HIGH PRECISION METABOLOMICS

Tharsana Mahendranathan Sellathurai

Master Thesis
Bioanalytical Chemistry

60 credits

Department of Chemistry
Faculty of Mathematics and Natural Sciences

UNIVERSITY OF OSLO

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Abstract

The human metabolome is a collection of small molecules (metabolites). Global metabolomics aims to study the whole metabolome, providing detailed human metabolic profiles. This may improve our understanding of the physiology of health and disease, discover novel metabolites, and identify new or better biomarkers of disease and the effect of treatment. Metabolite identifications are one of the biggest bottlenecks for obtaining reliable data of diagnostic relevance. Identification should ideally have the highest level of confidence to be of diagnostic value. This is obtained by using a reference in-house library, containing the fragmenting spectra (MS^2) and the retention time of metabolite standards analyzed on the same analytical platform operated under identical conditions

In this master thesis an in-house library, containing retention time and MS^2 for 442 standard metabolites, was created and tested on an untargeted metabolomics data processing workflow. The standard metabolites were obtained from a mass spectrometer metabolite library standard (MS-MLS) kit, consisting of 603 dry metabolites. These are important metabolites and intermediated in main metabolic pathways. Non-isobaric mixtures of the standard metabolites were made to save time and resources, prior to being analyzed on a developed in-house high-resolution LC-MS method. To extract MS^2 and RT for each metabolite computer software was used, and the MS^2 was verified with online MS^2 databases. The created in-house library consists mainly of $[M+H]^+$ and $[M-H]^-$. By grouping the metabolites according to their chemical classes, it was found that fatty acyls, aldehydes, and triphosphates metabolites were generally difficult to detect with the method used, and in another MS-MLS study using other separation principles. The in-house library, implemented in an untargeted metabolomics workflow, provided identifications with a confidence level of 1 when the MS^2 match factor was above 90%. With the created in-house library, it was shown that a biomarker for an inborn error of metabolism not diagnosed in Norway can be quantitatively detected. Hence, using the created in-house library with the developed high-resolution LC-MS method, patients with this inborn error of metabolism may be diagnosed, and other detectable biomarkers may also be highlighted.

PREFACE

This thesis was performed at the National Unit of Screening and Congenital Pediatric Metabolic Disorders at Oslo University Hospital, Rikshospitalet, from January 2020 to May 2022. My supervisors have been Katja B. P. Elgstøen at Rikshospitalet, and Steven R. H. Wilson at the Department of Chemistry, University of Oslo.

First and foremost, I would like to thank Katja for all her encouragement, insight, and knowledge during my time working on the thesis. I have learned a lot from her and the others in her research group. I am grateful that I had the opportunity to meet these amazing people and work on my master thesis with their guidance

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Thank you to everyone at IKB for being so welcoming, and for all the conversations and lunches, and thank you everyone at the BacH group at the Department of Chemistry for being so friendly.

Finally, I would like to give a huge thanks to my family and friends for supporting me throughout my time working with this thesis 😊

Oslo, Norway, May 2022

Tharsana Mahendranathan Sellathurai

Table of Content

1 ABBREVIATIONS

Abbreviations	Term
ACN	Acetonitrile
APCI	Atmospheric pressure chemical ionization
APPI	Atmospheric pressure photoionization
C	Concentation
Da	Dalton
DIA	Data-dependent acquisition
ddMS2	Data-dependent tandem mass spectrometry
DDA	Direct-dependent acquisition
DBS	Dried blood spots
ESI	Electrospray ionization
EIC	Extracted ion chromatogram
FA	Formic acid
FWHM	Full width at half maximum
GC	Gas chromatography
GC-MS	Gas chromatography
HR	High resolution
HPLC	High-performance liquid chromatography
HILIC	Hydrophilic interaction liquid chromatography
IEM	Inborn errors of metabolism
IPA	Isopropanol
LC	Liquid chromatography
LC-MS	Liquid chromatography - mass spectrometry
LR	Low resolution
m	Mass
MRM	Multiple reaction monitoring
MS-MLS	Mass Spectrometer - Metabolite Standard Library
MS	Mass Spectrometery

m/z	mass-to-charge ratio
MeOH	Methanol
MP	Mobile phase
Mm	Molar mass
M	Molecule
NBS	Newborn Screening
NMR	Nuclear magnetic resonance
N	Number of replicates
C18	Ocatadecyl
C8	Octyl
ppm	Part per million
q	quadrupol
Q-ToF	Quadrupole- time-of-flight
RF	Radio frequency
REC	Regional Commitee for Medical and Health Research Ethics
RT	Retention time
RPLC	Reverse phase liquid chromatography
rpm	Rotations per minute
SRM	Selected reaction monitoring
Std	Standard
SP	Stationary phase
R	The resolving power
ToF	Time-of-flight
TIC	Total ion chromatogram
TMA	Trimethylamineuria
TMAU	Trimethylamineuria
HMDB	Human metabolome database
SMILES	Simplified molecular-input line-entry system
RI	Relative intensity

2 INTRODUCTION

2.1 Metabolome – *A snapshot of the metabolic biochemistry*

The metabolome is the complete collection of all small molecules, typically under 1.5 kDa, present in body fluids, cells, tissues, organs, or an entire organism. These molecules are called metabolites. Organic acids, amino acids, lipids, and nucleic acids, carbohydrates are some examples (1-3).

Metabolites can be categorized into endogenous and exogenous metabolites. Endogenous metabolites are the results of the genome's downstream products and are produced by catabolism or anabolism. These metabolites are necessary for growth, development, and normal function**.** Exogenous metabolites are absorbed from the biological system's external environment, such as through diet, drugs, and pollutants, as well as from the intestinal microbiome (1).

Endogenous and exogenous metabolites are involved in biochemical pathways, which are usually catalyzed by enzymes. These are important for detoxification of xenobiotic metabolites, degradation of complex metabolites, and metabolite transformation to produce energy, anabolic substrates, or other necessary metabolites (4, 5). The biochemical pathways are further intertwined in a highly interconnected network with other biochemical pathways, as seen in **Figure 2.1**. Among metabolisms of different classes of metabolites, the figure illustrates how for example the metabolism of amino acids is connected to the metabolism of lipids (6)

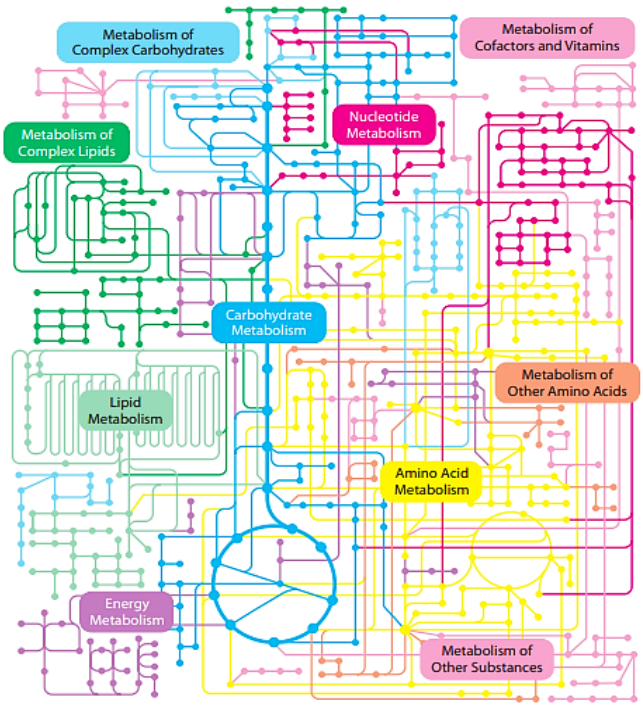


Figure 2.1: A part of the interconnected biochemical network of the metabolome (6)

The human metabolome is heterogeneous and complex. There is a large variety in molecular weight, hydrophobicity, charge, and concentrations (7). The metabolomes of body fluids, cells, tissues, and organs may differ distinctly. Each organ is specialized to produce or consume different levels of metabolites. In the heart and brain, for example, glucose consumption is highest, while urea and bile acids are only produced in the liver (1). Hence, the concentration of metabolites detected can vary from the ng/L to the g/L range (8).

The human metabolome's total size is not yet known or defined. The human metabolome database (HMDB) is a free database that contains information on endogenous and exogenous metabolites (3, 9). According to HMDB as of 2018, 114 109 unique metabolites were expected in the human metabolome, when considering metabolites detected in human samples, and those to be expected based on known genes and metabolic pathways, see **Figure 2.2**. However, HMDB is continually growing (10). In the latest version 5.0, the number of currently registered metabolites expected in the human body had increased to 217 920 unique metabolites (11). **Figure 2.2** shows the total number as of 2018 (10). Lipids constitute the majority of the expected metabolites, because of their well-known structure and biochemical pathways (3).

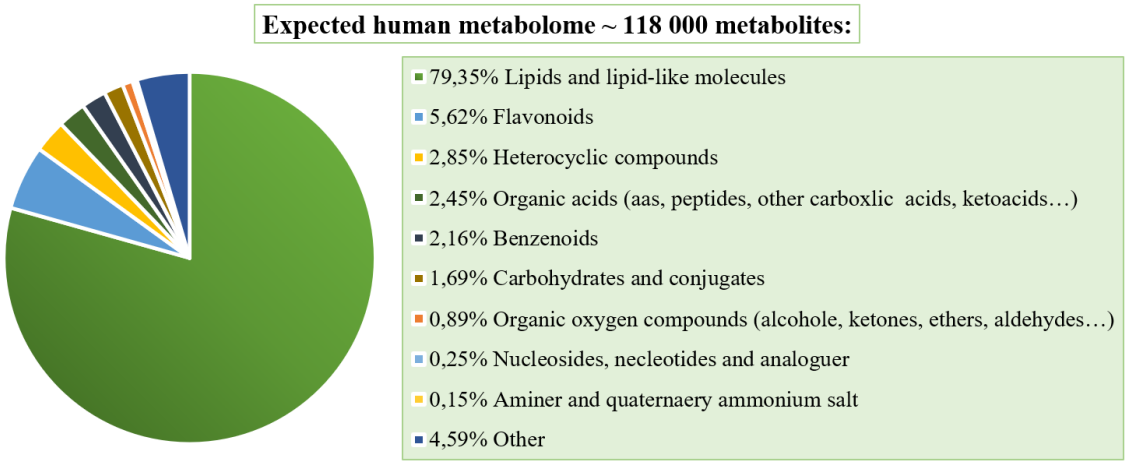


Figure 2.2: A distribution of unique metabolites, expected in the humane metabolome. *Of these, 114 109 are from HMDB, and the rest are unique metabolites registered in other databases (KEGG, VMH, HumanCyc) (10).*

According to Wishart et. al. (1) and Idle et. al. (12) the metabolome may consist of more than 1 million metabolites due to the huge exposure of metabolites humans experiences, such as through diet and environmental chemicals. With a computational tool called BioTransformer, that may consider biotransformations exogenous and endogenous metabolites can undergo, Wishart and his lab estimated that the human metabolome can potentially consist of a selection of 5 million metabolites (10).

The metabolome is highly sensitive to age, gender, diet-health, and genetic changes that can lead to a 10 000x change in the concentration of individual metabolites (1, 13, 14). Hence, a measurement of the metabolome is considered to give a better presentation of an individual's phenotype, than the genome and proteome (1, 15, 16). The observable characteristics of an individual, which are dependent on genetic and environmental factors, are referred to as the individuals' phenotype (17). **Figure 2.3** shows the environmental and physiological factors' level of influence on the metabolome and illustrates how a person's diet will give the most dynamic response, and thereby be a better indicator of environmental influence, compared to proteome and genome (1).

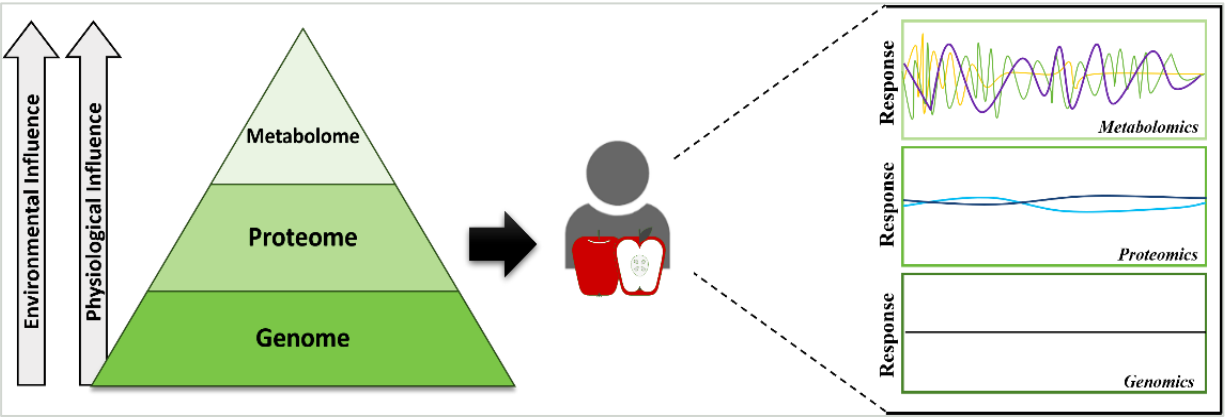


Figure 2.3: Environmental and physiological influence on genome, proteome, and metabolome. *Hierarchy with the genome, proteome, and metabolome organized relative to the increasing influence of environmental and physiological factors. This illustrates the dynamics in the responses seen in the metabolome, proteome, and genome after intake of food. The figure is adapted from (14).*

2.2 Inborn errors of metabolism

Inborn errors of metabolism (IEM) are defects in a metabolic pathway, giving pathological congenital disorders. These can cause irreversible neurological and/or other physical or mental damage, which can be life-threatening and even fatal (4, 18). IEMs can be divided into different groups of disorders, such as amino acid disorders, fatty acid oxidation defects, urea cyclic defects, and purine and pyrimidine metabolism disorders (19). These disorders can lead to intoxication, defects in energy metabolism, or defect degradation or synthesis of complex molecules (19, 20). Currently, more than 1500 IEM are known (21). IEMs are typically caused by autosomal recessive inherited single genetic mutations, that gives defective syntheses of relevant proteins like enzymes or membrane transporter, necessary for enzyme activity in metabolic pathways, transport, and/or excretion of metabolites. IEMs may also be a result of spontaneous mutations (22, 23).

Figure 2.4 gives a simplified presentation of a biochemical reaction with an IEM. Metabolite A is transformed into metabolite B. Because of a block between metabolite B and C, metabolite B accumulates and might also be transformed into another potentially toxic metabolite. Because of the block, there will also be a deficiency of metabolite C. This block is caused by an enzyme deficiency. In normal circumstances, without IEM, metabolite B would be transformed into the end metabolite C.

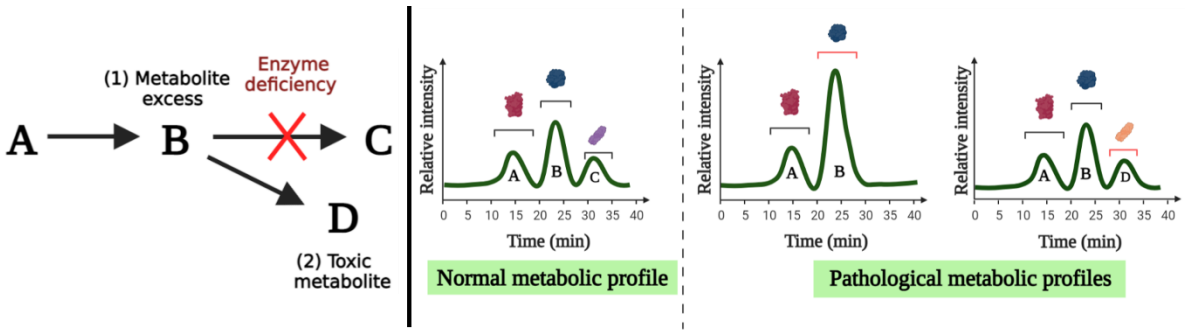


Figure 2.4: A biochemical pathway with IEM, illustration of a normal metabolic profile, and pathological metabolic profiles. *A, B, C, and D are different metabolites. Between metabolites B and C there is a block, preventing the transformation of metabolite B to metabolite C. On the right side, there is an illustration of a normal versus pathological metabolic profiles when including metabolite B, C, and D.*

A person with IEM will have a pathological metabolic profile, with metabolites that stand out in comparison with the metabolites in a normal metabolic profile. These metabolites are used as biomarkers in clinical diagnostics to determine if a patient has an IEM (23). Figure 4 illustrates the difference between a normal and two alternative pathological metabolic profiles. Here metabolites B, C, and D, or a ratio between them can be used as biomarkers.

Phenylketonuria

Phenylketonuria (PKU) is an example of an inherited autosomal recessive IEM (19). It is the most common disruption that may occur in the amino acid metabolism (1:10 000) (24). Here, a deficiency of the enzyme hepatic phenylalanine hydroxylase (PAH), reduces the transformation of phenylalanine to tyrosine and gives a toxic build-up in the blood and brain phenylalanine (1, 24), causing severe symptoms like reduced cognitive function, and seizures (23-25).

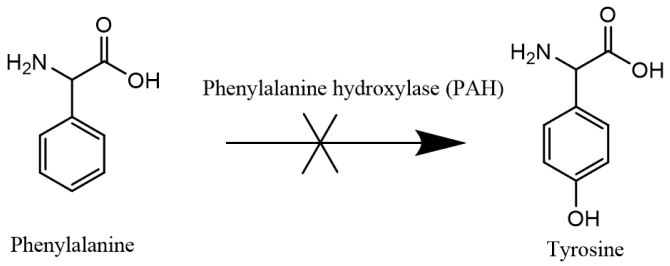


Figure 2.5: The biochemical reaction for metabolizing phenylalanine to tyrosine. *A deficiency of PAH inhibits the reaction, and phenylalanine accumulates.*

Patients with IEM can sometimes be treated by reducing the dietary intake of upstream metabolites of the enzymatic block or by the supplementation of downstream metabolites or giving them an enzyme replacement (22, 23, 26). For a patient with Phenylketonuria, the primary treatment is to limit the intake of proteins consisting of phenylalanine, i.e. protein restriction, and to supplement with other amino acids thus needed (27).

2.2.1 Diagnosis of IEMs

Early diagnosis can help avoid permanent outcomes, or reduce the severity of disruptive metabolic pathways (28). For these reasons, laboratories in different countries offer to do newborn screening (NBS) for a limited number of treatable IEM, and in some countries it is mandatory (19, 29).

For NBS, dried blood spots (DBS) are collected from a heel prick of newborns (19, 30), and biomarkers for a group of specified IEMs are analyzed. NBS was first introduced with the detection of PKU in the 1960s. Newborns with PKU don't show any noticeable symptoms before a few months, which already then may be irreversible if early treatment hasn't begun. With NBS newborns can early on be diagnosed and start treatment (31). In mid-1990s the development of instruments enhanced, and the inclusion of other IEMs in the NBS program expanded (32). The criteria for including a disease in the NBS program are strict, and are influenced by Wilson and Junger's criteria for screening, published in 1968 (33). Some of the criteria among these are acceptable treatment for the disease exists and the treatment is more efficient if begun quickly as possible. The IEMs selected in the NBS program, are also those with a high incidence of causing irreversible organ damage (18, 34).

Norway offers a newborn screening program consisting of 26 diseases, of which 21 are congenital metabolic disorders (35, 36). Phenylketonuria and Cystic Fibrosis are two of these (37). From NBS, 1-2 % of newborns are diagnosed with a serious illness (36). IEMs are usually present in infancy or childhood, but may present at any age (19). Patients suspected of having an IEM in Norway, which is not covered by the newborn screening, have their samples examined at the Norwegian National Unit for Screening and Diagnostics of Congenital Pediatric Metabolic Disorders at Oslo University Hospital. They have the national diagnostic function for congenital metabolic disorders and are a part of the newborn screening program (35). Each year, they perform about 2000 diagnostic investigations for patients suspected of having a congenital disease (38). For some of the congenital diseases, the samples have to be sent to specialist laboratories in other countries for analyses (39).

2.3 Metabolomics

Metabolomics is the comprehensive high-throughput study of metabolites in tissues, cells, and biofluids, such as plasma, serum, and urine (23, 40, 41). The workflow generally consists of an experimental design, sample collection and preparation, data acquisition, and data processing (42). Targeted metabolomics and untargeted metabolomics are the two different approaches metabolomics can be sorted into, see **Figure 2.6** (43).

Targeted metabolomics is hypothesis-driven and focuses on the identification and the absolute quantifications of tens to hundreds pre-determined metabolites. The sample preparation and/or the instrumentation are chosen to gain optimal sensitivity and achieve reliable identification and quantification of pre-determined metabolites (1, 7, 44-46).

Untargeted metabolomics is a holistic approach, trying to analyze and identify as many metabolites as possible on the metabolome, which may be more than 10 000. To obtain a wide coverage of the metabolites in a biological samples, the sample preparation and/or instrumentational setup are non-selective (44, 47). Untargeted metabolomics is mostly used to compare metabolomics data sets obtained from of different biological samples. Each metabolite in this approach is identified by correlating data results with data from reference databases. Unlike targeted metabolomics, which is based on a pre-defined hypothesis, the goal here is to generate a hypothesis after the sample has been analyzed, based on the results observed (47).

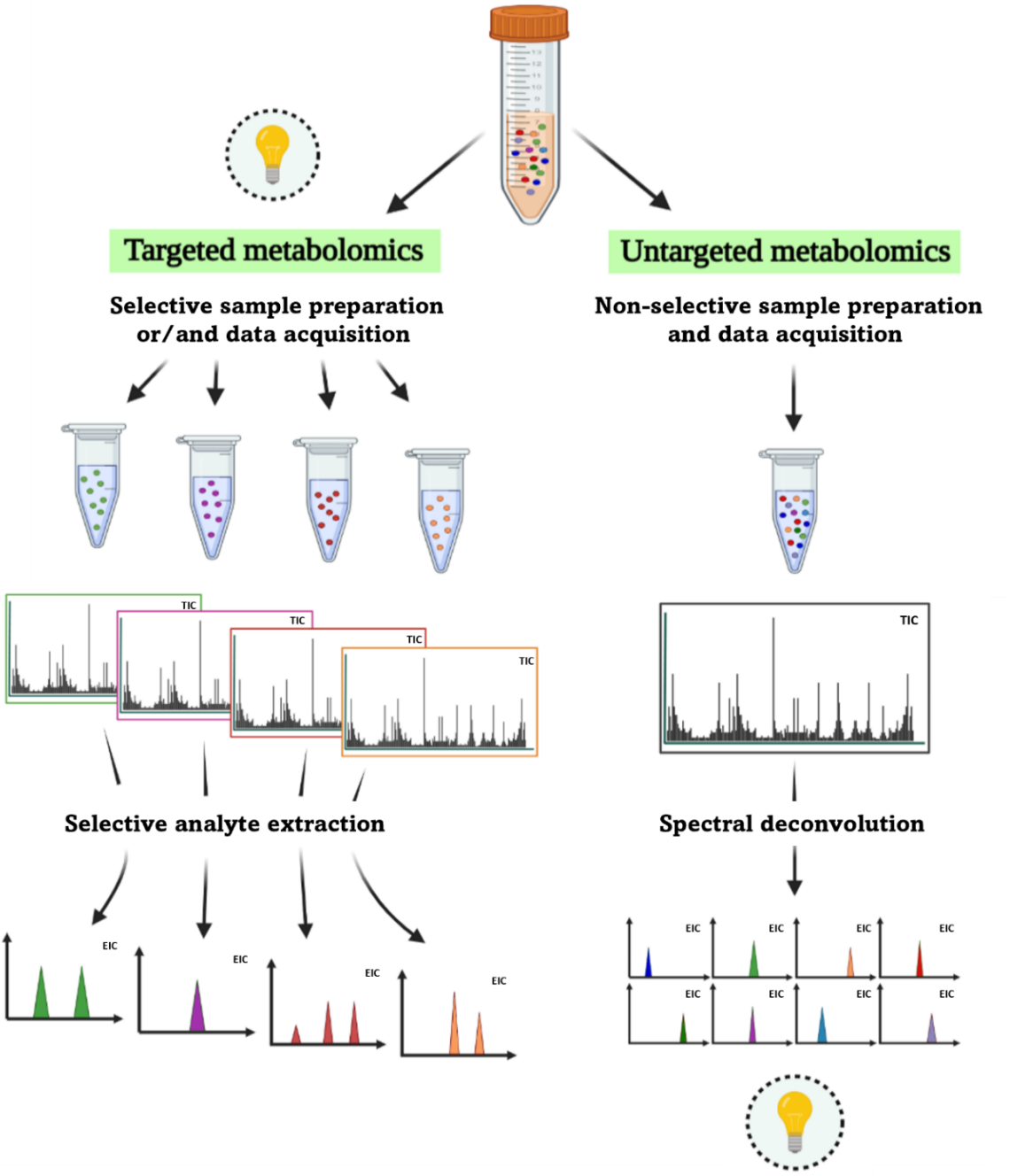


Figure 2.6: The difference between targeted and untargeted metabolomics. *In targeted metabolomics, there is selective sample preparation and/ or data acquisition, influenced by a prior hypothesis determining which analytes to measure. Each different-colored dots in each Eppendorf tube represents a group of analytes with similar physiochemical properties. In targeted, EIC is selectively obtained from the TIC. Untargeted metabolomics have non-selective sample preparation and data acquisition to include the detection of every metabolite in the sample.*

2.3.1 Targeted metabolomics – Today's widely used tool for laboratorial diagnosis of patients with IEM

Targeted metabolomics is highly standardized in clinical laboratories. It is used in NBS programs, and it is the most used approach for the laboratory diagnosis of IEM (47, 48). In laboratory diagnostic, targeted metabolic profiling is routinely performed with metabolite assays consisting of groups of metabolites with similar physiochemical properties or metabolites from the same metabolic pathway, as the targeted metabolites (41, 49). These assays can be groups of amino acids, long fatty acids, steroids, acylcarnitines, or organic acids. Each assay is targeted to different groups of disorders determined by the patients' clinical phenotype and the diagnostic armamentarium available (18, 40)

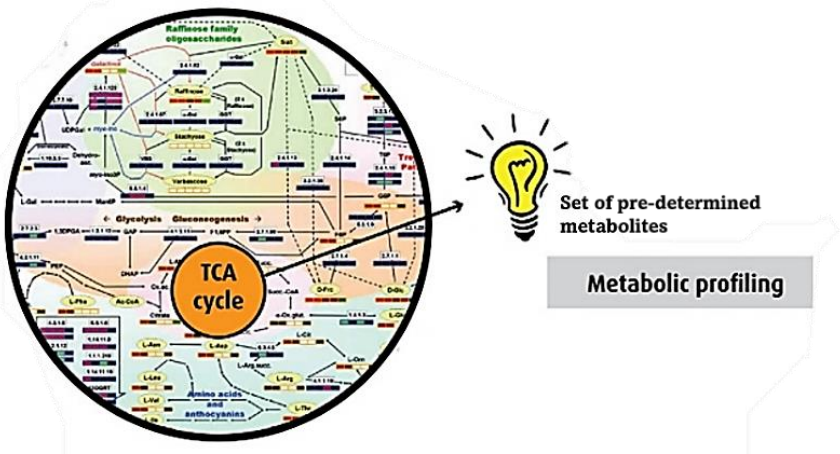


Figure 2.7: Illustration of targeted metabolic profiling of a pre-determined part of the metabolome (41)

Each assays have their own distinct protocol for sample preparation and instrumental platform, due to their targeted metabolites' various physicochemical properties. This will provide reliable data to diagnose a patient if the correct hypothesis for which diseases the patient has is proposed. If any false negative occurs, and it becomes necessary to test other hypothesis, with other assays, targeted metabolomics may be challenging. This is because with targeted metabolomics only a limited metabolomic profile is generate generated, as shown in **Figure 2.7**. False-negative results can occur if patients' clinical phenotype is incomplete, or they have asymptotic IEM (19). Here, untargeted metabolomics could have been used as a supplement to generate a hypothesis for which type of metabolic disorder the patient may have, providing the metabolites to analyze in a targeted metabolomics.

2.3.2 Global metabolomics – The future's tool for diagnosis and individualized treatment

Global metabolomics is an untargeted approach intending to simultaneously study all metabolites in a biological sample (15, 44).

The biochemical composition of the human metabolome is only partially known and there are still IEMs without known or robust biochemical biomarkers. Global metabolomics is emerging in the field of research (50, 51). The broad coverage obtained from global metabolomics can identify novel metabolites, discover new biomarkers for diseases, uncover underlying mechanisms and give a better knowledge of diseases, such as asymptomatic IEMs (4). This will have a major impact on the laboratory diagnosis of IEMs and treatment planning (52, 53). As an example, instead of treating the patient by a evidence based medicine, which is a treatment-failure approach, global metabolomics may allow for personalized medicine, in which the biochemical impact of individual diversity in genes, environment, and personal lifestyle is considered (34).

Figure 2.8 shows the workflow for biomarker discovery. It includes a study design, sample collection, sample preparation, data acquisition, statistical analysis, biomarker identifications, pathway analysis, and biological interpretations. In the statistical analysis, specific metabolites standing out in pathological samples, compared to healthy samples are observed. With an increasing number of healthy and pathological samples, the probability of achieving results of statistical significance is increased (42). In pathways analysis, metabolomic data are combined with data from genomics, transcriptomics, and proteomics to obtain more reliable data results. This is because of metabolomes' dynamic changes which can occur due to environmental factors, can sometimes hamper the quality of results (7, 40).

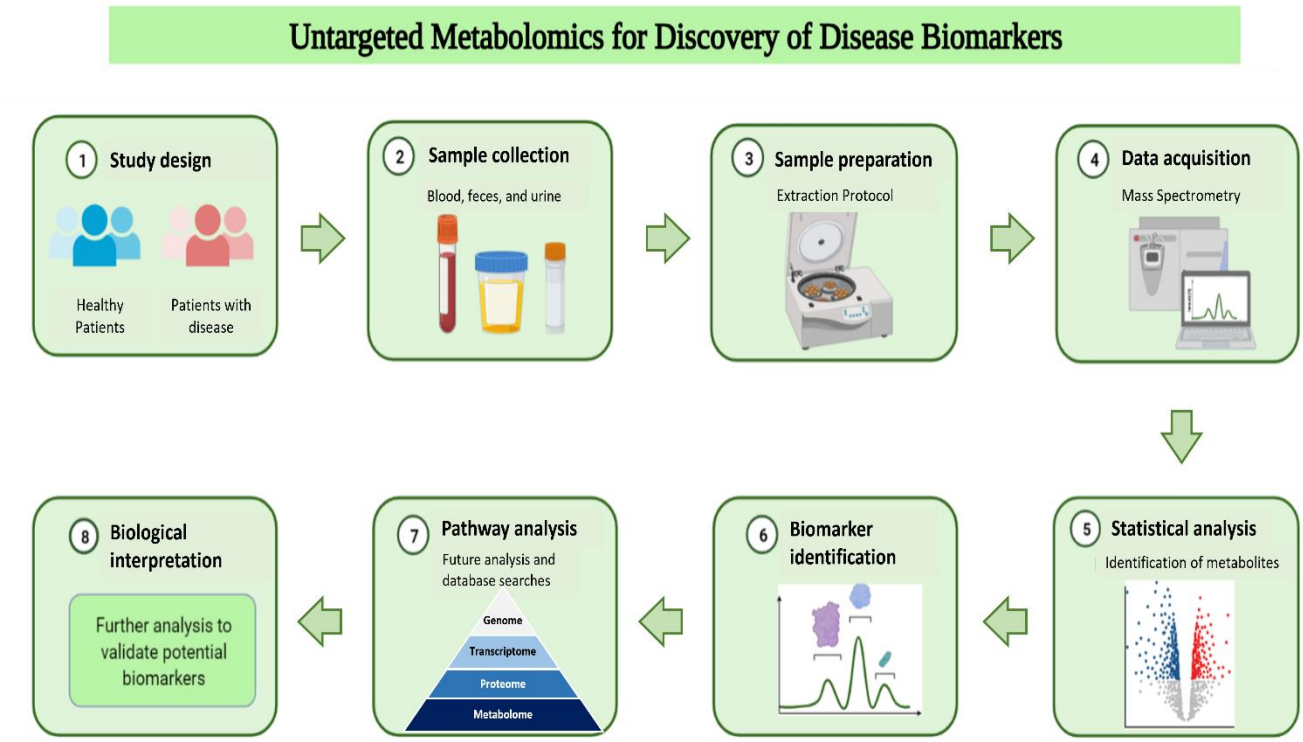


Figure 2.8: The workflow for biomarker discovery, using untargeted metabolomics. *The workflow consists of a study design, sample collection, sample preparation, data acquisition, statistical analysis, biomarker identification, pathway analysis, and biological interpretation.*

Challenges with global metabolomics

Due to the large physiochemical diversity in the human metabolome and the dynamic nature of metabolism, sample collection and preparation are faced with challenges. Temperature, light, metabolism quenching, and extraction solution need to be balanced equally for all the metabolites in the sample to ensure no contamination or degradation of metabolites, which could compromise results. (40). As an example, vitamins can degrade in direct light, while other metabolites can degrade dependent on factors such as temperature and the presence of oxygen (8). The individual variance must also be considered. Variations in hydration and diet will cause individuals' metabolite concentrations to differ. Here, urinary creatinine level is commonly used to normalize urine analysis. It is also important to consider the variation of exogenous interference between individuals (42). To minimize pre-analytical variation, quality control and standardization are performed by following standard operating procedures (SOP) (8, 54).

