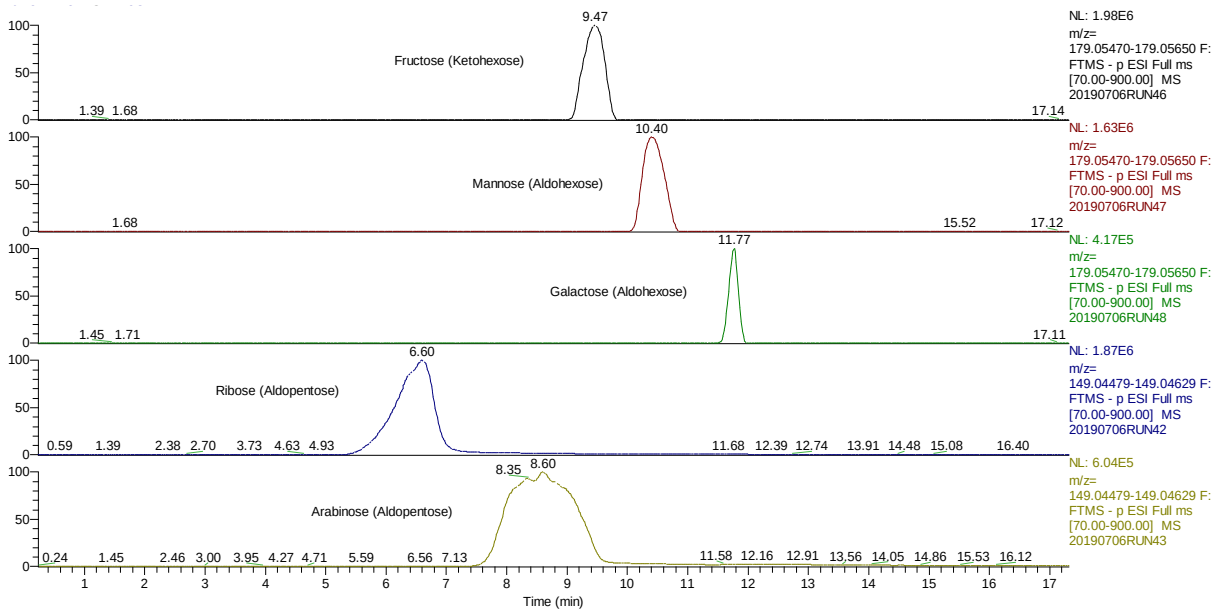

Table 1

Column type	**SeQuant ZIC-pHILIC 5µm polymer 150 x 2.1 mm**		
Solvent A	LC-MS grade H2O: ACN (95:5, v/v) 20 mM Ammonium acetate		
Solvent B	LC-MS grade Acetonitrile (ACN)		
Pressure limit	Maximum = 300 bar	Minimum = 0	
HPLC gradient program			
Time (minutes)	Flow rate (0.25 ml/min)	Compositions	
		A%	B%
0.5	0.25	5	95
26	0.25	40	60
30	0.25	70	30
31	0.25	70	30
31.1	0.4	5	95
43.9	0.4	5	95
44	0.25	5	95
45	0.25	5	95

Table 2

Full scan mass range (Dalton)	70-900
FTMS (Orbitrap analyzer) full scan resolution	6.0×10^4
FTMS (Orbitrap analyzer) HCD resolution	7500
FTMS full scan AGC target	1.0×10^6
FTMS MSn AGC target	5.0×10^4
Ion trap (LTQ) MSn AGC target	1.0×10^4
Ion Source type	Heated Electrospray Ionization
Capillary Temperature (°C)	275
Source heater Temperature (°C)	250
Sheath Gas Flow	10
Aux Gas flow	5

Table 3

Instrument polarity	Positive/Negative
Activation type	CID/HCD
Min. signal required	5000
Isolation Width	2
Normalized Collision energies for CID	35, 60
Normalized Collision energies for HCD	35, 45, 55
Default charge state	1
Activation time for CID (ms)	10, 30
Activation time for HCD (ms)	10
Number of MS/MS events in CID	Top 3, Top 5, Top 10
Number of MS/MS events in HCD	Top 5
Number of micro scans used in both HCD and CID	1

Instrument polarity	Positive/Negative
Activation type	CID/HCD
Min. signal required	5000
Isolation Width	2
Normalized Collision energies for CID	35, 60
Normalized Collision energies for HCD	35, 45, 55
Default charge state	1
Activation time for CID (ms)	10, 30
Activation time for HCD (ms)	10
Number of MS/MS events in CID	Top 3, Top 5, Top 10
Number of MS/MS events in HCD	Top 5
Number of micro scans used in both HCD and CID	1

Table 4

Std Metabolites	M.W. (g/mole)	[M+H]+1	[M-H]-1	Detection mode
glycine	75.03203	76.03931	74.02475	P
tryptophan	204.08988	205.09716	203.0826	P
phenylalanine	165.07898	166.08626	164.0717	P
arginine	174.11168	175.11896	173.1044	P
threonine*	119.05824	120.06552	118.05096	P
serine	105.04259	106.04987	104.03531	P
glutamate	147.05316	148.06044	146.04588	P
methionine	149.05105	150.05833	148.04377	P
aspartate	133.03751	134.04479	132.03023	P
valine	117.07898	118.08626	116.0717	P
isoleucine	131.09463	132.10191	130.08735	P
leucine	131.09463	132.10191	130.08735	P
histidine	155.06948	156.07676	154.0622	P
tyrosine	181.07389	182.08117	180.06661	P
lysine	146.10553	147.11281	145.09825	P
alanine	89.04768	90.05496	88.0404	P
proline	115.06333	116.07061	114.05605	P
cysteine	121.01975	122.02703	120.01247	P
asparagine	132.05349	133.06077	131.04621	P
glutamine	146.06914	147.07642	145.06186	P
guanine	151.04941	152.05669	150.04213	P
guanosine	283.09167	284.09895	282.08439	P
GMP	363.058	364.06528	362.05072	P
GDP	443.02434	444.03162	442.01706	P
GTP	522.99067	523.99795	521.98339	P
AMP	347.06309	348.07037	346.05581	P
ADP	427.02942	428.0367	426.02214	P
ATP	506.99575	508.00303	505.98847	P
NADH	665.12478	666.13206	664.1175	P
NAD+	663.10912	664.1164	662.10184	P
glucose**	180.06339	181.07067	179.05611	
fructose***	180.06339	181.07067	179.05611	N
mannose***	180.06339	181.07067	179.05611	N
galactose***	180.06339	181.07067	179.05611	N
ribose***	150.05283	151.06011	149.04555	N
arabinose***	150.05283	151.06011	149.04555	N
fructose-6-phosphate***	260.02972	261.037	259.02244	N

glucose-6-phosphate***	260.02972	261.037	259.02244	N
citric acid	192.12 g	193.034279	191.019726	N
malic acid	134.09	135.0288	133.014247	N
pyruvic acid	88.06	89.02332	87.008768	N

Lowest concentration detected (pmol/µL)
7.43E+00
5.56E-02
5.14E-02
7.14E-02
4.66E+00
4.20E-01
1.96E+00
3.75E+00
1.49E+00
1.84E+00
2.26E+00
2.53E+00
8.72E-02
1.43E-01
1.12E+00
1.05E+00
8.22E-01
1.08E+00
1.92E+00
5.47E+00
3.67E-01
7.17E-01
2.57E+00
2.27E+00
5.25E-01
1.32E+00
1.77E+00
1.47E+00
3.03E+00
5.67E-01
1.05E+00
9.00E-01
1.10E+00
1.23E+00
6.60E+00

4.27E+00
9.33E-01
1.23E+00
2.77E+00

Table of Materials

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Name of Material/ Equipment	Company	Catalog Number
Chemicals		
Acetonitrile	Fisher Scientific	A9554
Ammonium acetate	Fisher Scientific	A11450
Ammonium bicarbonate	Sigma	9830
Bactopeptone	Fisher Scientific	BP1420-2
Chloroform	Fisher Scientific	C297-4
Glucose	Fisher Scientific	D16-10
L-histidine	Sigma	H8125
L-leucine	Sigma	L8912
L-lysine	Sigma	L5501
Methanol	Fisher Scientific	A4564
Methanol	Fisher Scientific	A4564
Propidium iodide	Thermo Scientific	R37108
Uracil	Sigma	U0750
Yeast extract	Fisher Scientific	BP1422-2
Hardware equipment		
500 ml centrifuge bottles	Beckman	355664
Agilent 1100 series LC system	Agilent Technologies	G1312A
Beckman Coulter Centrifuge	Beckman	6254249
Beckman Coulter Centrifuge Rotor	Beckman	JA-10
Centra CL2 clinical centrifuge	Thermo Scientific	004260F
Digital thermometer	Omega	HH509
Foam Tube Holder Kit with Retainer	Thermo Scientific	02-215-388
SeQuant ZIC-pHILIC zwitterionic-phase column (5µm polymer 150 x 2.1 mm)	Sigma Milipore	150460
Thermo Orbitrap Velos MS	Fisher Scientific	ETD-10600
Ultrasonic sonicator	Fisher Scientific	15337416
Vortex	Fisher Scientific	2215365
ZORBAX Bonus-RP, 80Å, 2.1 x 150 mm, 5 µm	Agilent Technologies	883725-901
Laboratory materials		
2-mL Glass sample vials with Teflon lined caps	Fisher Scientific	60180A-SV9-1P
Glass beads (acid-washed, 425-600 µm)	Sigma-Aldrich	G8772
Hemacytometer	Fisher Scientific	267110
15-mL High-speed glass centrifuge tubes with Teflon lined caps	PYREX	05-550
Software		
Compound Discoverer 3.1	Fisher Scientific	V3.1
Yeast strain		
Yeast strain BY4742	Dharmacon	YSC1049

November 6, 2020

Nam Nguyen, Ph.D.
Manager of Review
JoVE

Dear Dr. Nguyen:

Please find attached a revised version of our manuscript, entitled "Quantitative Metabolomics of *Saccharomyces Cerevisiae* Using Liquid Chromatography Coupled with Tandem Mass Spectrometry" (manuscript reference number JoVE62061), which we would like to be considered for publication in the *JoVE*.

We have addressed each of the editorial and peer review comments and revised the manuscript accordingly. Provided below is a detailed description of the revisions we have made.

Editorial comments:

General:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.
Our response:
We thoroughly proofread the revised manuscript. We confirm that it does not have any spelling and grammar errors. We also confirm that all abbreviations in the revised manuscript are defined at first use.

2. Please make the title concise: Quantitative Metabolomics of Saccharomyces cerevisiae Using Liquid Chromatography Coupled with Tandem Mass Spectrometry.
Our response:
We revised the title of our manuscript, as requested. Please see lines 2 and 3 in the revised manuscript.

3. Unfortunately, there are sections of the manuscript that show overlap with previously published work. Please revise the following lines: 103-144 (…equipment for); 385-388; 465 (that can be...)-469 (…performed); 524-531.
Our response:
We modified all these overlapping sections in the revised manuscript.

4. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.
For example: Beckman Coulter, JA-10, Centra CL2 clinical centrifuge, Eppendorf tubes, LTQ Orbitrap Velos mass spectrometer, Compound Discoverer 3.1, Leica DM6000, Hamamatsu Orca ER camera, Plan Fluotar Lens (NA1.3), Zorbax Eclipse Plus, SeQuant ZIC-pHILIC, etc.

Our response:
In the revised manuscript, we removed all company names preceding the names of instruments, reagents and software.

5. Please convert centrifuge speeds to centrifugal force (x g) instead of revolutions per minute (rpm).
Our response:
We converted revolutions per minute to the relative centrifugal force in the revised manuscript.

6. As we are a methods journal, please revise the Discussion to add the following with citations:
a) Any limitations of the technique
b) The significance with respect to existing methods
Our response:
We revised the Discussion as requested. We outlined several advantages of our method over other methods of non-targeted metabolomics and cited relevant publications. We also described several limitations our method has.

7. Please sort the Materials Table alphabetically by the name of the material.
Our response:
We sorted the Tables of Materials as requested.

Reviewer #1:

1. What are advantages of this method compared to previous approach, what is precision and repeatability considering the complexity of yeast culture and fermentation. In the introduction, the author list six limitation of previous work, but I cannot find the information of the present approach to solve them all. the author should highlight them.
Our response:
We revised the Discussion section by outlining several advantages of our method over other non-targeted metabolomics methods and citing relevant publications. We also described several limitations of our method.

2. L471, why glucose cannot be identified?
Our response:
As we indicated in the legend for table 4, glucose cannot be identified because it creates multiple peaks during chromatography on the zwitterionic-phase ZIC-pHILIC column. Reducing sugars, such as glucose, fructose or any other monosaccharide (either an aldose or ketose), are known to undergo mutarotation in aqueous solutions. During mutarotation, a cyclic form of monosaccharide is in equilibrium with its α and β anomeric forms. Please see the following paper for a detailed discussion of this issue:

Zhang R, Watson DG, Wang L, Westrop GD, Coombs GH, Zhang T. Evaluation of mobile phase characteristics on three zwitterionic columns in hydrophilic interaction liquid chromatography mode for liquid chromatography-high resolution mass spectrometry based untargeted metabolite

profiling of Leishmania parasites. J Chromatogr A. 2014; 1362:168-79. doi: 10.1016/j.chroma.2014.08.039. PMID: 25160959.

Therefore, monosaccharides are separated by commercial ion-pairing columns, gas chromatography or other methods. Please see the following:

https://www.waters.com/nextgen/ca/en/library/application-notes/2009/analysis-of-carbohydrates-by-uplc-and-mass-spectrometry.html

Yang H, Shi L, Zhuang X, Su R, Wan D, Song F, Li J, Liu S. Identification of structurally closely related monosaccharide and disaccharide isomers by PMP labeling in conjunction with IM-MS/MS. Sci Rep. 2016; 6:28079. doi: 10.1038/srep28079. PMID: 27306514.

3. The author should prove the advantages of this approach, because it is well done in the metabolome of SC.
Our response:
We revised the Discussion section by outlining several advantages of our method over other non-targeted metabolomics methods and citing relevant publications. We also described several limitations of our method.

4. How you define the water-soluble metabolites?
Our response:
During metabolite extraction, we add chloroform, methanol, water and glass beads to cells. After disrupting the cells by vortexing, we centrifuge the tube. This centrifugation step allows separating the upper aqueous phase (which contains water-soluble metabolites) from the middle layer (which contains cell debris and proteins) and from the lower organic phase (which contains mostly lipids). We use a micropipette to recover the upper aqueous phase. We then subject this aqueous phase to the LC-MS/MS analysis. Thus, the water-soluble metabolites are the metabolites that are soluble in water.

5. There is less information of the present method compared to other methods.
Our response:
We revised the Discussion section by outlining several advantages of our method over other non-targeted metabolomics methods and citing relevant publications. We also described several limitations of our method.

Reviewer #2:

1. It will be useful to mention in the abstract that this paper includes an untargeted approach for metabolomics.
Our response:
We mentioned in the abstract that our manuscript describes an LC-MS/MS method of non-targeted metabolomics. Please see lines 47 and 52 in the revised manuscript.

2. Authors claim that the method can resolve and identify stereoisomers. Evidence for the same has not been provided. This can be shown as a figure.
Our response:
Figure 8 shows the efficient chromatographic separation of galactose and mannose. This figure also indicates that chromatography efficiently separates from each other ribose and arabinose. Galactose is a stereoisomer of mannose, whereas ribose is a stereoisomer of arabinose. The molecules within each of these two pairs have the same molecular formula and atomic connectivity but differ in atoms' spatial arrangement in the molecule.

3. It is definitely desirable to show SD and not SEM for all figures. Also, it is better to show individual data points within the bar graph (to see the spread).
Our response:
As requested, Figure 4 and Figure 5 in the revised manuscript show the values of SD (instead of SEM) and individual data points within the bar graph.

4. Since the authors use an Orbitrap type system (and not a triple-quad type MS) for quantification, it'll be useful to indicate a typical linear dynamic range that they can observe with this. This point need not be shown for all metabolites, but can be indicated for a small set of key metabolites in a table.
Our response:
We added Supplemental Table 5 to address this issue. The table shows a typical linear dynamic range that we observed when we measured the concentrations of various amino acids with the help of the Thermo Orbitrap Velos mass spectrometer. We mentioned this observation in the Representative Results section of the revised manuscript.

5. The authors make a major point of using a buffered, 60% methanol solution for quenching, to improve integrity of cell membranes. This however is not that unusual, and several varied studies use a 60% methanol solution for quenching. I think it is best if the authors just make the statement that this method is excellent (and include the data), instead of making a point about 'the most widely used method …' etc. (since that is not really true).
Our response:
We changed the relevant text of the Representative Results section and Figure 3 and Figure 5 legends as requested.

6. Although RP C18 columns are widely used, other mixed-separation columns (other than HILIC) are widely used, either in reverse phase or otherwise. For example, see PMID: 17940006 or 30345389. A useful comment (in the short discussion) of the use of non C18 columns (which can separate based on charge as well) could be useful for other practitioners.
Our response:
We comment on the use of these mixed-separation columns for resolving charged metabolites. Our comment can be found in the Discussion section of the revised manuscript.

7. line 129: OD600 value/cell mass not mentioned.
Our response:
We do not mention OD_{600} measurement in the revised manuscript.

8. line 223: 20 mM Ammonium acetate buffer pH not mentioned.
Our response:
We provide the value of pH for 20 mM ammonium acetate in the revised manuscript.

9. Figure 3: Scale bar not provided.
Our response:
We provide a scale bar in Figure 3 of the revised manuscript.

We are grateful to both Reviewers for constructive criticisms and suggestions for changes to be made in the text, all of which contributed to a substantial improvement of the original manuscript. We would also like to thank you for allowing us to address these criticisms and for handling our manuscript.

Yours sincerely,

Vladimir

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Metabolites	Reverse-phase Zorbax Eclipse Plus C18 column		Zwitterionic-phase ZIC-pHILIC column		*p* values
	Mean RT shift (seconds)	SD	Mean RT shift (seconds)	SD	
NAD+	7.8	0.45	19.2	0.108	9.80E-05
AMP	6.8	0.35	21.5	0.103	5.30E-07
GMP	8.4	0.43	16.5	0.102	2.40E-03
Tryptophan	9.7	0.43	9.2	0.47	7.50E-01
Arginine	7.8	0.34	16.3	0.115	2.90E-03
Glutamic acid	6.9	0.42	21	0.99	9.10E-07
Steric acid	14.6	0.84	7.7	0.47	2.70E-03
Lauric acid	13.8	0.74	6.1	0.37	1.60E-04
Decanoic acid	12.9	0.76	9.1	0.41	5.80E-02

Column type	**Zorbax Eclipse Plus C18**	
Solvent A	LC-MS grade water + 0.1 formic acid (v/v)	
Solvent B	LC-MS grade Acetonitrile (ACN) + 0.1 formic acid (v/v)	
Pressure limit	Maximum = 340 bar	Minimum = 0
HPLC gradient program		
Time (minutes)	Flow rate (0.25 ml/min)	Compositions
		A% / B%
0	0.25	100 / 0
2	0.25	100 / 0
14	0.25	40 / 60
16	0.25	10 / 90
18	0.25	10 / 90
18.1	0.25	100 / 0
30.1	0.25	100 / 0

Compound Name	MW	RT (min)	[M+H]+	[M-H]-
dodecatrienol	180.15141	1.4	181.15863	
4-Vinylcyclohexene	108.09381	1.43	109.10108	
Erucamide	337.33443	1.43	338.34177	
Oleamide	281.27181	1.446	282.27911	
Sphingosine (d18:1)	299.28237	1.47	300.28979	
Paradol	278.18712	1.59	279.19443	
16-Hydroxyhexadecanoic acid	272.23466	1.6	271.22726	
Triphenylphosphine oxide	278.08598	1.615	279.09326	
N,N-Dimethylaniline	121.08911	1.64	122.09638	
Triprolidine	278.17864	1.64	279.186	
Ethionine	163.06667	1.68	164.07396	
Palmitoyl ethanolamide	299.28237	1.761	300.28964	
nylon cyclic dimer	226.16844	1.78	227.17572	
7-Oxoheptanoic acid	144.07867	1.8	143.07133	
D-Sphingosine	299.5	1.839	300.28976	
Ripazepam	268.13249	1.89	269.13965	
(Z)-dehydrobutyrine	101.04757	1.91	102.05485	
apronalide	184.12113	1.91	185.12842	
gamma-Aminobutyric acid	103.06322	2.21	104.0705	
Phenethylamine	121.08908	2.26	122.09638	
Ureidoisobutyric acid	146.06914	3.15	147.07642	
trimethadione	143.05828	3.33	144.06546	
N5-METHYLGLUTAMINE	160.08479	3.33	161.0921	
7,8-Diaminononanoic acid	188.15244	3.33	189.15977	
Leucineamide	130.11061	3.51	131.11789	
Pyridoxine	169.07385	3.83	170.08116	
N,N-Dimethylacetamide	87.06825	3.85	88.07554	
5-Aminopentanamide	116.09492	3.89	117.10221	
6-Methyl-2-thiouracil	142.02007	4.33	143.02731	
Vorinostat	264.14748	4.4	265.15488	
1-Vinylimidazole	94.05297	4.746	95.06027	
4-Aminopyridine	94.05297	4.76	95.06024	
3-Hydroxystachydrine	159.08951	5.43	160.09674	
Triethanolamine	149.10508	5.83	150.11243	
2'-Deoxyadenosine	251.10198	6.002	252.10925	
Leu-Leu	244.17875	6.67	245.18605	
Eleganin	434.15865	7.06	435.16625	
Creatinine	113.05887	7.68	114.06615	
Choline O-Sulfate	183.05655	7.83	184.06384	
Adenine	135.05449	8.28	136.0618	
Pyridoxal	167.05829	8.8	168.06552	
Hypoxanthine	136.03853	8.995	137.0458	
Riboflavin	376.13863	9.02	377.1459	
4-Nitroaniline	138.04256	9.16	139.04984	
7-Methylguanine	165.06516	9.26	166.07246	
Biotin	244.08844	9.32	245.09538	

Octylamine	129.15193	9.33	130.15916
6-Aminocaproic acid	131.09461	9.789	132.10184
Anabasine	162.11585	10.21	163.12303
Betaine	117.07898	10.28	118.08626
Nornicotine	148.09921	10.604	149.10669
Betahistine	136.10005	10.96	137.10733
Leucylproline	228.14742	11.19	229.15474
4-Methylene-glutamine	158.0692	11.61	159.07648
1-Methylimidazoleacetic acid	140.0586	11.64	141.06584
Inosine	268.08077	11.94	269.08805
Adenosine	267.09667	12.04	268.1041
4'-Phosphopantetheine	358.09587	12.05	359.10272
Acetyl-carnitine	203.11566	12.16	204.12289
2,6-Diaminotoluene	122.08438	12.38	123.09162
Pyridostigmine	180.08997	12.45	181.09717
4-Amino-phenylalanine	180.08997	12.45	181.09726
Benzaldehyde	106.0418	12.86	107.0491
Cytosine	111.04328	12.9	112.05048
Leucine	131.09464	12.94	132.10181
Phenylalanine	165.07903	13.12	166.06623
Nikethamide	178.11069	13.26	179.11768
Amobarbital	226.13141	13.26	227.13869
pentobarbital	226.13192	13.26	227.13916
Guanine	151.04953	13.29	152.05664
Benzamidine	120.06899	13.33	121.07626
Aminocarb	208.12134	13.34	209.12831
Epiguanine	165.06534	13.35	166.07236
Isoleucine	131.09431	13.5	132.10156
AAD	161.06911	13.63	162.07625
Salsolinol	179.09465	14.2	180.10222
5-Hydroxy-tryptophan	220.08518	14.2	221.09253
Proline	115.06319	14.22	116.07058
lentiginosine	157.11035	14.41	158.11765
Methionine	149.05103	14.428	150.0584
2-Aminobutyric acid	103.06336	14.56	104.07043
Pheneturide	206.10565	14.6	207.11316
Indole	117.05797	14.66	118.06509
3-methyleneoxindole	145.05237	14.66	146.05965
8-Hydroxyquinoline	145.05272	14.66	146.05994
2-(Methylamino)isobutyric acid	117.07898	14.669	118.08627
naphthylamine	143.07332	14.69	144.08067
Tryptophan	204.08992	14.69	205.09703
3-Buten-1-amine	71.07329	14.73	72.08057
Valine	117.07862	14.94	118.0857
Crotonoside	283.09142	14.97	284.09875
Carnitine	161.1048	14.99	162.11194
Guanosine	283.09101	15.18	284.09845

Norchelerythrine	333.09938	15.37	334.10672
N-Acetyl-histidine	197.19	15.56	198.08731
Acetylcholine	145.11022	15.7	146.11739
Daminozide	160.08411	15.7	161.09138
Tiglic acid	100.0523	15.72	101.05962
Thiamine	264.10432	15.9	265.11172
Imidazolelactic acid	156.05345	16.15	157.06076
pirsidomine	330.16896	16.26	331.17533
N6-Acetyl-lysine	188.1158	16.55	189.12325
N-(4-Oxobutyl)-glutamine	216.11081	16.59	217.11751
Tyrosine	181.07347	16.75	182.08374
4-Hydroxybenzaldehyde	122.03662	16.85	123.04398
4-Coumaric acid	164.04736	16.85	165.05457
ACETYL PROLINE	157.17	16.99	158.08124
Biocytin	372.18334	17.05	373.19064
14(S)-HDHA	356.24227	17.21	257.24988
N-Acetylornithine	174.10056	17.45	175.10783
(E)-7-(methylsulfanyl)heptanal oxime	175.10369	17.45	176.11104
D-Erythrose 4-phosphate	200.00862	17.5	201.01595
Xanthosine	284.07614	17.54	285.08337
3-Hydroxy-proline	131.05836	17.58	132.06561
4-styrylpyridine	181.08527	17.59	182.09245
1-(4-Aminobutyl)urea	131.10584	17.64	132.11302
Caprolactam	113.08401	17.72	114.09129
Creatine	131.06948	17.77	132.07676
Alanine	89.04769	18	90.05482
Lactamide	89.04763	18	90.05483
Sarcosine	89.04768	18.05	90.05496
Homoserine	119.05824	18.14	120.06552
alpha-Glycerylphosphorylcholine	257.10282	18.24	258.11005
Threonine	119.05814	18.5	120.06546
Flavin mononucleotide (FMN)	456.10451	18.76	457.11179
nicotianamine	303.1429	18.79	304.15018
2-S-Glutathionyl acetate	365.08923	18.8	366.0961
Alanyl-proline	186.10044	18.84	187.10773
pentabamate	204.11072	18.84	205.11824
1-Hydroxycyclohexyl phenyl ketone	204.11101	18.84	205.11827
Acetylarginine	216.12232	18.9	217.12961
4-Guanidinobutyric acid	145.08531	18.93	146.09256
N-α-Acetyl-arginine	216.24	19.066	217.12961
3,4-Diaminopyridine	109.06384	19.17	110.07117
Imidazole-4-acetaldehyde	110.04787	19.17	111.05532
Histamine	111.07954	19.17	112.08686
Histidine	155.06937	19.17	156.07664
Fomepizole	82.05304	19.2	83.06023
2'-Deoxyadenosine 5'-monophosphate (dAMP)	331.06816	19.3	332.07617
S-Adenosyl-homocysteine	384.12159	19.53	385.12887

S-Adenosylhomocysteine	384.12147	19.63	385.12958
Glycine	75.03203	19.66	76.03931
glutaral	100.05225	19.76	101.05965
afegostat	147.08956	19.84	148.09674
Glutamine	146.06925	19.85	147.07651
Pyroglutamic acid	129.04264	19.86	130.04996
5-Aminolevulinate	131.05824	20.06	132.06552
5-Aminolevulinic acid	131.05822	20.14	132.06548
Asparagine	132.05347	20.37	133.06076
Serine	105.04259	20.533	106.0497
Benzimidazole	118.0531	20.62	119.06036
2-Hydroxyfelbamate	254.09039	20.62	255.09764
Nicotinamide	122.04796	20.63	123.05515
1,3-Diphenylacetone	227.13832	20.67	228.14561
Aceglutamide	188.07974	20.76	189.08694
Succinyl proline	215.07935	20.8	216.08685
N-(3-Carboxypropyl)-glutamine	232.10583	20.8	233.11331
Adenosine 5'-monophosphate (AMP)	347.06209	20.8	348.07007
flavin adenine dinucleotide (FAD+)	785.15714	20.94	786.16442
Citrulline	175.09577	21.06	176.10307
Vinylbital	224.11633	21.1	225.12369
Aprobarbital	210.10071	21.13	211.10791
sonedenoson	421.11592	21.34	422.12302
Dopamine 3-O-sulfate	233.03618	21.41	234.04332
Cysteine	121.01986	21.46	122.02703
l-hydroxyvaline	133.07389	21.9	134.08116
sulfacetamide	214.04131	21.94	215.04874
Cysteinylglycine	178.04138	21.96	179.04849
Glutathione (reduced)	307.08342	21.96	308.09094
Aceanthrenequinone	232.05196	21.97	233.05923
Pidotimod	244.05168	21.97	245.05902
Deoxycytidine monophosphate (dCMP)	307.197	21.97	308.064213
ACPC	101.04762	22.03	102.05489
Glutamic acid	147.05308	22.05	148.06038
O-Acetyl-serine	147.05316	22.05	148.06044
Carnosine	226.10675	22.44	227.11368
2-Aminoadipic acid	161.06872	22.49	162.07603
oxazolidinone	87.03181	22.52	88.0391
Nicotinamide adenine dinucleotide (NAD+)	663.10912	22.86	664.1164
β-Nicotinamide mononucleotide	334.05663	22.96	335.06384
inosine 5'-monophosphate (IMP)	348.0471	23.2	349.05438
Nicotinamide adenine dinucleotide (NADH)	665.12478	23.2	666.13206
Fosthiazate	283.04573	23.52	284.0531
Pyridoxamine 5-phosphate	248.05629	23.62	249.06358
D-Glucosamine 6-phosphate	259.04579	23.62	260.05301
3-hydroxykynurenine	224.07981	23.69	225.08701
gamma-Glu-gln	275.11183	23.69	276.11908

Nicotinic acid mononucleotide	335.04024	23.954	336.04788
Gonyautoxin V	379.08995	24.01	380.09653
Cytidine 5'-monophosphate	323.05142	24.15	324.05933
Cytidine 5'-diphosphocholine	488.1071	24.177	489.11462
R-(-)-Phenylephrine	167.09468	24.37	168.10197
1-phenethylamine	121.08901	24.4	122.09628
6-hydroxypseudooxynicotine	194.10576	24.4	195.11298
Butobarbital	212.11637	24.4	213.12381
(alpha)-Kainic Acid	213.10047	24.4	214.10744
Saccharopine	276.13233	24.4	277.13959
lysopine	218.12667	24.43	219.13376
pentahomomethionine	219.13016	24.46	220.13757
2'-Deoxyadenosine 5'-diphosphate (dADP)	411.2	24.71	412.041777
Scillabiose	326.12046	24.87	327.1282
Bromadiolone	526.07842	25.15	527.08612
b-Ala-Lys	217.14281	25.17	218.15009
beta-Alanyl-arginine	245.14897	25.22	246.15613
Guanosine monophosphate (GMP)	363.05755	25.357	364.06549
Fosfosal	217.998	25.5	219.00534
Adenosine diphosphate (ADP)	427.02926	25.6	428.03687
Cystathionine	222.0675	25.84	223.07489
N,N-dimethylarginine	202.25	25.883	203.15027
11-dehydro Thromboxane B2	368.21726	26.07	369.22464
PPK	340.21156	26.34	341.21838
Argininosuccinic acid	290.12291	26.49	291.13022
GPK	300.17989	26.55	301.18741
N6,N6,N6-Trimethyl-lysine	188.15248	26.65	189.15976
S-Adenosylmethionine	398.13744	26.7	399.14471
S-Adenosyl-methionine	398.13724	26.7	399.14452
N-Methylhexanamide	129.11551	26.85	130.12265
Deoxyadenosine 5'-triphosphate (dATP)	491.00151	27.2	492.00879
D-glucose-6-phosphate	260.02963	27.47	261.03708
Cadralazine	283.16446	27.53	284.17188
Phosphonoacetate	139.98746	27.76	140.99475
gamma-Glu-Glu	276.09586	27.8	277.10327
indospicine	173.11643	28.14	174.12372
7-(2-aminophenyl)heptanoic acid	203.12699	28.16	204.13438
Nicotinamide adenine dinucleotide phosphate (NADP+)	743.0755	28.51	744.0827
Adenosine triphosphate (ATP)	506.99584	28.522	508.0033
N6-Methyl-lysine	160.12118	28.92	161.12846
Cytidine diphosphate (CDP)	403.01819	29.12	404.02547
6-Phosphonoglucono-D-lactone	258.0139	29.22	259.02158
Nicotinamide adenine dinucleotide phosphate (NADPH	745.09111	29.83	746.09839
Pipecolic acid	129.07911	30.08	130.08652
Lysine	146.10559	30.08	147.11288
1-Piperideine	83.07343	30.09	84.08065
Arginine	174.11176	30.33	175.11905

Prolinamide	114.07945	30.68	115.08669	
1-Nitrosopiperidine (NPIP)	114.07949	30.68	115.08673	
Cytidine 5'-triphosphate	482.98546	30.69	483.99274	
Ornithine	132.08983	30.7	133.09723	
5-Hydroxylysine	162.10044	31.23	163.10757	
Uridine-5'-triphosphate (UTP)	484.14	31.25	484.9758	
Myristyl sulfate	294.18605	1.2		293.17877
2,3-dinor Prostaglandin E1	308.20183	1.21		307.19455
Gingerol	294.18587	1.23		293.1788
4-Dodecylbenzenesulfonic acid	326.19133	1.281		325.184
Cerotic acid	396.69	1.393		395.38959
Lignoceric acid	368.36476	1.4		367.35748
Pentacosanoic acid	382.38069	1.4		381.3732
11(E)-Eicosenoic Acid	310.2863	1.42		309.27936
Arachidic acid	312.5	1.43		311.29532
dilauroyl peroxide	398.33897	1.44		397.33127
Stearic acid	284.27104	1.44		283.26385
Putaminoxin	212.1409	1.45		211.13362
Houttuynin	198.1617	1.46		197.15466
(2Z)-2-Octyl-2-pentenedioic acid	242.15143	1.46		241.14449
Ethyl myristate	256.23967	1.46		255.23216
Palmitoleic acid	254.22409	1.46		253.21687
Margaric acid	270.45	1.468		269.24829
Proximadiol	240.20875	1.47		239.20116
Myristic Acid	228.20839	1.47		227.20132
pentadecanoic acid	242.22401	1.47		241.21674
Tridecylic acid	214.19302	1.493		213.18575
Cycloheximide	186.16176	1.52		185.15448
(R)-2-hydroxystearic acid	300.26567	1.52		299.25833
16-Oxohexadecanoic acid	270.21895	1.52		269.21167
lauric acid	200.17734	1.52		199.1702
Decanoic acid	172.14615	1.57		171.13887
4-Heptylphenol	192.15072	1.59		191.14398
Nonanoic acid	158.1306	1.603		157.12332
[1,1'-biphenyl]-2,2'-dicarboxylic acid	242.06111	1.61		241.05382
2,5-di-tert-Butylhydroquinone	222.1617	1.63		221.15442
Embelin	294.18306	1.63		293.17578
2-Ethylhexanoic acid	144.11517	1.64		143.10776
Caprylic acid	144.11502	1.657		143.10774
Clomipramine	314.15465	1.66		313.14713
2-Hydroxymyristic acid	244.20367	1.68		243.1964
10-oxocapric acid	186.12546	1.71		185.11818
n-heptanoic acid	130.09935	1.71		129.09207
12-Hydroxydodecanoic acid	216.17232	1.723		215.16504
3-oxolauric acid	214.1566	1.73		213.14938
3-Hydroxydecanoic acid	188.14117	1.755		187.1339
9-Oxononanoic acid	172.10982	1.76		171.10254