The biggest bottlenecks for global metabolomics are metabolite annotation and identification (45). Reasons for this are covered in chapters 2.4 and 2.5.

2.4 Analytical platforms applied in metabolomics

Nuclear magnetic resonance (NMR), gas chromatography-mass spectrometer (GC-MS), and liquid chromatography-mass spectrometer (LC-MS) are instruments applied for metabolomics (10). There is currently no single analytical platform that can fully cover the whole metabolome, due to the metabolomes' physiochemical diversity (41). **Figure 2.9** illustrates NMR, LC-MS, and GC-MS metabolite coverage (55). For a more complete metabolite coverage, different analytical platforms may accompany each other (56).

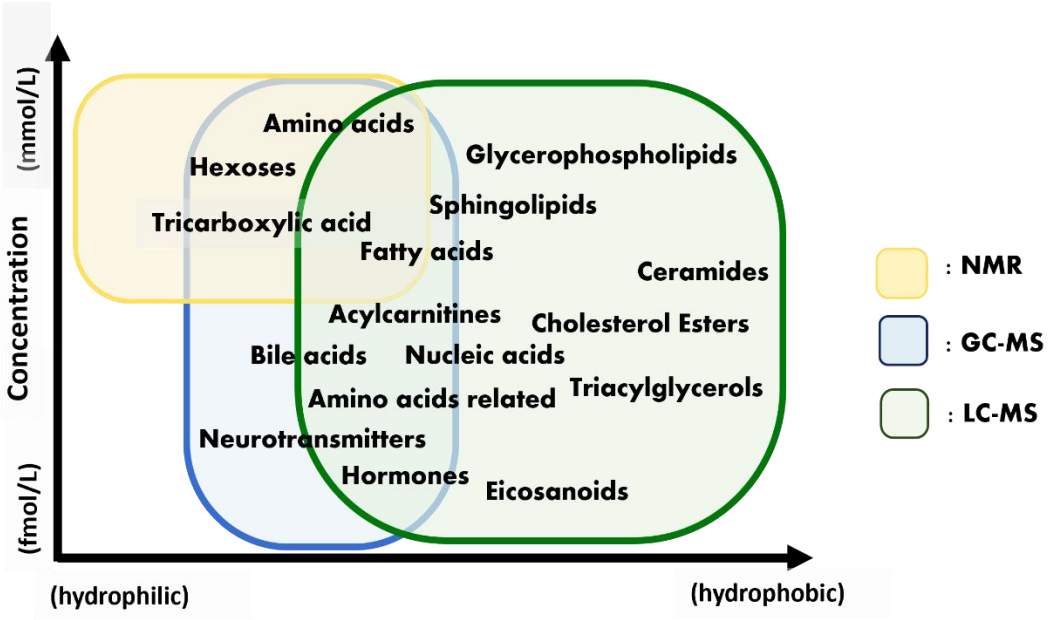


Figure 2.9: Illustration of NMR, GC-MS, LC-MS's metabolite coverage, with different concentration and hydrophobicity. *The figure was adapted from (55)*

In metabolomics, NMR was the pioneering technique. Unlike MS, NMR is non-destructive, requires little sample preparation, and produces highly reproducible data. However it has a limited metabolite coverage and the sensitivity only goes down to the micro molar ranges (57). MS has gained more interest because of its sensitivity (fM to aM) (10), wide dynamic range, and its' possibility to be coupled with separation techniques, such as LC or GC, providing the ability to analyze complex samples (41). Due to LC-MS' metabolite coverage of metabolites in a wide range of polarity and concentration, as well as continuous "technical improvements", it is now the dominating analytical platform for untargeted metabolomics (41, 43, 51, 58)

2.4.1 The Mass Spectrometer

A mass spectrometer (MS) detects mass-to-charge ratios (*m/z*) of molecular ions. MS is used to quantify and identify compounds in a sample (59). The mass spectrometer provides information through different types of mass spectra, such as MS^1 and MS^2. These are obtained in different MS scan modes. MS^1 is obtained from a full scan. Here, intact molecular ions are scanned in a specific time frame, providing *m/z* values of the detected ions on an x-axis and their intensity on a y-axis. MS^2 consists of the *m/z* values and intensities of fragment ions, and in some cases the fragment ions' precursor ion as well. MS^2 is obtained from tandem MS scans (MS/MS), this is more explained below (59, 60).

The main units of the mass spectrometer are an ion source, a mass analyzer, and a detector, see **Figure 2.10**. To avoid collision of ions and ensure a high mean free pathway through the mass spectrometer, the system operates at a high vacuum, up to 10^{-3} *bar*, obtained from instrumented vacuum pumps (61).

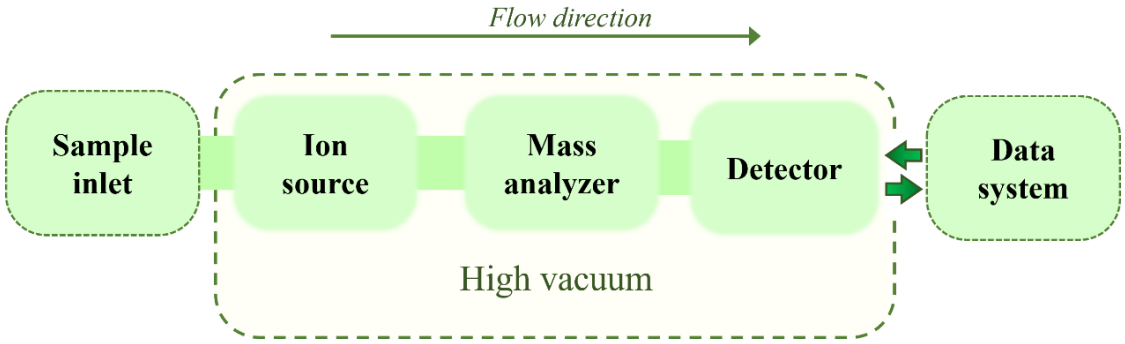


Figure 2.10: The different units of a mass spectrometer, aligned according to the direction of the flow. *The ion source, mass analyzer, and detector operate in a high vacuum. The figure was adapted from (62).*

Samples can be injected directly into the MS, or from a prior separation technique like LC or GC. Into the MS, the sample needs to be in its gas phase. For samples injected in a liquid state, such as in LC-MS, the ion source is a very important interface. The ion source evaporates the sample, as well as ionizing molecules in the sample (63). Electrospray ionization (ESI) is the most used ion source in LC-MS (64) and is also used in this thesis. ESI is further explained in **section 2.4.1.1**.

The mass analyzer filters and determines which ions can pass further to the detector (63). The scan speed, sensitivity, mass accuracy, and mass resolution determine the mass analyzer's performance. This performance categorizes them either as low-resolution (LR) mass analyzers or high-resolution (HR) mass analyzers (65).

- *Scan speed* is given in Hz, and it is the acquisition rate of mass spectra (59). With the measurement of 12-15 unique *m/z* values, quantitative peaks are obtained. As the number of measurements grows, the quality of peaks improves (63, 66)

- *The sensitivity* corresponds to the signal-to-noise ratio (SNR). The sensitivity increases as the SNR increases. Because noise may be removed from detection in tandem MS modes, the sensitivity of the MS may be higher than in a full scan (67). An analyte needs to obtain an SNR above 2 to be classified as detectable. And, for a detected analyte to give reliable quantification, the SNR needs to be above 10 (63).

- *The mass accuracy (E)* is calculated with ***Equation* 1** and is given in parts per million (ppm). It is the difference between the experimental measured *m/z* value and the theoretical (63).

$$E = \frac{\frac{m}{z}(experimental\ value) - \frac{m}{z}(theoretical\ value)}{\frac{m}{z}(theoretical\ value)} \times 10^6 \qquad \textit{Equation 1}$$

- *The mass resolving power (R)* is the mass analyzers' ability to precisely distinguish between different *m/z* values. The R is calculated by ***Equation 2***, and $\Delta m/z$ is the full width at half maximum height (FWHM) (63).

$$R = \frac{m/z}{\Delta m/z} \qquad \textit{Equation 2}$$

Figure 2.1 shows the resolution, mass accuracy, scan rate, sensitivity, and the detectable mass range of some mass analyzers used in metabolomics.

Table 2.1: *The mass resolution at full width at half maximum height, mass accuracy, scan rate and sensitivity for triple quadrupole, time-of-flight, and orbitrap. The values were adapted from (59, 68).*

Mass analyzer	Mass resolution (FWHM)	Mass accuracy (ppm)	Scan rate (Hz)	Sensitivity
Triple Quadrupole	1000 - 5000	100 – 1500	20	Attomole
Time-of-flight	10 000 - 20 000	5 – 50	10 000	Femtomole
Orbitrap	50 000 – 500 000	< 5	10	Femtomole

The triple quadrupole is a LR, while Time-of-Flight and Orbitrap are HR mass analyzers. ToF is one of the fastest mass analyzers, and the separation is based on the ions' velocity which corresponds to the ions' *m/z* value. An orbitrap separates ions by their axial oscillating frequency around a cylindrical electrode (62). An HR mass analyzer has an R above 10 000 and can distinguish compounds by their monoisotopic mass, while an LR analyzer can only distinguish compounds by their nominal mass. Nominal is the compounds' molecular mass calculated with the integer mass of atoms' most abundant isotope, while monoisotopic mass uses the exact mass of atoms' most abundant isotope in the calculation (59, 69).

Figure 2.11 illustrates 3 alternative chromatographic separations of two compounds with almost identical m/z values. The results in alternative A are from an LC-LR-MS analysis in a full scan mode. Because the compounds have identical nominal mass and almost the same retention time (RT), the MS can't distinguish them, and the MS detects one large peak. The retention time is obtained from the LC separation technique, this is explained further in chapter 2.4.1.1. To separate the peaks in LR, either the prior separation can be optimized, resulting in distinguishable RTs, or they can be analyzed in tandem MS scan mode, resulting in distinguishable MS^2 spectra. This is shown in alternative B. The results in alternative C are from an LC-HR-MS analysis in a full scan mode. Because of the mass analyzer's higher resolution and the high difference in compounds' monoisotopic mass the mass analyzer can differentiate them (70). Due to this, a mass analyzer with high resolution is favored when doing untargeted analysis of complex biological samples.

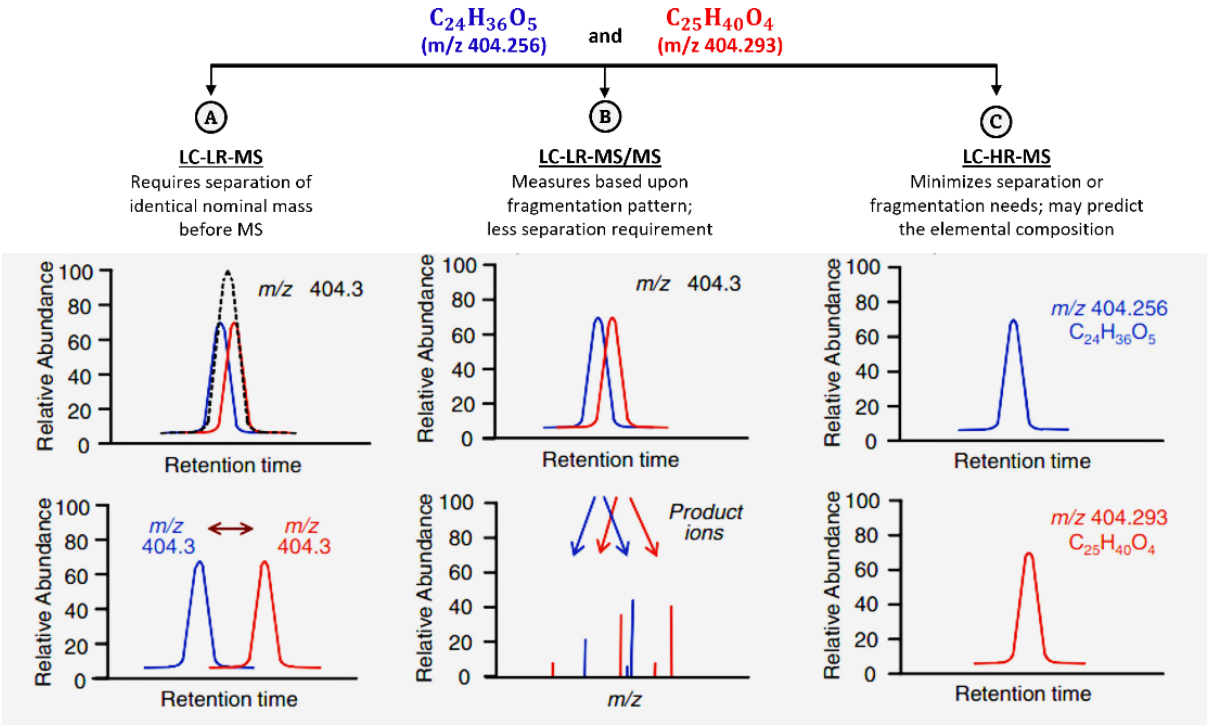


Figure 2.11: Compounds with the same nominal mass, but with different empirical formulas, analyzed with low- and high-resolution mass analyzers. *Alternative A: LC-LR-MS, full scan, Alternative B: LC-LR-MS, tandem MS. Alternative C: LC-HR-MS, full scan.*

Despite the limited resolution, a triple quadrupole can provide high specificity. A triple quadrupole consists of 3 quadrupoles. One quadrupole can be referred to as one mass analyzer. A quadrupole is made up of 4 parallel rods that onto which radio frequency (RF) voltage and direct current (DC) can be applied (61). As a result, an electric field will be established inside the quadrupole. Corresponding to the changes in the electric field, ions will experience an oscillation movement passing through the quadrupole. Separation in the quadrupole is based on the ions' trajectory. With certain electric fields, only ions with certain m/z values will pass through without colliding with the rods excluding them from the MS detection (59).

With two or more mass analyzers it is possible to do a tandem MS scan (MS/MS). In the first mass analyzer, precursor ions are selected and transferred to a collision cell for fragmentation. Fragments are further transferred to another mass analyzer where they are detected, this gives the ability to collect fragmentation spectra, such as MS^2 spectra (71, 72). There are different types of tandem MS modes. The triple quadrupole can perform single reaction monitoring (SRM) and multiple reaction monitoring (MRM) (72). These are tandem MS modes that quantify specific transitions of molecular ions (10). Here, the last mass analyzer, which is a

quadrupole, may also select which specific fragmentation products must be present to perform detection. This enhances specificity. Precursor ions may have the same nominally m/z value, but they might produce unique fragmentation products (62, 73). Due to triple quadrupole's specificity in tandem MS, it is one of the most commonly applied mass analyzers in targeted metabolomics, especially in clinical labs (10)

Hybrid instruments:

Because ToF and Orbitrap cannot perform tandem MS, hybrid instruments have been constructed exploiting the benefits of high-resolution mass analyzers and fragmentation spectra, (61, 62). Q-ToF and Q-orbitrap are some examples, in which a quadrupole has been coupled in front of the mass analyzer. For untargeted metabolomics, hybrid HR-mass analyzers providing high mass resolution, mass accuracy of 1-10 ppm, as well as fragmentation spectra are the most applied (10) For this thesis a Q-Orbirap is used. **Figure 2.12** shows an illustration of the mass analyzer, as well as demonstrating how MS^1 and MS^2 are collected.

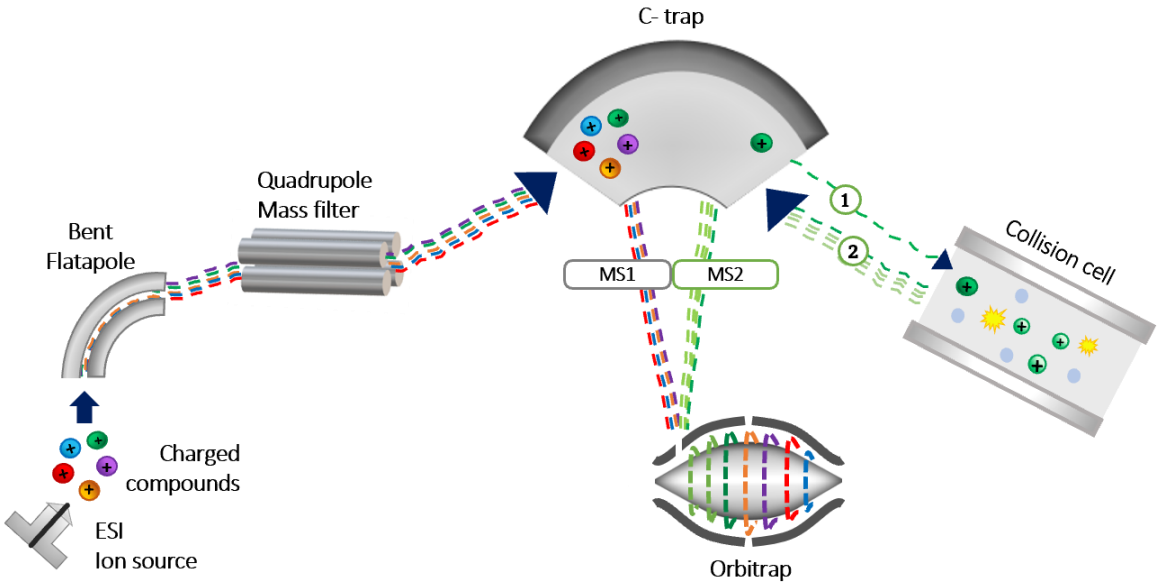


Figure 2.12: Illustration of a q-exactive-orbitrap, collecting MS^1 and MS^2 spectra*. The figure was inspired by (74).*

The ESI ion source positively charges compounds before they are focused to the quadrupole. The quadrupole works as a mass filter and transfers molecular ions within a m/z range further to the C-trap. If a full scan is performed, the C-trap focuses all the ions to the orbitrap. If an MS/MS is performed, the C-trap transfers molecule ions to the collision cell (74).

Two types of tandem MS are possible with Q-Orbitrap, a data-independent acquisition (DIA) and data-dependent acquisition (DDA). Compared to SRM/MRM, the acquired MS^2 from DDA or DIA are only influenced by predefined selections at one mass analyzer, and is therefore less specific (62). In DIA, all molecular ions are simultaneously transferred from the C-trap to the collision cell, and the acquired MS^2 consists of fragments originating from several precursor ion. In DDA, automatic switches from full scan to MS/MS are performed by the mass spectrometer, and MS^2 is collected from the top n (n=1, 2, 3, etc.) abundant molecular ions, see **Figure 2.13** (75).

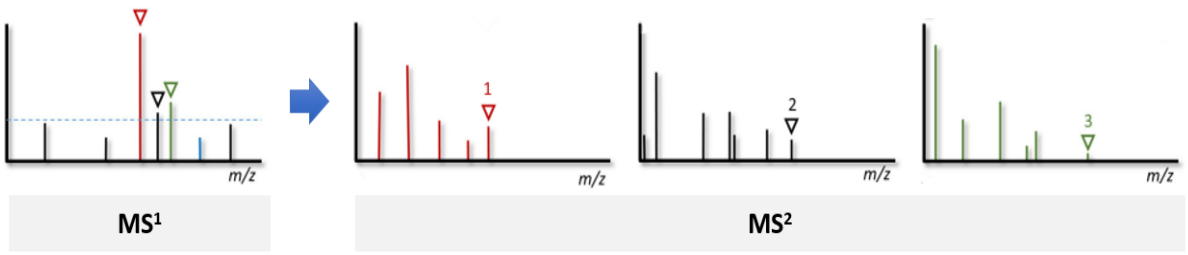


Figure 2.13: Data-dependent acquisition of top 3 abundant molecular ions (75).

DDA may give data results with low analytical reproducibility, because the collected MS^2 depends on which co-eluting molecular ions are the most abundant, which may vary in samples (75). To ensure MS^2 of molecular ions with low intensity, their molecular ions' m/z values can be added to an inclusion list ensuring fragmentation.

2.4.1.1 Electrospray ionization

ESI can positively or negatively charge compounds. The process of positively charging compounds is illustrated in **Figure 2.14**.

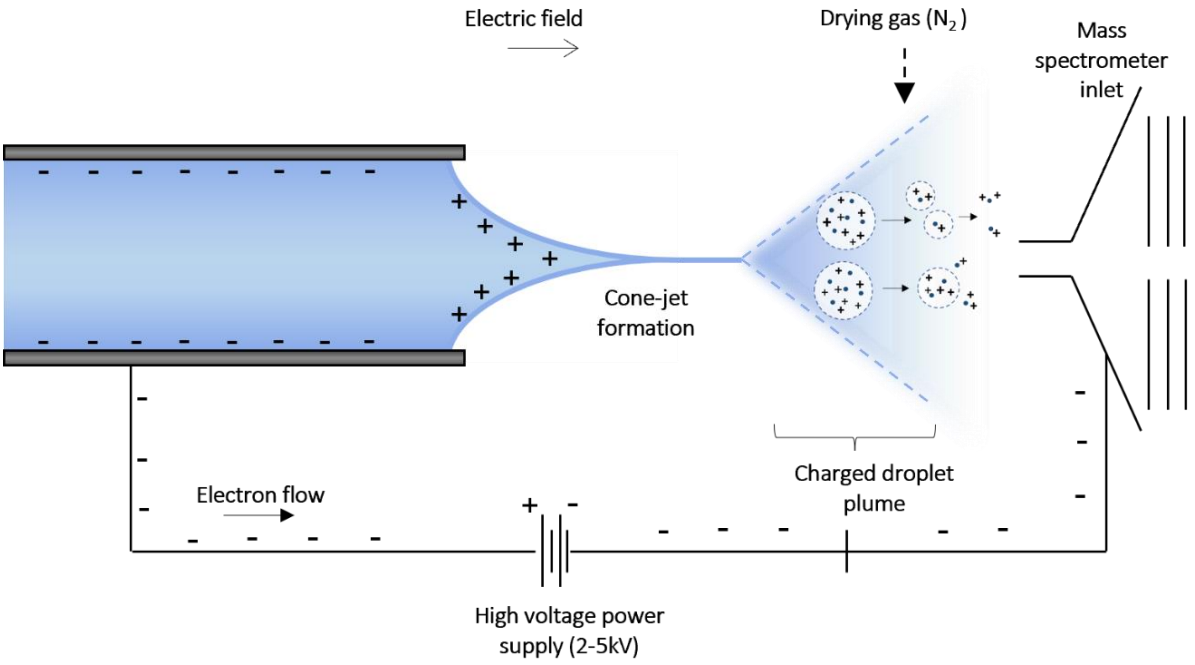


Figure 2.14: Illustration of the positive ionization process in electrospray ionization (ESI). *The figure was inspired by (63).*

Inside a small capillary with an inner diameter < 250 µm, analytes and mobile phases in a liquid phase passes through. Between the capillary tip and a counter electrode at the mass spectrometer inlet, a positive potential is applied. Positive particles in the capillary experience an electrostatic force towards the MS inlet. With a huge enough voltage, between 3 kV – 4.5 kV (76), a Taylor cone and a jet region will develop, as shown in the figure. A spray of charged droplets will develop from a stream in the jet region. Each droplet gradually resolves into smaller droplets due to one of the two mechanisms: A Columbic repulsion, that is shown in the figure, or drop jet fission. These mechanisms are a result of the droplets reaching the highest limit of charge density inside the droplet, also called the Rayleigh limit. Further, nebulizing of the droplets is facilitated by a drying gas from a secondary coaxial flow. N_2 is usually chosen as the drying gas. The mobile phase will eventually evaporate and ions in a gas phase are generated. Analytes can either be ionized in the electrospray, or they can already be in their ionized form in the ESI capillary, dependent on the pH of the mobile phase (61, 63)

Not all compounds will ionize in positive ionization. Typically, amines ionize in positive mode, while acids and alcohols ionize in negative mode. The process for ESI in negative ionization mode is the same, but the polarity of the electrodes is switched.

Protonated, $[M+H]^+$, and deprotonated, $[M-H]^-$ molecules, in the positive and negative ionization modes, respectively, are the most usual. Dependent on the voltage and solvent the charge can be more than 1 (z=2,3,4 etc.). Also, other types of ions can develop. With a thermal ion attachment, adducts can develop. $[M+Na]^+$ and $[M+OH]^-$ are two examples. With non-covalent interactions between neutral molecules and ions, cluster ions $[M+H+(M)_n]^+$ or $[M-H+(M)_n]^-$, can develop (76).

The application range for ESI, and other ionization sources:

ESI provides ionization efficiency for compounds with a wide range of molecular weight and polarity, from semi-polar to polar, see **Figure 2.15** (77).

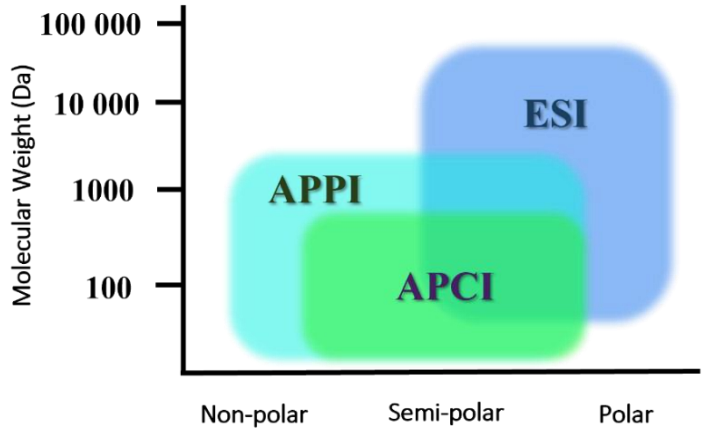


Figure 2.15: The application ranges for electrospray ionization, atmospheric pressure photoionization, and atmospheric pressure chemical ionization. *The figure was adapted from (77).*

With the huge range in polarity in the metabolome, the ion source is an essential part of the instrument, and a high performance and quality is wise to prioritize. ESI is a soft ionization source, ionizing intact molecules. Due to this, and its wide range of applications, it is the most commonly used ion source in metabolomics. One of the major challenges with ESI is the increasing chance of ion suppression if there are a lot of co-eluting compounds in the ion source. Complementary to ESI, there are atmospheric pressure photoionization (APPI) and atmospheric pressure chemical ionization (APCI). These can ionize less polar and non-polar compounds, and ion suppression is a lesser problem (76, 77).

2.4.2 Liquid Chromatography

The setup for high-pressure liquid chromatography is illustrated in **Figure 2.16**.

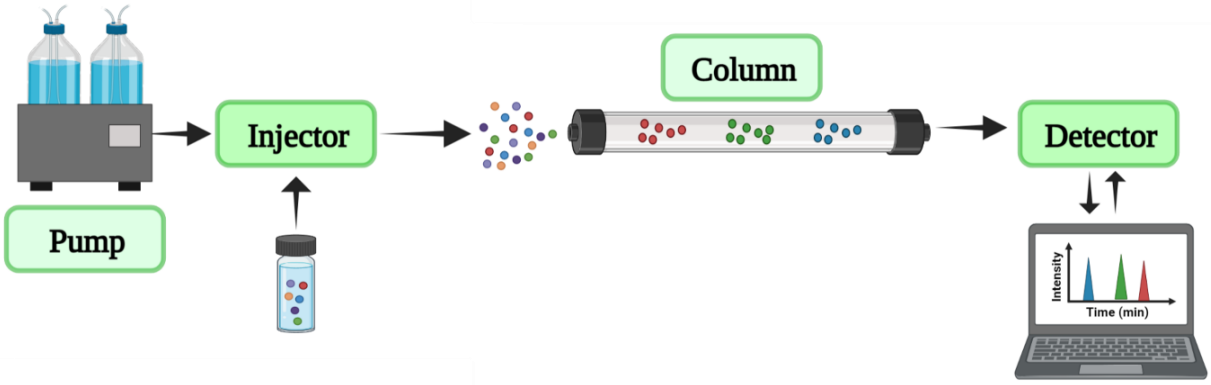


Figure 2.16: The setup of high-pressure liquid chromatography. *A pump, an injector, a column, and a detector. The figure was inspired by (63)*

The injector transfers a predetermined amount of the sample from a vial into the system. By a single mobile phase or a combination of mobile phases, the sample migrates through the LC system. The mobile phases are pumped into the system with a predetermined flow. The column is packed with particles covered with a stationary phase with properties that to a large extent determine affinities to the compounds in the sample injected. The level of affinity will determine the compounds' migration through the column, resulting in a separation of molecules with different hydrophobicity, charge, and size. The difference in migration time for compounds correlates to retention times (RT) provided in chromatograms. The detector can be a mass spectrometer. The combination of LC prior to a MS improves MS's sensitivity, mass accuracy, and acquisition. The additional RT information collected with LC may also allow for easier identification of compounds in complex samples (78).

Different types of separation principles may be applied in LC, such as reverse phase (RP), normal phase (NP), and hydrophilic interaction chromatography (HILIC). No single separation principle can separate all metabolites in the metabolome. Hence, for a wider coverage, untargeted metabolomics of a sample is usually performed in more than one LC-MS platform, using different separation principles (79).

Reverse phase chromatography has a nonpolar stationary phase, and a polar mobile phase, and separates nonpolar to semi-polar metabolites (80). Analytes are separated through direct hydrophobic interactions with the SP. In metabolomics, a reverse-phase column with silica-based C18 or C8 as the stationary phase is most commonly applied. Smaller particle sizes increase the efficiency of the chromatographic separation, but also increases back pressure (42, 51, 81). For conventional HPLC, the particle size is 3-5 µm (76). To utilize smaller particle sizes, and obtain efficient and faster separation (7.5 -30 min), with reduced peak width and increased peak capacity, ultra-high pressure liquid chromatography (UHPLC) has been introduced in metabolomics (2, 51). These systems operate with sub 2 µm particle size and can tolerate the high pressure needed to generate flow rate (10, 68).

The mobile phase in RP is usually a combination of water and organic solvent and, modifiers, like formic acid (FA), are added to the mobile phase to improve the separation efficiency (81). A flow gradient of mobile phases is mainly used in metabolomics to increase the selectivity of the diverse metabolites. The flow gradient starts with a low percentage of organic solvent, for example 2%, which gradually increases throughout the analysis, increasing the elution power (80).

For an improved separation of more polar metabolites, and to achieve a wider metabolome coverage, HILIC is often used complementary to RP (10). Here, the stationary phase is polar and develops hydrophilic interactions with analytes. The mobile phase is also polar, and it is a combination of organic solvent and water. Unlike the flow gradient in RP, the flow gradient here starts with a high percentage of organic mobile phase, which gradually decreases.

2.5 Reference standards

Compounds in a sample analyzed with a mass spectrometer can be identified by different characteristics. They can be identified with a unique m/z value, a molecular formula, or/and a structure. Biological interpretation of data depends on precise metabolite identifications (8). The identifications of compounds can be categorized into different levels of confidence. Five different levels are common. **Figure 2.17** shows a hierarchy of different levels of confidence, where 5 is the lowest and 1 is the highest (8).