10-Hydroxydecanoic acid	188.14146	1.761	187.13419
Dodecanedioic acid	230.15138	1.764	229.14413
Valeric acid	102.06806	1.773	101.06078
Sesamex	298.14119	1.8	297.13416
Hydrocinnamic acid	150.06799	1.8	149.06071
(Hydroxyethyl)methacrylate	130.06294	1.82	129.05574
8-Hydroxyoctanoic acid	160.21	1.843	159.10294
Ethyl acetate	88.05254	1.89	87.04539
Isobutyric acid	88.05258	1.9	87.04531
Sinapyl alcohol	210.0889	1.91	209.08188
2,5-Dioxopentanoic acid	130.02643	2.17	129.01915
Itaconic acid	130.02672	2.17	129.0193
2-Naphthalenesulfonic acid	208.01903	2.4	207.01175
Succinic anhydride	100.01619	2.59	99.00891
4-Thiapentanoic acid	120.02451	2.79	119.01744
Benzoic acid	122.03705	3.2	121.02964
Azelaic acid	188.10507	4.867	187.09764
4-Aminobenzoic acid	137.04725	5.315	136.04007
4-Nitrotoluene	137.04747	5.35	136.0401
Salicylamide	137.04745	5.35	136.04007
5-Aminonicotinic acid	138.04292	5.67	137.03532
N-Acetyl-phenylalanine	207.08916	6.6	206.0822
2-Aminooctanedioic acid	189.10021	6.93	188.09285
N-Acetyl-methionine	191.0614	7.2	190.05412
N-Acetyl-cysteine	163.03041	8.08	162.02309
(S)-(-)-pipecolic acid	129.07905	8.23	128.07178
Vigabatrin	129.07915	8.23	128.07172
Picolinic acid	123.03212	8.26	122.02492
Nicotinic acid	123.03208	8.32	122.02483
Mevalonic acid	148.07336	8.45	147.06642
Lactic acid	90.0319	8.64	89.02462
Uridine	244.06904	8.771	243.06197
Erythritol	122.05794	9.03	121.05066
Pantothenic acid	219.11064	9.18	218.10295
Xanthine	152.03317	10.34	151.02585
anthopleurine	177.09996	10.49	176.09283
Metronidazole	171.06433	10.86	170.05695
Ethenzamide	165.07886	12.87	164.0714
Succinic acid	118.02659	12.87	117.01929
Penicillamine	149.05099	14.26	148.0437
Dulcitol	182.07889	14.497	181.07159
Mannitol	182.07887	14.55	181.07158
5-Aminovaleric acid	117.07882	14.62	116.07164
2-Oxobutyric acid	102.03171	15.02	101.02443
metioprim	306.11522	15.06	307.1225
N-Acetyl-glutamine	188.18	15.438	187.07295
Dihydroorotic acid	158.03245	15.5	157.02518

Threonic acid	136.03705	16.18	135.02994
Glycyl-leucine	188.11578	16.42	187.10864
2-Hydroxyphenylalanine	181.107416	16.7	180.06656
Orotidine	288.05855	17.81	287.05164
3-Hydroxy-3-methylglutaric acid	162.05275	18.09	161.04536
Cytidine	243.08565	18.5	242.07907
2-Isopropylmalic acid	176.06814	18.6	175.06104
(Hydroxymethyl)phosphonic acid	111.99257	19.09	110.98529
Isoniazid	137.05879	19.31	136.05151
4-Aminonicotinic acid	138.04274	19.32	137.03549
D-Xylonic acid	166.04814	19.58	165.04066
5-Nitrilonorvaline	128.05879	19.86	127.05143
Glutamine	146.06903	19.86	145.0618
2'-Deoxyguanosine 5'-monophosphate (dGMP)	347.06245	20.278	346.05518
beta-D-Mannopyranose	180.06329	20.54	179.05595
Hexose	180.06332	20.54	179.05602
Sucrose	342.11573	21.09	341.10846
α,α-Trehalose	342.11579	21.09	341.10858
Citrulline	175.09558	21.29	174.0883
Prolylglycine	172.08471	21.48	171.07762
Nicotinuric acid	180.05359	21.89	179.04602
Zalcitabine	211.09544	21.91	210.08817
Glycerol 3-phosphate	172.01352	22.14	171.00632
Uridine monophosphate (UMP)	324.03486	22.15	323.02811
Aspartic acid	133.03742	22.2	132.03012
glyceraldehyde 3-phosphate	169.99794	22.45	168.99066
N-Acetyl-glutamic acid	189.0636	22.48	188.05632
Formylpyruvate	116.01092	22.5	115.00378
N-Acetyl-aspartic acid	175.0479	22.5	174.0406
Cyclic ADP-ribose	541.06044	22.53	540.05267
Malic acid	134.0214	24.31	133.01421
Tenuazonic acid	197.10486	24.43	196.09779
Maleic acid	116.01103	25.58	115.00376
Maleamic acid	115.02692	25.6	114.0197
Uridine 5'-diphosphate (UDP)	404.00166	27.3	402.99432
3-Phosphoglyceric acid	185.99305	27.76	184.98582
2-Furoic acid	112.01612	27.894	111.00881
Phosphoenolpyruvic acid	167.98225	28.37	166.97499
Orotidine 5'-monophosphate	368.02424	28.63	367.01715
Deoxyguanosine diphosphate (dGDP)	427.02775	28.646	426.02103
Glutathione oxidized	612.15122	29.4	611.14343
Guanosine 5'-diphosphate (GDP)	443.0236	29.4	442.01633
homoisocitric acid	206.04236	29.43	205.03508
Ornithine	132.08969	30.5	131.08244
α-D-Glucose-1,6-bisphosphate	339.99483	30.63	338.98752
Guanosine triphosphate (GTP)	522.98933	31.4	521.98224

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HCD_35_T5_10ms	HCD_45_T5_10ms
1-(4,5-dihydro-1H-imidazol-2-	12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acic	16-Hydroxyhexadecanoic acic
1-Nitrosopyrrolidine (NPYR)	1-Phenyl-1H-pyrazolo[3,4-d]p
1-Phenyl-1H-pyrazolo[3,4-d]p	1-Vinylimidazole
1-Vinylimidazole	2-(2-hydroxy-3-methylbutana
2-(2-hydroxy-3-methylbutana	2,5-di-tert-Butylhydroquinon
2,5-di-tert-Butylhydroquinon	2,6-Dimethylpyrazine
2,6-Dimethylpyrazine	2-Aminonicotinic acid
2-Aminoadipic acid	2-Aminooctanedioic acid
2-Aminonicotinic acid	2'-Deoxyadenosine
2-Aminooctanedioic acid	2-Furoic acid
2'-Deoxyadenosine	2-Hydroxycaproic acid
2'-Deoxyadenosine 5'-monop	2-Isopropylmalic acid
2'-Deoxyguanosine 5'-monop	2-Naphthalenesulfonic acid
2-Ethylhexanoic acid	2-Oxobutyric acid
2-Furoic acid	3-(2,6-Dioxocyclohexyl)propa
2-Hydroxycaproic acid	3-amino-1H-pyrazolo[4,3-c]py
2-Isopropylmalic acid	3-Amino-3-(4-hydroxyphenyl)
2-Naphthalenesulfonic acid	3-Hydroxy-3-methylglutaric a
2-Oxobutyric acid	3-Hydroxybutyric acid
3-(2,6-Dioxocyclohexyl)propa	3-Phenyllactic acid
3-Hydroxy-3-methylglutaric a	3-Phosphoglyceric acid
3-Hydroxybutyric acid	4-Dodecylbenzenesulfonic ac
3-Methylcrotonylglycine	4-Methyl-5-thiazoleethanol
3-Phenyllactic acid	4-Oxoproline
4-Aminonicotinic acid	5-Aminonicotinic acid
4-chloro-5-morpholino-2-quir	5-Aminovaleric acid
4-Dodecylbenzenesulfonic ac	5'-S-Methyl-5'-thioadenosine
4-Methyl-5-thiazoleethanol	Acetylarginine
4-Oxoproline	Acetylcholine
5-Aminovaleric acid	Acetyl-L-carnitine
5-Hydroxylysine	Adenine
5-morpholino-2,4(1H,3H)-pyr	Adenosine
5'-S-Methyl-5'-thioadenosine	Adenosine 5'-monophosphat
6-Aminocaproic acid	Adenosine diphosphate (ADP
7-(tert-butyl)-4-imino-1,2,3,4	Adenosine triphosphate (ATP
7-Methylguanine	Argininosuccinic acid
Acetylarginine	Asparagine
Acetylcholine	Azelaic acid
Acetyl-L-carnitine	Benzoic acid
Adenine	Betaine
Adenosine	Biocytin
Adenosine 5'-monophosphat	Biotin
Adenosine diphosphate (ADP	Bis(2-ethylhexyl) sebacate
Adenosine triphosphate (ATP	Bis(4-ethylbenzylidene)sorbit

Asparagine	Caprylic acid
Azelaic acid	Carnosine
Benzoic acid	Choline
Betaine	cis-4-Hydroxy-D-proline
Biocytin	Citric acid
Biotin	Creatinine
Chlormequat	Cyclic ADP-ribose
Choline	Cysteinylglycine
cis-4-Hydroxy-D-proline	Cytidine
Citric acid	Cytidine 5'-monophosphate (
Creatinine	Cytosine
Cyclic ADP-ribose	Decanoic acid
Cystathionine	Desthiobiotin
Cysteinylglycine	Disperse orange 3
Cytidine	DL-Alanine
Cytidine 5'-monophosphate (	DL-Arginine
Cytosine	DL-Carnitine
D-(-)-Mannitol	DL-Dihydroorotic acid
Debromohymenialdisine	DL-β-Leucine
Decanoic acid	D-Mannose 6-phosphate
Deoxyguanosine diphosphate	D-myo-Inositol 1,4-bisphosph
Desthiobiotin	D-Sedoheptulose 7-phosphat
Disperse orange 3	Dulcitol
DL-Alanine	Erucamide
DL-Carnitine	Ethyl myristate
DL-Dihydroorotic acid	Flavin mononucleotide (FMN)
DL-β-Leucine	Glycyl-L-leucine
D-Ribose-1-phosphate	Guanine
Ethyl myristate	Guanosine 5'-diphosphate (G
Flavin mononucleotide (FMN)	Guanosine monophosphate (
Glycyl-L-leucine	Guvacoline
GPK	Hypoxanthine
Guanine	Imidazolelactic acid
Guanosine 5'-diphosphate (G	Isobutyric acid
Guvacoline	Isoleucine
Imidazolelactic acid	L-(-)-Malic acid
Isobutyric acid	L-(-)-Methionine
Isoleucine	L-(+)-Lactic acid
L-(-)-Malic acid	L-Aspartic acid
L-(+)-Arginine	L-Cystathionine
L-(+)-Lactic acid	L-Glutamic acid
Lactamide	L-Glutathione (reduced)
L-Aspartic acid	L-Glutathione oxidized
L-Glutamic acid	L-Histidine
L-Glutamine	L-Lysine
L-Glutathione (reduced)	L-Phenylalanine
L-Glutathione oxidized	L-Pyroglutamic acid

L-Histidine	L-Saccharopine
L-Lysine	Methyl acetoacetate
L-Phenylalanine	Mevalonic acid
L-Pyroglutamic acid	Myristyl sulfate
L-Saccharopine	N-(2,4-Dimethylphenyl)-N'-m
L-threo-3-Phenylserine	N,N-Dimethylaniline
L-Threonic acid-1,4-lactone	N6,N6,N6-Trimethyl-L-lysine
L-Tyrosine	N6-Acetyl-L-lysine
Methyl acetoacetate	N-Acetylaspartic acid
Mevalonic acid	N-Acetyl-D-alloisoleucine
Myristyl sulfate	N-Acetyl-DL-glutamic acid
N,N-Dimethylaniline	N-Acetyl-L-leucine
N4-Acetyl sulfamethazine	N-Acetyl-L-methionine
N6,N6,N6-Trimethyl-L-lysine	N-Acetyl-L-phenylalanine
N6-Acetyl-L-lysine	N-Acetyl-α-D-glucosamine 1-p
N-Acetyl-D-alloisoleucine	N-Ethylglycine
N-Acetyl-DL-glutamic acid	Nicotinamide
N-Acetyl-L-methionine	Nicotinic acid
N-Acetyl-L-phenylalanine	Nicotinic acid mononucleotid
N-Acetyl-α-D-glucosamine 1-p	Nicotinuric acid
N-Ethylglycine	Nonanoic acid
Nicotinamide	Nornicotine
Nicotine	NP-001346
Nicotinic acid	N-Tigloylglycine
Nicotinic acid mononucleotid	Ornithine
Nicotinuric acid	Orotic acid
Nonanoic acid	Orotidine
NP-001346	Orotidine 5'-monophosphate
N-Tigloylglycine	Pantothenic acid
Ornithine	PEG n6
Orotic acid	Pentadecanoic acid
Orotidine	Phenylpyruvic acid
Orotidine 5'-monophosphate	Pipecolic acid
Pantothenic acid	PPK
PEG n7	Proline
Pentadecanoic acid	Prolylleucine
Phenylpyruvic acid	Propionylcarnitine
Pilocarpine	Pyridoxal
Pipecolic acid	Pyridoxine
PPK	Pyruvic acid
Proline	S-Adenosylhomocysteine
Prolylglycine	S-Adenosylmethionine
Propionylcarnitine	Stearic acid
Pyridoxal	Succinic acid
Pyridoxine	Sucrose
Pyruvic acid	Thiamine
S-Adenosylhomocysteine	Threonine

S-Adenosylmethionine	Thymidine
S-Lactoylglutathione	Thymidine 5'-monophosphate
Stearic acid	Tridecylic acid
Succinic acid	Triphenylphosphine oxide
Sucrose	Uracil
Thiamine	Uridine
Threonine	Uridine 5'-diphosphate (UDP)
Thymidine	Uridine monophosphate (UM
trans-3-Indoleacrylic acid	Urocanic acid
Tridecylic acid	Valeric acid
Uracil	Valine
Uridine	VLK
Uridine monophosphate (UM	Xanthine
Valeric acid	α,α-Trehalose
Valine	β-Nicotinamide mononucleot
Xanthine	δ-Valerolactam
α,α-Trehalose	Y-Glutamylcysteine
α-D-Glucose-1,6-bisphosphat	Y-L-Glutamyl-L-glutamic acid
β-Nicotinamide mononucleotide	
δ-Ribono-1,4-lactone	
δ-Valerolactam	
Y-Glutamylcysteine	
Y-L-Glutamyl-L-glutamic acid	

TOTAL # OF METABOLITES	**162**	**157**
TOTAL UNIQUE METABOLITES	**23**	**8**

HCD_55_T5_10ms	T3_35E_10ms	T3_35E_30ms
16-Hydroxyhexadecanoic acic	(+/-)-C75	(+/-)-C75
2-(Methylamino)isobutyric ac	10-Hydroxydecanoic acid	10-Hydroxydecanoic acid
2,5-di-tert-Butylhydroquinon	12-Hydroxydodecanoic acid	12-Hydroxydodecanoic acid
2,6-Dimethylpyrazine	16-Hydroxyhexadecanoic acic	16-Hydroxyhexadecanoic acic
2-Aminoadipic acid	2-Aminoadipic acid	2-Aminoadipic acid
2-Aminonicotinic acid	2-Aminonicotinic acid	2-Aminonicotinic acid
2'-Deoxyadenosine	2'-Deoxyadenosine	2'-Deoxyadenosine
2-Furoic acid	2-Furoic acid	2-Hydroxycaproic acid
2-Hydroxy-2-methylbutyric ac	2-Hydroxycaproic acid	2-Hydroxyvaleric acid
2-Hydroxycaproic acid	2-Hydroxyvaleric acid	2-Isopropylmalic acid
2-Hydroxyphenylalanine	2-Isopropylmalic acid	2-Oxobutyric acid
2-Isopropylmalic acid	2'-O-Methyladenosine	3'-Adenosine monophosphat
2-Naphthalenesulfonic acid	3'-Adenosine monophosphat	3-Hydroxy-3-methylglutaric a
2-Oxobutyric acid	3-Hydroxy-3-methylglutaric a	3-Hydroxybutyric acid
3-Hydroxy-3-methylglutaric a	3-Hydroxybutyric acid	3-Hydroxy-L-proline
3-Hydroxybutyric acid	3-Phenyllactic acid	3-Phenyllactic acid
3-Hydroxydecanoic acid	4-(3-Hydroxybutyl)phenyl β-D	3-Phosphoglyceric acid
3-Hydroxy-L-proline	4-Acetamidobutanoic acid	4-Acetamidobutanoic acid
3-Phenyllactic acid	4-Aminonicotinic acid	4-Aminonicotinic acid
4-Dodecylbenzenesulfonic ac	4-Coumaric acid	4-Coumaric acid
4-Methyl-5-thiazoleethanol	4-Hydroxybenzaldehyde	4-Hydroxybenzaldehyde
4-Oxoproline	4-Methylumbelliferone	5-Aminolevulinic acid
5-Aminovaleric acid	5-Aminolevulinic acid	5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine	5-Aminovaleric acid	5'-S-Methyl-5'-thioadenosine
Acetylcholine	5-Hydroxylysine	6-Aminocaproic acid
Acetyl-L-carnitine	5'-S-Methyl-5'-thioadenosine	Acetylcholine
Adenine	6-Aminocaproic acid	Acetyl-L-carnitine
Adenosine	Acetyl-L-carnitine	Adenosine
Adenosine 5'-monophosphat	Adenosine	Adenosine 5'-monophosphat
Adenosine diphosphate (ADP	Adenosine 5'-monophosphat	Adenosine diphosphate (ADP
Adenosine triphosphate (ATP	Adenosine diphosphate (ADP	Argininosuccinic acid
Anhydroecgonine	Adenosine triphosphate (ATP	Asparagine
Argininosuccinic acid	Argininosuccinic acid	Azelaic acid
Asparagine	Asparagine	Benzoic acid
Azelaic acid	Azelaic acid	Biocytin
Benzoic acid	Benzoic acid	Biotin
Betaine	Biocytin	Carnosine
Biocytin	Biotin	Choline
Biotin	Carnosine	cis-4-Hydroxy-D-proline
Bis(4-ethylbenzylidene)sorbit	Choline	Citric acid
Caprylic acid	Citric acid	Creatinine
Carnosine	CMPF	Cyclic ADP-ribose
Choline	Creatinine	Cystathionine
Citric acid	Cyclic ADP-ribose	Cysteinylglycine
Creatinine	Cystathionine	Cytidine 5'-diphosphocholine

Cyclic ADP-ribose	Cysteinylglycine	Cytidine 5'-monophosphate (
Cystathionine	D-(-)-Glutamine	D-(-)-Glutamine
Cytidine 5'-monophosphate (	D-(-)-Mannitol	D-(-)-Mannitol
Cytosine	D-(+)-Pyroglutamic Acid	D-Carnitine
D-(-)-Glutamine	D-Carnitine	DL-3-Aminoisobutyric acid
D-(-)-Mannitol	DL-3-Aminoisobutyric acid	DL-Alanine
D-(+)-Pipecolinic acid	DL-Arginine	DL-Arginine
Decanoic acid	DL-Dihydroorotic acid	DL-Dihydroorotic acid
Desthiobiotin	DL-Leucineamide	DL-Leucineamide
DL-Alanine	DL-Lysine	DL-Lysine
DL-Arginine	DL-Tryptophan	DL-Tryptophan
DL-Carnitine	D-Serine	D-Serine
DL-Dihydroorotic acid	Ethyl myristate	Flavin mononucleotide (FMN
DL-β-Leucine	Flavin mononucleotide (FMN	Guanosine 5'-diphosphate (G
D-Sedoheptulose 7-phosphat	Fumaric acid	Indole-3-acrylic acid
Ethyl myristate	Guanosine 5'-diphosphate (G	Isoniazid
Flavin mononucleotide (FMN	Guanosine 5'-diphospho-D-m	L-(-)-Malic acid
Glycyl-L-leucine	Guanosine monophosphate (	L(+)-2-Aminobutyric acid
Guanine	Imidazolelactic acid	L-(+)-Arginine
Guanosine 5'-diphosphate (G	Indole-3-acrylic acid	L-(+)-Citrulline
Hypoxanthine	L-(-)-Malic acid	L-Aspartic acid
Imidazolelactic acid	L(+)-2-Aminobutyric acid	Leucine
Isobutyric acid	L-(+)-Arginine	Leucylproline
L-(-)-Malic acid	L-(+)-Citrulline	Levulinic acid
L-(+)-Citrulline	L-Aspartic acid	L-Glutamic acid
L-(+)-Lactic acid	Leucine	L-Glutamine
Lactamide	Leucylproline	L-Glutathione (reduced)
L-Aspartic acid	Levulinic acid	L-Glutathione oxidized
L-Cystathionine	L-Glutamic acid	L-Histidine
Leucine	L-Glutamine	L-Lysine
L-Glutamic acid	L-Glutathione (reduced)	L-Norleucine
L-Glutathione (reduced)	L-Glutathione oxidized	L-Phenylalanine
L-Glutathione oxidized	L-Histidine	L-Pyroglutamic acid
L-Histidine	Lignoceric acid	L-Saccharopine
L-Lysine	L-Lysine	L-Serine
L-Norleucine	L-Norleucine	L-Threonine
L-Phenylalanine	L-Phenylalanine	L-Tyrosine
L-Pyroglutamic acid	L-Pyroglutamic acid	Maleic acid
L-Saccharopine	L-Saccharopine	Malonic acid
Mevalonic acid	L-Threonine	Methionine
N-(2,4-Dimethylphenyl)-N'-m	Maleic acid	Mevalonic acid
N,N-Dimethylaniline	Malonic acid	N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine	Methionine	N6-Acetyl-L-lysine
N-Acetylaspartic acid	Mevalonic acid	N8-Acetylspermidine
N-Acetyl-D-alloisoleucine	N6,N6,N6-Trimethyl-L-lysine	N-Acetylaspartic acid
N-Acetyl-DL-glutamic acid	N6-Acetyl-L-lysine	N-Acetyl-D-alloisoleucine
N-Acetyl-L-methionine	N8-Acetylspermidine	N-Acetyl-DL-glutamic acid

N-Acetyl-L-phenylalanine	N-Acetylaspartic acid	N-Acetyl-L-cysteine
N-Acetyl-α-D-glucosamine 1-p	N-Acetyl-D-alloisoleucine	N-Acetyl-L-methionine
N-Ethylglycine	N-Acetyl-DL-glutamic acid	N-Acetyl-L-phenylalanine
Nicotinamide	N-Acetyl-L-methionine	Nicotinic acid
Nicotinic acid	N-Acetyl-L-phenylalanine	Nicotinuric acid
Nicotinic acid mononucleotid	Nicotinuric acid	NP-008309
Nicotinuric acid	NP-018741	NP-018741
Nonanoic acid	NP-020205	NP-020205
Nornicotine	N-α-L-Acetyl-arginine	N-α-L-Acetyl-arginine
NP-001346	Oleamide	Ornithine
N-Tigloylglycine	Ornithine	Orotic acid
N-α-L-Acetyl-arginine	Orotic acid	Orotidine
Ornithine	Orotidine	Orotidine 5'-monophosphate
Orotic acid	Orotidine 5'-monophosphate	Palmitoyl ethanolamide
Orotidine	Palmitoyl ethanolamide	Pantothenic acid
Pantothenic acid	Pantothenic acid	Phenylpyruvic acid
Phenylpyruvic acid	Paracetamol	Picolinic acid
Pipecolic acid	Phenylpyruvic acid	Pipecolic acid
PPK	Picolinic acid	Proline
Prolinamide	Pipecolic acid	Propionylcarnitine
Proline	PPH	Pyridoxal
Prolylleucine	PPK	Pyridoxine
Propionylcarnitine	Proline	Pyruvic acid
Pyridoxal	Propionylcarnitine	Riboflavin
Pyridoxine	Pyridoxal	S-Adenosylhomocysteine
Pyruvic acid	Pyridoxine	S-Adenosylmethionine
S-Adenosylhomocysteine	Pyruvic acid	Salicylamide
S-Adenosylmethionine	Riboflavin	Succinic acid
Stearic acid	S-Adenosylhomocysteine	Thiamine
Succinic acid	S-Adenosylmethionine	Threonine
Thiamine	Salsolinol	trans-Aconitic acid
Threonine	Stearic acid	trans-Cinnamic acid
Thymidine	Succinic acid	Uridine
Thymidine 5'-monophosphat	Sucrose	Uridine monophosphate (UM
Triphenylphosphine oxide	Thiamine	Urocanic acid
Uracil	Threonine	Valine
Uridine	Thymidine 5'-monophosphat	Xanthine
Uridine monophosphate (UM	trans-Aconitic acid	α,α-Trehalose
Urocanic acid	Uridine	α-D-Glucose-1,6-bisphosphat
Valeric acid	Uridine monophosphate (UM	β-Alanine
VLK	Urocanic acid	β-Nicotinamide mononucleot
Xanthine	Valine	δ-Ribono-1,4-lactone
α,α-Trehalose	Xanthine	Y-Aminobutyric acid (GABA)
β-Nicotinamide mononucleot	α-D-Glucose-1,6-bisphosphat	Y-Glutamylcysteine
δ-Valerolactam	β-Alanine	
Y-Glutamylcysteine	β-Nicotinamide mononucleotide	
	Y-Aminobutyric acid (GABA)	

Y-Glutamylcysteine

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P)

ide

138	**140**	**136**
7	**3**	**0**

T3_60E_10ms	T3_60E_30ms	T5_35E_10ms
(+/-)-C75	(+/-)-C75	(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic a	(2Z)-2-Octyl-2-pentenedioic a	(2Z)-2-Octyl-2-pentenedioic a
10-Hydroxydecanoic acid	10-Hydroxydecanoic acid	10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid	12-Hydroxydodecanoic acid	12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acic	16-Hydroxyhexadecanoic acic	16-Hydroxyhexadecanoic acic
2-Aminoadipic acid	2-Aminoadipic acid	2-Aminoadipic acid
2-Aminonicotinic acid	2-Aminobutyric acid	2-Aminonicotinic acid
2'-Deoxyadenosine	2'-Deoxyadenosine	2'-Deoxyadenosine
2-Hydroxycaproic acid	2-Furoic acid	2-Furoic acid
2-Hydroxyvaleric acid	2-Hydroxycaproic acid	2-Hydroxycaproic acid
2-Isopropylmalic acid	2-Hydroxyvaleric acid	2-Hydroxyvaleric acid
2-Methylglutaric acid	2-Isopropylmalic acid	2-Isopropylmalic acid
3'-Adenosine monophosphat	3'-Adenosine monophosphat	3'-Adenosine monophosphat
3-Hydroxy-3-methylglutaric a	3-Hydroxy-3-methylglutaric a	3-Hydroxy-3-methylglutaric a
3-Hydroxybutyric acid	3-Hydroxybutyric acid	3-Hydroxybutyric acid
3-Phenyllactic acid	3-Hydroxy-L-proline	3-Phenyllactic acid
4-Acetamidobutanoic acid	3-Phenyllactic acid	4-(3-Hydroxybutyl)phenyl β-D
4-Aminonicotinic acid	4-Acetamidobutanoic acid	4-Acetamidobutanoic acid
4-Coumaric acid	4-Aminonicotinic acid	4-Aminonicotinic acid
4-Hydroxybenzaldehyde	4-Coumaric acid	4-Coumaric acid
5-Aminolevulinic acid	4-Hydroxybenzaldehyde	4-Hydroxybenzaldehyde
5-Hydroxylysine	5-Aminolevulinic acid	4-Methylumbelliferone
5'-S-Methyl-5'-thioadenosine	5-Hydroxylysine	5-Aminolevulinic acid
6-Aminocaproic acid	5'-S-Methyl-5'-thioadenosine	5-Hydroxylysine
Acetyl-L-carnitine	6-Aminocaproic acid	5'-S-Methyl-5'-thioadenosine
Adenosine	Acetylcholine	Acetyl-L-carnitine
Adenosine 5'-monophosphat	Acetyl-L-carnitine	Adenosine
Adenosine diphosphate (ADP	Adenosine	Adenosine 5'-monophosphat
Argininosuccinic acid	Adenosine 5'-monophosphat	Adenosine diphosphate (ADP
Asparagine	Adenosine diphosphate (ADP	Argininosuccinic acid
Benzoic acid	Adenosine triphosphate (ATP	Asparagine
Biocytin	Argininosuccinic acid	Azelaic acid
Biotin	Asparagine	Benzoic acid
Carnosine	Azelaic acid	Biocytin
Citric acid	Benzoic acid	Biotin
Cyclic ADP-ribose	Biocytin	Carnosine
Cystathionine	Biotin	Citric acid
Cysteinylglycine	Carnosine	Creatinine
Cytidine 5'-diphosphocholine	Choline	Cyclic ADP-ribose
Cytidine 5'-monophosphate (	Citric acid	Cystathionine
D-(-)-Glutamine	Cyclic ADP-ribose	Cysteinylglycine
D-(+)-Pyroglutamic Acid	Cystathionine	Cytidine 5'-diphosphocholine
D-Carnitine	Cysteinylglycine	D-(-)-Glutamine
Diacetyl	Cytidine 5'-diphosphocholine	D-(-)-Mannitol
DL-3-Aminoisobutyric acid	D-(-)-Glutamine	D-(+)-Pyroglutamic Acid

DL-Alanine	D-(-)-Mannitol	D-Carnitine
DL-Arginine	D-(+)-Pyroglutamic Acid	DL-3-Aminoisobutyric acid
DL-Dihydroorotic acid	D-Carnitine	DL-Arginine
DL-Leucineamide	DL-3-Aminoisobutyric acid	DL-Dihydroorotic acid
DL-Lysine	DL-Alanine	DL-Leucineamide
D-Sedoheptulose 7-phosphat	DL-Arginine	DL-Lysine
D-Serine	DL-Dihydroorotic acid	D-Mannose 6-phosphate
D-α-Hydroxyglutaric acid	DL-Leucineamide	D-Sedoheptulose 7-phosphat
Flavin mononucleotide (FMN	DL-Lysine	D-Serine
Guanosine 5'-diphosphate (G	DL-Tryptophan	D-α-Hydroxyglutaric acid
Guanosine 5'-diphospho-D-m	D-Sedoheptulose 7-phosphat	Ethyl myristate
Hypoxanthine	D-Serine	Flavin mononucleotide (FMN
Indole-3-acrylic acid	Flavin mononucleotide (FMN	Glucose 1-phosphate
L-(-)-Malic acid	Guanosine 5'-diphosphate (G	Guanosine 5'-diphosphate (G
L-(-)-Methionine	Guanosine monophosphate (	Guanosine 5'-diphospho-D-m
L-(+)-Arginine	Hypoxanthine	Hydrocinnamic acid
L-Aspartic acid	Indole-3-acrylic acid	Hypoxanthine
Leucine	L-(-)-Malic acid	Imidazolelactic acid
Levulinic acid	L-(-)-Methionine	Indole-3-acrylic acid
L-Glutamic acid	L-(+)-Arginine	L-(-)-Malic acid
L-Glutamine	L-(+)-Citrulline	L-(+)-Arginine
L-Glutathione (reduced)	L-Aspartic acid	L-(+)-Citrulline
L-Glutathione oxidized	Leucine	L-Aspartic acid
L-Histidine	Levulinic acid	Leucine
L-Lysine	L-Glutamic acid	Levulinic acid
L-Norleucine	L-Glutamine	L-Glutamic acid
L-Phenylalanine	L-Glutathione (reduced)	L-Glutamine
L-Pyroglutamic acid	L-Glutathione oxidized	L-Glutathione (reduced)
L-Saccharopine	L-Histidine	L-Glutathione oxidized
L-Serine	L-Lysine	L-Histidine
L-Threonine	L-Norleucine	Lignoceric acid
L-Tyrosine	L-Phenylalanine	L-Lysine
Maleic acid	L-Pyroglutamic acid	L-Norleucine
Malonic acid	L-Saccharopine	L-Phenylalanine
Mevalonic acid	L-Serine	L-Pyroglutamic acid
N6,N6,N6-Trimethyl-L-lysine	L-Threonine	L-Saccharopine
N6-Acetyl-L-lysine	L-Tyrosine	L-Threonine
N-Acetylaspartic acid	Maleic acid	L-Tyrosine
N-Acetyl-D-alloisoleucine	Malonic acid	Maleic acid
N-Acetyl-DL-glutamic acid	Methionine	Malonic acid
N-Acetyl-L-methionine	Mevalonic acid	Methionine
N-Acetyl-L-phenylalanine	N,N-Dimethylglycine	Mevalonic acid
N-Acetylornithine	N6,N6,N6-Trimethyl-L-lysine	N-[(1R,9S)-6-Oxo-11-(2-pyrim
Nicotinic acid	N6-Acetyl-L-lysine	N6,N6,N6-Trimethyl-L-lysine
Nicotinuric acid	N8-Acetylspermidine	N6-Acetyl-L-lysine
NP-008309	N-Acetylaspartic acid	N8-Acetylspermidine
NP-016212	N-Acetyl-D-alloisoleucine	N-Acetylaspartic acid

N-α-L-Acetyl-arginine	N-Acetyl-DL-glutamic acid	N-Acetyl-D-alloisoleucine
Ornithine	N-Acetyl-L-cysteine	N-Acetyl-DL-glutamic acid
Orotic acid	N-Acetyl-L-methionine	N-Acetyl-L-cysteine
Orotidine	N-Acetyl-L-phenylalanine	N-Acetyl-L-methionine
Orotidine 5'-monophosphate	Nicotinic acid	N-Acetyl-L-phenylalanine
Palmitoyl ethanolamide	Nicotinuric acid	Nicotinuric acid
Pantothenic acid	NP-016212	NP-018741
Paracetamol	NP-018741	NP-020205
Phenylpyruvic acid	N-α-L-Acetyl-arginine	N-α-L-Acetyl-arginine
Phosphoenolpyruvic acid	Ornithine	Ornithine
Picolinic acid	Orotic acid	Orotic acid
Pipecolic acid	Orotidine	Orotidine
PPH	Orotidine 5'-monophosphate	Orotidine 5'-monophosphate
PPK	Palmitoyl ethanolamide	Palmitoyl ethanolamide
Proline	Pantothenic acid	Pantothenic acid
Propionylcarnitine	Phenylpyruvic acid	Paracetamol
Pyridoxal	Picolinic acid	Phenylpyruvic acid
Pyridoxine	Pipecolic acid	Phosphoenolpyruvic acid
Pyruvic acid	PPH	Picolinic acid
Riboflavin	Proline	Pipecolic acid
S-Adenosylhomocysteine	Pyridoxal	PPH
S-Adenosylmethionine	Pyridoxine	PPK
Salicylamide	Pyruvic acid	Proline
Salsolinol	Riboflavin	Propionylcarnitine
Succinic acid	S-Adenosylhomocysteine	Pyridoxal
Sucrose	S-Adenosylmethionine	Pyridoxine
Thiamine	Salicylamide	Pyruvic acid
Threonine	Salsolinol	Riboflavin
trans-4-Hydroxy-L-proline	Succinic acid	S-Adenosylhomocysteine
trans-Aconitic acid	Sucrose	S-Adenosylmethionine
trans-Cinnamic acid	Thiamine	Salicylamide
Uridine	Threonine	Stearic acid
Uridine monophosphate (UM	trans-Aconitic acid	Stearoyl Ethanolamide
Urocanic acid	trans-Cinnamic acid	Succinic acid
Valine	Uridine	Thiamine
Xanthine	Uridine monophosphate (UM	Threonine
α-D-Glucose-1,6-bisphosphat	Urocanic acid	Thymidine 5'-monophosphate
β-Alanine	Valine	trans-4-Hydroxy-L-proline
β-Nicotinamide mononucleot	Xanthine	trans-Aconitic acid
Y-Aminobutyric acid (GABA)	α-D-Glucose-1,6-bisphosphat	Uridine
ide	β-Alanine	Uridine monophosphate (UM
	β-Nicotinamide mononucleot	Urocanic acid
	Y-Aminobutyric acid (GABA)	Valine
	Y-Glutamylcysteine	Xanthine
		α,α-Trehalose
		α-D-Glucose-1,6-bisphosphate
		β-Alanine