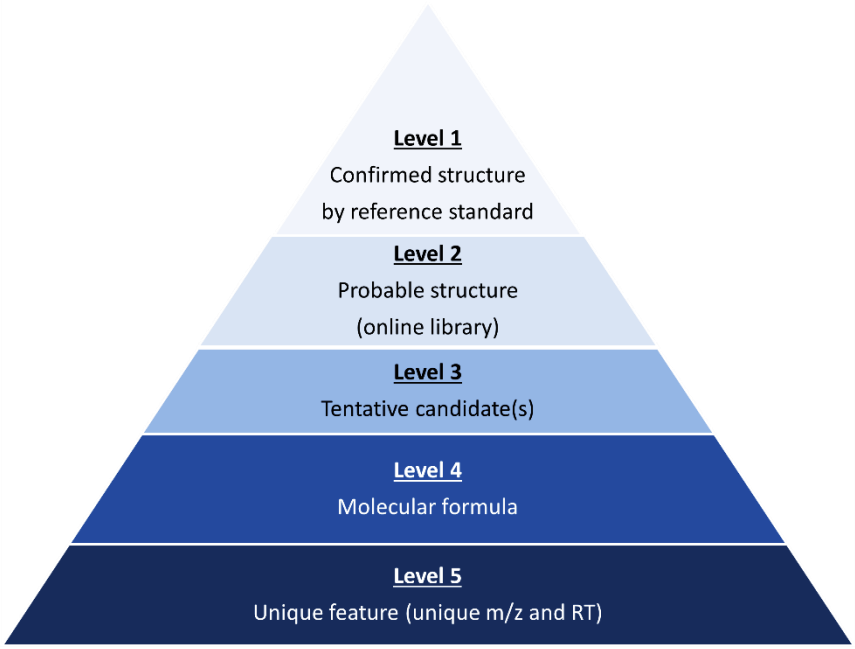


Figure 2.17: The level of confidence for the identification of compounds. *Level 5 is the lowest level, while level 1 is the highest. The figure was adapted from (8).*

If a compound is identified as a unique feature with a unique *m/z* and RT, the identification has a level of confidence 5. A level at 4 is defined as having a predicted molecular formula. With a level 3, the feature has tentative structure candidates, these are obtained by searching for the m/z values obtained from MS^1 in databases such as PubChem, where the high resolution from HR mass analyzers is exploited (8).

To achieve levels of 2 and 1 MS^2 from the analyzed sample needs to be comparable with other reference MS^2. With Q-Orbitrap, DDA collects cleaner MS^2 than DIA, making them easier to compare to reference MS^2. For a level 2, the MS^2 obtained from an analyzed compound is compared to MS^2 from an online database (METLIN, HMDB, MassBank, etc.) (82).

Figure 2.18 shows how MS^2 are obtained from a spectral deconvolution of an extracted ion chromatogram (EIC), as well as how the compounds are identified by comparing MS^2. EIC is obtained by extracting certain m/z values from a total ion chromatogram (TIC) (83).

Online libraries don't cover all metabolites in the metabolome. To overcome the amount of missing online MS^2, algorithms generating *in-silico* MS^2 have been developed (8, 84). *In-silico* refers to data that has been simulated. To generate *in-silico* MS^2, the algorithms use groups of experimental MS^2 obtained from other compounds with chemical similarity (85).

The data results obtained from different LC-MS instruments may not be directly comparable because instrumentation, settings and protocols are rarely standardized and it is virtually impossible to achieve identical calibration, and adjustment for analytical drift (58). For the identification with a level of confidence 1, compounds' MS^2 is validated with a reference MS^2 acquired from reference standards, analyzed on the same instrument with identical experimental conditions as the sample, and other orthogonal information such as RT(8). Data collected from reference standards are used to generate an in-house library providing a level of confidence 1 (86). Even though creating an in-house library is time-consuming, it is of great value for laboratories, especially those performing untargeted analysis of complex samples, and it is important for biological interpretation. It can reduce the impact of identification as the greatest bottleneck for data interpretation in untargeted metabolomics (42). An in-house library can also provide data for compounds that don't have their MS^2 listed at online libraries, as well as other additional information like RT and collision energy not provided by online libraries. This can help distinguish isometric compounds with similar MS^2 (82).

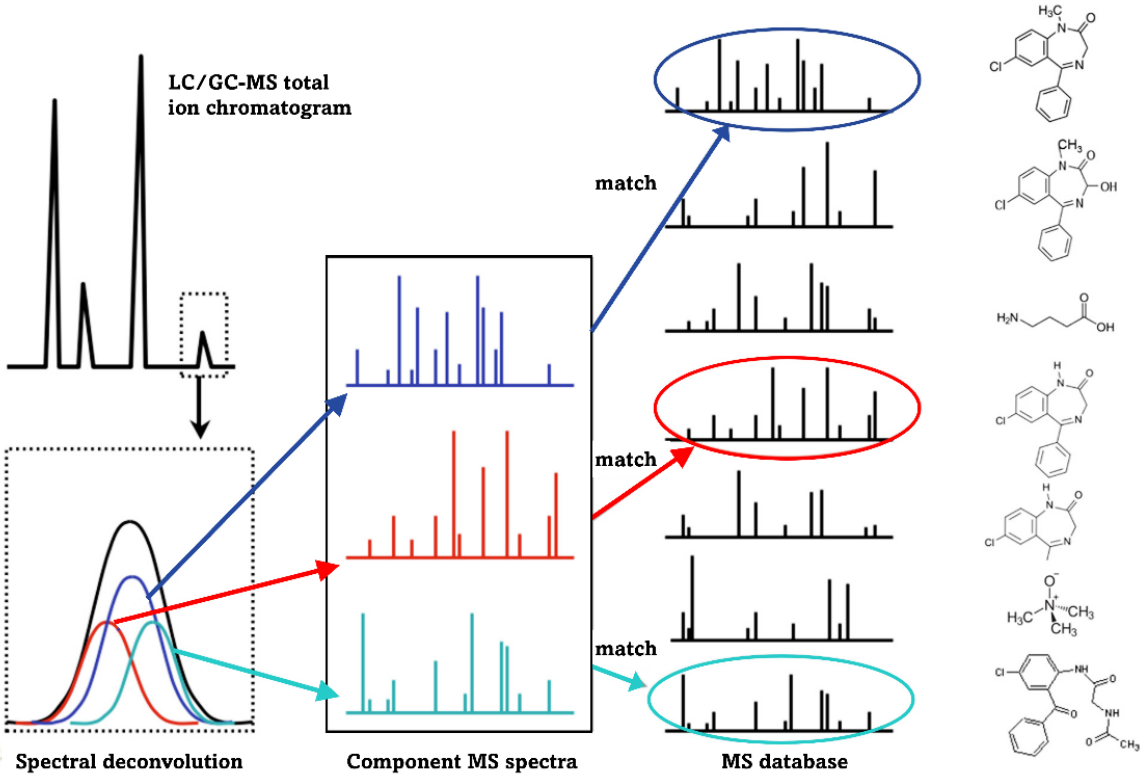


Figure 2.18: A metabolite identification of three different components, identified by comparing MS^2 spectra from the analyzed sample and reference MS^2 spectra from a database. *A peak from TIC is extracted before a spectral deconvolution is done to obtain the MS^2 spectra. If the MS database in not from an in-house library, this provides a level of 2 confidence. The figure was adapted from (1).*

2.6 Aim of Study

Global metabolomics aims to detect a wide spectrum of metabolomes in biological samples. One of the major bottlenecks of global metabolomics is that the identification of metabolites should ideally be of the highest level of confidence to transfer the metabolomics data to reliable data for metabolite annotations of highly quality necessary for diagnostic and therapeutic use. This is achieved by using an in-house library containing MS^2 and RT for reference metabolites.

This master thesis aimed to create an in-house library of 603 standard metabolites that will provide identifications with the highest level of confidence in global metabolomics of patient samples, as shown in ***Figure 2.19***. The in-house library was created by analyzing the 603 metabolite standards on a developed global metabolomics LC-HR-MS method. The experimental design was designed to prioritize time- and cost-efficiency. The data processing of the metabolite standards was performed with different computer software. The metabolite standard coverage on the developed global metabolomics method used was investigated by grouping the standards into log P intervals, and chemical classes, and comparing the metabolites detected with other separation principles. The performance of the in-house library identification was tested by comparing the MS^2 match factor of metabolite standards spiked in 5% MeOH and urine. The quantitatively of a standard metabolite that may be a biomarker was also tested.

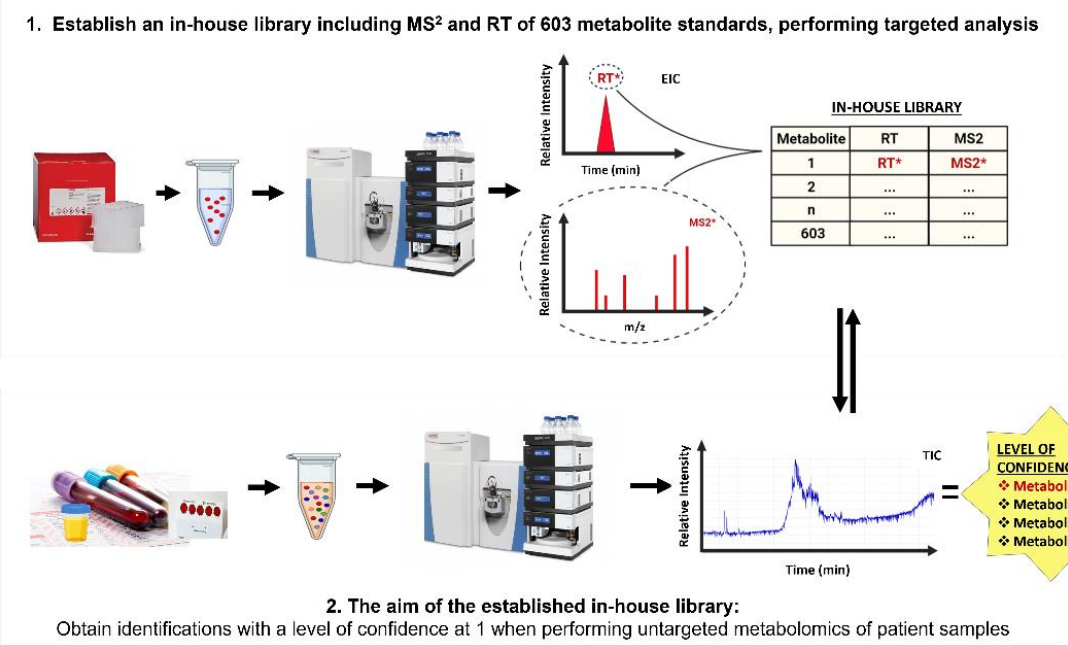


Figure 2.19: *The workflow for creating the in-house library and the untargeted metabolomics workflow using the created in-house library to obtain identification with a level of confidence 1.*

3 EXPERIMENTAL

3.1 Materials

3.1.1 Small equipment

All pipettes used was Fisherbrand Elite Adjustable-Volume pipettes from Fisher Scientific (Waltham, MA, USA). Tubes used were 0.25 mL EU 8-tube strips supplied by BIOplastics BV (Landgraaf, Netherlands), and 1.5 ml microtube supplied by Sarstedt AG & Co. KG (Nümbrecht, Germany). For filtration 0.22 µm Cellulose Acetate Spin Filter supplied by Agilent Technologies was used. The centrifuge used was a Heraeus Fresco 21 Centrifuge supplied by Thermo Scientific, and the mixers used were a Reax top from Heidolph, and a Themomixer Comfort from Eppendorf (Hamburg, Germany). The sonicator used was a Branson 3800 Cleaner supplied by Emerson (Missouri, USA). For LC analysis, Snap-top vial, Snap Ring Cap and 0.1 mL Micro-Insert, from Matriks AS (Oslo, Norway) were used.

3.1.2 Biological material

A urine sample, with a measured creatine value of 18.87 mM, was obtained with approval from the Regional Committee for Medical and Health Research Ethics (REC). See the informed consent in **Appendix 6.13**The creatine value was measured at the routine lab at the Department of Medical Biochemistry at Oslo University Hospital. Creatinine was measured with a Cobas c702 supplied by Roche Diagnostics AS (Basel, Switzerland).

The urine sample was stored at -80 °C.

3.2 Chemicals

3.2.1 Solvents

Methanol (MeOH) and acetonitrile (ACN) were supplied from Rathburn Chemicals Ltd. (Walkerburn, Scotland). Isopropanol (IPA) was supplied from Merck KGaA (Darmstadt, Germany). All these three solvents were of LC-MS grades. Formic acid (FA) (98%-100%) were supplied from Thermo Scientific (Rockford, USA). A Millipore Milli-Q purification system provided type 1 water with a resistivity of 18.2 MΩ•cm at 25 °C. It was instrumented with a quantum cartridge, and a 0.2 µm pore filter membrane purchased from Merck (Darmstadt, Germany). Type 1 water was the only type of water used for this thesis.

3.2.2 Reagents

A mass spectrometry metabolite library of standards (MS-MLS) kit, with a lot number 205-78 was obtained from IROA Technologies (Sea Girt, NJ, USA) (87). It is a collection of 603 unique dry metabolites (purity > 95%), distributed in seven 96-well plates. In each well it is 5 µg. The metabolites in the kit cover important metabolites and intermediates from main metabolic pathways. These are from many different metabolite superclasses, see **Figure 3.1**.

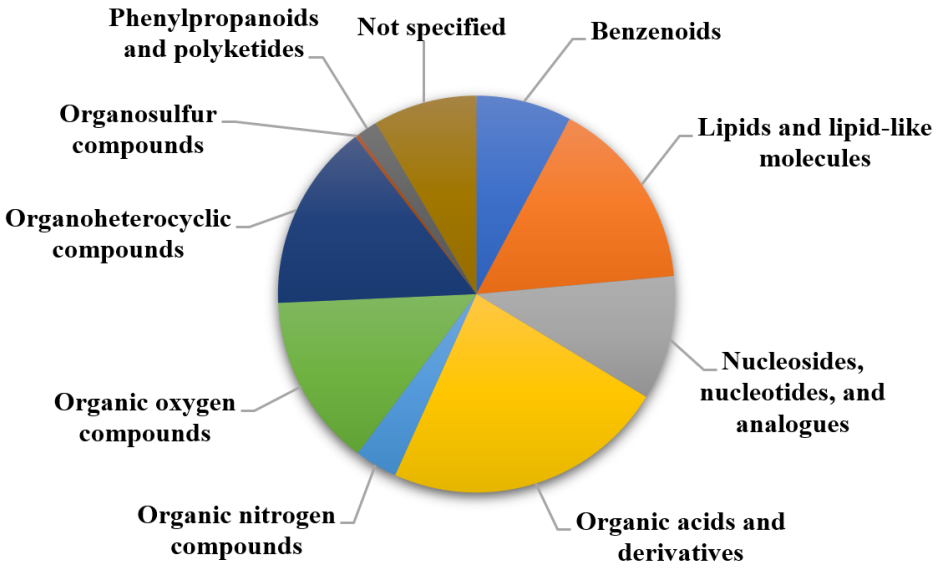


Figure 3.1: The percentage of metabolites from the MS-MLS kit divided into their superclasses. *The superclasses were obtained from HMDB (88)*

Plate 1-5 consists of polar metabolites. Plate 6 rows A-E consist of less polar metabolites, while rows F-G consists of polar sugar metabolites. Plate 7 consists of lipophile metabolites. The kit came along with a plate map, product sheet, and the software, MLSDiscovery. The plate map provided information, such as metabolites' position (plate, row, column), molecular formula, monoisotopic mass, and different IDs (KEGG, CAS, HMDB, etc.)

The MS-MLS kit was stored at -80 °C until sample preparation.

3.3 Solutions

Mobile phase:

In this thesis two mobile phases were used: Mobile phase A and mobile phase B. Mobile phase A was water with 0.1% FA. Mobile phase B was MeOH with 0.1% FA. The mobile phases were stored at room temperature.

Solvents used for MS-MLS:

Four solvents were used to solve the dry metabolites in the MS-MLS, see **Table 3.1**. These solutions will be referred to as solvents A, B, C, and D in the sample preparation.

Table 3.1*: Solutions classified as A, B, C, D.*

Solvent A	Water
Solvent B	MeOH
Solvent C	40% MeOH
Solvent D	1:1 ACN/IPA

3.4 Databases

HMDB (88) was used to obtain information on some of the metabolites in the MS-MLS kit, such as their superclass, class, water-solubility, log P, spectral MS^2 data, etc. Water solubilities were also obtained from Sigma Aldrich (89), and spectral MS^2 data were also obtained from METLIN (90), and PubChem (91).

3.5 Sample preparation of MS-MLS

The sample preparation of the 603 metabolites in the MS-MLS kit was designed to be time-efficient. It was supported by each metabolite's monoisotopic mass, the product sheet following the kit, their calculated concentrations in different volumes, and water solubility obtained from HMDB or Sigma Aldrich.

Table 3.2 shows the different solvents used for the different plates. For a detailed overview of the solvent(s) used for each metabolite, see **Appendix 6.1**.

Table 3.2: An overview of which solvent(s) were used for different plates, as well as the volume. *Some metabolites were solved in two solvents, while others only had one.*

No	Solvent 1	Solvent 2	Metabolites	Solvent volume [µL]
1	A	None	Specific metabolites in plates 1-5, as well as all metabolites in plate 6 rows F and G	A: 100 µL
2	B	A	Specific metabolites in plates 1-5	B: 5 µL A: 95 µL
3	C	None	Metabolites in plate 6 row A to E	C: 100 µL
4	D	A	Metabolites in plate 7	D: 40 µL A: 40 µL

In plates 1-6, solvent A was used for metabolites with a certainty of having a water solubility above 5 µg/100µL

The procedure for solving the metabolites in each plate was as followed: The volume of solvent 1, listed in table 2 was pipetted over to each well. When every well in the plate had been transferred solvent, the plate was covered with parafilm. The plate was mixed for 3 minutes at 850 rpm at room temperature with a Thermomixer Comfort. For plates containing metabolites that needed to be solved in an additional solvent, see table 2, the parafilm was removed. The volume of solvent 2 mentioned in table 2 was pipetted. Further, the plate was covered with parafilm again and mixed at 850 rpm at room temperature, but this time for 5 minutes. If it was observed unsolved metabolites in the wells, the plate was mixed further for 1-2 minutes, if this didn't work the unsolved metabolites were transferred to an 0.25 mL tube and sonicated.

As illustrated in **Figure 3.3,** each solved metabolite standard was transferred to 0.25 mL tubes. The tubes were named by the metabolites' plate number followed by their row letter, and column number. Metabolites being non-isobaric across their row were mixed, by pooling 10 µL of each in another 0.25 mL tube. The mixtures were named with the metabolites' plate number followed by the row letter. **Appendix 6.2** shows how metabolites being isobaric across their row were mixed before the LC-MS analysis.

Figure 3.2: Metabolites in plates 1-5, solved and contained in 1000 µL Eppendorf tubes. *These were stored at -80 °C.*

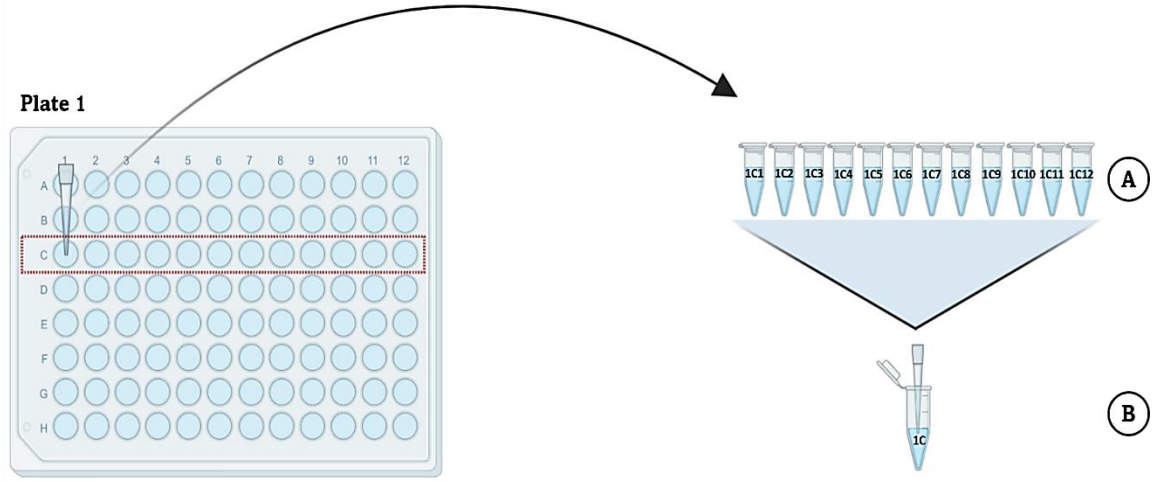


Figure 3.3: An illustration of the transfer of solved metabolites in plate 1, row C, to 0.25 mL tubes (A), prior to further mixing in an 0.25 mL tube 1C (B). *The mix is stored at 5 °C prior LC-MS analysis*

For LC-MS analysis, 10 µL of the mixtures were transferred to HPLC vials with inserts and caps.

3.6 Sample preparation – Urine spiked with metabolite standard(s)

Urine was spiked with metabolite standards for two separate experiments. The first one was to test computer software's identification performance of metabolite standard solutions spiked in urine. The second was to develop a proof of concept for the quantification of trimethylamine.

Figure 3.4 illustrates the sample preparation of urine spiked with metabolite standard(s). For these experiments, urine with18.87 mM creatinine was used. A centrifugation step for 10 min at 14.8 rpm at 4 °C was added for non-fresh urine samples, stored at -80 °C. Prior to the centrifugation 1000 mL of the non-fresh urine sample were transferred to a 1.5 ml microtube. Further, metabolite standard(s) and water were added to a certain amount of urine sample to obtain a normalized creatinine value at 2 mM and certain metabolite standard concentrations. See **Table 3.3** and **Table 3.4** for the volumes of urine, metabolite standard(s), and water used for the two experiments. A vortex of the sample was done before filtration with a 0.22 µm Cellulose Acetate Spin Filter, at 14.8 rpm at 4 °C for 10-30 minutes.

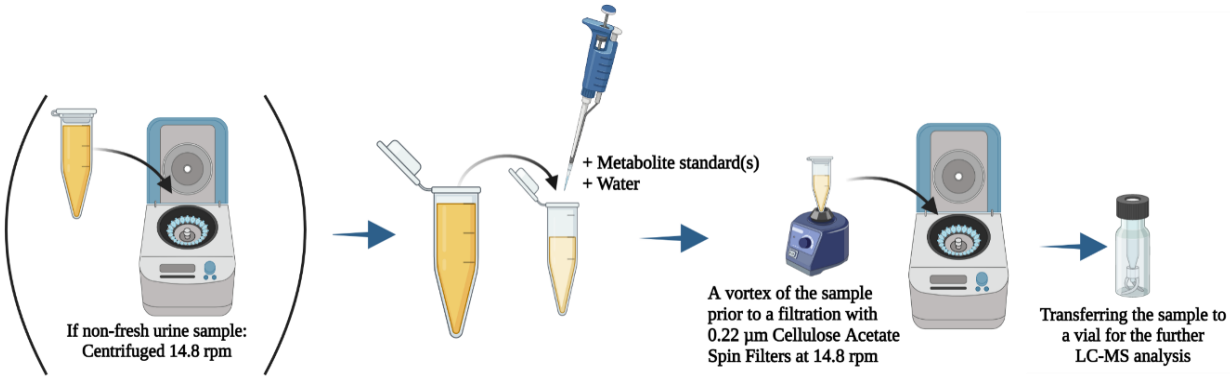


Figure 3.4: The sample preparation of urine spiked with metabolite standard(s). *In this procedure urine was normalized to 2 mM creatinine and filtered.*

1) The sample preparation of urine spiked with metabolite standards for the testing of a computer software's identification performance

***Table 3.3: The volumes of urine** with 18.87 mmol creatinine, **water**, and the **metabolite standards;** 1B10, 4A3, 4A7, 4A12, 4B2, and 4B4 mixed together.*

Sample	Volume [µL]
Urine	10.6
Water	29.4
1B10 Glycerol	10
4A3 N-Acetyl galactosamine	10
4A7 N-Acetylcysteine	10
4A12 S-Carboxymethyl cysteine	10
4B2 Thiamine pyrophosphate	10
4B4 Thymidine monophosphate	10

2) The sample preparation of urine spiked with trimethylamine to test is the detection is quantitative

Seven urine samples with 2 mM creatine and different concentration of trimethylamine were constructed. Prior to the sample preparation, the measured amount of TMA in children and adults with and without trimethylaminuria was obtained with a data search, see **Figure 4.21** in **Section 4.5**.The constructed concentration of TMA was supported by the data search, the limited amount of 0.846 mM TMA standard available, and urine dilution restrictions.

Table 3.4: The desired concentration of trimethylamine (TMA) to construct, and the volumes of urine, trimethylamine standard, and water used.

Sample No	*The desired concentration of TMA to construct*	*V (Urine) [µL]*	*V (Std. TMA) [µL]*	*V (H_2O) [µL]*
1	*0*	10.6	0.0	89.4
2	*2.6*	106	3.1	890.8
3	*12*	16.0	2.1	132.0
4	*30*	10.6	3.5	85.8
5	*50*	10.6	6.0	83.4
6	*84.64*	10.6	10	79.4
7	*253.92*	10.6	30	59.4

3.7 LC-MS analysis

For LC-MS analysis, a Dionex Ultimate 3000 UHPLC system coupled to a Q Exactive MS was used. The UHPLC included an autosampler, a column department, and a pump. Pursuit XRs C18-diphenyl (250 x 2.0 mm, particle size 3 µm) from Agilent Technologies (Santa Clara, CA, USA), was the column used. The ionization source used was ESI. The instrumental settings used in this thesis were obtained from previous projects, focusing on improving the signal intensity and selectivity, when analyzing metabolites in DBS, by respectively optimizing the parameters on the Q-Exactive MS and LC. (92). See **Tables 5-8** for these parameters. **Table 3.5** shows the settings used for the LC, and **Table 3.6** and **Figure 3.5** show the LC flow gradient. **Table 3.7** shows the settings used for MS. **Table 3.8** shows the settings applied for ESI.

Table 3.5: *The setup used for liquid chromatography.*

Parameter	Setting
Mobile phase A	Water + 0.1 % formic acid
Mobile phase B	Methanol + 0.1 % formic acid
Gradient	See table _ and figure
Injection volume	2 µl
Column temperature	30 °C
Flow rate	300 µL/min
Analysis time	27.5 min
Re-equilibration time	10 min

Table 3.6: Flow gradient *applied for the analysis.*

Time (min)	Mobile phase B (%)
0	2
6	10
8.5	75
25	100
32.5	100
37.51	2

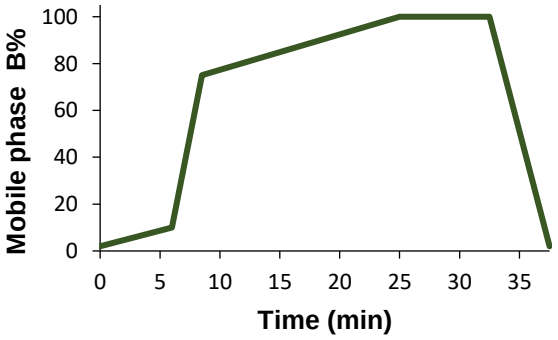


Figure 3.5: The LC flow gradient*. Starting and ending with 2% mobile phase B.*

Table 3.7: *The instrumental settings for the mass spectrometer*

Parameter	Settings
Scan types	Full MS, and data-dependent acquisition (top 5)
Scan ranges	50-750, 70-1050, and 1000-1500
Resolution at m/z 200	Full MS: 70 000 DDA (top 5): 17 500
Stepped normalized collision energy	20, 50 and 80
Polarity	Positive and negative
Micro scans	1
Lock masses	Off
Automatic gain control target value	Full MS: 1.00E+6 DDA (top 5): 5.00E+6
Maximum injection time	Full MS: 250 ms DDA (top 5): 100 ms
Analysis time	32.5 min
Re-equilibration time	10 min

The scan ranges listed in **Table 3.7** were developed by looking at the metabolites with the lowest and highest molecular mass in the mixtures prepared (**section 3.5**). See **Appendix 6.1** for scan ranges used for each metabolite.

Table 3.8*: The instrumental settings for the electrospray.*

Electrospray parameter	Settings
Sheath gas (N_2) flow rate	40 a.u.
Auxiliary gas (N_2) flow rate	10 a.u
Sweep gas (N_2) flow rate	2 a.u
Capillary temperature	250 C
S-lens RF level	50.0
Auxiliary gas heater temperature	300 °C
Electrospray voltage	+/- 3.5 kV
Electrospray needle position	C
Capillary temperature	250 °C

3.7.1 Applying molecular ions to an inclusion list

Metabolites were initially analyzed without adding their possible m/z values on an inclusion list. For those metabolites missing MS^2, from the initially method, were re-analyzed with their m/z value on an inclusion list. See **Table 3.9** and **Table 3.10** for metabolites analyzed again in positive and negative mode respectively.

Positive ionization:

Table 3.9: Parameters for metabolites analyzed with their positive ion's m/z value on an inclusion list. *Shows the metabolites' molecular formula, neutral monoisotopic molecular mass, and observed ion mass in an LC-MS analysis without inclusion list. As well as the RT interval, m/z scan range, and the sample injected when including an inclusion list.*

PLATE	NROW	NCOL	PRIMARY_NAME	MOLECULAR FORMULA	NEUTRAL MONOISOTOPIC MOLECULAR MASS	OBSERVED ION MASS	RT INTERVAL MS METHOD	M/Z SCAN RANGE	SAMPLE INJECTED
1	**C**	**11**	PHENYLALANINE	C9H11NO2	165.08	166.09	2-10	70-1050	1C
1	**F**	**11**	ASCORBATE	C6H8O6	176.03	177.04	1-4	70-1050	1F
1	**G**	**10**	CYSTEINE	C3H7NO2S	121.02	122,03	1-4	70-1050	1G
2	**D**	**9**	MELANIN	C18H10N2O4	318.06	319,07	4-8	70-1050	2D
2	**G**	**1**	NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE	C21H27N6O18P3	744.06	745,07	2-7	70-1050	2G1
2	**H**	**6**	NICOTINAMIDE HYPOXANTHINE DINUCLEOTIDE	C21H26N6O15P2	664.09	665,1	4-8	70-1050	2H6
3	**C**	**7**	FRUCTOSE 6-PHOSPHATE	C6H13O9P	260.03	283,02	1-4	70-1050	3C
3	**F**	**4**	GUANOSINE DIPHOSPHATE	C10H15N5O11P2	443.02	444,03	1-6	70-1050	3F4
4	**B**	**10**	RAFFINOSE	C18H32O16	504.17	527,16	1-5	70-1050	4B10
4	**C**	**4**	LACTOSE	C12H22O11	342.12	343,12	1-4	70-1050	4C
4	**E**	**6**	ADENOSINE DIPHOSPHATE RIBOSE	C15H23N5O14P2	559.07	560,08	2-6	70-1050	4E
4	**G**	**7**	DEOXYADENOSINE TRIPHOSPHATE	C10H16N5O12P3	491.00	492,01	3-10	70-1050	4G7
5	**E**	**10**	10-HYDROXYDECANOATE	C10H20O3	188.14	189,15	12-16	70-1050	5E
5	**G**	**9**	BENZYLAMINE	C7H9N	107.07	108,08	3-7	70-1050	5G

Negative ionization:

Table 3.10: Parameters for metabolites analyzed with their negative ion's m/z value on an inclusion list. *Shows the metabolites' molecular formula, neutral monoisotopic molecular mass, and observed ion mass in an LC-MS analysis without inclusion list. As well as the RT interval, m/z scan range, and the sample injected when including an inclusion list.*

PLATE	NROW	NCOL	PRIMARY_NAME	MOLECULAR FORMULA	NEUTRAL MONOISOTOPIC MOLECULAR MASS	OBSERVED ION MASS	RT INTERVAL MS METHOD	M/Z SCAN RANGE	SAMPLE INJECTED
1	**G**	**1**	PYRAZOLE	C3H4N2	68,0	67,0	1-4	50-750	1G
1	**G**	**10**	CYSTEINE	C3H7NO2S	121,0	120,0	1-4	50-750	1G
2	**F**	**12**	ALPHA-HYDROXYISOBUTYRATE	C4H8O3	104,0	103,0	1-7	50-750	2F
2	**G**	**12**	DGTP	C10H16N5O13P3	507,0	506,0	3-10	50-750	2G12
4	**C**	**2**	ADENOSINE TRIPHOSPHATE	C10H16N5O13P3	507,0	506,0	1-12	50-750	4C2
4	**C**	**8**	CYTIDINE TRIPHOSPHATE	C9H16N3O14P3	483,0	482,0	1-12	50-750	4C8
4	**F**	**8**	COENZYME A	C21H36N7O16P3S	767,1	766,1	8-14	70-1050	4F8
4	**F**	**10**	INOSINE TRIPHOSPHATE	C10H15N4O14P3	508,0	507,0	1-12	50-750	4F10
5	**H**	**1**	BUTANOATE	C4H8O2	88,1	87,0	7-11	50-750	5H
5	**H**	**5**	DEHYDROASCORBATE	C6H6O6	174,0	173,0	1-4	50-750	5H
6	**B**	**12**	GERANYL-PP	C10H20O7P2	314,1	313,1	4-8	70-1050	6B

3.8 Computer software

Xcalibur (version 4.2.47) was used to control LC-MS parameters and acquire data. To calibrate and monitor mass spectrometric parameters Tune (version 2.11) was utilized. Xcalibur and Tube were supplied by Thermo Scientific.