β-Nicotinamide mononucleot
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

132	**136**	**142**
0	**1**	**0**

T5_35E_30ms	T5_60E_10ms	T5_60E_30ms
(+/-)-C75	(+/-)-C75	(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic a	(2Z)-2-Octyl-2-pentenedioic a	(2Z)-2-Octyl-2-pentenedioic a
10-Hydroxydecanoic acid	10-Hydroxydecanoic acid	10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid	12-Hydroxydodecanoic acid	12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acic	16-Hydroxyhexadecanoic acic	16-Hydroxyhexadecanoic acic
2-Aminoadipic acid	2-Aminoadipic acid	2-Aminoadipic acid
2'-Deoxyadenosine	2-Aminoisobutyric acid	2-Aminoisobutyric acid
2-Furoic acid	2'-Deoxyadenosine	2-Aminonicotinic acid
2-Hydroxycaproic acid	2-Furoic acid	2'-Deoxyadenosine
2-Hydroxyvaleric acid	2-Hydroxycaproic acid	2-Hydroxycaproic acid
2-Isopropylmalic acid	2-Hydroxyvaleric acid	2-Hydroxyvaleric acid
2-Oxobutyric acid	2-Isopropylmalic acid	2-Isopropylmalic acid
3'-Adenosine monophosphat	3'-Adenosine monophosphat	2-Methylglutaric acid
3-Hydroxy-3-methylglutaric a	3-Hydroxy-3-methylglutaric a	3'-Adenosine monophosphat
3-Hydroxybutyric acid	3-Hydroxybutyric acid	3-Hydroxy-3-methylglutaric a
3-Phenyllactic acid	3-Hydroxy-L-proline	3-Hydroxybutyric acid
4-Aminonicotinic acid	3-Phenyllactic acid	3-Phenyllactic acid
4-Coumaric acid	4-Acetamidobutanoic acid	4-Acetamidobutanoic acid
4-Hydroxybenzaldehyde	4-Aminonicotinic acid	4-Aminobenzoic acid
4-Oxoproline	4-Coumaric acid	4-Aminonicotinic acid
5-Aminolevulinic acid	4-Hydroxybenzaldehyde	4-Coumaric acid
5-Hydroxylysine	5-Aminolevulinic acid	4-Hydroxybenzaldehyde
5'-S-Methyl-5'-thioadenosine	5-Hydroxylysine	5-Aminolevulinic acid
6-Aminocaproic acid	5'-S-Methyl-5'-thioadenosine	5-Hydroxylysine
Acetyl-L-carnitine	6-Aminocaproic acid	5'-S-Methyl-5'-thioadenosine
Adenosine	Acetylcholine	6-Azidohexanoic acid
Adenosine 5'-monophosphat	Acetyl-L-carnitine	Acetylcholine
Adenosine diphosphate (ADP	Adenosine	Acetyl-L-carnitine
Adenosine triphosphate (ATP	Adenosine 5'-monophosphat	Adenosine
Argininosuccinic acid	Adenosine diphosphate (ADP	Adenosine 5'-monophosphat
Asparagine	Argininosuccinic acid	Adenosine diphosphate (ADP
Azelaic acid	Asparagine	Argininosuccinic acid
Benzoic acid	Azelaic acid	Asparagine
Biocytin	Benzoic acid	Azelaic acid
Biotin	Biocytin	Benzoic acid
Carnosine	Biotin	Biocytin
Choline	Carnosine	Biotin
Citric acid	Citric acid	Carnosine
Creatinine	CMPF	Choline
Cyclic ADP-ribose	Cyclic ADP-ribose	cis-4-Hydroxy-D-proline
Cystathionine	Cystathionine	Citric acid
Cysteinylglycine	Cysteinylglycine	Cyclic ADP-ribose
Cytidine 5'-diphosphocholine	Cytidine 5'-diphosphocholine	Cystathionine
Cytidine 5'-monophosphate (	Cytidine 5'-monophosphate (	Cysteinylglycine
D-(-)-Mannitol	D-(-)-Glutamine	Cytidine 5'-diphosphocholine

D-Carnitine	D-(-)-Mannitol	Cytidine 5'-monophosphate (
DL-3-Aminoisobutyric acid	D-(+)-Pyroglutamic Acid	D-(-)-Glutamine
DL-Arginine	D-Carnitine	D-(-)-Mannitol
DL-Dihydroorotic acid	DL-3-Aminoisobutyric acid	D-(+)-Pyroglutamic Acid
DL-Leucineamide	DL-Alanine	D-Carnitine
DL-Lysine	DL-Arginine	DL-3-Aminoisobutyric acid
DL-Tryptophan	DL-Dihydroorotic acid	DL-Alanine
Dodecanedioic acid	DL-Leucineamide	DL-Arginine
D-Ribose-1-phosphate	DL-Lysine	DL-Dihydroorotic acid
D-Sedoheptulose 7-phosphat	DL-Tryptophan	DL-Leucineamide
D-Serine	D-Sedoheptulose 7-phosphat	DL-Lysine
D-α-Hydroxyglutaric acid	D-Serine	DL-Tryptophan
Flavin mononucleotide (FMN	D-α-Hydroxyglutaric acid	D-Sedoheptulose 7-phosphat
Glucose 1-phosphate	Flavin mononucleotide (FMN	D-Serine
Guanosine 5'-diphosphate (G	Glucose 1-phosphate	D-α-Hydroxyglutaric acid
Guanosine triphosphate (GTP	Guanosine 5'-diphosphate (G	Erucamide
Indole-3-acrylic acid	Guanosine monophosphate (	Flavin mononucleotide (FMN
L-(-)-Malic acid	Hydrocinnamic acid	Guanosine 5'-diphosphate (G
L(+)-2-Aminobutyric acid	Indole-3-acrylic acid	Indole-3-acrylic acid
L-(+)-Arginine	L-(-)-Malic acid	L-(-)-Malic acid
L-(+)-Citrulline	L-(-)-Methionine	L-(-)-Methionine
L-Aspartic acid	L-(+)-Arginine	L-(+)-Arginine
Leucine	L-Aspartic acid	L-Aspartic acid
Levulinic acid	Leucine	L-Cystathionine
L-Glutamic acid	Levulinic acid	Leucine
L-Glutamine	L-Glutamic acid	Leucylproline
L-Glutathione (reduced)	L-Glutamine	Levulinic acid
L-Glutathione oxidized	L-Glutathione (reduced)	L-Glutamic acid
L-Histidine	L-Glutathione oxidized	L-Glutamine
L-Lysine	L-Histidine	L-Glutathione (reduced)
L-Norleucine	L-Lysine	L-Glutathione oxidized
L-Phenylalanine	L-Norleucine	L-Histidine
L-Pyroglutamic acid	L-Phenylalanine	L-Lysine
L-Saccharopine	L-Pyroglutamic acid	L-Norleucine
L-Serine	L-Saccharopine	L-Phenylalanine
L-Threonine	L-Threonine	L-Pyroglutamic acid
L-Tyrosine	Maleic acid	L-Saccharopine
Maleic acid	Malonic acid	L-Serine
Malonic acid	Mevalonic acid	L-Threonine
Methionine	N6,N6,N6-Trimethyl-L-lysine	L-Tyrosine
Mevalonic acid	N6-Acetyl-L-lysine	Maleic acid
N-[(1R,9S)-6-Oxo-11-(2-pyrim	N-Acetylaspartic acid	Malonic acid
N6,N6,N6-Trimethyl-L-lysine	N-Acetyl-D-alloisoleucine	Methionine
N6-Acetyl-L-lysine	N-Acetyl-DL-glutamic acid	Mevalonic acid
N8-Acetylspermidine	N-Acetylhistamine	N6,N6,N6-Trimethyl-L-lysine
N-Acetylaspartic acid	N-Acetyl-L-methionine	N6-Acetyl-L-lysine
N-Acetyl-D-alloisoleucine	N-Acetyl-L-phenylalanine	N8-Acetylspermidine

N-Acetyl-DL-glutamic acid	Nicotinic acid	N-Acetylaspartic acid
N-Acetyl-L-cysteine	Nicotinuric acid	N-Acetyl-D-alloisoleucine
N-Acetyl-L-methionine	NP-016212	N-Acetyl-DL-glutamic acid
N-Acetyl-L-phenylalanine	NP-020205	N-Acetylhistamine
Nicotinic acid	N-α-L-Acetyl-arginine	N-Acetyl-L-cysteine
Nicotinuric acid	Ornithine	N-Acetyl-L-methionine
NP-016212	Orotic acid	N-Acetyl-L-phenylalanine
NP-018741	Orotidine	Nicotinic acid
NP-020205	Orotidine 5'-monophosphate	Nicotinuric acid
N-α-L-Acetyl-arginine	Pantothenic acid	NP-020205
Ornithine	Paracetamol	N-α-L-Acetyl-arginine
Orotic acid	Phenylpyruvic acid	Ornithine
Orotidine	Picolinic acid	Orotic acid
Orotidine 5'-monophosphate	Pipecolic acid	Orotidine
Palmitoyl ethanolamide	PPK	Orotidine 5'-monophosphate
Pantothenic acid	Proline	Palmitoyl ethanolamide
Phenylpyruvic acid	Prolylleucine	Pantothenic acid
Picolinic acid	Propionylcarnitine	Paracetamol
Pipecolic acid	Pyridoxal	Phenylpyruvic acid
Proline	Pyridoxamine 5-phosphate	Phosphoenolpyruvic acid
Pyridoxal	Pyridoxine	Picolinic acid
Pyridoxine	Riboflavin	Pipecolic acid
Pyruvic acid	S-Adenosylhomocysteine	PPH
Riboflavin	S-Adenosylmethionine	Proline
S-Adenosylhomocysteine	Succinic acid	Pyridoxal
S-Adenosylmethionine	Sucrose	Pyridoxine
Salicylamide	Thiamine	Pyruvic acid
Succinic acid	Threonine	Riboflavin
Thiamine	Thymidine 5'-monophosphat	S-Adenosylhomocysteine
Threonine	trans-4-Hydroxy-L-proline	S-Adenosylmethionine
trans-4-Hydroxy-L-proline	trans-Aconitic acid	Succinic acid
trans-Aconitic acid	trans-Cinnamic acid	Sucrose
trans-Cinnamic acid	Uridine	Thiamine
Uridine	Uridine 5'-diphosphate (UDP)	Threonine
Uridine monophosphate (UM	Uridine monophosphate (UM	trans-4-Hydroxy-L-proline
Urocanic acid	Urocanic acid	trans-Aconitic acid
Valine	Valine	trans-Cinnamic acid
Xanthine	Xanthine	Uridine
α,α-Trehalose	α-D-Glucose-1,6-bisphosphat	Uridine monophosphate (UM
α-D-Glucose-1,6-bisphosphat	β-Alanine	Urocanic acid
β-Alanine	β-Nicotinamide mononucleot	Valine
β-Nicotinamide mononucleot	Y-Aminobutyric acid (GABA)	Xanthine
Y-Aminobutyric acid (GABA)	Y-Glutamylcysteine	α-D-Glucose-1,6-bisphosphat
		β-Alanine
		β-Nicotinamide mononucleot
		Y-Aminobutyric acid (GABA)
		Y-Glutamylcysteine

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ide

135	**135**	**139**
0	**1**	**2**

T10_35E_10ms	T10_35E_30ms	T10_60E_10ms
(+/-)-C75	(+/-)-C75	(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic a	10-Hydroxydecanoic acid	(2Z)-2-Octyl-2-pentenedioic a
10-Hydroxydecanoic acid	12-Hydroxydodecanoic acid	10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid	16-Hydroxyhexadecanoic acic	12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acic	2-Aminoadipic acid	16-Hydroxyhexadecanoic acic
2-Aminoadipic acid	2-Aminonicotinic acid	2-Aminoadipic acid
2'-Deoxyadenosine	2'-Deoxyadenosine	2-Aminobutyric acid
2-Furoic acid	2-Furoic acid	2-Aminonicotinic acid
2-Hydroxycaproic acid	2-Hydroxycaproic acid	2'-Deoxyadenosine
2-Hydroxyvaleric acid	2-Hydroxyvaleric acid	2-Hydroxycaproic acid
2-Isopropylmalic acid	2-Isopropylmalic acid	2-Hydroxyvaleric acid
2-Oxobutyric acid	2-Oxobutyric acid	2-Isopropylmalic acid
3'-Adenosine monophosphat	3'-Adenosine monophosphat	3'-Adenosine monophosphat
3-Hydroxy-3-methylglutaric a	3-Hydroxy-3-methylglutaric a	3-Hydroxy-3-methylglutaric a
3-Hydroxybutyric acid	3-Hydroxybutyric acid	3-Hydroxybutyric acid
3-Hydroxy-L-proline	3-Phenyllactic acid	3-Hydroxy-L-proline
3-Phenyllactic acid	4-Acetamidobutanoic acid	3-Phenyllactic acid
4-(3-Hydroxybutyl)phenyl β-D	4-Aminonicotinic acid	4-Acetamidobutanoic acid
4-Aminonicotinic acid	4-Coumaric acid	4-Aminonicotinic acid
4-Coumaric acid	4-Hydroxybenzaldehyde	4-Coumaric acid
4-Hydroxybenzaldehyde	5-Aminolevulinic acid	4-Hydroxybenzaldehyde
5-Aminolevulinic acid	5-Hydroxylysine	4-Methylumbelliferone
5-Hydroxylysine	5'-S-Methyl-5'-thioadenosine	5-Aminolevulinic acid
5'-S-Methyl-5'-thioadenosine	Acetohydroxamic acid	5-Hydroxylysine
Acetylcholine	Acetylcholine	5'-S-Methyl-5'-thioadenosine
Acetyl-L-carnitine	Acetyl-L-carnitine	Acetylcholine
Adenosine	Adenosine	Acetyl-L-carnitine
Adenosine 5'-monophosphat	Adenosine 5'-monophosphat	Adenosine
Adenosine diphosphate (ADP	Adenosine diphosphate (ADP	Adenosine 5'-monophosphat
Adenosine triphosphate (ATP	Argininosuccinic acid	Adenosine diphosphate (ADP
Argininosuccinic acid	Asparagine	Adenosine triphosphate (ATP
Asparagine	Benzoic acid	Argininosuccinic acid
Azelaic acid	Biocytin	Asparagine
Benzoic acid	Biotin	Azelaic acid
Biocytin	Carnosine	Benzoic acid
Biotin	Choline	Biocytin
Carnosine	Citric acid	Biotin
Citric acid	Creatinine	Carnosine
Creatinine	Cyclic ADP-ribose	Choline
Cyclic ADP-ribose	Cystathionine	Citric acid
Cystathionine	Cysteinylglycine	Cyclic ADP-ribose
Cytidine 5'-diphosphocholine	Cytidine 5'-diphosphocholine	Cystathionine
D-(-)-Glutamine	D-(-)-Glutamine	Cysteinylglycine
D-(-)-Mannitol	D-(-)-Mannitol	Cytidine 5'-diphosphocholine
D-Carnitine	D-Carnitine	Cytidine 5'-monophosphate (

DL-3-Aminoisobutyric acid	Dihydrothymine	D-(-)-Glutamine
DL-Arginine	DL-3-Aminoisobutyric acid	D-(-)-Mannitol
DL-Dihydroorotic acid	DL-Alanine	D-(+)-Pyroglutamic Acid
DL-Leucineamide	DL-Arginine	D-Carnitine
DL-Lysine	DL-Dihydroorotic acid	DL-3-Aminoisobutyric acid
DL-Tryptophan	DL-Leucineamide	DL-Alanine
Dodecanedioic acid	DL-Lysine	DL-Arginine
D-Ribose-1-phosphate	DL-Tryptophan	DL-Dihydroorotic acid
D-Sedoheptulose 7-phosphat	Dodecanedioic acid	DL-Leucineamide
D-Serine	D-Sedoheptulose 7-phosphat	DL-Lysine
D-α-Hydroxyglutaric acid	D-Serine	DL-Tryptophan
Ethyl myristate	Flavin mononucleotide (FMN	Dodecanedioic acid
Flavin mononucleotide (FMN	Glutaconic acid	D-Sedoheptulose 7-phosphat
Guanosine 5'-diphosphate (G	Guanosine 5'-diphosphate (G	D-Serine
Guanosine monophosphate (	Guanosine monophosphate (	D-α-Hydroxyglutaric acid
Hydrocinnamic acid	Hypoxanthine	Flavin mononucleotide (FMN
Hypoxanthine	Indole-3-acrylic acid	Guanosine 5'-diphosphate (G
Indole-3-acrylic acid	L-(-)-Malic acid	Guanosine monophosphate (
Isoniazid	L(+)-2-Aminobutyric acid	Hypoxanthine
L-(-)-Malic acid	L-(+)-Arginine	Indole-3-acrylic acid
L(+)-2-Aminobutyric acid	L-(+)-Citrulline	L-(-)-Malic acid
L-(+)-Arginine	L-Aspartic acid	L-(-)-Methionine
L-(+)-Citrulline	Leucine	L-(+)-Arginine
L-Aspartic acid	Leucylproline	L-(+)-Citrulline
Leucine	Levulinic acid	L(+)-Ornithine
Levulinic acid	L-Glutamic acid	L-Aspartic acid
L-Glutamic acid	L-Glutamine	Leucine
L-Glutamine	L-Glutathione (reduced)	Leucylproline
L-Glutathione (reduced)	L-Glutathione oxidized	Levulinic acid
L-Glutathione oxidized	L-Histidine	L-Glutamic acid
L-Histidine	L-Lysine	L-Glutamine
Lignoceric acid	L-Norleucine	L-Glutathione (reduced)
L-Lysine	L-Phenylalanine	L-Glutathione oxidized
L-Norleucine	L-Pyroglutamic acid	L-Histidine
L-Phenylalanine	L-Saccharopine	L-Lysine
L-Pyroglutamic acid	L-Serine	L-Norleucine
L-Saccharopine	L-Threonine	L-Phenylalanine
L-Serine	L-Tyrosine	L-Pyroglutamic acid
L-Threonine	Maleic acid	L-Saccharopine
L-Tyrosine	Malonic acid	L-Threonine
Maleic acid	Methionine	L-Tyrosine
Malonic acid	Mevalonic acid	Maleic acid
Methionine	N6,N6,N6-Trimethyl-L-lysine	Malonic acid
Mevalonic acid	N6-Acetyl-L-lysine	Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine	N8-Acetylspermidine	N-[(1R,9S)-6-Oxo-11-(2-pyrim
N6-Acetyl-L-lysine	N-Acetylaspartic acid	N6,N6,N6-Trimethyl-L-lysine
N8-Acetylspermidine	N-Acetyl-D-alloisoleucine	N6-Acetyl-L-lysine

N-Acetylaspartic acid	N-Acetyl-DL-glutamic acid	N8-Acetylspermidine
N-Acetyl-D-alloisoleucine	N-Acetyl-L-cysteine	N-Acetylaspartic acid
N-Acetyl-DL-glutamic acid	N-Acetyl-L-methionine	N-Acetyl-D-alloisoleucine
N-Acetyl-L-methionine	N-Acetyl-L-phenylalanine	N-Acetyl-DL-glutamic acid
N-Acetyl-L-phenylalanine	Nicotinuric acid	N-Acetylhistamine
N-Acetylornithine	N-α-L-Acetyl-arginine	N-Acetyl-L-methionine
Nicotinuric acid	Ornithine	N-Acetyl-L-phenylalanine
NP-008309	Orotic acid	Nicotinic acid
NP-016212	Orotidine	Nicotinuric acid
NP-018741	Orotidine 5'-monophosphate	NP-016212
NP-020205	Palmitoyl ethanolamide	NP-020205
N-α-L-Acetyl-arginine	Pantothenic acid	N-α-L-Acetyl-arginine
Ornithine	Paracetamol	Ornithine
Orotic acid	Phenylpyruvic acid	Orotic acid
Orotidine	Phosphoenolpyruvic acid	Orotidine
Orotidine 5'-monophosphate	Picolinic acid	Orotidine 5'-monophosphate
Palmitoyl ethanolamide	Pipecolic acid	Pantothenic acid
Pantothenic acid	PPH	Paracetamol
Paracetamol	Proline	Phenethylamine
Phenylpyruvic acid	Propionylcarnitine	Phenylpyruvic acid
Picolinic acid	Pyridoxal	Phosphoenolpyruvic acid
Pipecolic acid	Pyridoxine	Picolinic acid
PPH	Pyruvic acid	Pipecolic acid
PPK	Riboflavin	PPH
Proline	S-Adenosylhomocysteine	PPK
Prolylleucine	S-Adenosylmethionine	Proline
Propionylcarnitine	Salicylamide	Propionylcarnitine
Pyridoxal	Stearoyl Ethanolamide	Pyridoxal
Pyridoxine	Succinic acid	Pyridoxine
Pyruvic acid	Thiamine	Pyruvic acid
Riboflavin	Threonine	Riboflavin
S-Adenosylhomocysteine	Thymidine 5'-monophosphat	S-Adenosylhomocysteine
S-Adenosylmethionine	trans-4-Hydroxy-L-proline	S-Adenosylmethionine
Stearic acid	trans-Aconitic acid	Succinic acid
Succinic acid	trans-Cinnamic acid	Sucrose
Succinic semialdehyde	Uridine	Thiamine
Sucrose	Uridine 5'-diphosphate (UDP)	Threonine
Thiamine	Uridine monophosphate (UM	Thymidine 5'-monophosphat
Threonine	Urocanic acid	trans-4-Hydroxy-L-proline
Thymidine 5'-monophosphat	Valine	trans-Aconitic acid
trans-4-Hydroxy-L-proline	Xanthine	Uridine
trans-Aconitic acid	α,α-Trehalose	Uridine 5'-diphosphate (UDP)
Uridine	α-D-Glucose-1,6-bisphosphat	Uridine monophosphate (UM
Uridine monophosphate (UM	β-Alanine	Urocanic acid
Urocanic acid	β-Nicotinamide mononucleot	Valine
Valine	Y-Aminobutyric acid (GABA)	Xanthine
Xanthine	Y-Glutamylcysteine	α-D-Glucose-1,6-bisphosphat

α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

β-Alanine
β-Nicotinamide mononucleot
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

144	**139**	**143**
1	**2**	**1**

T10_60E_30ms
(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminobutyric acid
2-Aminoisobutyric acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
cis-4-Hydroxy-D-proline
Citric acid
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
Cytidine 5'-monophosphate (hydrate)

D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
Diacetyl
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Flavin mononucleotide (FMN)
Glutaconic acid
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Guanosine triphosphate (GTP)
Hypoxanthine
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine

N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetylhistamine
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Pantothenic acid
Paracetamol
Phenethylamine
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
PPH
Proline
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Succinic acid
Sucrose
Thiamine
Threonine
Thymidine
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)

Y-Glutamylcysteine
ide

140
0

T3_35E_10ms
(+/-)-C75
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2'-O-Methyladenosine
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-(3-Hydroxybutyl)phenyl β-D-glucopyranoside
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
4-Methylumbelliferone
5-Aminolevulinic acid
5-Aminovaleric acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Adenosine triphosphate (ATP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
Citric acid
CMPF
Creatinine
Cyclic ADP-ribose
Cystathionine

Cysteinylglycine
D-(-)-Glutamine
D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
DL-3-Aminoisobutyric acid
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Serine
Ethyl myristate
Flavin mononucleotide (FMN)
Fumaric acid
Guanosine 5'-diphosphate (GDP)
Guanosine 5'-diphospho-D-mannose
Guanosine monophosphate (GMP)
Imidazolelactic acid
Indole-3-acrylic acid
L-(-)-Malic acid
L(+)-2-Aminobutyric acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
Lignoceric acid
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Threonine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine

N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinuric acid
NP-018741
NP-020205
N-α-L-Acetyl-arginine
Oleamide
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
PPH
PPK
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salsolinol
Stearic acid
Succinic acid
Sucrose
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-Aconitic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)

Y-Glutamylcysteine

TOTAL # OF METABOLITES	**140**
TOTAL UNIQUE METABOLITES	**22**

T3_35E_30ms

(+/-)-C75
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2-Oxobutyric acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Hydroxy-L-proline
3-Phenyllactic acid
3-Phosphoglyceric acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
cis-4-Hydroxy-D-proline
Citric acid
Creatinine
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine

Cytidine 5'-monophosphate (hydrate)
D-(-)-Glutamine
D-(-)-Mannitol
D-Carnitine
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Serine
Flavin mononucleotide (FMN)
Guanosine 5'-diphosphate (GDP)
Indole-3-acrylic acid
Isoniazid
L-(-)-Malic acid
L(+)-2-Aminobutyric acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid

N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-008309
NP-018741
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Succinic acid
Thiamine
Threonine
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α,α-Trehalose
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
δ-Ribono-1,4-lactone
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

136
18

T5_35E_10ms
(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-(3-Hydroxybutyl)phenyl β-D-glucopyranoside
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
4-Methylumbelliferone
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Citric acid
Creatinine
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
D-(-)-Glutamine
D-(-)-Mannitol
D-(+)-Pyroglutamic Acid

D-Carnitine
DL-3-Aminoisobutyric acid
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
D-Mannose 6-phosphate
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Ethyl myristate
Flavin mononucleotide (FMN)
Glucose 1-phosphate
Guanosine 5'-diphosphate (GDP)
Guanosine 5'-diphospho-D-mannose
Hydrocinnamic acid
Hypoxanthine
Imidazolelactic acid
Indole-3-acrylic acid
L-(-)-Malic acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
Lignoceric acid
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N-[(1R,9S)-6-Oxo-11-(2-pyrimidinyl)-7,11-diazatricyclo[7
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine
N-Acetylaspartic acid

N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinuric acid
NP-018741
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Phosphoenolpyruvic acid
Picolinic acid
Pipecolic acid
PPH
PPK
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Stearic acid
Stearoyl Ethanolamide
Succinic acid
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-4-Hydroxy-L-proline
trans-Aconitic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α,α-Trehalose
α-D-Glucose-1,6-bisphosphate
β-Alanine

β-Nicotinamide mononucleotide	
Y-Aminobutyric acid (GABA)	
Y-Glutamylcysteine	
TOTAL # OF METABOLITES	**142**
TOTAL UNIQUE METABOLITES	**22**

T5_35E_30ms

(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2-Oxobutyric acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
4-Oxoproline
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Adenosine triphosphate (ATP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
Citric acid
Creatinine
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
Cytidine 5'-monophosphate (hydrate)
D-(-)-Mannitol

D-Carnitine
DL-3-Aminoisobutyric acid
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
Dodecanedioic acid
D-Ribose-1-phosphate
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Flavin mononucleotide (FMN)
Glucose 1-phosphate
Guanosine 5'-diphosphate (GDP)
Guanosine triphosphate (GTP)
Indole-3-acrylic acid
L-(-)-Malic acid
L(+)-2-Aminobutyric acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N-[(1R,9S)-6-Oxo-11-(2-pyrimidinyl)-7,11-diazatricyclo[7.3.1.02,7]trideca-2,4-dien-5-yl]isonicotinamide
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine

N-Acetyl-DL-glutamic acid
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-016212
NP-018741
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
Proline
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Succinic acid
Thiamine
Threonine
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α,α-Trehalose
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)

135
15

T10_35E_10ms
(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2-Oxobutyric acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Hydroxy-L-proline
3-Phenyllactic acid
4-(3-Hydroxybutyl)phenyl β-D-glucopyranoside
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Adenosine triphosphate (ATP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Citric acid
Creatinine
Cyclic ADP-ribose
Cystathionine
Cytidine 5'-diphosphocholine
D-(-)-Glutamine
D-(-)-Mannitol
D-Carnitine

DL-3-Aminoisobutyric acid
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
Dodecanedioic acid
D-Ribose-1-phosphate
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Ethyl myristate
Flavin mononucleotide (FMN)
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Hydrocinnamic acid
Hypoxanthine
Indole-3-acrylic acid
Isoniazid
L-(-)-Malic acid
L(+)-2-Aminobutyric acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
Lignoceric acid
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine

N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
N-Acetylornithine
Nicotinuric acid
NP-008309
NP-016212
NP-018741
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
PPH
PPK
Proline
Prolylleucine
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Stearic acid
Succinic acid
Succinic semialdehyde
Sucrose
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-4-Hydroxy-L-proline
trans-Aconitic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine

α-D-Glucose-1,6-bisphosphate	
β-Alanine	
β-Nicotinamide mononucleotide	
Y-Aminobutyric acid (GABA)	
Y-Glutamylcysteine	
TOTAL # OF METABOLITES	**144**
TOTAL UNIQUE METABOLITES	**21**

T10_35E_30ms

(+/-)-C75
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2-Oxobutyric acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
Acetohydroxamic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
Citric acid
Creatinine
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
D-(-)-Glutamine
D-(-)-Mannitol
D-Carnitine

Dihydrothymine
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
Dodecanedioic acid
D-Sedoheptulose 7-phosphate
D-Serine
Flavin mononucleotide (FMN)
Glutaconic acid
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Hypoxanthine
Indole-3-acrylic acid
L-(-)-Malic acid
L(+)-2-Aminobutyric acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine

N-Acetyl-DL-glutamic acid
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinuric acid
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Phosphoenolpyruvic acid
Picolinic acid
Pipecolic acid
PPH
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Stearoyl Ethanolamide
Succinic acid
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine 5'-diphosphate (UDP)
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α,α-Trehalose
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

139
16

T3_60E_10ms
(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2-Methylglutaric acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Benzoic acid
Biocytin
Biotin
Carnosine
Citric acid
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
Cytidine 5'-monophosphate (hydrate)
D-(-)-Glutamine
D-(+)-Pyroglutamic Acid
D-Carnitine
Diacetyl
DL-3-Aminoisobutyric acid

DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Flavin mononucleotide (FMN)
Guanosine 5'-diphosphate (GDP)
Guanosine 5'-diphospho-D-mannose
Hypoxanthine
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
N-Acetylornithine
Nicotinic acid
Nicotinuric acid
NP-008309
NP-016212

N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Phosphoenolpyruvic acid
Picolinic acid
Pipecolic acid
PPH
PPK
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Salsolinol
Succinic acid
Sucrose
Thiamine
Threonine
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)

TOTAL # OF METABOLITES **132**

TOTAL UNIQUE METABOLITES	**13**

T3_60E_30ms

(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminobutyric acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Hydroxy-L-proline
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Adenosine triphosphate (ATP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
Citric acid
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
D-(-)-Glutamine

D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Sedoheptulose 7-phosphate
D-Serine
Flavin mononucleotide (FMN)
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Hypoxanthine
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N,N-Dimethylglycine
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine

N-Acetyl-DL-glutamic acid
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-016212
NP-018741
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
PPH
Proline
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Salsolinol
Succinic acid
Sucrose
Thiamine
Threonine
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

T5_60E_10ms
(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminoisobutyric acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Hydroxy-L-proline
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Citric acid
CMPF
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
Cytidine 5'-monophosphate (hydrate)
D-(-)-Glutamine

D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Flavin mononucleotide (FMN)
Glucose 1-phosphate
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Hydrocinnamic acid
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Threonine
Maleic acid
Malonic acid
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetylhistamine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine

Nicotinic acid
Nicotinuric acid
NP-016212
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
PPK
Proline
Prolylleucine
Propionylcarnitine
Pyridoxal
Pyridoxamine 5-phosphate
Pyridoxine
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Succinic acid
Sucrose
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine 5'-diphosphate (UDP)
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

TOTAL # OF METABOLITES	**135**
TOTAL UNIQUE METABOLITES	**14**

T5_60E_30ms

(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminoisobutyric acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2-Methylglutaric acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminobenzoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Azidohexanoic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
cis-4-Hydroxy-D-proline
Citric acid
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine

Cytidine 5'-monophosphate (hydrate)
D-(-)-Glutamine
D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Erucamide
Flavin mononucleotide (FMN)
Guanosine 5'-diphosphate (GDP)
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-Aspartic acid
L-Cystathionine
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine

N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetylhistamine
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Phosphoenolpyruvic acid
Picolinic acid
Pipecolic acid
PPH
Proline
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Succinic acid
Sucrose
Thiamine
Threonine
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

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18

T10_60E_10ms
(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminobutyric acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Hydroxy-L-proline
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
4-Methylumbelliferone
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Adenosine triphosphate (ATP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
Citric acid
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
Cytidine 5'-monophosphate (hydrate)

D-(-)-Glutamine
D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
Dodecanedioic acid
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Flavin mononucleotide (FMN)
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Hypoxanthine
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-(+)-Citrulline
L(+)-Ornithine
L-Aspartic acid
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Mevalonic acid
N-[(1R,9S)-6-Oxo-11-(2-pyrimidinyl)-7,11-
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine

N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetylhistamine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-016212
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Pantothenic acid
Paracetamol
Phenethylamine
Phenylpyruvic acid
Phosphoenolpyruvic acid
Picolinic acid
Pipecolic acid
PPH
PPK
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Succinic acid
Sucrose
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-4-Hydroxy-L-proline
trans-Aconitic acid
Uridine
Uridine 5'-diphosphate (UDP)
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α-D-Glucose-1,6-bisphosphate

β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

TOTAL # OF METABOLITES	**143**
TOTAL UNIQUE METABOLITES	**16**

T10_60E_30ms

(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminobutyric acid
2-Aminoisobutyric acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
cis-4-Hydroxy-D-proline
Citric acid
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
Cytidine 5'-monophosphate (hydrate)

D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
Diacetyl
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Flavin mononucleotide (FMN)
Glutaconic acid
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Guanosine triphosphate (GTP)
Hypoxanthine
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine

N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetylhistamine
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Pantothenic acid
Paracetamol
Phenethylamine
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
PPH
Proline
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Succinic acid
Sucrose
Thiamine
Threonine
Thymidine
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)

Y-Glutamylcysteine

140
13

T3_35E_10ms
(+/-)-C75
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2'-O-Methyladenosine
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-(3-Hydroxybutyl)phenyl β-D-glucopyranoside
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
4-Methylumbelliferone
5-Aminolevulinic acid
5-Aminovaleric acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Adenosine triphosphate (ATP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
Citric acid
CMPF
Creatinine
Cyclic ADP-ribose
Cystathionine

Cysteinylglycine
D-(-)-Glutamine
D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
DL-3-Aminoisobutyric acid
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Serine
Ethyl myristate
Flavin mononucleotide (FMN)
Fumaric acid
Guanosine 5'-diphosphate (GDP)
Guanosine 5'-diphospho-D-mannose
Guanosine monophosphate (GMP)
Imidazolelactic acid
Indole-3-acrylic acid
L-(-)-Malic acid
L(+)-2-Aminobutyric acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
Lignoceric acid
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Threonine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine

N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinuric acid
NP-018741
NP-020205
N-α-L-Acetyl-arginine
Oleamide
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
PPH
PPK
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salsolinol
Stearic acid
Succinic acid
Sucrose
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-Aconitic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)

Y-Glutamylcysteine	
TOTAL # OF METABOLITES	**140**
TOTAL UNIQUE METABOLITES	**28**

T3_60E_10ms

(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2-Methylglutaric acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Benzoic acid
Biocytin
Biotin
Carnosine
Citric acid
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
Cytidine 5'-monophosphate (hydrate)
D-(-)-Glutamine
D-(+)-Pyroglutamic Acid
D-Carnitine
Diacetyl
DL-3-Aminoisobutyric acid

DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Flavin mononucleotide (FMN)
Guanosine 5'-diphosphate (GDP)
Guanosine 5'-diphospho-D-mannose
Hypoxanthine
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
N-Acetylornithine
Nicotinic acid
Nicotinuric acid
NP-008309
NP-016212

N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Phosphoenolpyruvic acid
Picolinic acid
Pipecolic acid
PPH
PPK
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Salsolinol
Succinic acid
Sucrose
Thiamine
Threonine
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)

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T3_35E_30ms
(+/-)-C75
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2-Oxobutyric acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Hydroxy-L-proline
3-Phenyllactic acid
3-Phosphoglyceric acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
cis-4-Hydroxy-D-proline
Citric acid
Creatinine
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine

Cytidine 5'-monophosphate (hydrate)
D-(-)-Glutamine
D-(-)-Mannitol
D-Carnitine
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Serine
Flavin mononucleotide (FMN)
Guanosine 5'-diphosphate (GDP)
Indole-3-acrylic acid
Isoniazid
L-(-)-Malic acid
L(+)-2-Aminobutyric acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid

N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-008309
NP-018741
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Succinic acid
Thiamine
Threonine
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α,α-Trehalose
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
δ-Ribono-1,4-lactone
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

TOTAL # OF METABOLITES **136**

TOTAL UNIQUE METABOLITES	**14**

T3_60E_30ms

(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminobutyric acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Hydroxy-L-proline
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Adenosine triphosphate (ATP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
Citric acid
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
D-(-)-Glutamine

D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Sedoheptulose 7-phosphate
D-Serine
Flavin mononucleotide (FMN)
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Hypoxanthine
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N,N-Dimethylglycine
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine

N-Acetyl-DL-glutamic acid
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-016212
NP-018741
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
PPH
Proline
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Salsolinol
Succinic acid
Sucrose
Thiamine
Threonine
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

T5_35E_10ms
(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-(3-Hydroxybutyl)phenyl β-D-glucopyranoside
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
4-Methylumbelliferone
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Citric acid
Creatinine
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
D-(-)-Glutamine
D-(-)-Mannitol
D-(+)-Pyroglutamic Acid

D-Carnitine
DL-3-Aminoisobutyric acid
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
D-Mannose 6-phosphate
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Ethyl myristate
Flavin mononucleotide (FMN)
Glucose 1-phosphate
Guanosine 5'-diphosphate (GDP)
Guanosine 5'-diphospho-D-mannose
Hydrocinnamic acid
Hypoxanthine
Imidazolelactic acid
Indole-3-acrylic acid
L-(-)-Malic acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
Lignoceric acid
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N-[(1R,9S)-6-Oxo-11-(2-pyrimidinyl)-7,11-diazatr
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine
N-Acetylaspartic acid

N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinuric acid
NP-018741
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Phosphoenolpyruvic acid
Picolinic acid
Pipecolic acid
PPH
PPK
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Stearic acid
Stearoyl Ethanolamide
Succinic acid
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-4-Hydroxy-L-proline
trans-Aconitic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α,α-Trehalose
α-D-Glucose-1,6-bisphosphate
β-Alanine