Figure 3.6 shows a workflow for the main software used to create the in-house library, as well as the software including in-house libraries for untargeted metabolomics data processing. MLSDiscovery (version 3.1B.27), supplied by IROA Technologies, and Freestyle (version 1.7 SP2), supplied by Thermo Fischer Scientific, were used to process, and collect mass spectrometric data. To store metabolite data mzVault (version 2.3). Compound Discoverer (version 3.1), supplied from Thermo Fisher Scientific, was used to perform an untargeted metabolomics with the created in-house library (stored in mzVault) for metabolite identifications.

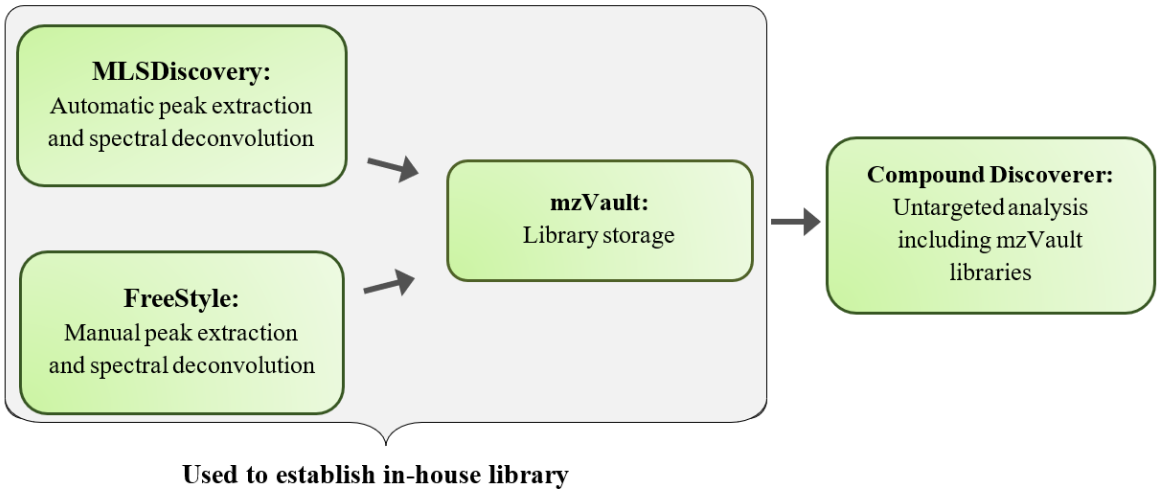


Figure 3.6: *A simplified workflow of the data processing for creating an in-house library and implementing it for untargeted analysis.*

3.8.1 Creating the in-house library

The procedure for using the MLSDiscovery to create the in-house library was as followed: A project setup for generating libraries on MLSDiscovery was applied. This setup included two tabs, "multiplex designer" and "data analysis", see **Appendix 6.3**.

In the *"multiplex designer"*, groups of the multiplexed samples were created, these contained the metabolites in the mixtures analyzed with LC-MS. A method to do a data search and extract peaks with RT, MS1, and MS^2 was made in the software. Here, the polarity mode

(positive/negative) was specified, and MS level at 2 was applied. Raw files obtained from the DDA (top 5) were converted into mxXML files by using ProteoWizard MSConvert. These were loaded into MLSDiscovery and coupled to their belonging multiplexed sample. A database library specifying polarity and name was created and coupled with the established data search method. A molecular ion list editor, containing 73 different types of molecular ions was set to the default settings, including all molecular ion types in the data search. For preference default settings applied in MLSDiscovery, see **Figure 4.7**.

Table 3.11: The applied preference settings in MLSDiscovery

Parameter	Default settings
Minimum peaks intensity	100 000
Maximum hits per compound	2
Retention time	0.00 -100.00 min
Minimal peak width	0.01 min
Filter width	5
Maximal charge	2
Minimum # of ions in adduct	2
Max isotope pattern deviation	20 %
m/z tolerance	30 ppm
Retention time tolerance	0.15 min
MS mode	Positive and negative

In *"data analysis"*, suggestions of metabolite identities on the extracted peaks with RT, adducts, MS^1, and MS^2 were shown.

A manual elimination of the suggested identities was performed based on peak shape, intensity, m/z error, and MS^2. Suggestions with *m/z* error >10 ppm, and MS^2 being non-comparable with online MS^2 were eliminated. Online reference MS^2 was obtained from HMDB, Metlin, and PubChem. Metabolites with no comparable MS^2 spectra with online MS^2 were manually processed at Freestyle.

The suggested identities kept were exported to the built-in database library at MLS Discovery. Here, identities missing MS^2 were deleted before exporting the library database to a NIST msp file (with a 5% cut off), that could be imported to Themo mzVault.

Freestyle

Freestyle was used to manually extract ion chromatograms and obtain MS^2 spectra, for metabolites not detected at MLSDiscovery and for metabolites analyzed with their *m/z* value on an inclusion list. MS^2 spectra's scan number for metabolites detected were added to mzVault

mzVault

Data from MLSDiscovery were imported to mzVault by loading the NIST msp files saved. Data imported to mzVault were compound names, MS^2 spectra, and the precursor ion m/z values. For some metabolites, InChi Key and SMILES were added by selecting fill missing data. Retention times, molecular formula, and neutral molecular mass were manually added. Due to the NIST msp file not specifying if metabolites were found in positive or negative mode, two separate mzVault libraries files distinguishing difference polarity were created.

Metabolites found with Freestyle were listed in mzVault by manually writing their name, molecular formula, and neutral mass. MS^2 were added by opening the RAW files corresponding to each metabolite, adding the MS^2 spectra's correct scan number. Here the polarity is also added from the RAW file, hence only one mzVault library containing metabolites found in positive and negative mode was created.

3.8.2 Importing the mzVault in-house library to an existing untargeted metabolomics workflow

Compound Discoverer is used for untargeted metabolomics analysis at the metabolomic research group. The created mzVault libraries were imported to this software and a template including a mzVault search, with defined settings, to an existing untargeted workflow was created. See **Appendix 6.3**, for the workflow and the settings applied for the mzVault search.

With the created workflow template, the identification performance of Compound Discovery coupled with mzVault was tested by analyzing some RAW files used to create the in-house library, as well as urine samples spiked with metabolite standards.

4 RESULTS AND DISCUSSION

In a metabolomics workflow, metabolite identification is often the bottleneck for transferring metabolomics data into reliable data for laboratory diagnostics. Identifications with a high level of confidence may be achieved by comparing MS^2 with reference MS^2. Online library databases, containing reference MS^2 obtained from different sources, can be used for identifications. However, due to the lack of repeatability and standardization of MS^2 amongst instruments, these reference MS^2 won't provide identification with the highest level of confidence, required for laboratory diagnostic. Hence, an in-house library containing reference MS^2 is necessary. An in-house library may also consist of additional information like RTs, that can distinguish isobaric metabolites with the same MS^2. In global metabolomics, an in-house library providing the highest level of confidence has great value and can be applied to discover new or enhanced biomarkers, or in precision medicine.

The metabolomic research group at the Norwegian National Unit for Screening and Diagnostics of Congenital Paediatric Metabolic Disorders has developed an LC-HR-MS method, optimized to detect a wide range of metabolites. The next step is to establish an in-house library and implement it into an existing data processing workflow currently used for untargeted metabolomics. For this purpose, an MS-MLS kit containing metabolites with a wide range of hydrophobicity was prepared and analyzed.

This chapter is divided into four parts. The first part, **4.1 - 4.2,** focuses on how metabolites from the MS-MLS kit were mixed and analyzed, as well as how MS^2 obtained from MLSDiscovery and Freestyle were validated with online MS^2. Since there are thousands of defined metabolites in the metabolome from various chemical classes (1), establishing an in-house library containing all of these will be time- and resource-consuming. Hence the second part, **4.3**, focuses on highlighting which chemical classes of metabolites may be easier to detect with the developed LC-HR-MS method. The third part, **4.4**, focuses on the results obtained from implementing the established in-house library into an untargeted metabolomics workflow and evaluating its identification performance. The last part **4.5**, focuses on identifying trimethylamine in urine with its reference MS^2 and RT included in the created in-house library and doing a quantitative study.

4.1 Non-isobaric metabolite mixtures were prepared and analyzed to save time and resources

A total of 603 metabolites were going to be prepared and analyzed with the developed LC-HR-MS. The instrument had a 32.5-minute-long analysis time and 10 minutes re-equilibrium time for each injection. Each metabolite was going to be injected four times; in a full scan MS mode, and an MS^2 DDA (top 5) mode, both in a positive and negative ionization. It would take 71 days, 4 hours, and 30 minutes to analyze every metabolite if they were individually injected into the instrument. The experimental design, establishing the procedure for preparing and analyzing the MS-MLS, focused on being time-efficient. Thus, it was determined to prepare and analyze mixtures of a group of pre-defined metabolites from the MS-MLS kit. The selections of metabolites that were going to be mixed were based on the metabolites' monoisotopic mass, hydrophobicity, and concentration. To ensure that the mass spectrometric data for metabolites in the prepared mixtures were distinguishable, they were controlled to be non-isobaric, providing distinguishable m/z values. The starting point was to mix every metabolite across each row or column. There were 49 of 51 rows with only non-isobaric metabolites, which was more than columns with non-isobaric metabolites. It was planned to analyze 49 mixtures containing all the metabolites across the non-isobaric metabolite rows, and 8 metabolite mixtures from the rest of the rows in the MS-MLS kit. This would take 6 days, 17 hours, and 30 minutes to analyze them on the instrument, and hence be 90.5 % more efficient than injecting them all individually. This strategy of mixing them was therefore used.

The mixing of all the metabolites across the 48 rows was dependent on the small amount of dry metabolite in each well at 5 µg, and the MS-MLS product sheet's recommendation of solubilizing each metabolite in approximately 100 µL solvent. Each row consisted of 12 metabolites. Two separate alternative procedures for mixing them were discussed:

Alternative 1: Solubilize the metabolites in each their 100 µL solvent and obtain a stock solution for each metabolite before pooling together 5-10 µL.

Alternative 2: Solubilize all the 12 metabolites in a row in the same 100 µL solvent.

With alternative 2, no pure stock solution of each metabolite would be obtained. After the establishment of the in-house library, it was a desire to spike metabolite standards in biological material to investigate matrix effects. To study the matrix effects, it is ideal to spike the biological material with only a small volume of standard, reducing the dilution volume and

obtaining most of the biological materials' properties. Hence, a concentrated metabolite standard was desirable, to achieve sensitive mass spectrometric detections of the standard metabolites, even if a small volume was spiked into the biological material. The metabolite concentration was calculated to be too low if mixtures from alternative 1 were used and the biological material and the standard metabolite mixture ratio were above 8:2. Therefore, alternative 2 was proposed to obtain mixtures with high enough metabolite concentration. Drawbacks would be if some of the metabolites weren't solved in the mixtures and hampered the detection of other metabolites in the mixture. To have the possibility to re-analyze metabolites, if necessary, and ensure an in-house library of hundreds of metabolites, alternative 1 was chosen.

The choice of solvents used for metabolites in plates 1-7 was the same solvents recommended by the MS-MLS product sheet, except for some metabolites in plates 1-5. Most of the polar metabolites were in plates 1-5. The MS-MLS product sheet recommended firstly solubilizing every metabolite in plates 1-5 with 5 µL MeOH, mixing the plates for some minutes, and lastly adding water before one final mixing. It was discussed that MeOH could evaporate before water was added, due to its volatility and the small volume, hence preventing metabolites to solve if both solvents had to be present to achieve complete solubilization. The water solubility for metabolites in plates 1-5 was therefore extracted from HMDB and Sigma Aldrich, and those with a water solubility above 5 µg/100 µL were only solved in 100 µL water.

The MS can only acquire mass spectrometric data for ions within an applied m/z scan range. The m/z scan range's highest m/z value is limited to be 15 levels higher than the range's lowest m/z value. No single m/z interval could include all the metabolites from the kit. Therefore 3 m/z intervals were developed. Only one row contained metabolites that couldn't be included in one m/z range. Here the metabolite with the highest monoisotopic mass was analyzed individually in another m/z range.

An overview of how many metabolites were detected and not with the procedure developed in the experimental design, and how many were detected after an optimized re-analysis are shown in

Table 4.1. Only some of the metabolites not detected in the initial procedure were re-analyzed, see **Section 4.2.2** for which.

Table 4.1: The numbers of MS-MLS detected with the initial procedure and with an optimized procedure for sample preparation and data acquisition, as well as the number of not detected MS-MLS

Metabolites from location:	*Detected without optimization*	*Detected after an optimization*	*Not detected*	*TOTAL*
Plate 1-5 (polar metabolites)	343	10	91	444
Plate 6, row A-E (less polar metabolites)	41	-	19	60
Plate 6, row F-G (polar sugar molecules)	24	-	0	24
Plate 7 (lipophilic metabolites)	24	-	51	75
TOTAL	432	10	161	603

Summary: *The procedure for sample preparation and analyzing the metabolites was created to be both time-efficient and to ensure metabolite detection. Non-isobaric mixtures were prepared, and pure metabolite stock solutions were stored in case of the metabolites were to be re-analyzing. Metabolites with a water solubility above 5 µg/100 µL, in plates 1-5, were solved in only water to assure solubilization. One metabolite was determined to be analyzed individually to obtain detection. Analyzing most of the metabolites in mixtures instead of individually was 90.5 % more time-efficient.*

4.2 The data processing at MLSDiscovery and Freestyle

Initially, the MLSDiscovery software was used to process large batches of raw data results files obtained from the LC-MS analysis of MS-MLS metabolite mixtures. According to metabolite ions' m/z values ± the default ppm, the software extracted ion chromatograms (EIC) from a total ion chromatogram (TIC). The EIC could contain peaks that could be classified as metabolite ions originating from a certain metabolite. The m/z values were calculated by the metabolites' monoisotopic mass and a list containing all the molecular ions to search for. The molecular ion list included possible ion formation of the metabolites, such as traditional protonated or deprotonated molecules, or adducts. Metabolites' monoisotopic mass was not possible to add manually into the MLSDiscovery software, these were already listed in the software by the producer.

The Freestyle software was used to control some data obtained from MLSDiscovery, and to process smaller batches of raw data files. Due to the ability to manually add which m/z to extract in Freestyle, this software was easier to use when processing data for a few metabolites.

Ideally, when analyzing standards, it should be possible to easily pinpoint which peak with $MS^{2,}$ and RT belongs to which standard. However, since the metabolite standards in this thesis were mixed with other standards, and analyzed in low concentration, online MS^2 was necessary to confirm the MS^2 for each standard.

The next **sections, 4.2.1** and **4.2.2,** will show data processing of the metabolites from plates 1-5, analyzed in mixtures.

Summary: *The MLSDiscovery software was initially used for data processing of big batches of raw data files. The freestyle software was used to control some data result obtained from the MLSDiscovery, and for smaller batches of raw data files.*

4.2.1 MLSDiscovery: Metabolite ions with various adducts were proposed, the majority were traditional protonated or deprotonated metabolites

In MLSDiscovery, a total of 2595 peaks were obtained for metabolites in plates 1-5. Many of these were eliminated due to poor peak shape as well as a high m/z error. A total of 73 molecular ions were in the molecular ion list, of these 63 molecular ions were extracted. The top 11 types of molecular ions extracted are shown in **Figure 4.1**. Here, protonated, and deprotonated metabolites were the most common.

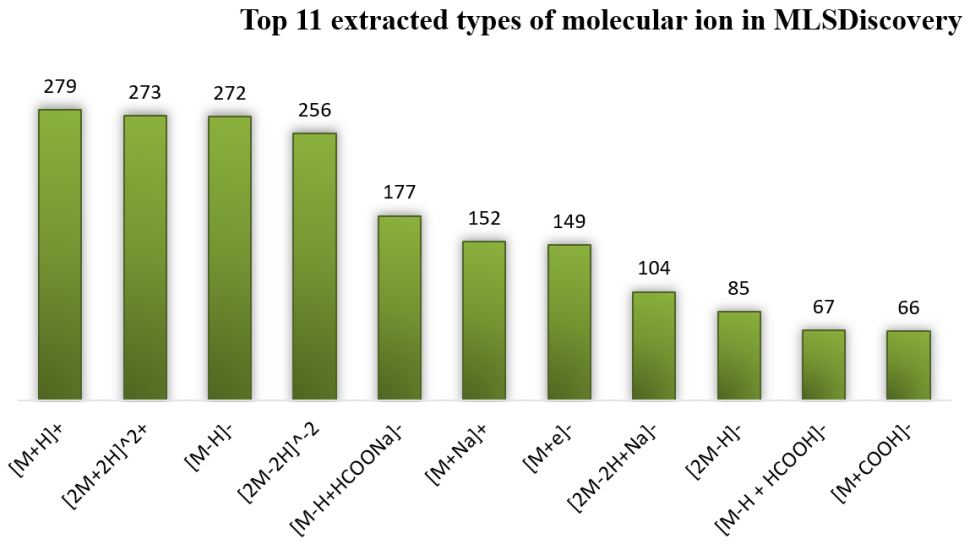


Figure 4.1: The top 11 extracted molecular ions for metabolites in plates 1-5*. Deprotonated and protonated metabolites are the most abundant molecular ions. Most of the metabolites have been extracted with more than one molecular ion type.*

Figure 4.1 shows that in addition to protonated and deprotonated molecules, a huge number of $[2M+2H]^{2+}$ and $[2M-2H]^{2-}$ were extracted, these could be $[M+H]^{+}$ and $[M-H]^{-}$, respectively, because they have the same m/z values.

4.2.1.1 The lack of online MS2 for metabolites with other adducts than H+ and H- makes the validation of some identifications challenging

Even though many peaks were obtained from the MLSDiscovery software, they didn't cover all the metabolites analyzed. Some peaks also didn't have an MS^2. After a data processing on MLSDiscovery of metabolites in plates 1-5, the metabolites were either classified as not found, unsure, or secure. **Figure 4.2** gives a schematic overview of the classification and how not found and unsure metabolites were processed further. The computer software, Freestyle was used to manually control if metabolites classified as not found or unsure were present in the analyzed sample. Metabolites classified as secure had an MS^2 that was comparable to an online reference MS^2.

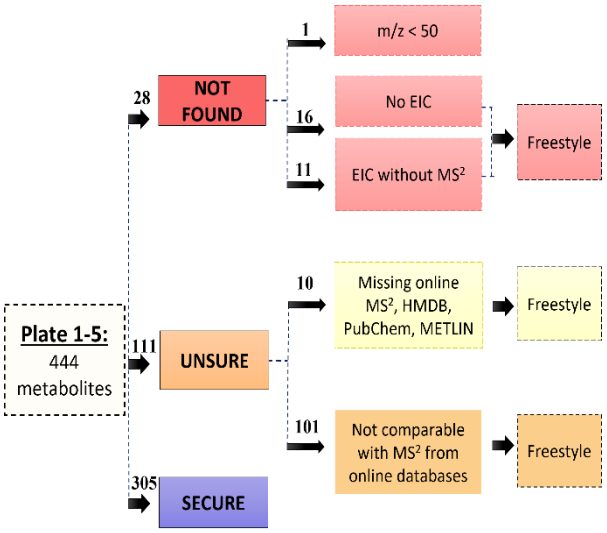


Figure 4.2: From plates 1-5, the 444 metabolites were classified as not found, unsure or secure metabolites after a data processing at MLSDiscovery. *Metabolites not found or unsure were further processed at Freestyle*

Sections **4.2.1.1.1-4.2.1.1.2** will explain further which metabolites were classified as not found or unsure, and how they were processed further.

Summary: *The metabolites processed at MLSDiscovery were classified as not found, unsure, or secure. Their classification determined their further data processing workflow.*

4.2.1.1.1 MLSDiscovery – Metabolites that were not found in the analyzed mixture:

Metabolites classified as not found in MLSDiscovery were either metabolites with an *m/z* below the orbitrap's lowest detectable mass at *m/z* 50 or metabolites with no extracted peak or a peak with no detected MS^2.

A peak may miss an MS^2 if its extracted *m/z* value wasn't included in the DDA (top 5) mode applied, which only provided MS^2 of the 5 most abundant molecular ions during a certain time frame. To ensure a collection of MS^2 for low abundant molecular ions, their *m/z* can be added to an inclusion list, see **Section 4.2.2.**

4.2.1.1.2 MLSDiscovery – Metabolite ions, unsure if are present in analyzed samples:

Metabolites classified as unsure were either those with an obtained MS^2 that wasn't comparable with the metabolite's online MS^2 or metabolites that only had obtained MS^2 with no available online MS^2 at HMDB, PubChem, or METLIN. **Figure 4.3** and **Figure 4.4** show some examples of these two types of unsure metabolites, respectively.

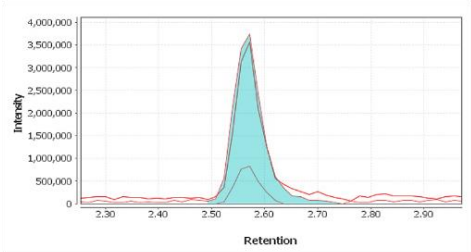
Some of the MS^2 obtained for metabolites differed from online MS^2 and were found in the blank sample

Before analyzing MS-MLS metabolite mixtures, their blank sample was analyzed. A blank sample only consisted of the solvent used to solubilize the metabolites. Some metabolites, with an obtained MS^2 from MLSDiscovery, which differed from their online MS^2, also had a similar peak in the blank sample as in their metabolite sample. The peak from these two samples was compared by using Freestyle. If it was similar in the metabolite sample and the blank sample, the observed MS^2 was not from the identified metabolite. Of all the obtained peaks for metabolites in plates 1-5, in MLSDiscovery, 48 were in the blank. Mostly the peaks with an RT between 13-17 minutes were in the blank sample. The q-orbitrap can have a mass accuracy below 5 ppm. MLSDiscovery had a default mass accuracy set to 30 ppm. Changing this parameter would have provided fewer peaks that also were in the blank sample, but it would still be some peaks from the blank sample.

Figure 4.3 shows peaks suggested by the MLSDiscovery to originate from the metabolites, glycerol, and 6-phosphogluconate. These have mass accuracies (m/z error) below 5 ppm. MLSDiscovery shows the MS^2 for the molecular ion with the highest relative intensity. For glycerol and 6-phosphogluconate, which is shown in the figure, the MS^2 were obtained for $[M+Na]^+$ and $[M-2H+K]^{-2}$, respectively. As shown in the figure, both had an m/z error below 5 ppm and a peak with a nice shape and high intensity. When extracting these m/z values from the blank and the sample, with Freestyle, they were shown to also be in the blank sample

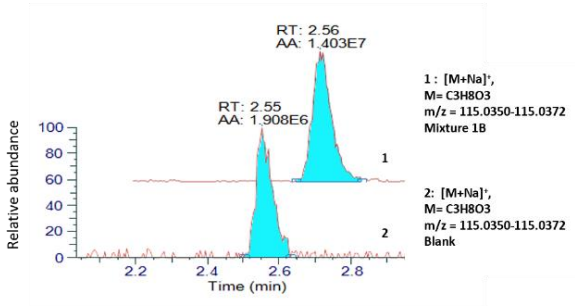
GLYCEROL:

MLSDiscovery:



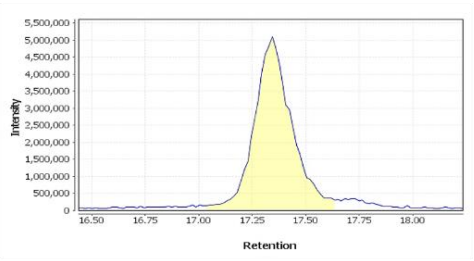
Type	Name	MZ observed	M/Z error, ppm	Relative intensity observed
ADDUCT	M+H	94.058	-3.093	2.363
ADDUCT	M+H	95.059	-1.071	0.710
ADDUCT	M+Na	117.041	-1.773	0.739
ADDUCT	M+Na	115.036	-1.699	100
ADDUCT	2M+2Na-H	229.056	-1.516	22.213
ADDUCT	M+Na	116.040	-0.736	3.613
ADDUCT	M+H	93.055	-0.593	95.234
ADDUCT	2M+2H	93.055	-0.593	95.234
ADDUCT	2M+2Na-H	230.069	-0.550	1.077
ADDUCT	2M+2Na-H	231.071	3.027	0.624
ADDUCT	2M+2H	94.058	9.602	2.363

Freestyle:



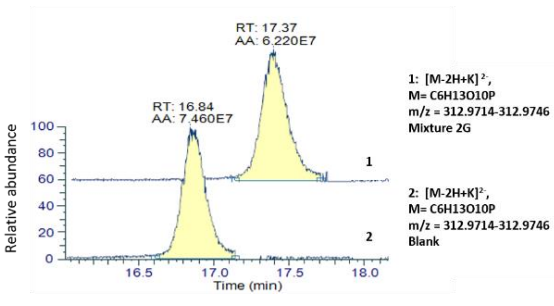
6- PHOSPHOGLUCONATE:

MLSDiscovery:



Type	Name	MZ observed	M/Z error, ppm	Relative intensity observed
ADDUCT	M-2H+K	312.973	-2.153	100
ADDUCT	M-2H+K	313.977	-0.124	6.495
ADDUCT	M-2H+K	314.971	-6.138	1.686
ADDUCT	M-2H+K	315.974	-6.459	0.164
ADDUCT	M-2H+K	316.989	32.774	1.744

Freestyle:



.

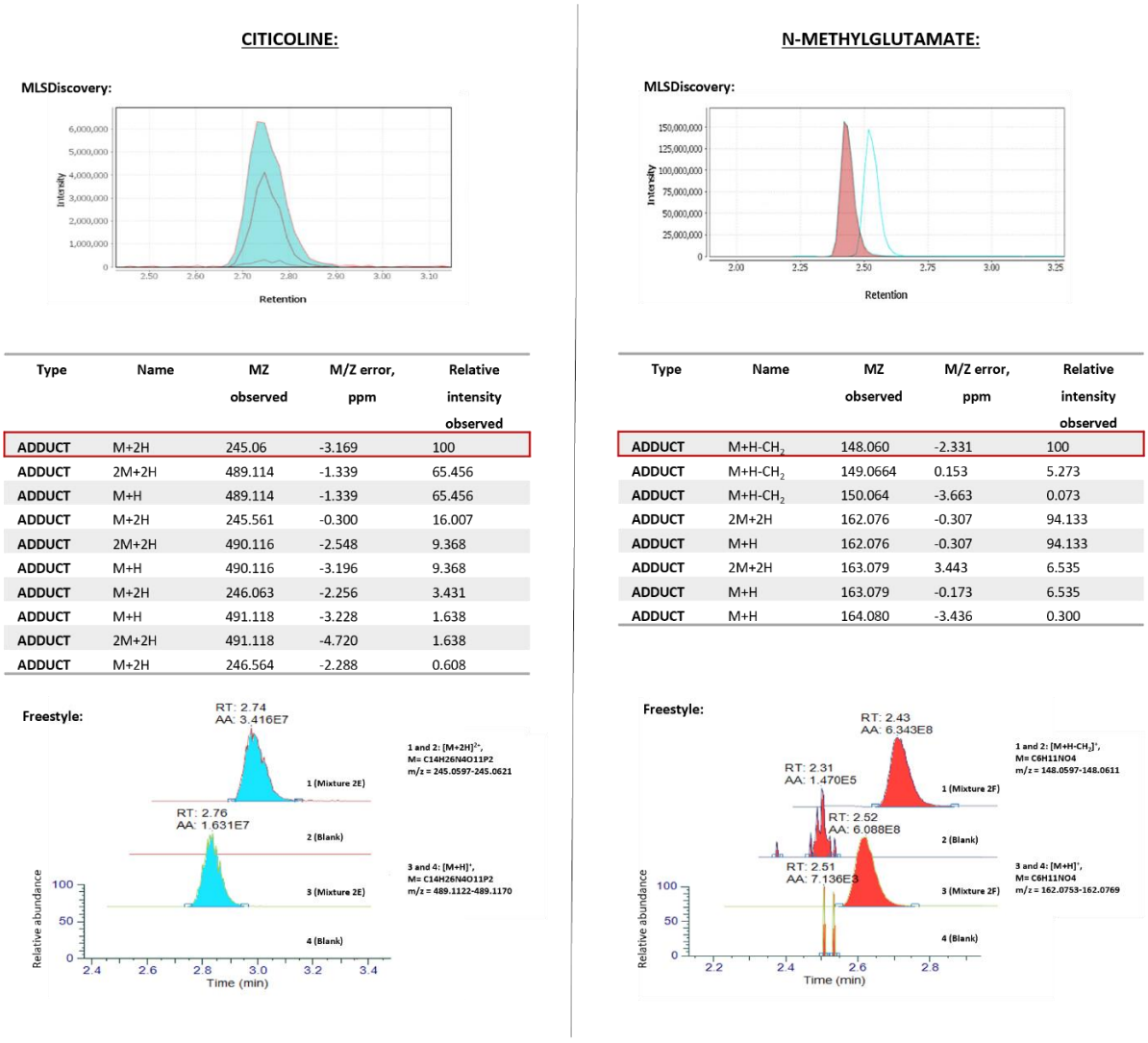
Figure 4.3: Data results for glycerol and 6-phosphogluconate, which are found in the blank sample, to the left and right respectively. *The data are obtained from MLSDiscovery and Freestyle. At the top, EIC is shown for the m/z values noted in the adduct list, the highest marked peak is for the m/z value marked in the adduct list. At the bottom, the EIC for the m/z value with the highest relative intensity is shown, extracted from the sample mixture, and the blank sample.*

Using online MS^2 for validation prevented in-correctly validations of peaks with nice peak shapes and high intensities. If all the obtained peaks for a metabolite were in the blank sample, the true peak for the metabolite was attempted to be found by searching for m/z values of different molecular ions in Freestyle.

***Summary**: MLSDiscovery didn't filter out peaks that were in the blank samples. Hence, even though molecular ions had a peak with a nice shape, high intensity, and a low m/z error these couldn't be classified as secure. If metabolites were analyzed individually and only MLSDiscovery was used, it may still have been necessary to compare their MS^2 to online MS^2 to distinguish which metabolites are from the blank sample.*

Some metabolite ions detected without available online reference MS^2 at HMDB, METLIN, or PubChem, were correctly identified

If MLSDiscovery only extracted metabolite ions without available online MS^2 to compare their MS^2 with, the MS^2 of another adduct with an online MS^2 was extracted from Freestyle. Citicoline ionized as $[M+2H]^{2+}$ and, N-methyl glutamate ionized as $[M-H+CH_2]^{+}$, are two examples, see **Figure 4.4**.



Type	Name	MZ observed	M/Z error, ppm	Relative intensity observed
ADDUCT	M+2H	245.06	-3.169	100
ADDUCT	2M+2H	489.114	-1.339	65.456
ADDUCT	M+H	489.114	-1.339	65.456
ADDUCT	M+2H	245.561	-0.300	16.007
ADDUCT	2M+2H	490.116	-2.548	9.368
ADDUCT	M+H	490.116	-3.196	9.368
ADDUCT	M+2H	246.063	-2.256	3.431
ADDUCT	M+H	491.118	-3.228	1.638
ADDUCT	2M+2H	491.118	-4.720	1.638
ADDUCT	M+2H	246.564	-2.288	0.608

Type	Name	MZ observed	M/Z error, ppm	Relative intensity observed
ADDUCT	M+H-CH_2	148.060	-2.331	100
ADDUCT	M+H-CH_2	149.0664	0.153	5.273
ADDUCT	M+H-CH_2	150.064	-3.663	0.073
ADDUCT	2M+2H	162.076	-0.307	94.133
ADDUCT	M+H	162.076	-0.307	94.133
ADDUCT	2M+2H	163.079	3.443	6.535
ADDUCT	M+H	163.079	-0.173	6.535
ADDUCT	M+H	164.080	-3.436	0.300

Figure 4.4: Data results for citicoline and n-methyl glutamate, to the left and right, respectively. *The data are obtained from MLSDiscovery and Freestyle. At the top, EIC is shown for the m/z value marked in the molecular ion list below. At the bottom, the EIC for the same m/z, as well as for the m/z value of* $[M+H]^{+}$*, is extracted from the sample mixture (1 and 3), and the blank sample (2 and 4),*

In the molecular ion list for citicoline, it is shown that $[M+2H]^{2+}$ has the highest relative intensity, hence MLSDiscovery provided the MS^2 for this molecular ion. To obtain the MS^2 of a molecular ion with less relative intensity, a new data search was necessary to perform in MLSDiscovery. There was no online MS^2 for diprotonated citicoline in HMDB, METLIN, or PubChem. Most of the available online MS^2 are for protonated or deprotonated metabolites. Hence, manual extraction of protonated citicoline's MS^2 was obtained in Freestyle. The MS^2 for the protonated citicoline obtained was comparable to the online MS^2. This MS^2 along with the RT was added to the in-house library. The MS^2 for $[M+2H]^+$ was also added to the in-house library because it had similar RT, with a difference below 1 minute, and similar peak intensity as the protonated metabolite.