β-Nicotinamide mononucleotide	
Y-Aminobutyric acid (GABA)	
Y-Glutamylcysteine	
TOTAL # OF METABOLITES	**142**
TOTAL UNIQUE METABOLITES	**25**

T5_60E_10ms

(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminoisobutyric acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Hydroxy-L-proline
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Citric acid
CMPF
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
Cytidine 5'-monophosphate (hydrate)
D-(-)-Glutamine

D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Flavin mononucleotide (FMN)
Glucose 1-phosphate
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Hydrocinnamic acid
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Threonine
Maleic acid
Malonic acid
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetylhistamine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine

Nicotinic acid
Nicotinuric acid
NP-016212
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
PPK
Proline
Prolylleucine
Propionylcarnitine
Pyridoxal
Pyridoxamine 5-phosphate
Pyridoxine
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Succinic acid
Sucrose
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine 5'-diphosphate (UDP)
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

135
18

T5_35E_30ms
(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2-Oxobutyric acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
4-Oxoproline
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Adenosine triphosphate (ATP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
Citric acid
Creatinine
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
Cytidine 5'-monophosphate (hydrate)
D-(-)-Mannitol

D-Carnitine
DL-3-Aminoisobutyric acid
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
Dodecanedioic acid
D-Ribose-1-phosphate
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Flavin mononucleotide (FMN)
Glucose 1-phosphate
Guanosine 5'-diphosphate (GDP)
Guanosine triphosphate (GTP)
Indole-3-acrylic acid
L-(-)-Malic acid
L(+)-2-Aminobutyric acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N-[(1R,9S)-6-Oxo-11-(2-pyrimidinyl)-7,11-diaz
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine

N-Acetyl-DL-glutamic acid
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-016212
NP-018741
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
Proline
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Succinic acid
Thiamine
Threonine
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α,α-Trehalose
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)

TOTAL # OF METABOLITES	**135**
TOTAL UNIQUE METABOLITES	**17**

T5_60E_30ms

(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminoisobutyric acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2-Methylglutaric acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminobenzoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Azidohexanoic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
cis-4-Hydroxy-D-proline
Citric acid
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine

Cytidine 5'-monophosphate (hydrate)
D-(-)-Glutamine
D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Erucamide
Flavin mononucleotide (FMN)
Guanosine 5'-diphosphate (GDP)
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-Aspartic acid
L-Cystathionine
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine

N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetylhistamine
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Phosphoenolpyruvic acid
Picolinic acid
Pipecolic acid
PPH
Proline
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Succinic acid
Sucrose
Thiamine
Threonine
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

139
21

T10_35E_10ms
(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2-Oxobutyric acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Hydroxy-L-proline
3-Phenyllactic acid
4-(3-Hydroxybutyl)phenyl β-D-glucopyranoside
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Adenosine triphosphate (ATP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Citric acid
Creatinine
Cyclic ADP-ribose
Cystathionine
Cytidine 5'-diphosphocholine
D-(-)-Glutamine
D-(-)-Mannitol
D-Carnitine

DL-3-Aminoisobutyric acid
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
Dodecanedioic acid
D-Ribose-1-phosphate
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Ethyl myristate
Flavin mononucleotide (FMN)
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Hydrocinnamic acid
Hypoxanthine
Indole-3-acrylic acid
Isoniazid
L-(-)-Malic acid
L(+)-2-Aminobutyric acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
Lignoceric acid
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine

N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
N-Acetylornithine
Nicotinuric acid
NP-008309
NP-016212
NP-018741
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
PPH
PPK
Proline
Prolylleucine
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Stearic acid
Succinic acid
Succinic semialdehyde
Sucrose
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-4-Hydroxy-L-proline
trans-Aconitic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine

α-D-Glucose-1,6-bisphosphate	
β-Alanine	
β-Nicotinamide mononucleotide	
Y-Aminobutyric acid (GABA)	
Y-Glutamylcysteine	
TOTAL # OF METABOLITES	**144**
TOTAL UNIQUE METABOLITES	**19**

T10_60E_10ms

(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminobutyric acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Hydroxy-L-proline
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
4-Methylumbelliferone
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Adenosine triphosphate (ATP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
Citric acid
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
Cytidine 5'-monophosphate (hydrate)

D-(-)-Glutamine
D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
Dodecanedioic acid
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Flavin mononucleotide (FMN)
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Hypoxanthine
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-(+)-Citrulline
L(+)-Ornithine
L-Aspartic acid
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Mevalonic acid
N-[(1R,9S)-6-Oxo-11-(2-pyrimidinyl)-7,11-diazatricyclo[7.3.1.02,7]trideca-2,4-dien-5-yl]isonicotinamide
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine

N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetylhistamine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-016212
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Pantothenic acid
Paracetamol
Phenethylamine
Phenylpyruvic acid
Phosphoenolpyruvic acid
Picolinic acid
Pipecolic acid
PPH
PPK
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Succinic acid
Sucrose
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-4-Hydroxy-L-proline
trans-Aconitic acid
Uridine
Uridine 5'-diphosphate (UDP)
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α-D-Glucose-1,6-bisphosphate

β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

143
18

T10_35E_30ms
(+/-)-C75
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
2-Oxobutyric acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
Acetohydroxamic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
Citric acid
Creatinine
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
D-(-)-Glutamine
D-(-)-Mannitol
D-Carnitine

Dihydrothymine
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
Dodecanedioic acid
D-Sedoheptulose 7-phosphate
D-Serine
Flavin mononucleotide (FMN)
Glutaconic acid
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Hypoxanthine
Indole-3-acrylic acid
L-(-)-Malic acid
L(+)-2-Aminobutyric acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine

N-Acetyl-DL-glutamic acid
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinuric acid
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Phosphoenolpyruvic acid
Picolinic acid
Pipecolic acid
PPH
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Stearoyl Ethanolamide
Succinic acid
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine 5'-diphosphate (UDP)
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α,α-Trehalose
α-D-Glucose-1,6-bisphosphate
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)
Y-Glutamylcysteine

TOTAL # OF METABOLITES	**139**
TOTAL UNIQUE METABOLITES	**17**

T10_60E_30ms

(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminobutyric acid
2-Aminoisobutyric acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
Acetylcholine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Choline
cis-4-Hydroxy-D-proline
Citric acid
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
Cytidine 5'-monophosphate (hydrate)

D-(-)-Mannitol
D-(+)-Pyroglutamic Acid
D-Carnitine
Diacetyl
DL-3-Aminoisobutyric acid
DL-Alanine
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
DL-Tryptophan
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Flavin mononucleotide (FMN)
Glutaconic acid
Guanosine 5'-diphosphate (GDP)
Guanosine monophosphate (GMP)
Guanosine triphosphate (GTP)
Hypoxanthine
Indole-3-acrylic acid
L-(-)-Malic acid
L-(-)-Methionine
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Leucylproline
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Serine
L-Threonine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine

N8-Acetylspermidine
N-Acetylaspartic acid
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetylhistamine
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinic acid
Nicotinuric acid
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Pantothenic acid
Paracetamol
Phenethylamine
Phenylpyruvic acid
Picolinic acid
Pipecolic acid
PPH
Proline
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Succinic acid
Sucrose
Thiamine
Threonine
Thymidine
trans-4-Hydroxy-L-proline
trans-Aconitic acid
trans-Cinnamic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
β-Alanine
β-Nicotinamide mononucleotide
Y-Aminobutyric acid (GABA)

Y-Glutamylcysteine

140

18

HCD_35_T5_10ms
1-(4,5-dihydro-1H-imidazol-2-yl)-3,5-dim
16-Hydroxyhexadecanoic acid
1-Nitrosopyrrolidine (NPYR)
1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-
1-Vinylimidazole
2-(2-hydroxy-3-methylbutanamido)-4-m
2,5-di-tert-Butylhydroquinone
2,6-Dimethylpyrazine
2-Aminoadipic acid
2-Aminonicotinic acid
2-Aminooctanedioic acid
2'-Deoxyadenosine
2'-Deoxyadenosine 5'-monophosphate (
2'-Deoxyguanosine 5'-monophosphate (
2-Ethylhexanoic acid
2-Furoic acid
2-Hydroxycaproic acid
2-Isopropylmalic acid
2-Naphthalenesulfonic acid
2-Oxobutyric acid
3-(2,6-Dioxocyclohexyl)propanenitrile
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Methylcrotonylglycine
3-Phenyllactic acid
4-Aminonicotinic acid
4-chloro-5-morpholino-2-quinoxalin-2-yl
4-Dodecylbenzenesulfonic acid
4-Methyl-5-thiazoleethanol
4-Oxoproline
5-Aminovaleric acid
5-Hydroxylysine
5-morpholino-2,4(1H,3H)-pyrimidinedio
5'-S-Methyl-5'-thioadenosine
6-Aminocaproic acid
7-(tert-butyl)-4-imino-1,2,3,4,5,6-hexahy
7-Methylguanine
Acetylarginine
Acetylcholine
Acetyl-L-carnitine
Adenine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Adenosine triphosphate (ATP)

Asparagine
Azelaic acid
Benzoic acid
Betaine
Biocytin
Biotin
Chlormequat
Choline
cis-4-Hydroxy-D-proline
Citric acid
Creatinine
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine
Cytidine 5'-monophosphate (hydrate)
Cytosine
D-(-)-Mannitol
Debromohymenialdisine
Decanoic acid
Deoxyguanosine diphosphate (dGDP)
Desthiobiotin
Disperse orange 3
DL-Alanine
DL-Carnitine
DL-Dihydroorotic acid
DL-β-Leucine
D-Ribose-1-phosphate
Ethyl myristate
Flavin mononucleotide (FMN)
Glycyl-L-leucine
GPK
Guanine
Guanosine 5'-diphosphate (GDP)
Guvacoline
Imidazolelactic acid
Isobutyric acid
Isoleucine
L-(-)-Malic acid
L-(+)-Arginine
L-(+)-Lactic acid
Lactamide
L-Aspartic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized

L-Histidine
L-Lysine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-threo-3-Phenylserine
L-Threonic acid-1,4-lactone
L-Tyrosine
Methyl acetoacetate
Mevalonic acid
Myristyl sulfate
N,N-Dimethylaniline
N4-Acetyl sulfamethazine
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
N-Acetyl-α-D-glucosamine 1-phosphate
N-Ethylglycine
Nicotinamide
Nicotine
Nicotinic acid
Nicotinic acid mononucleotide
Nicotinuric acid
Nonanoic acid
NP-001346
N-Tigloylglycine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Pantothenic acid
PEG n7
Pentadecanoic acid
Phenylpyruvic acid
Pilocarpine
Pipecolic acid
PPK
Proline
Prolylglycine
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
S-Adenosylhomocysteine

S-Adenosylmethionine
S-Lactoylglutathione
Stearic acid
Succinic acid
Sucrose
Thiamine
Threonine
Thymidine
trans-3-Indoleacrylic acid
Tridecylic acid
Uracil
Uridine
Uridine monophosphate (UMP)
Valeric acid
Valine
Xanthine
α,α-Trehalose
α-D-Glucose-1,6-bisphosphate
β-Nicotinamide mononucleotide
δ-Ribono-1,4-lactone
δ-Valerolactam
Y-Glutamylcysteine
Y-L-Glutamyl-L-glutamic acid

TOTAL # OF METABOLITES	**162**
TOTAL UNIQUE METABOLITES	**81**

CID_T5_35E_10ms

(+/-)-C75
(2Z)-2-Octyl-2-pentenedioic acid
10-Hydroxydecanoic acid
12-Hydroxydodecanoic acid
16-Hydroxyhexadecanoic acid
2-Aminoadipic acid
2-Aminonicotinic acid
2'-Deoxyadenosine
2-Furoic acid
2-Hydroxycaproic acid
2-Hydroxyvaleric acid
2-Isopropylmalic acid
3'-Adenosine monophosphate (3'-AMP)
3-Hydroxy-3-methylglutaric acid
3-Hydroxybutyric acid
3-Phenyllactic acid
4-(3-Hydroxybutyl)phenyl β-D-glucopyranoside
4-Acetamidobutanoic acid
4-Aminonicotinic acid
4-Coumaric acid
4-Hydroxybenzaldehyde
4-Methylumbelliferone
5-Aminolevulinic acid
5-Hydroxylysine
5'-S-Methyl-5'-thioadenosine
Acetyl-L-carnitine
Adenosine
Adenosine 5'-monophosphate
Adenosine diphosphate (ADP)
Argininosuccinic acid
Asparagine
Azelaic acid
Benzoic acid
Biocytin
Biotin
Carnosine
Citric acid
Creatinine
Cyclic ADP-ribose
Cystathionine
Cysteinylglycine
Cytidine 5'-diphosphocholine
D-(-)-Glutamine
D-(-)-Mannitol
D-(+)-Pyroglutamic Acid

D-Carnitine
DL-3-Aminoisobutyric acid
DL-Arginine
DL-Dihydroorotic acid
DL-Leucineamide
DL-Lysine
D-Mannose 6-phosphate
D-Sedoheptulose 7-phosphate
D-Serine
D-α-Hydroxyglutaric acid
Ethyl myristate
Flavin mononucleotide (FMN)
Glucose 1-phosphate
Guanosine 5'-diphosphate (GDP)
Guanosine 5'-diphospho-D-mannose
Hydrocinnamic acid
Hypoxanthine
Imidazolelactic acid
Indole-3-acrylic acid
L-(-)-Malic acid
L-(+)-Arginine
L-(+)-Citrulline
L-Aspartic acid
Leucine
Levulinic acid
L-Glutamic acid
L-Glutamine
L-Glutathione (reduced)
L-Glutathione oxidized
L-Histidine
Lignoceric acid
L-Lysine
L-Norleucine
L-Phenylalanine
L-Pyroglutamic acid
L-Saccharopine
L-Threonine
L-Tyrosine
Maleic acid
Malonic acid
Methionine
Mevalonic acid
N-[(1R,9S)-6-Oxo-11-(2-pyrimidinyl)-7,11-diazatricyclo[7.3.1.02,7]trideca-2,4-dien-5-yl]isonicotinamide
N6,N6,N6-Trimethyl-L-lysine
N6-Acetyl-L-lysine
N8-Acetylspermidine
N-Acetylaspartic acid

N-Acetyl-D-alloisoleucine
N-Acetyl-DL-glutamic acid
N-Acetyl-L-cysteine
N-Acetyl-L-methionine
N-Acetyl-L-phenylalanine
Nicotinuric acid
NP-018741
NP-020205
N-α-L-Acetyl-arginine
Ornithine
Orotic acid
Orotidine
Orotidine 5'-monophosphate
Palmitoyl ethanolamide
Pantothenic acid
Paracetamol
Phenylpyruvic acid
Phosphoenolpyruvic acid
Picolinic acid
Pipecolic acid
PPH
PPK
Proline
Propionylcarnitine
Pyridoxal
Pyridoxine
Pyruvic acid
Riboflavin
S-Adenosylhomocysteine
S-Adenosylmethionine
Salicylamide
Stearic acid
Stearoyl Ethanolamide
Succinic acid
Thiamine
Threonine
Thymidine 5'-monophosphate
trans-4-Hydroxy-L-proline
trans-Aconitic acid
Uridine
Uridine monophosphate (UMP)
Urocanic acid
Valine
Xanthine
α,α-Trehalose
α-D-Glucose-1,6-bisphosphate
β-Alanine

β-Nicotinamide mononucleotide

Y-Aminobutyric acid (GABA)

Y-Glutamylcysteine

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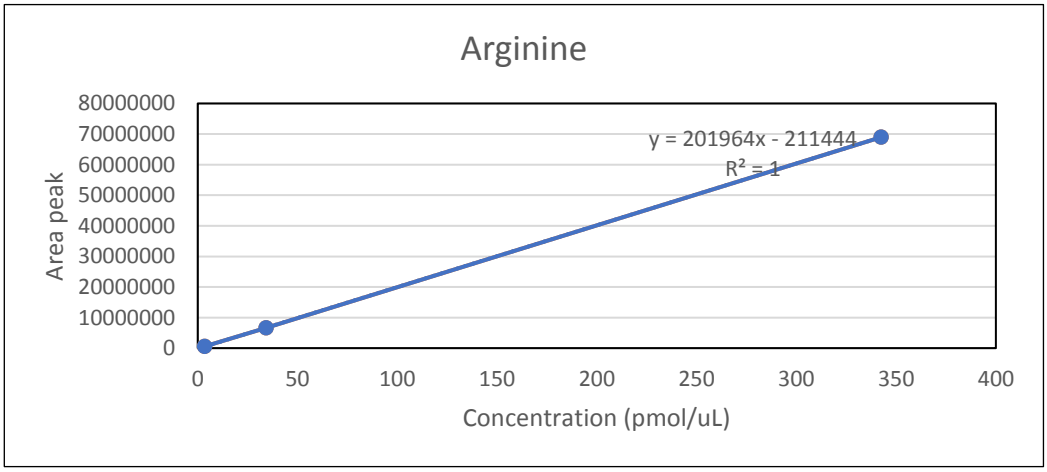
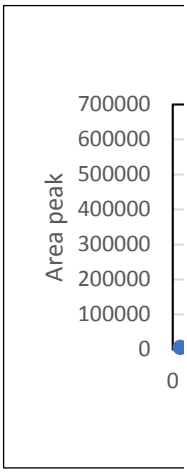
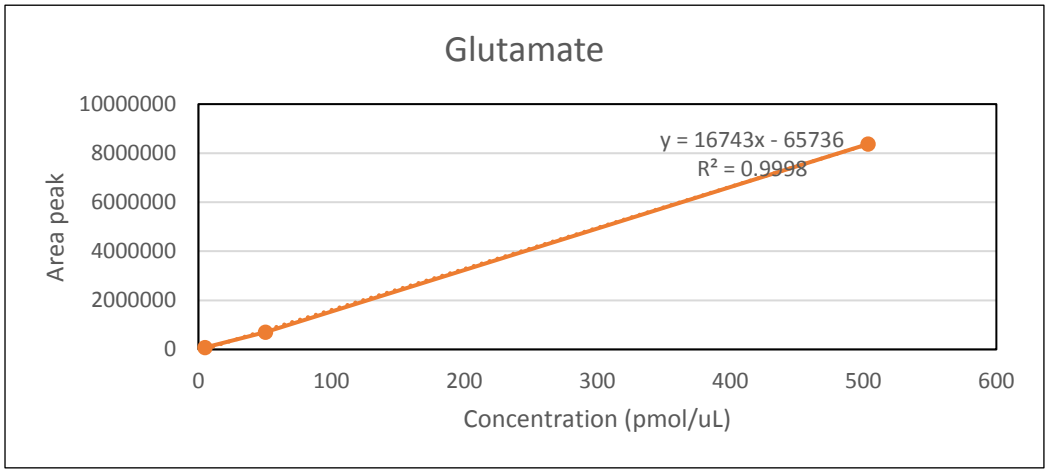
Supplemental File (Figures, Permissions, etc.)