The ion $[M+H-CH_2]^+$ of N-methyl glutamate was controlled in the same way as citicoline, and its MS^2 and RT were likewise added to the in-house library

Summary: *Some metabolite ions with no online MS^2 to compare their MS^2 with had their MS^2 added into the library if another molecular ion, originating from the same metabolite, had similar RT and an MS^2 that was comparable to an online MS^2. Most of the online MS^2 were for protonated and deprotonated metabolites.*

4.2.2 Optimization steps - Inclusion list or/and an elevated concentration:

Some metabolites that only had extracted molecular ions without any MS^2 were re-analyzed. If the observed m/z value for a metabolite ion had a mass accuracy below 5 ppm, its m/z value was added to an inclusion list before the LC-MS analysis. **Appendix 6.5** shows the metabolites having their m/z value on an inclusion list, their calculated mass accuracy, and if they were detected with an MS^2 that was comparable with an online MS^2. Their mass accuracy was calculated with **Equation 1** in **Section 2.4.1** and with the observed and theoretical m/z values listed in **Appendix 6.5**.

Metabolites that weren't detected with a concentration below 10 µM in the mixture analyzed, were analyzed once more with an elevated concentration. **Appendix 6.6** shows which were

detected with a concentration below 10 µM, and which of the non-detected metabolites with a concentration below 10 µM were detected when re-analyzing them at a higher concentration.

Figure 4.5 illustrates how many metabolites were found and how many were not throughout the data processing and after optimization processes for plates 1-5.

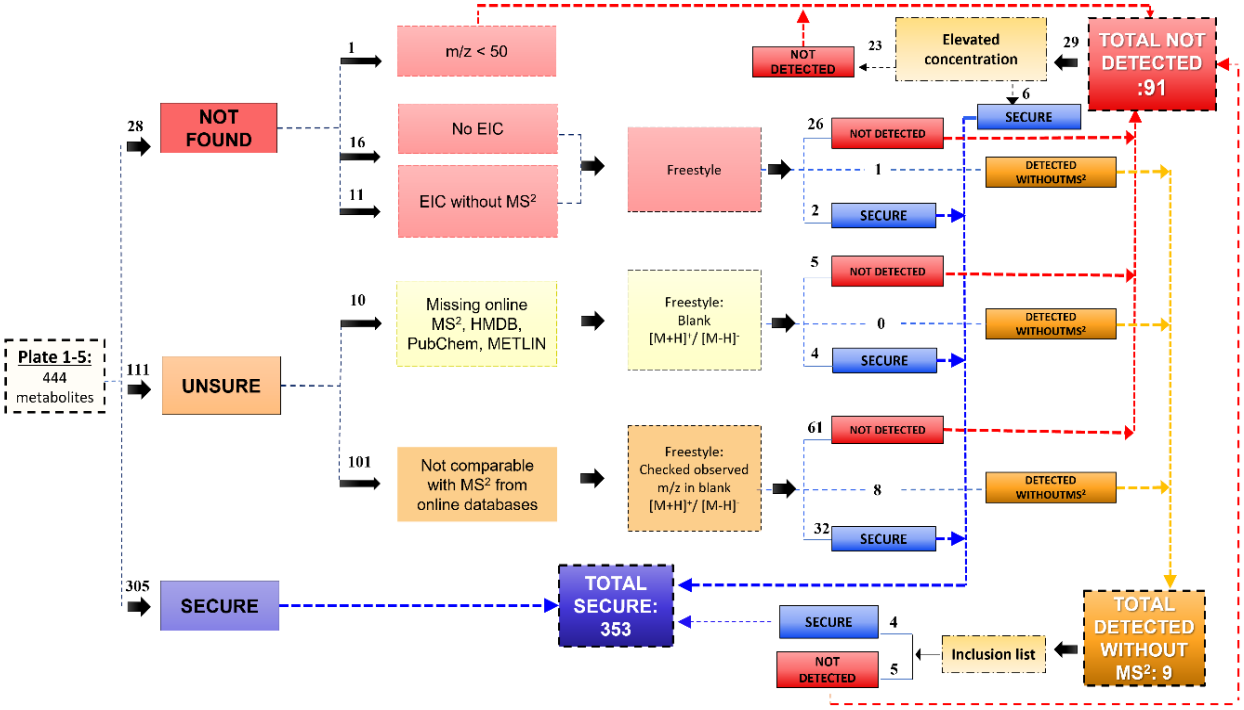


Figure 4.5 All the steps throughout the data processing at MLSDiscovery and Freestyle and optimizations steps, obtaining validated MS² and RT for metabolites in plates 1-5.

Appendix 6.7 shows how validated MS^2 and RT were obtained by data processing the raw files to metabolites in plates 6 and 7.

4.2.3 Most of the metabolites detected are deprotonated or protonated. Alternative adducts with high quantities could be present

Figure 4.6 shows that most metabolite ions with MS^2 and RT added to the in-house library are $[M+H]^+$ or $[M-H]^-$. These are the most traditional molecular ions detected when the ion source is ESI (93), and most of the online MS^2 are from these molecular ions.

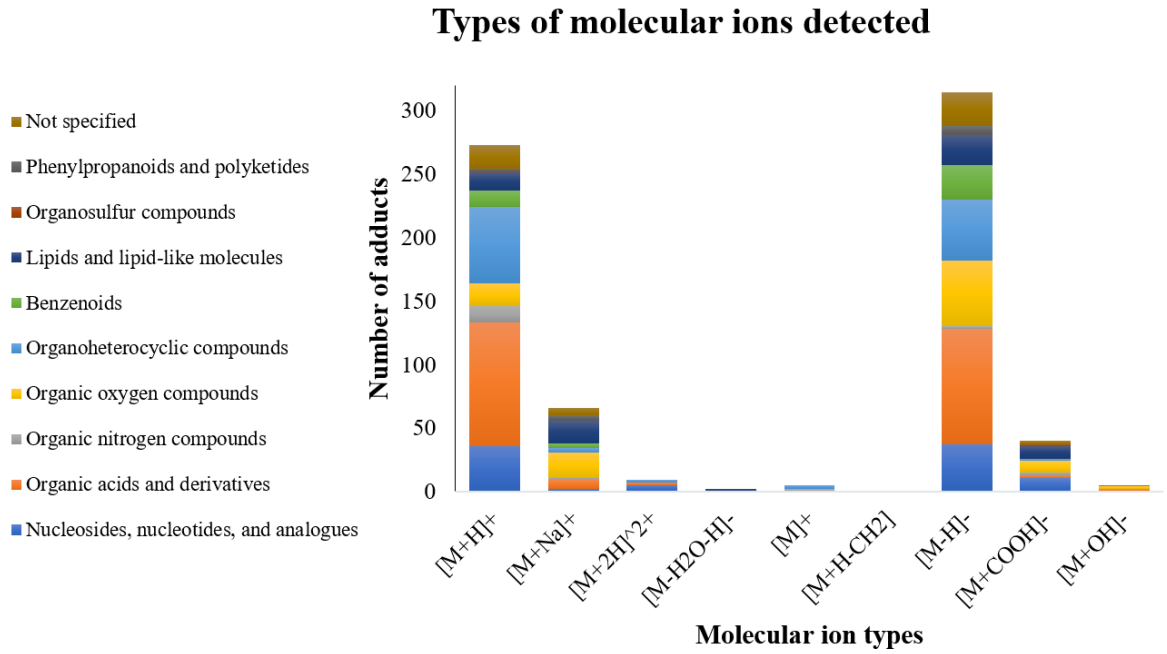


Figure 4.6: The distribution of molecular ions added into the in-house library. *The diagram also pinpoints which superclasses the metabolites with different molecular ion types were from.*

Because of the lack of online experimental MS^2 of other adducts at MLSDiscovery, these adducts' MS^2 wasn't added to the in-house library. *In-silico* MS2 might have been a solution to validate MS^2 for the adducts missing online experimental MS^2 and establish an in-house library with a bigger variance of molecular ion types. One can either obtain *in-silico* MS^2 by using certain databases and generate *in-silico* MS^2 for ions with certain adducts, or these can be obtained from databases such as PubChem and HMDB, these *in-silico* MS^2 have been generated and uploaded by others. Most of the *in-silico* MS^2 at HMDB was observed to commonly be for protonated and deprotonated metabolites. In this thesis, *in-silico* MS^2, from HMDB, for $[M+H]^+$, $[M-H]^-$, or $[M+Na]^+$ were only used to verify metabolites if no other obtained MS^2 had an online experimental MS^2 at HMDB, PubChem, or METLIN. If MS^2 for protonated or deprotonated were found and compared with online MS^2, some of the proposed MS^2 for molecular ions with other adducts weren't added to the in-house library. This was done to

increase the chance of correctly identifying metabolites with a level of confidence 1 when using the established in-house library later.

If the metabolite standards were analyzed individually instead of in a mixture, it could have been easier to identify peaks as the correct metabolite. Nicholas et. al (94) also performed an MS-MLS study and established an in-house library by analyzing mixtures of metabolites from an MS-MLS kit. Here some of the adducts observed were $[M-2H+Na]^{2-}$, $[M+H+CH_3COOH]^{+}$, $[M+Na-H2O]^{+}$, $[M+Na-H]^{+}$, $[M+H-H_2O]^{+}$, and $[M-H-H2O]^{-}$

The uncertainty of which types of adducts might exist for the different metabolites could be the reason why some of the metabolites aren't detected. Three peaks with unknown identities were accidentally observed in the metabolite mixtures with Freestyle. **Figure 4.7** shows the peak and MS^1 for one of them. It was extracted with the m/z value 362.0509 from the mixture 3F when analyzed in negative ionization. No metabolites in the mixture will get an m/z value at 362.0509 if their molecular ions are of the molecular ion types in **Figure 4.6**.

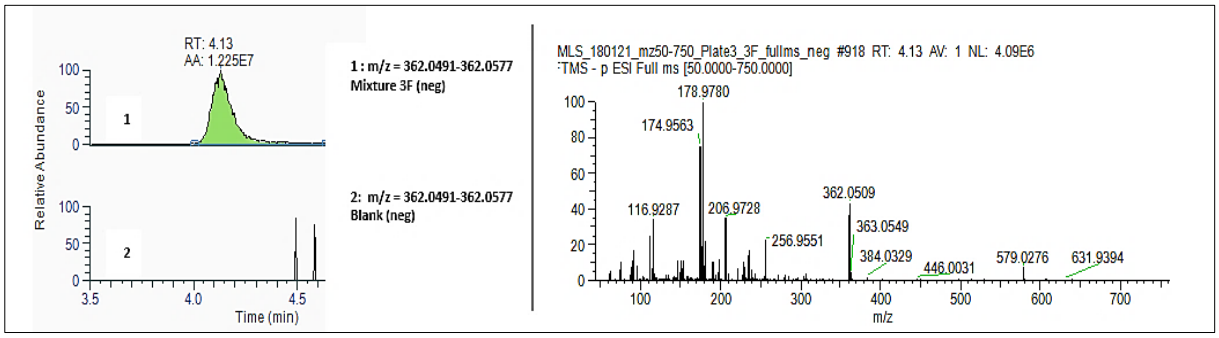


Figure 4.7: The EIC of an unknown feature with a unique m/z value at 362.0491-362.0577, having a peak in mixture 3F and not in the blank sample. *The MS^1 for the peak in the 3F mixture shows a signal at 362.0509, which could be the m/z value of the peak shown. The signal m/z 174.9563 and 178.9700 were noise.*

Summary: *Most of the metabolite ions added to the in-house library were protonated and deprotonated metabolites, this is because most of the reference online MS^2 were for protonated and deprotonated metabolites. It might be other molecular ions present for metabolites, as $[M-2H+Na]^{2-}$, $[M+H+CH3COOH]^{+}$, $[M+Na-H2O]^{+}$, $[M+Na-H]^{+}$, etc.*

4.3 A chemical overview

4.3.1 The number of detected metabolites was depending on their hydrophobicity and not the concentrations

As seen in figure 8, some of the metabolites in a row mixed together had a wide range of concentrations. Because each metabolite has a different monoisotopic mass and it was given 5 µg of each metabolite, the concentration varies among metabolites in mixtures.

The concentration of the metabolites in their stock solution was calculated with ***Equation 3***. While the concentration of the metabolites in mixtures was calculated with ***Equation 4***. The data used to calculate the concentration is found in **Appendix 6.1** and **Table 3.2** in **Section 3.5**.

$$C_1 = \frac{m(metabolite,well)}{Mm(metabolite,well) \times V(solvent,well)} \qquad \textit{Equation 3}$$

$$C_2 = \frac{C_1}{Dilution\ factor} \qquad \textit{Equation 4}$$

In addition to illustrating the different concentrations in mixtures, **Figure 4.8** shows the hydrophobicity in different plates, and which metabolites concentrations were detected without any optimization of the initial procedure for preparing and analyzing the metabolites in the mixtures.

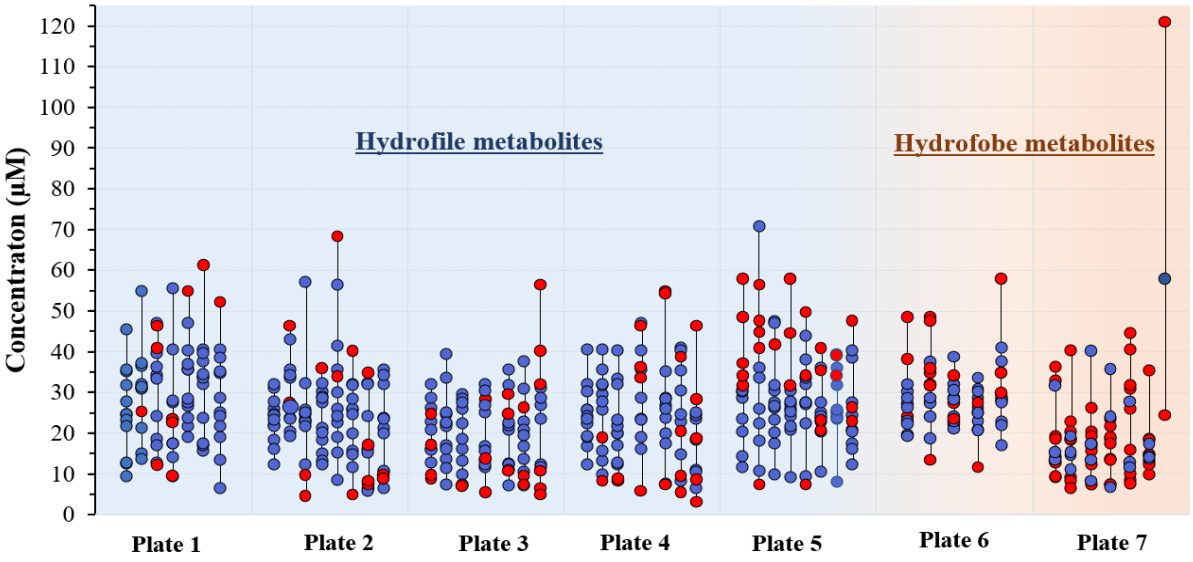


Figure 4.8: The ranges of metabolite concentrations in mixtures. *The blue dots are the metabolites detected, and the red dots are the metabolites not detected prior to any optimization. As the background changes from blue to orange the polarity increases. Mixtures created of metabolites in plate 6, rows F and G, are not included in the diagram.*

There was no significant relationship between the concentrations among the detected and not detected metabolites, as seen in **Figure 4.8**. It existed metabolites that weren't detected down to 4.57 µM, as well as up to 121.02 µM. It was suspected that the majority of the not detected metabolites weren't detected due to a low concentration. Hence, metabolites not detected with a concentration below 10 µM, which included 63 %, were re-analyzed with an elevated concentration. A total of 41 metabolites were analyzed with an elevated concentration, and 17 % of these were detected and obtained a validated MS^2 and RT. **Figure 4.9** shows one metabolite detected with elevated concentration.

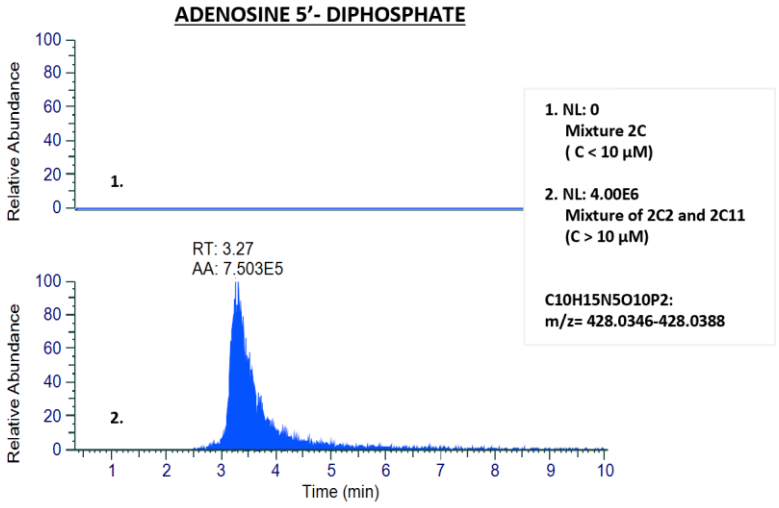


Figure 4.9: The protonated peak for adenosine 5'- diphosphate extracted from a mixture with adenosine 5'- diphosphate concentration < 10 µM (1), and a mixture with adenosine 5'- diphosphate concentration > 10 µM (2)

Summary: *The metabolites had a wide concentration range. The majority of the not detected wasn't due to low concentrations. When re-analyzing metabolites with a higher concentration than 10 µM, only 17 % were detected.*

4.3.1.1 Metabolites' log P, monoisotopic mass, and chemical class influence the chromatographic separation

To conclude if hydrophobicity influenced the detected metabolites, experimental log P, classes and subclass were extracted from HMDB. Experimental log P values were obtained for 194 of the 603 metabolites, whereas 144 of these were detected. A scatter plot of the detected metabolites, with different log P ranges, is shown in **Figure 4.10**. This visualizes how log P, as well as the monoisotopic mass, may influence the RT value.

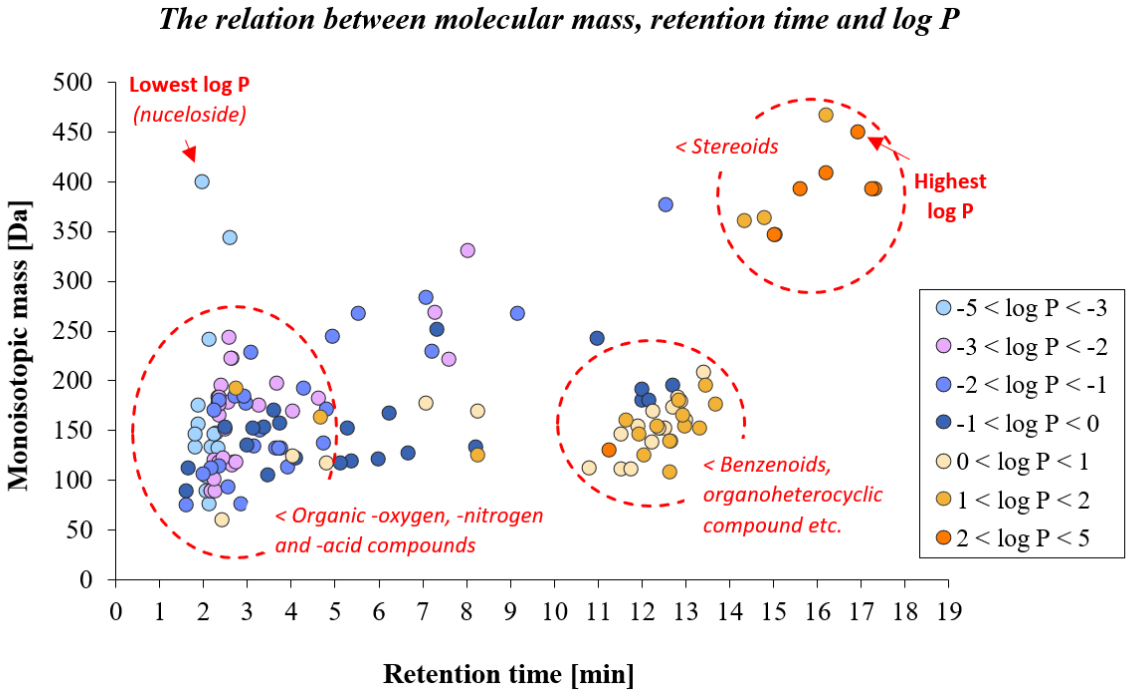


Figure 4.10: A scatter plot, visualizing the relation between monoisotopic mass, and RT of 144 metabolites grouped into log P ranges. *Metabolites in the plot are from plates 1-7, with a registered experimental log P at HMDB. The metabolites with the lowest and highest registered log P are marked, as well as the dominating superclasses/classes at the different clustered spots.*

The detected metabolite with the highest registered log P was chenodeoxycholate. This is a steroid, which is one of the lipids and lipid-like molecules' classes. Chenodeoxycholate has a log P 4.15. The detected metabolite with the lowest log P was s-adenosylmethionine, it is a 5'-deoxyribonucleosides, belonging to nucleosides, nucleotides, and analogues. S-adenosylmethionine has a log P at -5.3. This is the metabolite with the lowest registered experimental log P in the whole MS-MLS kit. It was 10 metabolites with registered experimental log P above 4.15, where 6 of these were fatty acyls. The metabolite with the

highest registered experimental log P in the kit, at 16.26, was glyceryl trimyristate, this belongs to the glycerolipids, one of the classes of lipids, and lipid-like molecules.

When looking at some of the data points within each log P range, on the scatter plot, metabolites get a higher RT as the monoisotopic mass increases. Those with a lower monoisotopic mass may have lower RT, because it is easier for these to migrate through the packed column, compared to bigger metabolites. When looking at the different colored dots, the RT mostly increases as the log P increases.

Most of the metabolites with the highest RT were steroids. In the RT range of 11 – 15 minutes, most of the eluted metabolites were either a benzenoid, organoheterocyclic compound or phenylpropanoids, and polyketides. Benzenoids, phenylpropanoids and polyketides are superclasses including compounds that consist of at least one aromatic ring (95). While steroids and organoheterocyclic compounds consist of either aromatic or non-aromatic rings (96). As well as having a high log P, metabolites from these four classes mentioned above may have high RTs, because they may experience π-π interactions with the diphenyl column. π-π interactions are forces made between cyclic molecules that are unsaturated, like aromatic molecules (97).

Those making π-π interactions with the stationary phase are eluted when their affinity for the mobile phase increases. For those with a high log P value, the affinity to the mobile phase will increase as the percent of the organic mobile phase (MeOH+0.1 % FA) increases. Some of the not detected metabolites may have experienced a higher affinity for the stationary phase in the column compared to the increasing % of organic mobile phase, preventing them to be detected. This may have happened to bilirubin and biliverdin, which are molecules with many unsaturated cyclic hydrocarbons. To investigate if this was the reason why they weren't detected, the organic mobile phase could have been changed to acetonitrile, which also can form π-π interactions with analytes.

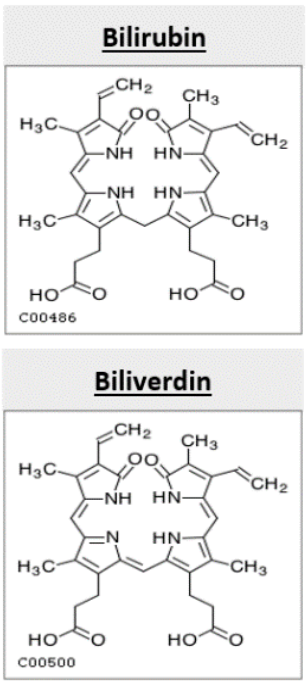


Figure 4.11: The stucture of bilirubin and biliverdin.

Of all the 194 metabolites with a registered experimental log P, 88 metabolites had a log P above 0, and 106 metabolites had a log P below 0, and of these 49% and 8.5% weren't detected, respectively.

Polar metabolites have a lower RT because they don't experience polar interaction with the diphenyl and have a greater affinity to the starting mobile phase which consists of 98%.

In the scatter plot, some metabolites stand out. In the log P range 1 – 2, there are two yellow dots on the left side of the plot. These are quinate and 4-methyl catechol with RT 2.76 and 4.709, respectively. The log P range at 1 – 2 range, mostly consists of benzenoids, lipids, and lipid-like molecules, organoheterocyclic compounds, phenylpropanoids, and polyketides. Quinate is the only organic oxygen compound, and it is a saturated cyclic metabolite with 4 hydroxy groups and 1 acid.

In the log P range 0 – 1, trimethylamine, nicotinate, and furumate, with RT 2.451, 4.05, and 4.82, respectively, have lower RT than the rest of the metabolites in the same range. Trimethylamine is the only organic nitrogen compound. Nicotinate and furumate is one of the three metabolites from organoheterocyclic compound and organic acids and derivatives, respectively, and have the lowest log P of their class. The rest of the metabolites in this range includes benzenoids and one lipid.

Summary: *Metabolites' chromatographic separation depends more on their log P than their monoisotopic mass, but it may depend on the metabolites' chemical class. The metabolites' affinity to the MP and SP also depends on the metabolites' chemical structure, which is related to the chemical class they belong to. Hence, a metabolite can have a higher log P and still migrate faster through the column compared to another metabolite from another chemical class with a lower log P, as was observed for quinate.*

4.3.1.2 Many lipids and lipid-like molecules were not detected, especially fatty acyls

Diagrams in **Figure 4.12** and **Figure 4.13** show the ratio of detected and not detected among each chemical class in plates 7 and 6 were shown respectively. A similar diagram for plates 1-5 is shown in **Appendix 6.8**.

Plate 6:

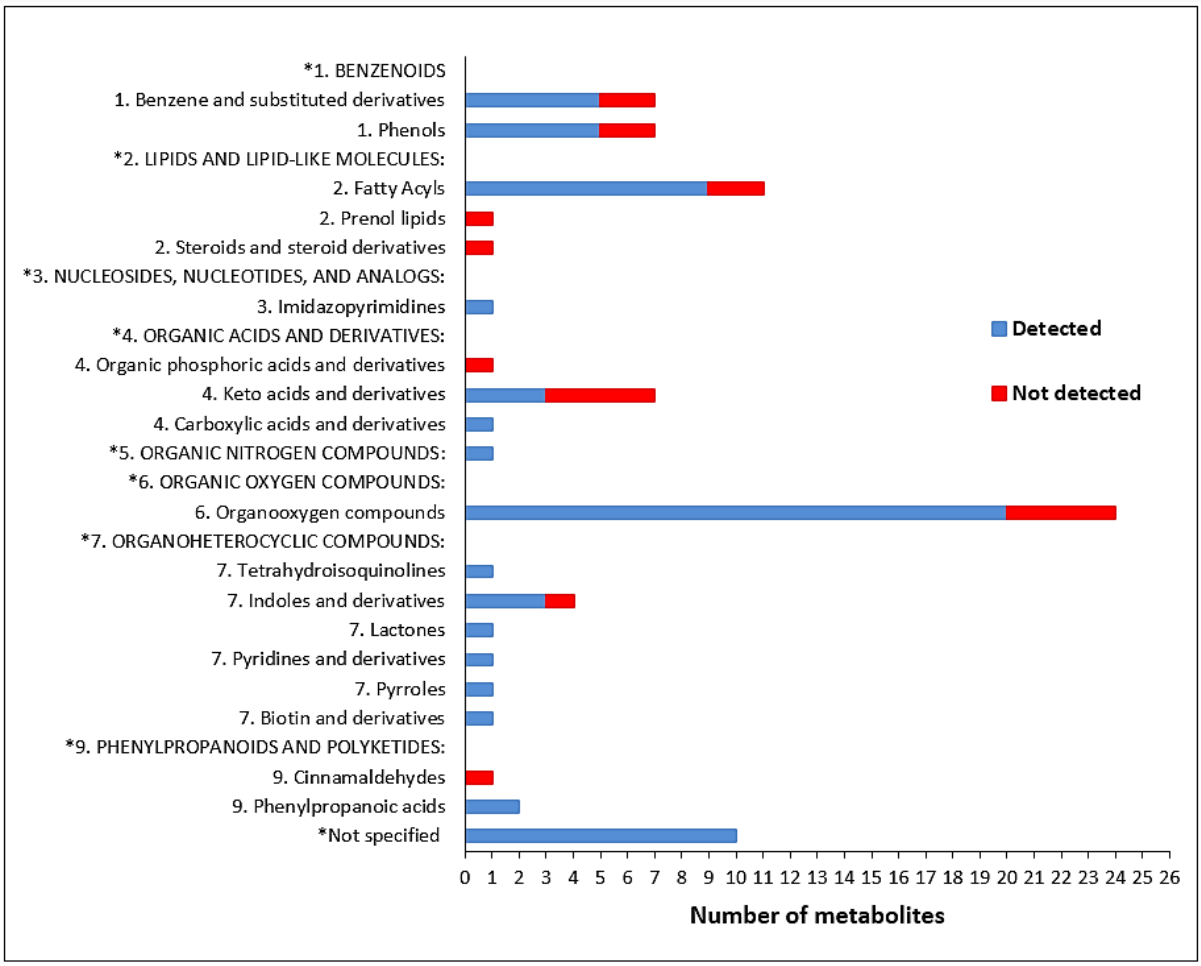


Figure 4.12: Metabolites from plate 6, categorized into their classes, showing the number of detected (blue) and not detected (red). *Those with a * are the superclasses, and those without * are the classes. The classes are extracted from HMDB. Other = metabolites with no registered class on HMDB.*

Organooxygen compounds are the biggest chemical class in plate 6, all the 20 detected metabolites here are sugar molecules from rows F and G. These are the most polar in plate 6 and are only solved in water, are unlike the other metabolites here, which are solved in 40% MeOH.

Plate 7:

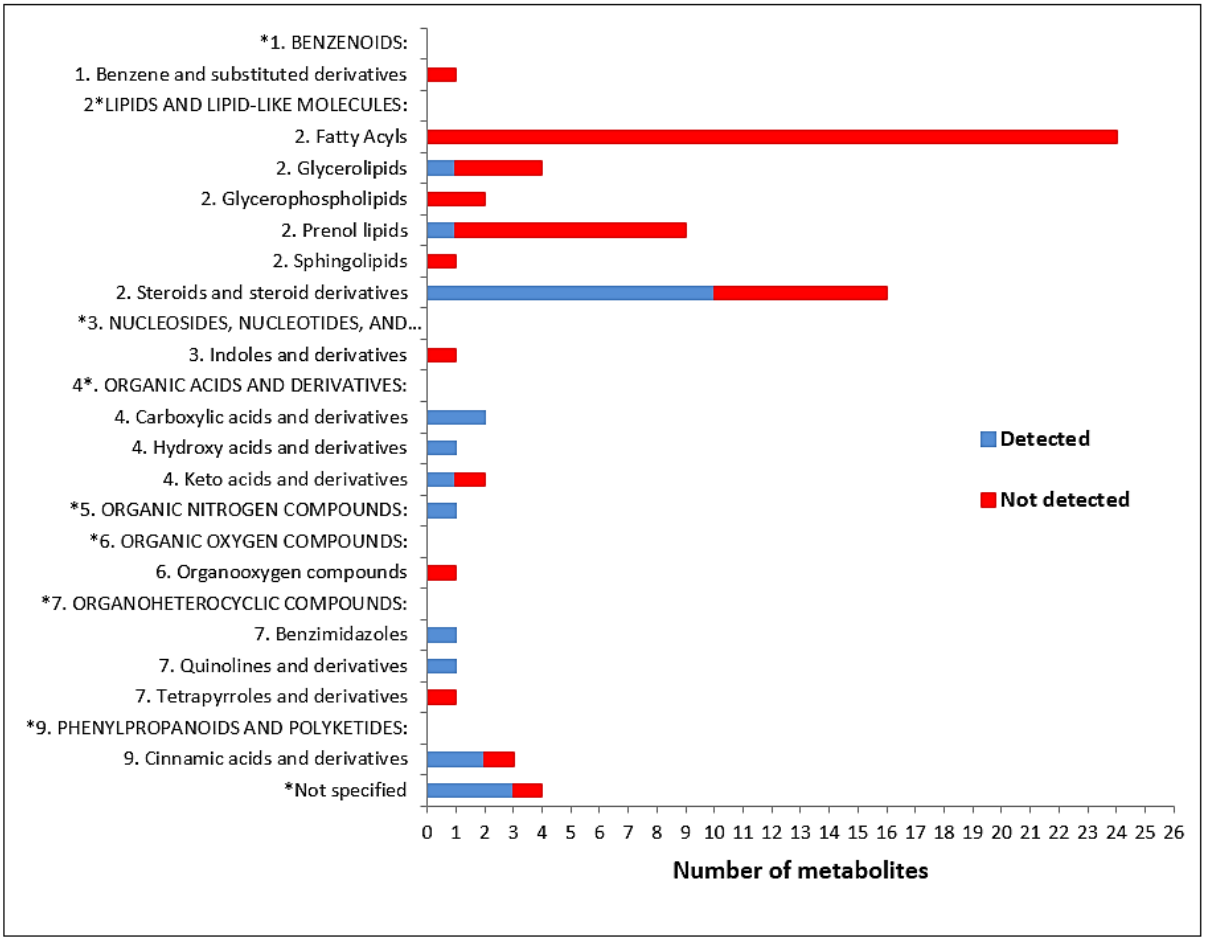


Figure 4.13: Metabolites from plate 7, categorized into their classes, showing the number of detected (blue) and not detected (red). *Those with a * are the superclasses, and those without * are the classes. The classes are extracted from HMDB. The classes are extracted from HMDB. Other = metabolites with no registered class on HMDB.*

Plate 7 consisted of the most lipophilic metabolites, compared to the other plates. These metabolites were solved in 50% 1:1 ACN: IPA. As shown in figure 13, the majority of not detected metabolites in plate 7 were from the superclass; lipid and lipid-like molecules. Within this superclass, fatty acyls dominated. No fatty acyls were detected. In plate 6 there were fatty acyls that were detected.

Some metabolites might not have been detected because they weren't solved in the solvent used, or the separation principle used isn't suited for them or they had a poor ionization efficiency in the ESI. Nichols et. al. *(94)* analyzed metabolites from an MS-MLS kit and also concluded that some of the not detected metabolites could be due to metastable dissociation in the tubes.

Fatty acyls may have experienced poor ionization efficiency:

Fatty acyls may not have been detected due to poor ionization. **Appendix 6.9** shows all the fatty acyls. Fatty acyls consist of a saturated or unsaturated hydrocarbon chain attached to a terminal acyl group (98). Fatty acyls in plates 6 and 7 have registered to have a pKa 4-5.5 If a fatty acyl is in an acidic pH environment, which is 2 pH below its pKa, all its acyl groups may be protonated, giving a fatty acid instead. See **Figure 4.14**.

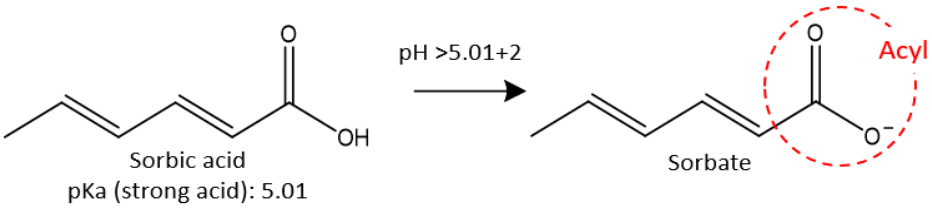


Figure 4.14: Sorbic acid completely deprotonates to sorbate when the pH is above its pKa + 2.

Fatty acyls are detected due to the negative charge. No experimental pKa was available. All fatty acids with a registered predicted pKa had a pKa for the terminal carboxylic acid between 4-5.5. For these to be 100% ionized the pH in the mobile phase should be 2 pH above the fatty acids' pKa. The pH in the mobile phase was approximately 2, hence few or no fatty acyls with a negative charge may have been present in the mass spectrometer to be detected.

Most of the fatty acyls detected consisted of a basic group in addition to the terminal acidic group. This basic group had a lower pKa than the mobile phase's pH and thus could have been charged and ensured a MS detection. Fatty acyls which weren't detected had no basic groups that could have ensured detection.

Aldehydes may have experienced poor ionization efficiency:

Metabolites containing aldehydes have also been difficult to detect. Of 11 only 1 was detected. Aldehydes are usually derivatized before LC-MS analysis. This is because they, among other things, have natural high volatility and biochemical instability, giving analytical challenges (99). They may also have experienced poor ionization efficiency. Succinate semialdehyde is one metabolite from the MS-MLS kit that wasn't detected. According to Pan Deng et. al. (100), derivatization of this metabolite is necessary to ensure sensitivity and detection. Few other articles (99, 101, 102) say the same for aldehydes generally.

Summary: *No fatty acyls were detected from plate 7; this could be because of a poor ionization due to a low pH in the mobile phase. Aldehydes were also difficult to detect. For a more coverage of the metabolites in the M-MLS kit, some metabolites, like fatty acyls, should be re-analyzed with mobile phase with a higher pH.*

4.3.2 A portion of the metabolites that are not detected are necessary not detected with other separation principles

To achieve a wider detectable coverage of a group of metabolites with a wide range of hydrophobicity, analyzing some metabolites in other separation principles may be a solution. Pezzatti et. al (45) did an MS-MLS study, analyzing mixtures of metabolites from an MS-MLS kit, on a UHPLC with different separation principles. RPLC with a C18 column and a HILIC with an amide column was two of them, see **Table 4.2**. They also used ESI in positive and negative modes.

Table 4.2: MS-MLS studies using different separation principles*. Alternative 1, was used for this thesis, alternatives 2 and 3 are from the MS-MLS study performed in another laboratory.*

MS-MLS study from this thesis	**MS-MLS study done by others (45)**	
1: Diphenyl column	**2: RPLC C18 column**	**3: HILIC Amide column**
MP A: H2O + 0.1 % FA MP B: MeOH + 0.1 % FA *Starting gradient: 2% MP B*	MP A: H2O + 0.1 % FA MP B: ACN + 0.1 % FA *Starting gradient: 2% MP B*	MP A: ACN: H2O 95:5 v/v MP B: ACN: H2O 30:70 v/v (H2O adjusted to pH 6.5) *Starting gradient: 2% MP B*

In their MS-MLS kit, they had 274 of the same metabolites which they had solubilized in the same solvent as in this thesis. These were from plates 1-5. **Figure 4.15** shows how many of these 274 metabolites they have detected and not detected with RP C18 and HILIC, compared to how many have been detected with diphenyl in this thesis. The diagram includes 61.7 % of the metabolites in plates 1-5, within these 55 % of the not detected metabolites from plates 1-5 are present.

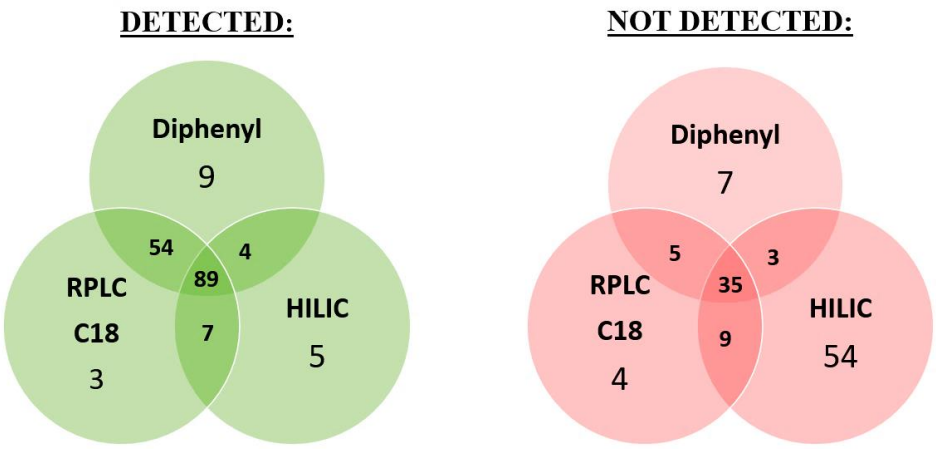


Figure 4.15: A comparison of 274 of the metabolites in plates 1-5, analyzed with a diphenyl column, and with a C18 column and HILIC. *The results from the two last separation principles are from Pezzatti et. al (45) MSMLS study. The green diagram shows a comparison of metabolites detected and the red diagram shows a comparison of metabolites not detected.*

The different numbers of detected and not detected metabolites in each separation principle could be due to their different affinities for different metabolites. Biliverdin was detected with RP C18 and HILIC, this may be because they don't experience π-π interactions as they do in the diphenyl column. It was a wide range of chemical classes among the 274 metabolites, and only a few metabolites of each chemical class, so it wasn't possible to determine whether any specific chemical classes worked better on a certain separation principle.

Of these three separation principles RP C18 separates the most hydrophobic metabolites, diphenyl columns separate less hydrophobic metabolites, and HILIC separates the most hydrophilic metabolites. The diphenyl column and C18 were both operated on an RPLC system, which may explain why these two have detected most of the same metabolites.

As seen in **Figure 4.15**, overall, 35 metabolites weren't detected in either one of the separation principles. This could be due to a low ionization efficiency in the ion source or metabolite instability, rather than a poor chromatographic separation. It was observed that alternatives 2 and 3 also had difficulties detecting metabolites with aldehydes. **Appendix 6.10** shows aldehyde.

In addition, metabolites containing 3 phosphates weren't detectable with C18 and HILIC. Using these two separation principles, Pezzatti et. al (45) analyzed 13 of the same metabolites containing 3 phosphate, as analyzed during this thesis. None of these were detected with either one of the separation principles. There were 15 metabolites with 3 phosphates in this thesis. Of these, only one metabolite, NADP, was detected in positive ionization

Summary: *Using more than one separation principle may cover more of the metabolites in the MS-MLS kit. RP C18 and HILIC detected some metabolites that weren't detected with the Diphenyl column, which could be due to different interactions in the columns. Some metabolites were also not detected with either of the separation principles, this might be due to poor ionization. Aldehydes and metabolites with 3 phosphates are some examples. For a wider coverage of the metabolites in the MS-MLS kit the non-detected metabolites should be analyzed with other separation principles.*

4.3.3 Subclasses with very high or very low probability of being detected with the developed LC-MS method:

Table 4.3 summarizes which subclasses of metabolites may be easy or difficult to detect with the developed LC-MS method. **Appendix 6.11** shows the whole table.

Table 4.3: The subclasses where less than 40% or more than 60% have been detected. *The total number of each metabolite in the MS-MLS kit, as well as their class and superclass*

SUBCLASS	TOTAL	DETECTED (%)	CLASS	SUPERCLASS
Amino acids, peptides, and analogues	99	96 %	Carboxylic acids and derivatives	Organic acids and derivatives
Carbohydrates and carbohydrate conjugates	64	88 %	Organooxygen compounds	Organic oxygen compounds
Amines	15	80 %	Organonitrogen compounds	Organic nitrogen compounds
Purines and purine derivatives	13	92 %	Imidazopyrimidines	Organoheterocyclic compounds
Benzoic acids and derivatives	12	83 %	Benzene and substituted derivatives	Benzenoids
Purine ribonucleotides	12	67 %	Purine nucleotides	Nucleosides, nucleotides, and analogues
Carbonyl compounds	12	17 %	Organooxygen compounds	Organic oxygen compounds
Benzenediols	11	82 %	Phenols	Benzenoids
Pyrimidines and pyrimidine derivatives	10	90 %	Diazines	Organoheterocyclic compounds

4.4 A part of the established in-house library needs further optimization before implementing it into an untargeted metabolomics data processing workflow

MS^2 and RT for metabolite ions were acquired from MLSDiscovery or Freestyle or both, and further added to mzVault, for storage and for the possibility to implement the library into an existing untargeted data processing workflow in the metabolomics software Compound Discoverer. Data from MLSDiscovery was saved in a file prior to importing it into mzVault. MS^2 highlighted for metabolites with Freestyle was directly added into mzVault by opening the raw files and finding the correct scan number for the MS^2. **Figure 4.16** shows how many metabolites had data collected from MLSDiscovery, Freestyle, or both. Those collected with both have been found with more than one metabolite ion. For the RT and type of ion detected for the metabolites, see **Appendix 6.12**.

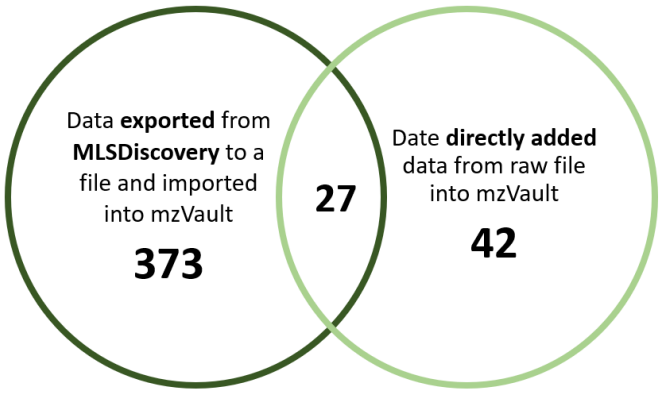


Figure 4.16: The number of identified metabolites with their data imported to mzVault from MLSDiscovery, or directly added from raw files in mzVault, or both

Three different mzVault files were established - One mzVault file contained data directly added from the raw file obtained from the LC-MS, including both positive and negative polarities. Two files contained data imported from MLSDiscovery. Using data from negative and positive ionization, respectively, were separated into two files because the polarity isn't integrated into the data result files extracted from MLSDiscovery.

The three libraries were implemented into the existing untargeted metabolomics workflow, and the computer software's identification performance was tested by performing an untargeted analysis including the in-house library. With the library established with MLSDiscovery imported data didn't give any MS^2 match when trying to identify metabolites in an analyzed sample. A further optimization of this library is necessary to obtain MS^2 matches when implementing it to an untargeted metabolomics data processing workflow, see **Appendix 6.3.2**.

Summary: *An informatic optimization of the established in-house library containing data exported from MLSDiscovery is necessary to ensure high confidence metabolite identification in Compound Discoverer with the whole established in-house library.*

4.4.1 Evaluating the implemented in-house library that works

The mzVauly file containing data directly added from RAW files, identified compounds when it was included in an untargeted analysis at Compound Discoverer. Two untargeted analyses were performed with the implemented in-house library. The first one screened mixtures 1B, 4A and 4B, 5B, which are some of the same mixtures used to acquire a part of the data added to the in-house library files. Hence, it was predicted to get a match for some of the metabolites, and with a high match factor. See **Table 4.4** for the results. The second untargeted analysis screened urine spiked with some of the metabolites in mixtures 4A and 4B, see **Table 4.5.**

4.4.1.1 Metabolites identified correctly with the in-house library have a match factor above 90%

There are no metabolites in mixture 1B added to the working in-house library. From mixtures 4A, 4B, and 5B, 7 metabolites are included in the working in-house library and should be identified.

Table 4.4: The metabolites predicted and not predicted to be identified after an untargeted analysis, matching data with a part of the established in-house library. *The mixtures, 4A, 4B, and 5B, that were used to gather data for the in-house library were the samples with "unknown" content, done an unknown data processing of. The adducts were predicted to or were found in the reference in-house library. Which mixture the metabolites are identified from, and the match factor is also listed.*

FROM MIXTURE ANALYZED	METABOLITE	ADDUCT	MATCH/ NO MATCH	IN MIXTURE DETECTED	MATCH FACTOR
4A	*N-ACETYLGALACTOSAMINE*	Na^+	No match	None	-
4A	*N-ACETYLCYSTEINE*	H^+	Match	4A	99.6
4A	*S-CARBOXYMETHYL CYSTEINE*	H^+	Match	4A	94.3
4B	*THIAMINE PYROPHOSPHATE*	H^+	Match	4B	99.0
4B	*THYMIDINE-MONOPHOSPHATE*	H^+	Match	4B	96.6
5B	*TRIMETHYLAMINE*	-	No match	None	-
5B	*NICOTINE*	H^+	Match	5B	99.3
None	*BETAINE(2B1)*	H^+	Match	1B	71.8
None	*HYDROCORTISONE(5F11)*	Na^+	Match	5B	59.7

In **Table 4.4,** we can see that all the correctly identified metabolites got a match factor above 90%. Two of the metabolites predicted to be identified, N-acetylgalactosamine and trimethylamine, weren't identified. Two metabolites, betaine and hydrocortisone acetate, weren't in the RAW files included in the untargeted analysis and were identified incorrectly. These two have match factors below 90%

N-acetylgalactosamine and trimethylamine had both their reference MS^2 in the working in-house library but weren't identified. The same MS^2s are also in the RAW file data processed at Compound Discoverer. **Figure 4.17** shows both of their MS^2. These only contain molecular ion. The settings on Compound Discoverer may need an optimization to match MS^2 with no fragments.

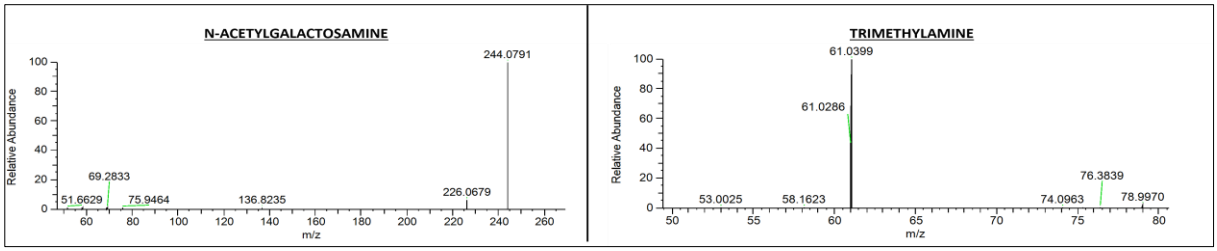


Figure 4.17: The MS^2 obtained for N-acetylgalactosamine and trimethylamine, to the left and right, respectively.

The in-house library managed to distinguish a metabolite incorrectly identified as its isomeric metabolites by giving a low match factor

A peak in mixture 1B, was incorrectly identified as betaine, with a match factor of 71.8%. The peak identified peak was valine, and not betaine. The MS^2 and RT for valine were not in the implemented mzVault library file, and the peak was therefore not identified as valine. Valine has been identified as betaine because they are structure isomers, sharing the same elemental composition, $C_5H_{11}NO_2$, and both are protonated in positive ionization. **Figure 4.18** shows the MS^2 of betaine and valine matched together to the left, and to the right, it shows the extracted peaks of $[C_5H_{11}NO_2+H]^+$ m/z value, from mixture 1B and mixture 1H. Betaine and valine also have similar RT, if they had a difference at 2 RT, this MS^2 match would have been excluded due to the RT tolerance value set to 2 minutes.

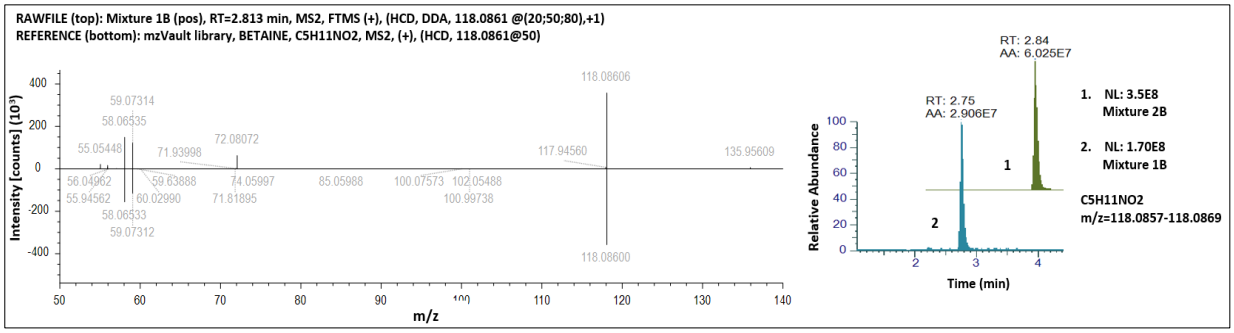


Figure 4.18: The extracted MS^2 from the RAW file, for m/z = 118.0861, matched with the reference MS^2 to betaine, from the in-house library to the left. The extracted EIC for the same m/z value, from the mixture 2B (betaine) and 1B (valine) to the right.

<u>Summary:</u> *Most of the metabolites anticipated to be identified in an untargeted data processing workflow, were identified by matching with the implemented in-house library. They had a match factor above 90%. Those who weren't identified may be due to the chosen settings. Metabolites that were identified incorrectly, including a structure isomeric metabolite, had a match factor below 72%. In future works it would be interesting to see if the in-house library may distinguish other isomeric metabolites with a low match factor.*

4.4.1.2 Metabolites identified in urine with a match factor above 90% provide a level of confidence of 1

Table 4.5: The metabolites expected to be identified from spiked urine samples when performing an untargeted data processing and matching data with a part of the established in-house library. *The table shows which metabolites were spiked in urine, with which adduct they were identified, if there were a match with the in-house library, and what the match factor was. Identified metabolites not spiked in urine are also shown.*

METABOLITE	SPIKED / NOT SPIKED	ADDUCT	MATCH/ NO MATCH	MATCH FACTOR
N-ACETYLGALACTOSAMINE	*Spiked*	Na^+	No match	-
N-ACETYLCYSTEINE	*Spiked*	H^+	Match	90.9
S-CARBOXYMETHYL CYSTEINE	*Spiked*	H^+	No match	-
THIAMINE PYROPHOSPHATE	*Spiked*	H^+	No match	-
THYMIDINE-MONOPHOSPHATE	*Spiked*	H^+	Match	92.2
BETAINE	*Not spiked*	H^+	Match	91.0
DIHYDROBIOPTERIN	*Not spiked*	H^+	Match	96.3

Two of the four identified with a match greater than 90% in the first untargeted data processing, as shown in **Section 4.4.1.1**, were found when spiked in urine. Their match factor when spiked in urine was lower, as seen in **Table 4.5**, but still above 90%. Other fragments and noise from the matrix may have been included in the MS^2 for these metabolites, resulting in a lower match factor. Two metabolites, betaine, and dihydrobiopterin, which weren't spiked in the urine sample, were identified with a match factor above 90%. Both were present in the spiked and non-spiked urine sample. **Figure 4.19** shows the peak for betaine in the non-spiked urine sample and spiked urine sample. Betaine is also, according to HMDB, expected to be present in urine (103).

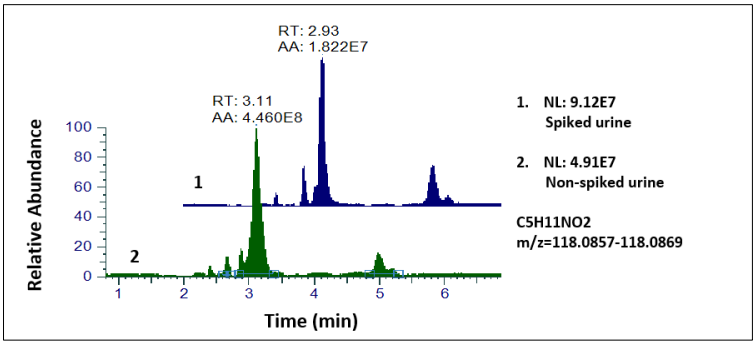


Figure 4.19: The peaks for protonated betaine extracted from the spiked urine sample (top), as well as the non-spiked urine sample (bottom)

MS^2 isn't collected for some spiked metabolites in urine, preventing the identification of them with the in-house library:

The two metabolites that were expected to be identified with the in-house library, but weren't, hadn't acquired their MS^2 from the MS. **Figure 4.20** shows that they were present at the spiked urine sample. MS^2 might not have been acquired because the metabolites' molecular ions weren't included as one of the top 5 abundant ions among other co-eluting ions. Due to the complex matrix of urine, there is a higher possibility for high intensity co-eluting compounds, being selected for MS^2. In future work the spiked metabolites' m/z values can be added to an inclusion list, ensuring the collection of their MS^2, or the DDA can be optimized to collect MS^2 for the top 10 abundant molecular ions.

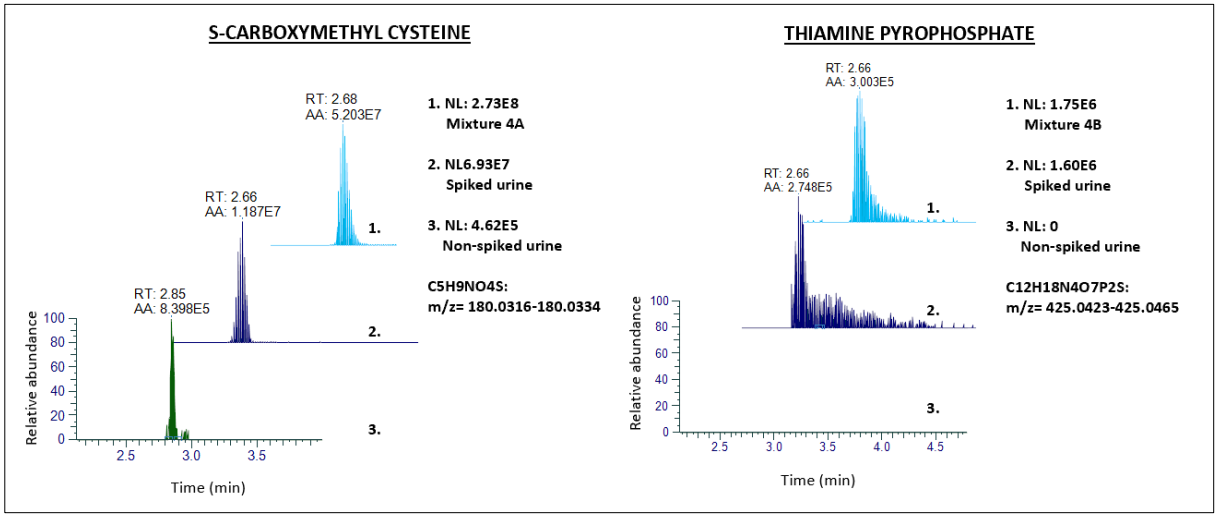


Figure 4.20: The peaks for protonated s-carboxymethyl cysteine (left) and thiamine pyrophosphate (right) extracted from their standard MS-MLS mixture (1), spiked urine sample (2), as well as the non-spiked urine sample (3).

Summary: *When spiked urine samples were processed with the untargeted data process workflow, including the established in-house library, two expected metabolites were identified with a match factor above 90%. The match factor of these two was less than their match factor when not spiked in urine. Four of two metabolites expected to be identified were identified. Some of the metabolites were unidentified metabolites because they hadn't acquired their MS^2, necessary for identification with the in-house library. Two metabolites that weren't spiked in the urine, were also identified, with a match factor above 90%. In a future work the match factors achieved when identifying metabolites in other biospecimen should be investigated.*

4.5 The establishment of an in-house library has highlighted the possibility of detecting a biomarker for an IEM in Norway

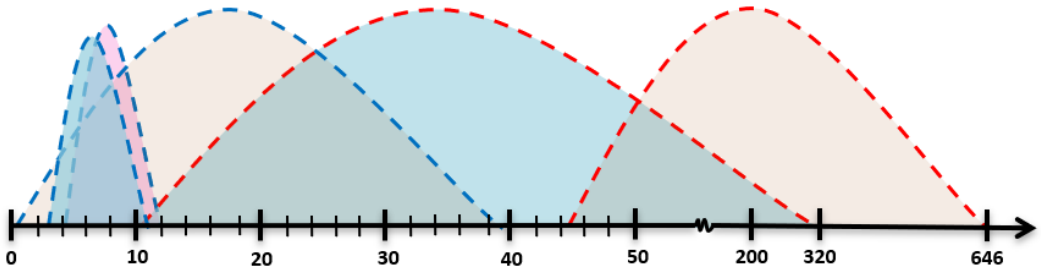
Trimethylaminuria (TMAU) is an IEM in which an excess of trimethylamine (TMA) accumulates in urine and sweat rather than being metabolized into trimethylamine-N-oxide (TMOA). A deficiency in the enzyme flavin monooxygenase, which oxidizes TMA into trimethylamine-N-oxide (TMAO), causes TMAU. This disease is also called the fish-smelling syndrome. Due to body smells, patients with TMAU can experience social difficulties, isolation, and depression (104, 105).

Patients in Norway who are suspected of having TMAU have their urine samples transferred to the University Clinic Heidelberg in Germany for laboratory diagnosis. This is because the Norwegian National Unit for Screening and Diagnostics of Congenital Paediatric Metabolic Disorders doesn't have a laboratory diagnostic test for TMAU, since its biomarkers aren't detectable in the diagnostic laboratory methods used.

The amount of TMA in urine and the ratio of TMAO/(TMA+TMOA) in normalized urine is usually used as biomarkers for this IEM (104).

During the establishment of the in-house library, it was highlighted that the LC-MS instrument in the research lab can detect TMA. The MS^2 and RT for TMA were included in the created in-house library. Hence, TMA in samples can obtain an identification with a level of confidence 1. To determine if TMA can be used as a quantitative biomarker for TMAU, a proof of concept was performed by investigate if the detected TMA signals correlates with the concentrations of TMA. For this urine samples containing pathological and normal concentration of TMA were used.

Figure 4.21 shows the published interval ranges of TMA concentration for newborns without TMAU, and for children and adults with and without TMAU. These concentrations are obtained from HMDB.



Newborn (0-30 days old)		Children (1-13 years old)		Adult (>18 years old)	
"Normal" concentration [µmol/2mmol creatinine]	Pathological concentration [µmol/2mmol creatinine]	"Normal" concentration [µmol/2mmol creatinine]	Pathological concentration [µmol/2mmol creatinine]	"Normal" concentration [µmol/2mmol creatinine]	Pathological concentration [µmol/2mmol creatinine]
< 12.0	-	< 10.7	10.0 <	< 38.8	44.4 <

Figure 4.21: Reported normal and pathological concentrations of TMA in newborns, children, and adults. *These measurements were obtained from HMDB. The peaks show the ranges registered for each category. The red and blues stippled lines are the pathological and "normal" registered values, respectively. The pink, blue, and brown peaks are for newborns, children, and adults, respectively. The normal concentrations in the table, are the highest normal registered at HMDB. The pathological concentrations in the table, are the lowest pathological value registered at HMDB. There were no data on pathological concentrations of TMA in newborns (106).*

There were no samples from patients diagnosed with TMAU, in the diagnostic biobank at OUS. This may be because their patient samples have been transferred to Heidelberg for diagnosis. Therefore, for this experiment, patient samples were constructed by spiking pathological amounts of TMA in children, and adults. The urine spiked was obtained from a person that is not diagnosed with TMAU. TMA solution spiked into the urine samples was the stock solution of TMA prepared from the MS-MLS kit. A urine sample was also spiked to achieve normal reported concentration of TMA in newborns, children, and adults. Seven urine samples were analyzed, where one was a non-spiked urine sample, and the rest were spiked. The concentrations in the spiked in urine were 2.62, 11.84, 29.65, 50.78, 84.64 and 253.92 µmol standard TMA/2mmol creatinine.

Figure 4.22 shows that it is a linear correlation between the concentration of TMA and the detected signal of TMA. Hence, it may be possible to do a quantitative analysis of a patient sample suspected to have TMAU.