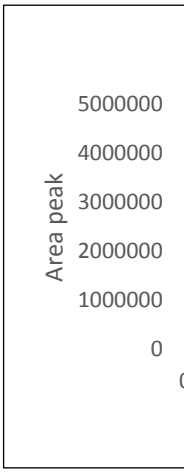
Click here to access/download;Supplemental File (Figures, Permissions, etc.);Supplemental Table 5.xlsx

Standards of amino acids	pmol/µl (1)	area (1)	pmol/µl (2)	area (2)
arginine	342.5181783	68975611.8	34.25181783	6586474.68
aspartate	375.0313048	589880.09	37.50313048	53449.968
glutamate	503.5229616	8370998.15	50.35229616	708464.311
glycine	742.6402025	4204003	74.26402025	409231.24
histidine	757.8198849	3649965.65	75.78198849	335932.327
isoleucine	551.4472313	6389811.14	55.14472313	585925.643
leucine	678.5088054	7662647.14	67.85088054	789436.398
lysine	342.5906925	67427310.2	34.25906925	7142717.02
methionine	294.3279047	10596384.9	29.43279047	1095386.5
phenylalanine	246.6856347	46151068.6	24.66856347	4679971.94
serine	466.2670092	1445639.42	46.62670092	150445.735
tryptophan	266.8559957	6079448.14	26.68559957	530178.98
tyrosine	209.2646761	60680158.9	20.92646761	5397861.96
valine	447.4320672	4227266.63	44.74320672	381341.162



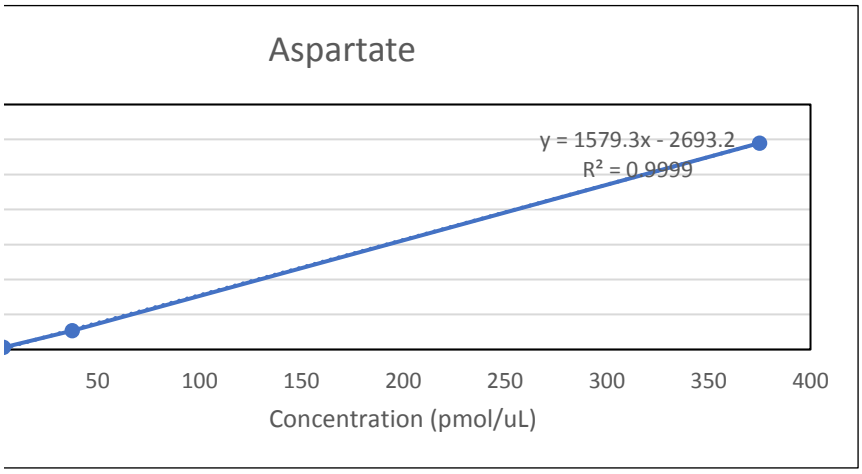


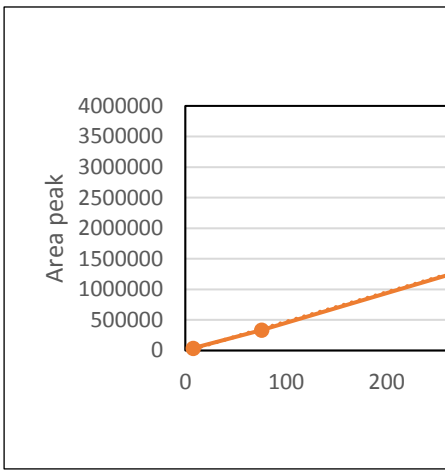


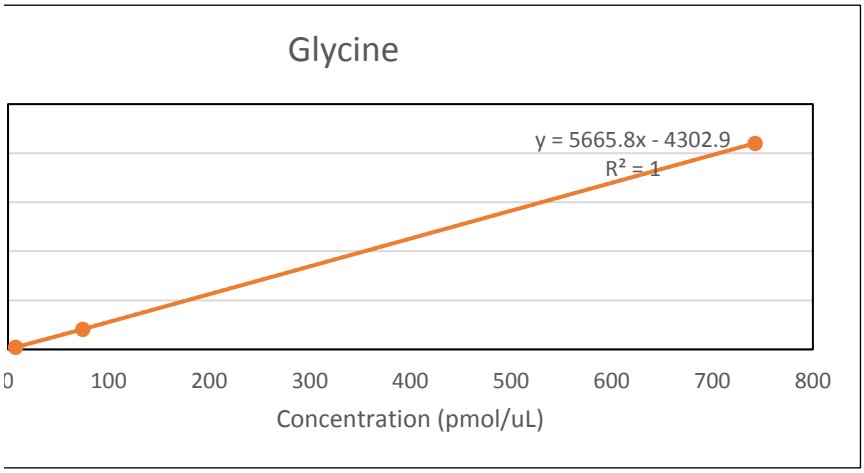


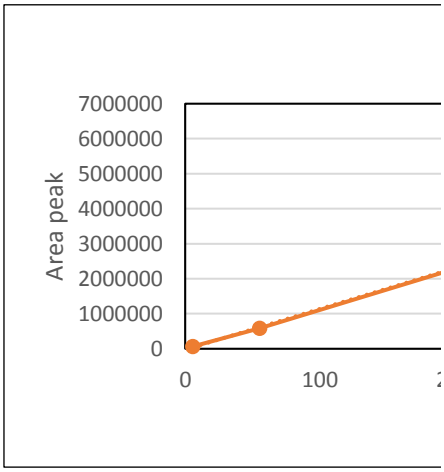
pmol/µl (3)	area (3)
3.425181783	589134.3127
3.750313048	6035.330608
5.035229616	81156.6871
7.426402025	44346.88481
7.578198849	38136.00322
5.514472313	62893.45098
6.785088054	81555.44206
3.425906925	700842.3171
2.943279047	112158.969
2.466856347	415581.5151
4.662670092	13424.07599
2.668559957	57209.12043
2.092646761	605386.0766
4.474320672	36127.28033

Arginine		Aspartate	
342.518178	68975611.8	375.031305	589880.09
34.2518178	6586474.68	37.5031305	53449.968
3.42518178	589134.313	3.75031305	6035.33061

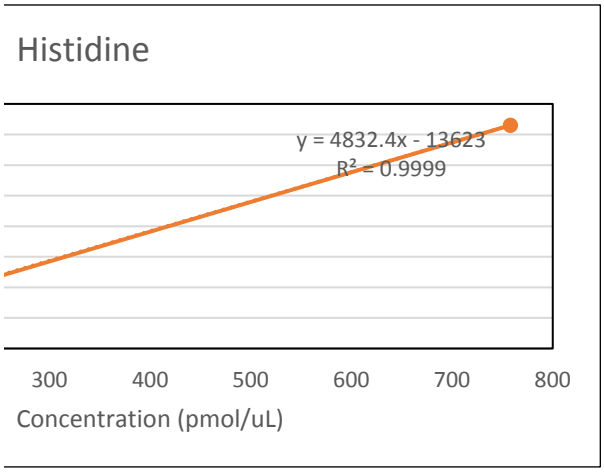


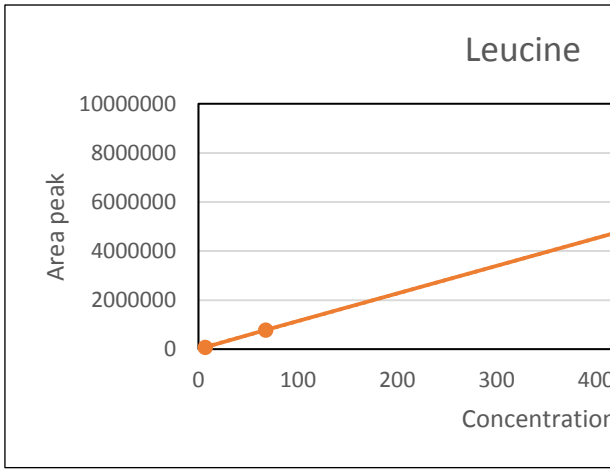


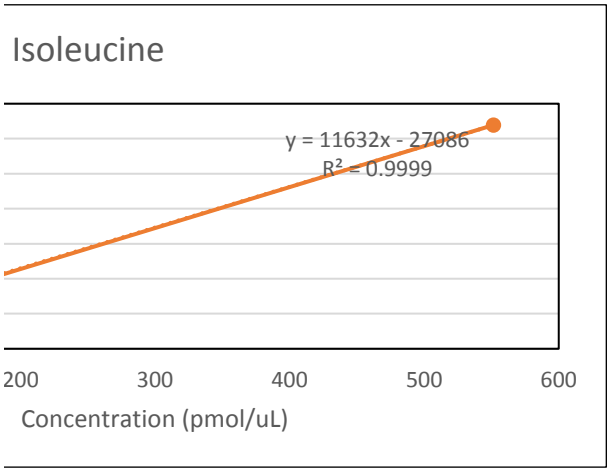


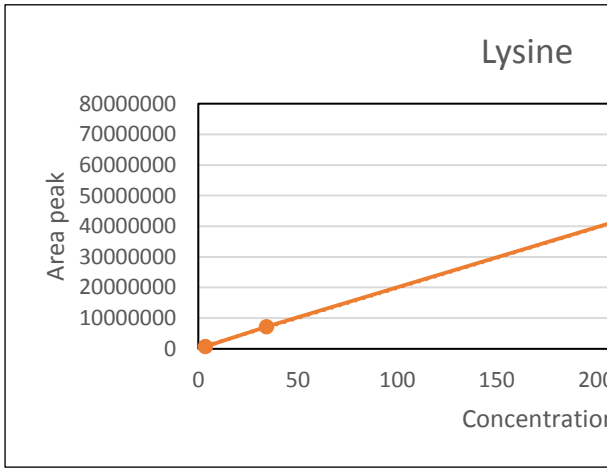


Glutamate		Glycine		Histidine		Isoleucine
503.522962	8370998.15	742.640202	4204003	757.819885	3649965.65	551.447231
50.3522962	708464.311	74.2640202	409231.24	75.7819885	335932.327	55.1447231
5.03522962	81156.6871	7.42640202	44346.8848	7.57819885	38136.0032	5.51447231

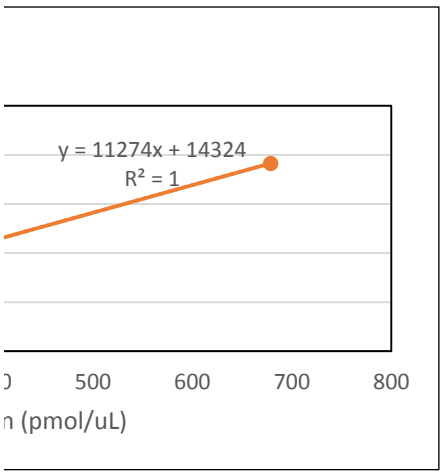


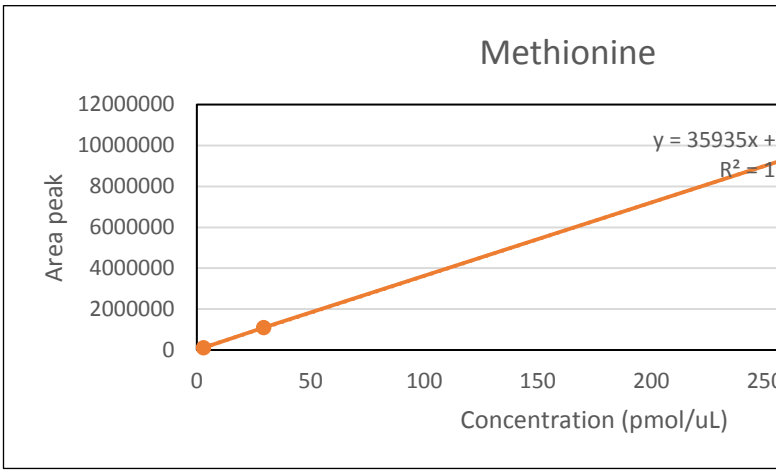


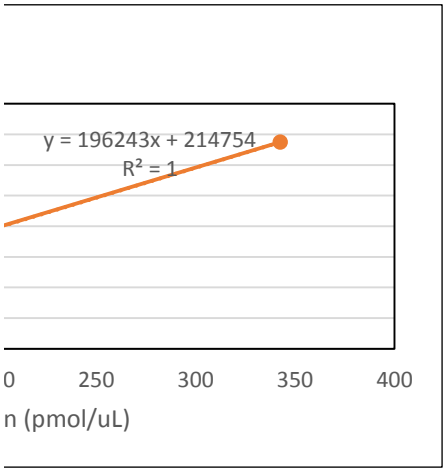


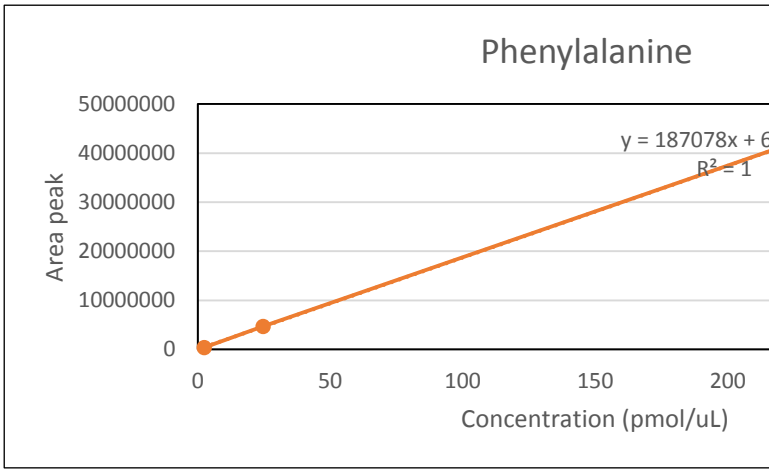


	Leucine		Lysine		Methionine	
6389811.14	678.508805	7662647.14	342.590692	67427310.2	294.327905	10596384.9
585925.643	67.8508805	789436.398	34.2590692	7142717.02	29.4327905	1095386.5
62893.451	6.78508805	81555.4421	3.42590692	700842.317	2.94327905	112158.969

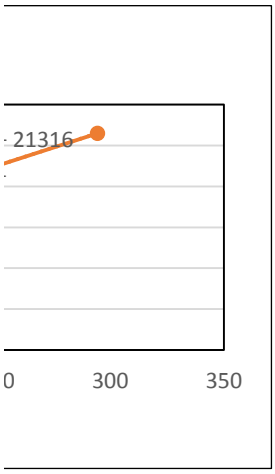


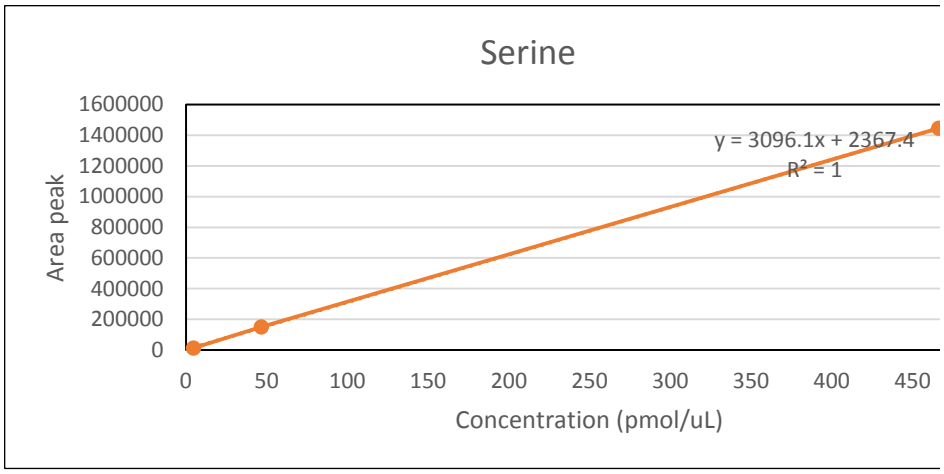


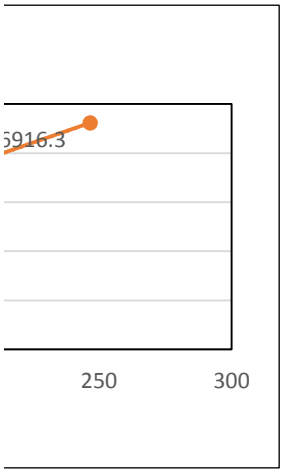


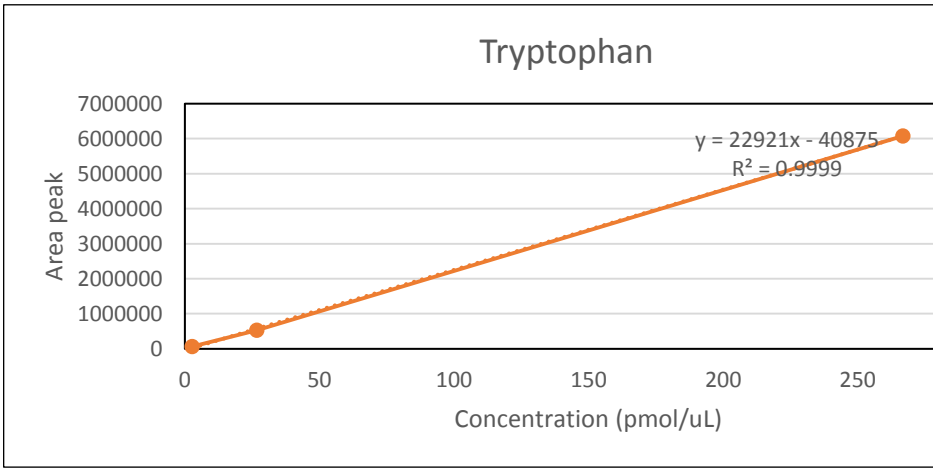


Phenylalanine		Serine		Typtophan		Tyrosine
246.685635	46151068.6	466.267009	1445639.42	266.855996	6079448.14	209.264676
24.6685635	4679971.94	46.6267009	150445.735	26.6855996	530178.98	20.9264676
2.46685635	415581.515	4.66267009	13424.076	2.66855996	57209.1204	2.09264676









	Valine

60680158.9	447.432067	4227266.63
5397861.96	44.7432067	381341.162
605386.077	4.47432067	36127.2803