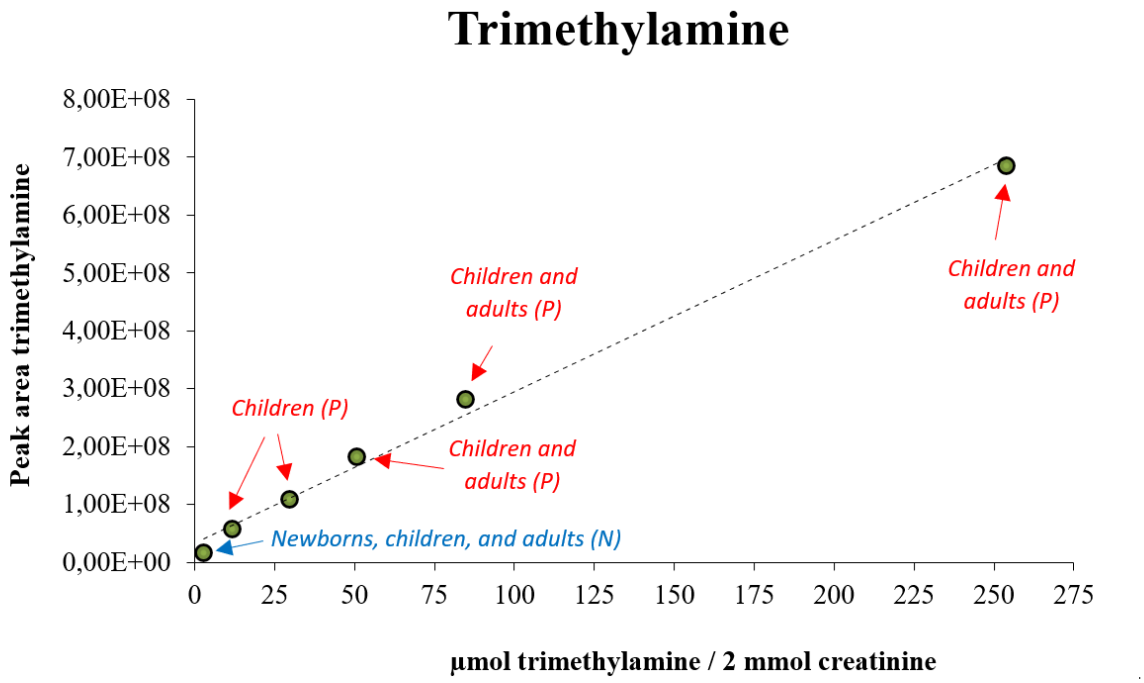


***Figure 4.22**: **The peak area for protonated TMA and the concentration of spiked standard TMA in the y-axis and the x-axis, respectively.** Those marked with (P) represent pathological amount TMA in children or adults or both. The data point marked with (N) represent the normal amount TMA in urine for newborn, children, and adults.*

The data for the non-spiked urine was not included in **Figure 4.22** because the observed peak for protonated TMA has an SNR below 10, as shown in **Figure 4.23**. SNR 10 is the lowest limit of quantification, as mentioned in **section 2.4.1**. It is uncertain what the TMA concentration in the non-spiked urine sample was, and therefore how low it is possible to quantitatively detect TMA but given that the urine sample came from a person without TMAU, the detected amount of TMA should be low. Because the SNR for the urine sample spiked with the least amount of standard TMA was 24.99, a standard addition of less standard TMA may have been spiked and still provided quantitative results.

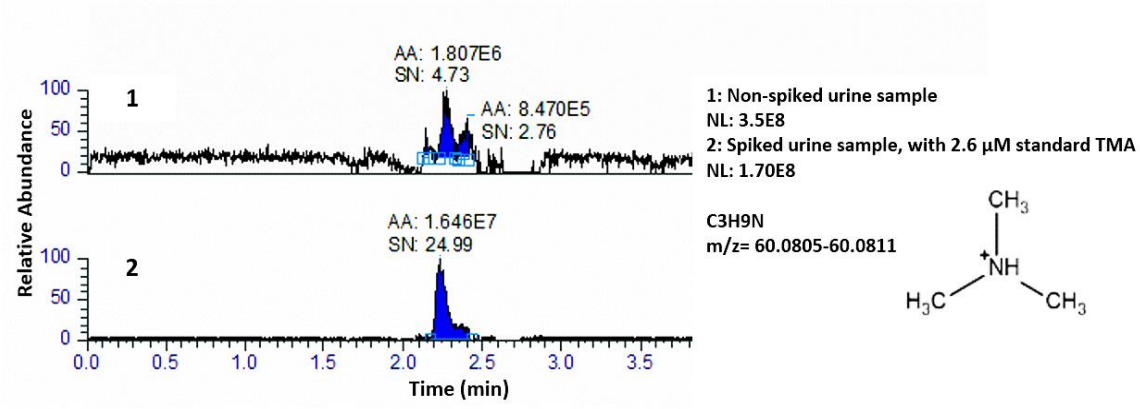


Figure 4.23: The extracted peaks for the m/z value of protonated TMA from the non-spiked urine (1), and the standard TMA spiked urine (2), showing the SNR.

Summary: *TMA, which is a biomarker for TMAU, was discovered to be detectable using the developed LC-MS. As a result, patients with TMAU may be possible to diagnose using the metabolomics method in combination with the in-house library from this thesis. With a proof of concept, it was demonstrated that TMA should be quantitatively detectable within a certain TMA concentration range. A future work would be to quantify TMA in samples obtained from patients with TMAU.*

5 CONCLUSION AND FUTURE WORK

From a kit of 603 commercially available metabolites, an in-house library of 442 metabolite standards with MS^2 and RT was created, using computer software MLSDiscovery, Freestyle and mzVault. Of these 63 metabolite standards were implemented in an untargeted metabolomics data processing workflow that provides identification of unknown metabolites in urine. Analyzing non-isobaric mixtures of the metabolite standards, instead of individually analyzing every single metabolite, was 90.5% more time-efficient. Most of the metabolites were added to the in-house library as protonated or deprotonated metabolites. With the initial method for sample preparation and data acquisition, 432 were detected. When 41 metabolites with a concentration below 10 µM were reanalyzed at a higher concentration, 7 metabolites were detected. Furthermore, when analyzing 24 metabolites with their m/z value on an inclusion list 6 metabolites were detected.

The coverage was dependent on the metabolites' log P, chemical classes, and pKa. The majority of the metabolites incorporated into the in-house library were carboxylic acids. The majority of the metabolites not covered were lipids, especially fatty acyls. This could be because the mobile phase had a too low pH, preventing ionization. Metabolites containing aldehydes and three phosphates were also difficult to detect. These two last groups of metabolites were also shown to be difficult to detect using the separation principles, RP C18 and HILIC. Of the 91 metabolites not detected in plates 1-5, 35 metabolites weren't detected with RP C18 or HILIC, and 15 metabolites were detected in one of these two separation principles or both. Using additional separation principles or using a mobile phase with higher pH may enhance the coverage of metabolite standards in the in-house library.

A part of the in-house library was included in an untargeted metabolomics workflow and enabled correct identification when the MS^2 match factor was above 90%, identifying spiked metabolites in non-biological samples and biological samples. For the complete established in-house library to identify metabolites in the used untargeted metabolomics workflow, an informatics optimization is necessary for future work.

Using the established in-house library and the developed global metabolomics LC-HR-MS method, it was shown that trimethylamine can be quantitatively detected in urine samples, enabling patients with Trimethylaminuria to be diagnosed in Norway.

For the metabolomics research group at the National Unit for Screening and Diagnosis of Congenital Pediatric Metabolic Disorders at Oslo University, the created in-house library will enable identification with the highest level of confidence at 1 for more than 400 metabolites, and the in-house library can help discover new IEMs and expand the knowledge of known diseases. The overview of which chemical classes of metabolites are detectable will also be useful when analyzing samples with the same LC-MS method.

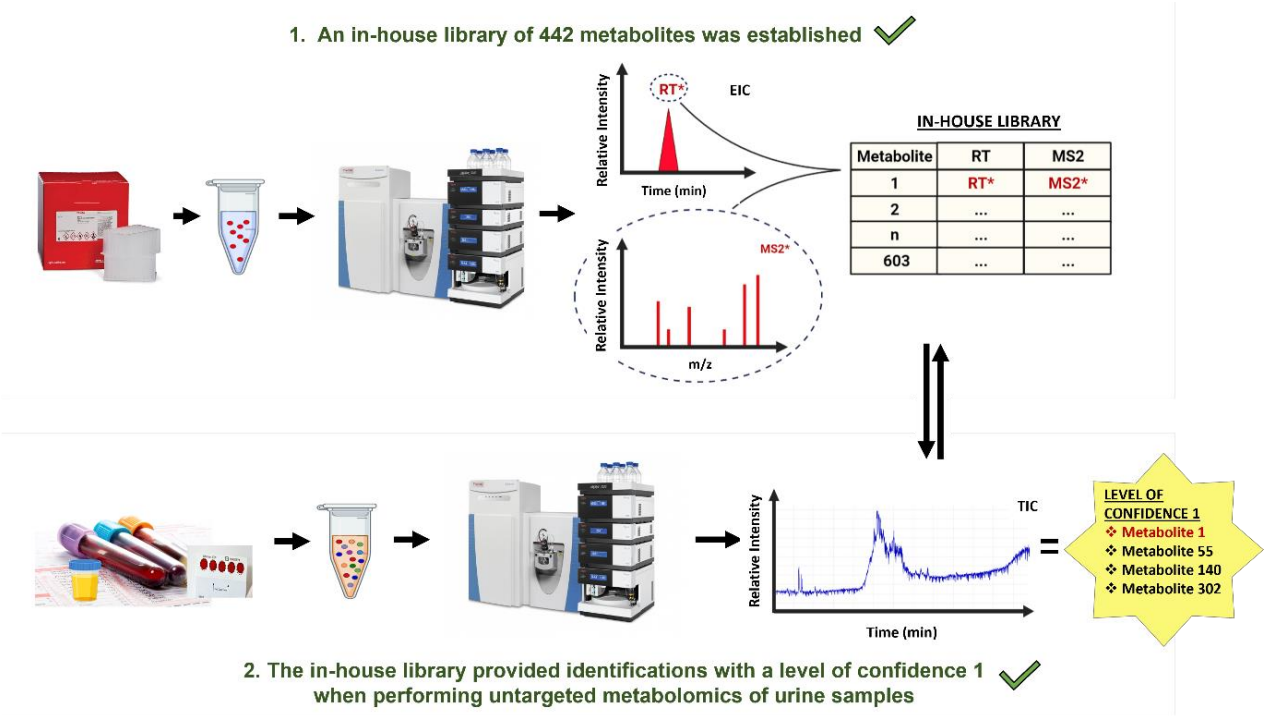


Figure 5.1: An in-house library of 442 metabolites were established and the in-house library provided identifications with a level of confidence 1 when performing untargeted metabolomics.

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6 APPENDIX

6.1 MS-MLS information provided by the MS-MLS product sheet and HMDB, as well as experimental condition chosen

Table 6.1: *The metabolite's position in the MS-MLS kit, their primary name, CAS ID, molecular formula, monoisotopic mass, the solvent used to solve them, the m/z scan range used to acquire MSMS, and their experimental log P, superclass, and class obtained from HMDB. The table continues to page 109.*

PLATE	NROW	NCOL	PRIMARY_NAME	CAS ID	MOLECULAR FORMULA	NEUTRAL MONOISOTOPIC MOLECULAR MASS	SOLVENT	m/z SCAN RANGE	Log P (HMDB)	SUPERCLASS	CLASS
1	A	1	EMPTY								
1	A	2	EMPTY								
1	A	3	EMPTY								
1	A	4	EMPTY								
1	A	5	EMPTY								
1	A	6	EMPTY								
1	A	7	EMPTY								
1	A	8	EMPTY								
1	A	9	EMPTY								
1	A	10	EMPTY								
1	A	11	EMPTY								
1	A	12	EMPTY								
1	B	1	3-METHYL-L-HISTIDINE	368-16-1	C7H11N3O2	169.085	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
1	B	2	NICOTINAMIDE MONONUCLEOTIDE	1094-61-7	C11H15N2O8P	334.057	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyridine nucleotides
1	B	3	FOLATE	59-30-3	C19H19N7O6	441.140	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
1	B	4	DEOXYADENOSINE MONOPHOSPHATE	653-63-4	C10H14N5O6P	331.068	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
1	B	5	PYRIDOXINE	65-23-6	C8H11NO3	169.074	H2O	50-750	-0.77	Organoheterocyclic compounds	Pyridines and derivatives
1	B	6	HOMOSERINE	672-15-1	C4H9NO3	119.058	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
1	B	7	GUANINE	73-40-5	C5H5N5O	151.049	H2O	50-750	-0.91	Organoheterocyclic compounds	Imidazo pyrimidines
1	B	8	VALINE	72-18-4	C5H11NO2	117.079	H2O	50-750	-2.26	Organic acids and derivatives	Carboxylic acids and derivatives
1	B	9	ASPARAGINE	70-47-3	C4H8N2O3	132.053	H2O	50-750	-3.82	Organic acids and derivatives	Carboxylic acids and derivatives

1	B	10	GLYCEROL	56-81-5	C3H8O3	92.047	H2O	50-750	-1.76	Organic oxygen compounds	Organooxygen compounds
1	B	11	TYROSINE	60-18-4	C9H11NO3	181.074	H2O	50-750	-2.26	Organic acids and derivatives	Carboxylic acids and derivatives
1	B	12	ISOCITRATE	320-77-4	C6H8O7	192.027	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
1	C	1	MALATE	636-61-3	C4H6O5	134.022	H2O	50-750	-1.26	Organic acids and derivatives	Hydroxy acids and derivatives
1	C	2	DIHYDROURACIL	504-07-4	C4H6N2O2	114.043	5 % MeOH	50-750		Organoheterocyclic compounds	Diazines
1	C	3	GUANOSINE	118-00-3	C10H13N5O5	283.092	H2O	50-750	-1.9	Nucleosides, nucleotides, and analogues	Purine nucleosides
1	C	4	L-DOPA	59-92-7	C9H11NO4	197.069	H2O	50-750	-2.39	Organic acids and derivatives	Carboxylic acids and derivatives
1	C	5	CREATINE	57-00-1	C4H9N3O2	131.069	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
1	C	6	HYPOXANTHINE	68-94-0	C5H4N4O	136.039	H2O	50-750	-1.11	Organoheterocyclic compounds	Imidazo pyrimidines
1	C	7	DEOXYCYTIDINE MONOPHOSPHATE	1032-65-1	C9H14N3O7P	307.057	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
1	C	8	ASPARTATE	56-84-8	C4H7NO4	133.038	H2O	50-750	-3.89	Organic acids and derivatives	Carboxylic acids and derivatives
1	C	9	THIOUREA	62-56-6	CH4N2S	76.010	H2O	50-750	-1.08	Organosulfur compounds	Thioureas
1	C	10	URACIL	66-22-8	C4H4N2O2	112.027	H2O	50-750	-1.07	Organoheterocyclic compounds	Diazines
1	C	11	PHENYLALANINE	63-91-2	C9H11NO2	165.079	H2O	50-750	-1.38	Organic acids and derivatives	Carboxylic acids and derivatives
1	C	12	SUCCINATE	110-15-6	C4H6O4	118.027	H2O	50-750	-0.59	Organic acids and derivatives	Carboxylic acids and derivatives
1	D	1	SHIKIMATE	138-59-0	C7H10O5	174.053	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
1	D	2	NICOTINAMIDE	98-92-0	C6H6N2O	122.048	H2O	50-750	-0.37	Organoheterocyclic compounds	Pyridines and derivatives
1	D	3	CARNOSINE	305-84-0	C9H14N4O3	226.107	H2O	50-750		Organic acids and derivatives	Peptidomimetics
1	D	4	FRUCTOSE BISPHOSPHATE	488-69-7	C6H14O12P2	339.996	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
1	D	5	URIDINE	58-96-8	C9H12N2O6	244.070	5 % MeOH	50-750	-1.98	Nucleosides, nucleotides, and analogues	Pyrimidine nucleosides
1	D	6	LACTATE	79-33-4	C3H6O3	90.032	5 % MeOH	50-750		Organic acids and derivatives	Hydroxy acids and derivatives
1	D	7	SUCCINATE SEMIALDEHYDE	692-29-5	C4H6O3	102.032	5 % MeOH	50-750		Lipids and lipid-like molecules	Fatty Acyls
1	D	8	THYMINE	65-71-4	C5H6N2O2	126.043	H2O	50-750	-0.62	Organoheterocyclic compounds	Diazines
1	D	9	PROLINE	147-85-3	C5H9NO2	115.063	H2O	50-750	-2.54	Organic acids and derivatives	Carboxylic acids and derivatives
1	D	10	URIDINE MONOPHOSPHATE	58-97-9	C9H13N2O9P	324.036	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
1	D	11	DIETHANOLAMINE	111-42-2	C4H11NO2	105.079	H2O	50-750	-1.43	Organic nitrogen compounds	Organonitrogen compounds
1	D	12	L-ALANINE	56-41-7	C3H7NO2	89.048	H2O	50-750	-2.85	Organic acids and derivatives	Carboxylic acids and derivatives
1	E	1	CYSTINE	56-89-3	C6H12N2O4S2	240.024	H2O	50-750	-5.08	Organic acids and derivatives	Carboxylic acids and derivatives
1	E	2	DIHYDROFOLATE	4033-27-6	C19H21N7O6	443.155	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
1	E	3	XANTHINE	69-89-6	C5H4N4O2	152.033	H2O	50-750	-0.73	Organoheterocyclic compounds	Imidazopyrimidines
1	E	4	GULOSE	6027-89-0	C6H12O6	180.063	5 % MeOH	50-750	-1.88	Organic oxygen compounds	Organooxygen compounds
1	E	5	AMINO ISOBUTANOATE	144-90-1	C4H9NO2	103.063	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
1	E	6	CYS-GLY	19246-18-5	C5H10N2O3S	178.041	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives

1	E	7	THYMIDINE	50-89-5	C10H14N2O5	242.090	H2O	50-750	-0.93	Nucleosides, nucleotides, and analogues	Pyrimidine nucleosides
1	E	8	METHYL THIOADENOSINE	2457-80-9	C11H15N5O3S	297.090	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	5'-deoxyribo nucleosides
1	E	9	2-PHOSPHO GLYCERATE	2553-59-5	C3H7O7P	185.993	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
1	E	10	TETRA HYDROFOLATE	135-16-0	C19H23N7O6	445.171	H2O	50-750	-2.7	Organoheterocyclic compounds	Imidazo pyrimidines
1	E	11	METHIONINE	63-68-3	C5H11NO2S	149.051	H2O	50-750	-1.87	Organic acids and derivatives	Carboxylic acids and derivatives
1	E	12	GLYCINE	56-40-6	C2H5NO2	75.032	H2O	50-750	-3.21	Organic acids and derivatives	Carboxylic acids and derivatives
1	F	1	FORMAMIDE	75-12-7	CH3NO	45.021	H2O	50-750	-1.51	Organic acids and derivatives	Carboximidic acids and derivatives
1	F	2	GUANIDINO ACETATE	352-97-6	C3H7N3O2	117.054	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
1	F	3	MALONATE	141-82-2	C3H4O4	104.011	H2O	50-750	-0.81	Organic acids and derivatives	Carboxylic acids and derivatives
1	F	4	DIHYDROOROTATE	5988-19-2	C5H6N2O4	158.033	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
1	F	5	QUINATE	77-95-2	C7H12O6	192.063	H2O	50-750	1.19	Organic oxygen compounds	Organooxygen compounds
1	F	6	CREATININE	60-27-5	C4H7N3O	113.059	H2O	50-750	-1.76	Organic acids and derivatives	Carboxylic acids and derivatives
1	F	7	SARCOSINE	107-97-1	C3H7NO2	89.048	H2O	50-750	-2.78	Organic acids and derivatives	Carboxylic acids and derivatives
1	F	8	GLYCOLATE	79-14-1	C2H4O3	76.016	5 % MeOH	50-750	-1.11	Organic acids and derivatives	Hydroxy acids and derivatives
1	F	9	N-ACETYL GLUCOSAMINE	7512-17-6	C8H15NO6	221.090	H2O	50-750	-2.1	Organic oxygen compounds	Organooxygen compounds
1	F	10	P-HYDROXY PHENYLACETATE	156-38-7	C8H8O3	152.047	H2O	50-750	0.75	Benzenoids	Phenols
1	F	11	ASCORBATE	50-81-7	C6H8O6	176.032	H2O	50-750	-1.85	Organoheterocyclic compounds	Dihydrofurans
1	F	12	GLUTAMATE	56-86-0	C5H9NO4	147.053	H2O	50-750	-3.69	Organic acids and derivatives	Carboxylic acids and derivatives
1	G	1	PYRAZOLE	288-13-1	C3H4N2	68.037	5 % MeOH	50-750			[NULL]
1	G	2	ISOLEUCINE	73-32-5	C6H13NO2	131.095	H2O	50-750	-1.7	Organic acids and derivatives	Carboxylic acids and derivatives
1	G	3	CYTOSINE	71-30-7	C4H5N3O	111.043	H2O	50-750	-1.73	Organoheterocyclic compounds	Diazines
1	G	4	GAMMA-AMINOBUTYRATE	56-12-2	C4H9NO2	103.063	H2O	50-750	-3.17	Organic acids and derivatives	Carboxylic acids and derivatives
1	G	5	INOSINE	58-63-9	C10H12N4O5	268.081	H2O	50-750	-2.1	Nucleosides, nucleotides, and analogues	Purine nucleosides
1	G	6	NICOTINATE	59-67-6	C6H5NO2	123.032	H2O	50-750	0.36	Organoheterocyclic compounds	Pyridines and derivatives
1	G	7	N-ACETYL TRYPTOPHAN	1218-34-4	C13H14N2O3	246.100	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
1	G	8	TAURINE	107-35-7	C2H7NO3S	125.015	H2O	50-750		Organic acids and derivatives	Organic sulfonic acids and derivatives
1	G	9	CITRULLINE	372-75-8	C6H13N3O3	175.096	H2O	50-750	-3.19	Organic acids and derivatives	Carboxylic acids and derivatives
1	G	10	CYSTEINE	52-90-4	C3H7NO2S	121.020	H2O	50-750	-2.49	Organic acids and derivatives	Carboxylic acids and derivatives
1	G	11	SERINE	56-45-1	C3H7NO3	105.043	H2O	50-750	-3.07	Organic acids and derivatives	Carboxylic acids and derivatives
1	G	12	CYTIDINE	65-46-3	C9H13N3O5	243.086	5 % MeOH	50-750	-2.51	Nucleosides, nucleotides, and analogues	Pyrimidine nucleosides
1	H	1	URATE	69-93-2	C5H4N4O3	168.028	H2O	50-750	-2.17	Organoheterocyclic compounds	Imidazo pyrimidines
1	H	2	TRANS-ACONITATE	4023-65-8	C6H6O6	174.016	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
1	H	3	PYRIMIDINE	25247-63-6	C4H4N2	80.037	H2O	50-750	-0.4	Organoheterocyclic compounds	Diazines

1	H	4	N-ACETYL MANNOSAMINE	7772-94-3	C8H15NO6	221.090	5 % MeOH	50-750	-3.22	Organic oxygen compounds	Organooxygen compounds
1	H	5	N-ACETYL NEURAMINATE	131-48-6	C11H19NO9	309.106	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
1	H	6	PURINE	120-73-0	C5H4N4	120.044	H2O	50-750	-0.37	Organoheterocyclic compounds	Imidazo pyrimidines
1	H	7	THREONINE	72-19-5	C4H9NO3	119.058	H2O	50-750	-2.94	Organic acids and derivatives	Carboxylic acids and derivatives
1	H	8	CITRATE	77-92-9	C6H8O7	192.027	H2O	50-750	-1.64	Organic acids and derivatives	Carboxylic acids and derivatives
1	H	9	N-METYL-ALANINE	NA	C4H9NO2	103.063	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
1	H	10	HYPOTAURINE	300-84-5	C2H7NO2S	109.020	5 % MeOH	50-750		Organic acids and derivatives	Sulfinic acids and derivatives
1	H	11	GLUTAMINE	56-85-9	C5H10N2O3	146.069	H2O	50-750	-3.64	Organic acids and derivatives	Carboxylic acids and derivatives
1	H	12	BETA-NICOTINAMIDE ADENINE DINUCLEOTIDE	53-84-9	C21H27N7O14P2	663.109	H2O	50-750		Nucleosides, nucleotides, and analogues	(5'->5')-dinucleotides
2	A	1	DIAMINOPIMELATE	583-93-7	C7H14N2O4	190.095	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	A	2	AMINOADIPATE	542-32-5	C6H11NO4	161.069	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	A	3	DEOXYCYTIDINE	951-77-9	C9H13N3O4	227.091	H2O	50-750	-1.77	Nucleosides, nucleotides, and analogues	Pyrimidine nucleosides
2	A	4	NORADRENALINE	51-41-2	C8H11NO3	169.074	H2O	50-750	-1.24	Benzenoids	Phenols
2	A	5	GLUCOSAMINE 6-PHOSPHATE	3616-42-0	C6H14NO8P	259.046	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
2	A	6	TARTRATE	147-71-7	C4H6O6	150.016	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
2	A	7	3-DEHYDR OSHIKIMATE	27655-56-7	C7H8O5	172.037	5 % MeOH	50-750			[NULL]
2	A	8	CAFFEINE	58-08-2	C8H10N4O2	194.080	H2O	50-750	-0.07	Organoheterocyclic compounds	Imidazo pyrimidines
2	A	9	HOMOCYSTEINE	454-29-5	C4H9NO2S	135.035	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	A	10	THEOPHYLLINE	58-55-9	C7H8N4O2	180.065	H2O	50-750	-0.02	Organoheterocyclic compounds	Imidazo pyrimidines
2	A	11	LEUCINE	61-90-5	C6H13NO2	131.095	H2O	50-750	-1.52	Organic acids and derivatives	Carboxylic acids and derivatives
2	A	12	TREHALOSE	99-20-7	C12H22O11	342.116	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
2	B	1	BETAINE	107-43-7	C5H11NO2	117.079	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	B	2	TRYPTOPHAN	153-94-6	C11H12N2O2	204.090	5 % MeOH	50-750		Organoheterocyclic compounds	Indoles and derivatives
2	B	3	3-SULFINOALANINE	1115-65-7	C3H7NO4S	153.010	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	B	4	O-SUCCINYL-HOMOSERINE	1492-23-5	C8H13NO6	219.074	5 % MeOH	50-750			[NULL]
2	B	5	ALLANTOIN	97-59-6	C4H6N4O3	158.044	H2O	50-750		Organoheterocyclic compounds	Azoles
2	B	6	GLYCERALDEHYDE	56-82-6	C3H6O3	90.032	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
2	B	7	D-GLUCURONOLACTONE	32449-92-6	C6H8O6	176.032	H2O	50-750		Organoheterocyclic compounds	Furofurans
2	B	8	(2-AMINOETHYL) PHOSPHONATE	2041-14-7	C2H8NO3P	125.024	5 % MeOH	50-750		Organic acids and derivatives	Organic phosphonic acids and derivatives
2	B	9	2,5-DIHYDRO BENZOIC ACID	490-79-9	C7H6O4	154.027	H2O	50-750	1.74	Benzenoids	Benzene and substituted derivatives
2	B	10	MALEIMIDE	541-59-3	C4H3NO2	97.016	5 % MeOH	50-750			[NULL]
2	B	11	THREITOL	2418-52-2	C4H10O4	122.058	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
2	B	12	GLUCOSAMINE	3416-24-8	C6H13NO5	179.079	H2O	50-750		Organic oxygen compounds	Organooxygen compounds

2	C	1	PARAXANTHINE	611-59-6	C7H8N4O2	180.065	5 % MeOH	70-1050		Organoheterocyclic compounds	Imidazo pyrimidines
2	C	2	ADENOSINE 5'-DIPHOSPHATE	58-64-0	C10H15N5O10P2	427.029	5 % MeOH	70-1050		Nucleosides, nucleotides, and analogues	Purine nucleotides
2	C	3	2-DEOXY-D-GLUCOSE	7512-17-6	C6H12O5	164.068	H2O	70-1050	-2.1	Organic oxygen compounds	Organooxygen compounds
2	C	4	1-METHYL-L-HISTIDINE	332-80-9	C7H11N3O2	169.085	H2O	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
2	C	5	GALACTITOL	608-66-2	C6H14O6	182.079	H2O	70-1050	-3.1	Organic oxygen compounds	Organooxygen compounds
2	C	6	OXOPROLINE	4042-36-8	C5H7NO3	129.043	H2O	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
2	C	7	4-PYRIDOXATE	82-82-6	C8H9NO4	183.053	5 % MeOH	70-1050		Organoheterocyclic compounds	Pyridines and derivatives
2	C	8	QUINOLINATE	89-00-9	C7H5NO4	167.022	H2O	70-1050		Organoheterocyclic compounds	Pyridines and derivatives
2	C	9	METHYL GUANIDINE	471-29-4	C2H7N3	73.064	H2O	70-1050		Organic nitrogen compounds	Organonitrogen compounds
2	C	10	DEOXYGUANOSINE-MONOPHOSPHATE	902-04-5	C10H14N5O7P	347.063	5 % MeOH	70-1050		Nucleosides, nucleotides, and analogues	Purine nucleotides
2	C	11	3-HYDROXY-3-METHYLGLUTARYL-COA	1553-55-5	C27H44N7O20P3S	911.157	5 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
2	C	12	GLUCURONATE	6556-12-3	C6H10O7	194.043	H2O	70-1050	-2.57	Organic oxygen compounds	Organooxygen compounds
2	D	1	1-METHYL ADENOSINE	15763-06-1	C11H15N5O4	281.112	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleosides
2	D	2	DEOXYURIDINE	951-78-0	C9H12N2O5	228.075	5 % MeOH	50-750	-1.51	Nucleosides, nucleotides, and analogues	Pyrimidine nucleosides
2	D	3	GLUCONATE	526-95-4	C6H12O7	196.058	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
2	D	4	UROCANATE	104-98-3	C6H6N2O2	138.043	H2O	50-750		Organoheterocyclic compounds	Azoles
2	D	5	KYNURENINE	2922-83-0	C10H12N2O3	208.085	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
2	D	6	PYROGLUTAMATE	98-79-3	C5H7NO3	129.043	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	D	7	4-ACETAMIDO BUTANOATE	3025-96-5	C6H11NO3	145.074	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	D	8	TRANS-1,2-CYCLO HEXANEDIOL	1460-57-7	C6H12O2	116.084	5 % MeOH	50-750			[NULL]
2	D	9	MELANIN	8049-97-6	C18H10N2O4	318.064	5 % MeOH	50-750		Benzenoids	Anthracenes
2	D	10	DOPAMINE	62-31-7	C8H11NO2	153.079	H2O	50-750	-0.98	Benzenoids	Phenols
2	D	11	ADENOSINE-MONOPHOSPHATE	61-19-8	C10H14N5O7P	347.063	H2O	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
2	D	12	LYSINE	56-87-1	C6H14N2O2	146.106	H2O	50-750	-3.05	Organic acids and derivatives	Carboxylic acids and derivatives
2	E	1	CITICOLINE	987-78-0	C14H26N4O11P2	488.107	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
2	E	2	1,3-DIAMINO PROPANE	109-76-2	C3H10N2	74.084	5 % MeOH	50-750	-1.43	Organic nitrogen compounds	Organonitrogen compounds
2	E	3	PHOSPHOSERINE	407-41-0	C3H8NO6P	185.009	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	E	4	1-AMINOCYCLO PROPANECARBOXYLATE	22059-21-8	C4H7NO2	101.048	5 % MeOH	50-750	-2.78	Organic acids and derivatives	Carboxylic acids and derivatives
2	E	5	GLUTARYL CARNITINE	102636-82-8	C12H21NO6	275.137	5 % MeOH	50-750		Lipids and lipid-like molecules	Fatty Acyls
2	E	6	CYSTATHIONINE	56-88-2	C7H14N2O4S	222.067	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	E	7	NORVALINE	760-78-1	C5H11NO2	117.079	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	E	8	3-HYDROXYMETHYL GLUTARATE	503-49-1	C6H10O5	162.053	5 % MeOH	50-750		Lipids and lipid-like molecules	Fatty Acyls
2	E	9	PHOSPHONO ACETATE	4408-78-0	C2H5O5P	139.987	H2O	50-750		Organic acids and derivatives	Organic phosphonic acids and derivatives

2	E	10	PICOLINATE	98-98-6	C6H5NO2	123.032	H2O	50-750	0.72	Organoheterocyclic compounds	Pyridines and derivatives
2	E	11	ETHANOLAMINE	141-43-5	C2H7NO	61.053	H2O	50-750	-1.31	Organic nitrogen compounds	Organonitrogen compounds
2	E	12	ARGININE	74-79-3	C6H14N4O2	174.112	H2O	50-750	-4.2	Organic acids and derivatives	Carboxylic acids and derivatives
2	F	1	TRANS-4-HYDROXY-L-PROLINE	51-35-4	C5H9NO3	131.058	H2O	70-1050	-3.17	Organic acids and derivatives	Carboxylic acids and derivatives
2	F	2	FUCOSE	2438-80-4	C6H12O5	164.068	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
2	F	3	HOMOCYSTINE	870-93-9	C8H16N2O4S2	268.055	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
2	F	4	N-METHY LGLUTAMATE	6753-62-4	C6H11NO4	161.069	5 % MeOH	70-1050			[NULL]
2	F	5	D-ORNITHINE	348-66-3	C5H12N2O2	132.090	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
2	F	6	XANTHOSINE	146-80-5	C10H12N4O6	284.076	5 % MeOH	70-1050		Nucleosides, nucleotides, and analogues	Purine nucleosides
2	F	7	3-METHYL CROTONYL-COA	793193-48-3	C26H42N7O17P3S	849.157	5 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
2	F	8	THYROTROPIN RELEASING HORMONE	NA	C16H22N6O4	362.170	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
2	F	9	CYSTEATE	498-40-8	C3H7NO5S	169.004	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
2	F	10	N-METHYL ASPARTATE	6384-92-5	C5H9NO4	147.053	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
2	F	11	GALACTARATE	526-99-8	C6H10O8	210.038	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
2	F	12	ALPHA-HYDROXYISOBUTYRATE	594-61-6	C4H8O3	104.047	5 % MeOH	70-1050	-0.36	Organic acids and derivatives	Hydroxy acids and derivatives
2	G	1	NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE	6450-77-7	C21H27N6O18P3	665.101	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	(5'->5')-dinucleotides
2	G	2	N-ACETYL ASPARAGINE	4033-40-3	C6H10N2O4	174.064	5 % MeOH	50-750	-2.6	Organic acids and derivatives	Carboxylic acids and derivatives
2	G	3	PIPECOLATE	3105-95-1	C6H11NO2	129.079	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	G	4	GLUCOSE 6-PHOSPHATE	56-73-5	C6H13O9P	260.030	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
2	G	5	NADP	53-59-8	C21H28N7O17P3	743.075	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	(5'->5')-dinucleotides
2	G	6	6-PHOSPHO GLUCONATE	921-62-0	C6H13O10P	276.025	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
2	G	7	ISOPENTENYL PYROPHOSPHATE	358-71-4	C5H12O7P2	246.006	5 % MeOH	50-750		Lipids and lipid-like molecules	Prenol lipids
2	G	8	GUANOSINE TRIPHOSPHATE	86-01-1	C10H16N5O14P3	522.991	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
2	G	9	DTDP-D-GLUCOSE	2196-62-5	C16H26N2O16P2	564.076	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
2	G	10	AGMATINE SULFATE	306-60-5	C5H14N4	130.122	5 % MeOH	50-750		Organic nitrogen compounds	Organonitrogen compounds
2	G	11	GLYCOL ALDEHYDE	141-46-8	C2H4O2	120.042	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
2	G	12	DGTP	2564-35-4	C10H16N5O13P3	506.996	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
2	H	1	N-ACETYLGLYCINE	543-24-8	C4H7NO3	117.043	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	H	2	N-ACETYL ASPARTATE	997-55-7	C6H9NO5	175.048	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
2	H	3	INOSINE 5'-DIPHOSPHATE	86-04-4	C10H14N4O11P2	428.013	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
2	H	4	PALMITOYL CARNITINE	2364-67-2	C23H45NO4	399.335	5 % MeOH	50-750		Lipids and lipid-like molecules	Fatty Acyls
2	H	5	NORSPERMIDINE	56-18-8	C6H17N3	131.142	5 % MeOH	50-750		Organic nitrogen compounds	Organonitrogen compounds
2	H	6	NICOTINAMIDE HYPOXANTHINE DINUCLEOTIDE	1851-07-6	C21H26N6O15P2	664.093	5 % MeOH	50-750			[NULL]

2	H	7	S-ADENOSYL METHIONINE	485-80-3	C15H22N6O5S	399.145	H2O	50-750	-5.3	Nucleosides, nucleotides, and analogues	5'-deoxyribo nucleosides
2	H	8	ERYTHRITOL	149-32-6	C4H10O4	122.058	H2O	50-750	-2.29	Organic oxygen compounds	Organooxygen compounds
2	H	9	GLUCOSAMINATE	3646-68-2	C6H13NO6	195.074	5 % MeOH	50-750			[NULL]
2	H	10	URIDINE TRIPHOSPHATE	63-39-8	C9H15N2O15P3	483.969	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
2	H	11	2-KETO-3-DEOXY-D-GLUCONIC ACID	17510-99-5	C6H10O6	178.048	5 % MeOH	50-750		Organoheterocyclic compounds	Keto acids and derivatives
2	H	12	D-SEDOHEPTULOSE	3019-74-7	C7H14O7	210.074	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
3	A	1	1,4-DIAMINOBUTANE DIHYDROCLORIDE	333-93-7	C4H12N2 (PUTRESCINE)	88.100	5 % MeOH	50-750	-0.7	Organic nitrogen compounds	Organonitrogen compounds
3	A	2	DEOXYCARNITINE	407-64-7	C7H15NO2	145.110	5 % MeOH	50-750		Lipids and lipid-like molecules	Fatty Acyls
3	A	3	ADENOSINE 2',3'-CYCLIC PHOSPHATE	634-01-5	C10H12N5O6P	329.053	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
3	A	4	MEVALOLACTONE	503-48-0	C6H10O3	130.063	5 % MeOH	50-750		Organoheterocyclic compounds	Lactones
3	A	5	GALACTOSE 1-PHOSPHATE	2255-14-3	C6H13O9P	260.030	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
3	A	6	GAMMA,GAMMA-DIMETHYLALLYL PYROPHOSPHATE	358-72-5	C5H12O7P2	246.006	5 % MeOH	50-750		Lipids and lipid-like molecules	Prenol lipids
3	A	7	DEOXYURIDINE TRIPHOSPHATE	1173-82-6	C9H15N2O14P3	467.974	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
3	A	8	PHOSPHORYL CHOLINE	3616-04-4	C5H14NO4P	184.074	5 % MeOH	50-750		Organic nitrogen compounds	Organonitrogen compounds
3	A	9	O-ACETYL CARNITINE	3040-38-8	C9H17NO4	203.116	5 % MeOH	50-750		Lipids and lipid-like molecules	Fatty Acyls
3	A	10	6-HYDROXYDOPAMINE	1199-18-4	C8H11NO3	169.074	5 % MeOH	50-750		Benzenoids	Phenols
3	A	11	THIAMINE	59-43-8	C12H17N4OS	265.112	H2O	50-750		Organoheterocyclic compounds	Diazines
3	A	12	DGDP	3493-09-2	C10H15N5O10P2	427.029	H2O	50-750		Phenylpropanoids and polyketides	Cinnamic acids and derivatives
3	B	1	5-METHYL CYTOSINE	554-01-8	C5H7N3O	125.059	H2O	50-750		Organoheterocyclic compounds	Diazines
3	B	2	GLYCERATE	473-81-4	C3H6O4	106.027	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
3	B	3	CYTIDINE 2',3'-CYCLIC PHOSPHATE	15718-51-1	C9H12N3O7P	305.041	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
3	B	4	N,N,N-TRIMETHYLLYSINE	19253-88-4	C9H20N2O2	188.152	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
3	B	5	RIBOFLAVIN	83-88-5	C17H20N4O6	376.138	H2O	50-750	-1.46	Organoheterocyclic compounds	Pteridines and derivatives
3	B	6	URIDINE DIPHOSPHATE GLUCOSE	133-89-1	C15H24N2O17P2	566.055	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
3	B	7	METHYL GALACTOSIDE	709-50-2	C7H14O6	194.079	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
3	B	8	PYRIDOXAL-PHOSPHATE	54-47-7	C8H10NO6P	247.025	H2O	50-750		Organoheterocyclic compounds	Pyridines and derivatives
3	B	9	DIHYDROXYACETONE PHOSPHATE	57-04-5	C3H7O6P	169.998	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
3	B	10	PHOSPHOENOLPYRUVATE	138-08-9	C3H5O6P	167.982	5 % MeOH	50-750		Organic acids and derivatives	Organic phosphoric acids and derivatives
3	B	11	MANNOSE 6-PHOSPHATE	3672-15-9	C6H13O9P	260.030	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
3	B	12	3-PHOSPHO GLYCERATE	820-11-1	C3H7O7P	185.993	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
3	C	1	L-CARNITINE	541-15-1	C7H15NO3	161.105	5 % MeOH	50-750		Organic nitrogen compounds	Organonitrogen compounds
3	C	2	O-PHOSPHOETHANOLAMINE	1071-23-4	C2H8NO4P	141.019	H2O	50-750		Organic acids and derivatives	Organic phosphoric acids and derivatives
3	C	3	O-ACETYLSERINE	5147-00-2	C5H9NO4	147.053	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives

3	C	4	CYTIDINE MONOPHOSPHATE	63-37-6	C9H14N3O8P	323.052	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
3	C	5	GUANOSINE DIPHOSPHATE MANNOSE	3123-67-9	C16H25N5O16P2	605.077	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
3	C	6	ADP-GLUCOSE	2140-58-1	C16H25N5O15P2	589.082	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
3	C	7	FRUCTOSE 6-PHOSPHATE	643-13-0	C6H13O9P	260.030	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
3	C	8	ADENOSINE 3',5'-DIPHOSPHATE	1053-73-2	C10H15N5O10P2	427.029	H2O	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
3	C	9	3-NITRO-L-TYROSINE	621-44-3	C9H10N2O5	226.059	5 % MeOH	50-750			[NULL]
3	C	10	P-OCTOPAMINE	104-14-3	C8H11NO2	153.079	5 % MeOH	50-750	-0.9	Benzenoids	Phenols
3	C	11	N-ALPHA-ACETYLLYSINE	1946-82-3	C8H16N2O3	188.116	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
3	C	12	URIDINE DIPHOSPHATEGALACTOSE	2956-16-3	C15H24N2O17P2	566.055	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
3	D	1	DIHYDROXYFUMARATE	133-38-0	C4H4O6	148.001	H2O	70-1050		Organoheterocyclic compounds	Keto acids and derivatives
3	D	2	PYRIDOXAMINE	85-87-0	C8H12N2O2	168.090	H2O	70-1050		Organoheterocyclic compounds	Pyridines and derivatives
3	D	3	5-AMINO LEVULINATE	106-60-5	C5H9NO3	131.058	H2O	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
3	D	4	DEOXYURIDINE-MONOPHOSPHATE	964-26-1	C9H13N2O8P	308.041	5 % MeOH	70-1050		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
3	D	5	5'-DEOXY ADENOSINE	4754-39-6	C10H13N5O3	251.102	5 % MeOH	70-1050		Nucleosides, nucleotides, and analogues	5'-deoxyribo nucleosides
3	D	6	RIBOSE 1,5-BISPHOSPHATE	14689-84-0	C5H12O11P2	309.985	5 % MeOH	70-1050		Organic oxygen compounds	Organooxygen compounds
3	D	7	XANTHOSINE-MONOPHOSPHATE	523-98-8	C10H13N4O9P	364.042	5 % MeOH	70-1050		Nucleosides, nucleotides, and analogues	Purine nucleotides
3	D	8	FAD	146-14-5	C27H33N9O15P2	785.157	H2O	70-1050		Nucleosides, nucleotides, and analogues	Flavin nucleotides
3	D	9	DEOXYGUANOSINE	961-07-9	C10H13N5O4	267.097	5 % MeOH	70-1050	-1.3	Nucleosides, nucleotides, and analogues	Purine nucleosides
3	D	10	OROTATE	65-86-1	C5H4N2O4	156.017	H2O	70-1050	-0.83	Organoheterocyclic compounds	Diazines
3	D	11	LAUROYL CARNITINE	25518-54-1	C19H37NO4	343.272	5 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
3	D	12	1-METHYL NICOTINAMIDE	3106-60-3	C7H9N2O	137.071	H2O	70-1050		Organoheterocyclic compounds	Pyridines and derivatives
3	E	1	SPERMINE	71-44-3	C10H26N4	202.216	5 % MeOH	50-750		Organic nitrogen compounds	Organonitrogen compounds
3	E	2	N-ACETYL METHIONINE	65-82-7	C7H13NO3S	191.062	5 % MeOH	50-750	-0.03	Organic acids and derivatives	Carboxylic acids and derivatives
3	E	3	CARBAMOYL PHOSPHATE	590-55-6	CH4NO5P	140.983	5 % MeOH	50-750		Organic acids and derivatives	Organic phosphoric acids and derivatives
3	E	4	PHOSPHORIBOSYL PYROPHOSPHATE	7540-64-9	C5H13O14P3	389.952	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
3	E	5	AICAR	3031-94-5	C9H15N4O8P	338.063	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Imidazole ribonucleosides and ribonucleotides
3	E	6	URIDINE DIPHOSPHATE-N-ACETYLGALACTOSAMINE	7277-98-7	C17H27N3O17P2	607.082	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
3	E	7	GLYCERALDEHYDE 3-PHOSPHATE	142-10-9	C3H7O6P	169.998	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
3	E	8	CYCLIC GMP	7665-99-8	C10H12N5O7P	345.047	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
3	E	9	HOMOCYSTEINE THIOLACTONE	3622-59-1	C4H7NOS	117.025	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
3	E	10	O-PHOSPHOSERINE	17885-08-4	C3H8NO6P	185.009	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
3	E	11	S-ADENOSYL HOMOCYSTEINE	979-92-0	C14H20N6O5S	384.122	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	5'-deoxyribonucleosides

3	E	12	L-ORNITHINE	3184-13-2	C5H12N2O2	132.090	H2O	50-750	-4.22	Organic acids and derivatives	Carboxylic acids and derivatives
3	F	1	ADENINE	73-24-5	C5H5N5	135.054	H2O	50-750	-0.09	Organoheterocyclic compounds	Imidazopyrimidines
3	F	2	NORMETANEPHRINE	97-31-4	C9H13NO3	183.090	5 % MeOH	50-750	-1.05	Benzenoids	Phenols
3	F	3	URIDINE DIPHOSPHATE-N-ACETYLGLUCOSAMINE	528-04-1	C17H27N3O17P2	607.082	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
3	F	4	GUANOSINE DIPHOSPHATE	146-91-8	C10H15N5O11P2	443.024	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
3	F	5	GLUTATHIONE REDUCED	70-18-8	C10H17N3O6S	307.084	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
3	F	6	URIDINE DIPHOSPHATE GLUCURONIC ACID	2616-64-0	C15H22N2O18P2	580.034	5 % MeOH	50-750		Organoheterocyclic compounds	Lactones
3	F	7	N,N-DIMETHYLARGININE	30315-93-6	C8H18N4O2	202.143	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
3	F	8	CYTIDINE DIPHOSPHATE	63-38-7	C9H15N3O11P2	403.018	5 % MeOH	50-750	2.44	Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
3	F	9	SELENO CYSTAMINE	2697-61-2	C4H12N2Se2	247.933	5 % MeOH	50-750			[NULL]
3	F	10	HISTAMINE	51-45-6	C5H9N3	111.080	5 % MeOH	50-750	-0.7	Organic nitrogen compounds	Organonitrogen compounds
3	F	11	INDOXYL SULFATE	487-94-5	C8H7NO4S	213.010	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
3	F	12	ETHYL 3-UREIDO PROPIONATE	NA	C6H12N2O3	160.085	5 % MeOH	50-750			[NULL]
3	G	1	DEOXYRIBOSE	533-67-5	C5H10O4	134.058	5 % MeOH	70-1050		Organic oxygen compounds	Organooxygen compounds
3	G	2	PHYTATE	83-86-3	C6H18O24P6	659.861	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
3	G	3	THIAMINE MONOPHOSPHATE	495-23-8	C12H17N4O4PS	344.071	5 % MeOH	70-1050		Organoheterocyclic compounds	Diazines
3	G	4	URACIL 5-CARBOXYLATE	23945-44-0	C5H4N2O4	156.017	5 % MeOH	70-1050			[NULL]
3	G	5	S-HEXYL-GLUTATHIONE	24425-56-7	C16H29N3O6S	391.178	5 % MeOH	70-1050			[NULL]
3	G	6	GLYOXYLATE	298-12-4	C2H2O3	74.000	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
3	G	7	GUANOSINE MONOPHOSPHATE	85-32-5	C10H14N5O8P	363.058	H2O	70-1050		Nucleosides, nucleotides, and analogues	Purine nucleotides
3	G	8	N-ACETYL ALANINE	97-69-8	C5H9NO3	131.058	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
3	G	9	4-GUANIDINO BUTANOATE	463-00-3	C5H11N3O2	145.085	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
3	G	10	HYDROXY PYRUVATE	1113-60-6	C3H4O4	104.011	5 % MeOH	70-1050		Organic acids and derivatives	Hydroxy acids and derivatives
3	G	11	D-MANNOSAMINE	NA	C6H13NO5	179.079	5 % MeOH	70-1050			[NULL]
3	G	12	CYTOCHROME C	9007-43-6	C42H52FeN8O6S2	884.280	5 % MeOH	70-1050			[NULL]
3	H	1	EMPTY								
3	H	2	EMPTY								
3	H	3	EMPTY								
3	H	4	EMPTY								
3	H	5	EMPTY								
3	H	6	EMPTY								
3	H	7	EMPTY								
3	H	8	EMPTY								

3	H	9	EMPTY								
3	H	10	EMPTY								
3	H	11	EMPTY								
3	H	12	EMPTY								
4	A	1	DEOXYADENOSINE	958-09-8	C10H13N5O3	251.102	5 % MeOH	50-750	-0.55	Nucleosides, nucleotides, and analogues	Purine nucleosides
4	A	2	N-ACETYL PUTRESCINE	18233-70-0	C6H14N2O	130.111	5 % MeOH	50-750		Organic acids and derivatives	Carboximidic acids and derivatives
4	A	3	N-ACETYL GALACTOSAMINE	1811-31-0	C8H15NO6	221.090	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
4	A	4	N-ACETYL GLUTAMATE	1188-37-0	C7H11NO5	189.064	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
4	A	5	2,4-DIHYDROXY PTERIDINE	487-21-8	C6H4N4O2	164.033	5 % MeOH	50-750			[NULL]
4	A	6	6-HYDROXY NICOTINATE	5006-66-6	C6H5NO3	139.027	5 % MeOH	50-750		Organoheterocyclic compounds	Pyridines and derivatives
4	A	7	N-ACETYL CYSTEINE	616-91-1	C5H9NO3S	163.030	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
4	A	8	INOSINE-MONOPHOSPHATE	131-99-7	C10H13N4O8P	348.047	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
4	A	9	PANTOTHENATE	79-83-4	C9H17NO5	219.111	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
4	A	10	2-AMINO ISOBUTYRATE	62-57-7	C4H9NO2	103.063	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
4	A	11	ANILINE-2-SULFONATE	88-21-1	C6H7NO3S	173.015	5 % MeOH	50-750			[NULL]
4	A	12	S-CARBOXY METHYLCYSTEINE	2387-59-9	C5H9NO4S	179.025	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
4	B	1	RHAMNOSE	3615-41-6	C6H12O5	164.068	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
4	B	2	THIAMINE PYROPHOSPHATE	154-87-0	C12H18N4O7P2S	424.037	5 % MeOH	50-750		Organoheterocyclic compounds	Diazines
4	B	3	HISTIDINOL	4836-52-6	C6H11N3O	141.090	5 % MeOH	50-750		Organic nitrogen compounds	Organonitrogen compounds
4	B	4	THYMIDINE-MONOPHOSPHATE	365-07-1	C10H15N2O8P	322.057	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
4	B	5	UREIDO PROPIONATE	462-88-4	C4H8N2O3	132.053	H2O	50-750		Organic acids and derivatives	Organic carbonic acids and derivatives
4	B	6	5-AMINO PENTANOATE	660-88-8	C5H11NO2	117.079	H2O	50-750	-2.63	Organic acids and derivatives	Carboxylic acids and derivatives
4	B	7	NORLEUCINE	327-57-1	C6H13NO2	131.095	H2O	50-750	-1.53	Organic acids and derivatives	Carboxylic acids and derivatives
4	B	8	N-FORMYL GLYCINE	2491-15-8	C3H5NO3	103.027	5 % MeOH	50-750			[NULL]
4	B	9	ADENOSINE	58-61-7	C10H13N5O4	267.097	H2O	50-750	-1.05	Nucleosides, nucleotides, and analogues	Purine nucleosides
4	B	10	RAFFINOSE	512-69-6	C18H32O16	504.169	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
4	B	11	MESO-TARTRATE	147-73-9	C4H606	150.016	5 % MeOH	50-750			[NULL]
4	B	12	2-ACETAMIDO-2-DEOXY-BETA-D-GLUCOSYLAMINE	4229-38-3	C8H16N2O5	220.106	5 % MeOH	50-750			[NULL]
4	C	1	SACCHARATE	87-73-0	C6H10O8	210.038	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
4	C	2	ADENOSINE TRIPHOSPHATE	56-65-5	C10H16N5O13P3	506.996	H2O	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
4	C	3	3-METHOXY TYROSINE	7636-26-2	C10H13NO4	211.084	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
4	C	4	LACTOSE	63-42-3	C12H22O11	342.116	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
4	C	5	3-HYDROXY BUTANOATE	300-85-6	C4H8O3	104.047	5 % MeOH	50-750		Organic acids and derivatives	Hydroxy acids and derivatives

4	C	6	4-IMIDAZOLE ACETATE	645-65-8	C5H6N2O2	126.043	5 % MeOH	50-750		Organoheterocyclic compounds	Azoles
4	C	7	GALACTURONATE	685-73-4	C6H10O7	194.043	5 % MeOH	50-750			[NULL]
4	C	8	CYTIDINE TRIPHOSPHATE	65-47-4	C9H16N3O14P3	482.985	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
4	C	9	CYCLIC AMP	60-92-4	C10H12N5O6P	329.053	H2O	50-750	-2.96	Nucleosides, nucleotides, and analogues	Purine nucleotides
4	C	10	METHIONINE SULFOXIMINE	1982-67-8	C5H12N2O3S	180.057	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
4	C	11	CIS-4-HYDROXY-D-PROLINE	2584-71-6	C5H9NO3	131.058	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
4	C	12	N1-ACETYL SPERMINE	25593-72-0	C12H28N4O	244.226	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
4	D	1	GLUCOSAMINE 6-SULFATE	91674-26-9	C6H13NO8S	259.036	5 % MeOH	70-1050		Organic oxygen compounds	Organooxygen compounds
4	D	2	NADPH	53-57-6	C21H30N7O17P3	745.091	5 % MeOH	70-1050		Nucleosides, nucleotides, and analogues	(5'->5')-dinucleotides
4	D	3	3-METHYL HISTAMINE	644-42-8	C6H11N3	125.095	5 % MeOH	70-1050		Organic nitrogen compounds	Organonitrogen compounds
4	D	4	MALEAMATE	557-24-4	C4H5NO3	115.027	5 % MeOH	70-1050			[NULL]
4	D	5	CHOLINE	62-49-7	C5H14NO	104.108	5 % MeOH	70-1050		Organic nitrogen compounds	Organonitrogen compounds
4	D	6	METHYL 4-AMINOBUTYRATE	56-12-2	C5H11NO2	117.079	H2O	70-1050	-3.17	Organic acids and derivatives	Carboxylic acids and derivatives
4	D	7	N-FORMYL-L-METHIONINE	4289-98-9	C6H11NO3S	177.046	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
4	D	8	ACETYLCHOLINE	51-84-3	C7H16NO2	146.118	5 % MeOH	70-1050		Organic nitrogen compounds	Organonitrogen compounds
4	D	9	OXALATE	144-62-7	C2H2O4	89.995	H2O	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
4	D	10	5-HYDROXY-L-TRYPTOPHAN	4350-09-8	C11H12N2O3	220.085	5 % MeOH	70-1050	-2.051	Organoheterocyclic compounds	Indoles and derivatives
4	D	11	D-ALANINE	338-69-2	C3H7NO2	89.048	H2O	70-1050	-2.912	Organic acids and derivatives	Carboxylic acids and derivatives
4	D	12	THEOBROMINE	83-67-0	C7H8N4O2	180.065	H2O	70-1050	-0.78	Organoheterocyclic compounds	Imidazo pyrimidines
4	E	1	GUANIDINO SUCCINATE	6133-30-8	C5H9N3O4	175.059	5 % MeOH	50-750	-2.068	Organic acids and derivatives	Carboxylic acids and derivatives
4	E	2	HISTIDINE	71-00-1	C6H9N3O2	155.069	H2O	50-750	-3.32	Organic acids and derivatives	Carboxylic acids and derivatives
4	E	3	ALLOTHREONINE	24830-94-2	C4H9NO3	119.058	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
4	E	4	PHOSPHO CREATINE	67-07-2	C4H10N3O5P	211.036	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
4	E	5	SPERMIDINE	124-20-9	C7H19N3	145.158	5 % MeOH	50-750		Organic nitrogen compounds	Organonitrogen compounds
4	E	6	ADENOSINE DIPHOSPHATE RIBOSE	20762-30-5	C15H23N5O14P2	559.072	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
4	E	7	2-METHOXY ETHANOL	109-86-4	C3H8O2	76.052	5 % MeOH	50-750			[NULL]
4	E	8	CITRAMALATE	597-44-4	C5H8O5	148.037	5 % MeOH	50-750		Lipids and lipid-like molecules	Fatty Acyls
4	E	9	ANSERINE	584-85-0	C10H16N4O3	240.122	5 % MeOH	50-750		Organic acids and derivatives	Peptidomimetics
4	E	10	BILIVERDIN	114-25-0	C33H34N4O6	582.248	5 % MeOH	50-750		Organoheterocyclic compounds	Tetrapyrroles and derivatives
4	E	11	5-HYDROXYLYSINE	1190-94-9	C6H14N2O3	162.100	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
4	E	12	CYSTEAMINE	60-23-1	C2H7NS	77.030	5 % MeOH	50-750		Organosulfur compounds	Thiols
4	F	1	OPHTHALMATE	495-27-2	C11H19N3O6	289.127	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
4	F	2	MESOXALATE	473-90-5	C3H2O5	117.990	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives

4	F	3	TRIGONELLINE	535-83-1	C7H7NO2	137.048	5 % MeOH	70-1050			[NULL]
4	F	4	EPINEPHRINE	51-43-4	C9H13NO3	183.090	H2O	70-1050	-1.37	Benzenoids	Phenols
4	F	5	3,4-DIHYDROXY PHENYLGLYCOL	28822-73-3	C8H10O4	170.058	5 % MeOH	70-1050	-1.01	Benzenoids	Phenols
4	F	6	CADAVERINE	462-94-2	C5H14N2	102.116	5 % MeOH	70-1050		Organic nitrogen compounds	Organonitrogen compounds
4	F	7	2-HYDROXY BUTYRATE	600-15-7	C4H8O3	104.047	5 % MeOH	70-1050		Organic acids and derivatives	Hydroxy acids and derivatives
4	F	8	COENZYME A	85-61-0	C21H36N7O16P3S	767.115	5 % MeOH	70-1050		Nucleosides, nucleotides, and analogues	Purine nucleotides
4	F	9	OXALOMALATE	89304-26-7	C6H6O8	206.006	5 % MeOH	70-1050			[NULL]
4	F	10	INOSINE TRIPHOSPHATE	132-06-9	C10H15N4O14P3	507.980	H2O	70-1050		Nucleosides, nucleotides, and analogues	Purine nucleotides
4	F	11	CDP-ETHANOLAMINE	3036-18-8	C11H20N4O11P2	446.060	5 % MeOH	70-1050		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
4	F	12	2,5-DIMETHYL PYRAZINE	123-32-0	C6H8N2	108.069	5 % MeOH	70-1050	0.63	Organoheterocyclic compounds	Diazines
4	G	1	STACHYOSE	470-55-3	C24H42O21	666.222	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
4	G	2	DEOXYCYTIDINE-DIPHOSPHATE	800-73-7	C9H15N3O10P2	387.023	5 % MeOH	50-750		Organic oxygen compounds	Organic oxoanionic compounds
4	G	3	2,3-BUTANEDIOL	24347-58-8	C4H10O2	90.068	5 % MeOH	50-750	0.88	Organic oxygen compounds	Organooxygen compounds
4	G	4	D-RIBOSE 5-PHOSPHATE	3615-55-2	C5H11O8P	230.019	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
4	G	5	HYDROXYKYNURENINE	484-78-6	C10H12N2O4	224.080	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
4	G	6	GALACTOSAMINE	7535-00-4	C6H13NO5	179.079	5 % MeOH	50-750			[NULL]
4	G	7	DEOXYADENOSINE TRIPHOSPHATE	1927-31-7	C10H16N5O12P3	491.001	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Purine nucleotides
4	G	8	GLYCEROL 3-PHOSPHATE	57-03-4	C3H9O6P	172.014	H2O	50-750		Lipids and lipid-like molecules	Glycerophospholipids
4	G	9	CYANOCOBALAMIN	32462-30-9	C8H9NO3	167.058	H2O	50-750		Organoheterocyclic compounds	Tetrapyrroles and derivatives
4	G	10	4-HYDROXY-L-PHENYLGLYCINE	16354-58-8	C5H9NO4	147.053	5 % MeOH	100-1500			[NULL]
4	G	11	N-ACETYLSERINE	58-98-0	C9H14N2O12P2	404.002	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
4	G	12	URIDINE 5'-DIPHOSPHATE	68-19-9	C63H89CoN14O14P	1355.575	5 % MeOH	50-750		Nucleosides, nucleotides, and analogues	Pyrimidine nucleotides
4	H	1	EMPTY								
4	H	2	EMPTY								
4	H	3	EMPTY								
4	H	4	EMPTY								
4	H	5	EMPTY								
4	H	6	EMPTY								
4	H	7	EMPTY								
4	H	8	EMPTY								
4	H	9	EMPTY								
4	H	10	EMPTY								
4	H	11	EMPTY								

4	H	12	EMPTY								
5	A	1	METHY GLUTARATE	626-51-7	C6H10O4	146.058	5 % MeOH	50-750		Lipids and lipid-like molecules	Fatty Acyls
5	A	2	SORBATE	110-44-1	C6H8O2	112.052	H2O	50-750	1.33	Lipids and lipid-like molecules	Fatty Acyls
5	A	3	MONOETHYL MALONATE	1071-46-1	C5H8O4	132.042	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
5	A	4	GLUCONO LACTONE	90-80-2	C6H10O6	178.048	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
5	A	5	4-HYDROXY BENZOATE	99-96-7	C7H6O3	138.032	H2O	50-750	1.58	Benzenoids	Benzene and substituted derivatives
5	A	6	TYRAMINE	51-67-2	C8H11NO	137.084	H2O	50-750		Benzenoids	Benzene and substituted derivatives
5	A	7	CORTISOL	50-23-7	C21H30O5	362.209	H2O	50-750	1.61	Lipids and lipid-like molecules	Steroids and steroid derivatives
5	A	8	PRENOL	556-82-1	C5H10O	86.073	5 % MeOH	50-750		Organic oxygen compounds	Organooxygen compounds
5	A	9	3-HYDROXY BENZALDEHYDE	100-83-4	C7H6O2	122.037	5 % MeOH	50-750			[NULL]
5	A	10	XANTHURENATE	59-00-7	C10H7NO4	205.038	5 % MeOH	50-750		Organoheterocyclic compounds	Quinolines and derivatives
5	A	11	2-METHYL PROPANAL	78-84-2	C4H8O	72.058	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
5	A	12	INDOXYL ?-GLUCOSIDE	487-60-5	C14H17NO6	295.106	5 % MeOH	50-750		Organic acids and derivatives	Organic sulfuric acids and derivatives
5	B	1	TRIMETHYLAMINE	75-50-3	C3H9N	59.073	H2O	50-750	0.16	Organic nitrogen compounds	Organonitrogen compounds
5	B	2	MELATONIN	73-31-4	C13H16N2O2	232.121	5 % MeOH	50-750		Organoheterocyclic compounds	Indoles and derivatives
5	B	3	MALEATE	110-16-7	C4H4O4	116.011	H2O	50-750	-0.48	Organic acids and derivatives	Carboxylic acids and derivatives
5	B	4	PENTANOATE	109-52-4	C5H10O2	102.068	H2O	50-750	1.39	Lipids and lipid-like molecules	Fatty Acyls
5	B	5	PROPANOATE	79-09-4	C3H6O2	74.037	H2O	50-750	0.33	Organic acids and derivatives	Carboxylic acids and derivatives
5	B	6	BILIRUBIN	635-65-4	C33H36N4O6	584.263	5 % MeOH	50-750		Organoheterocyclic compounds	Tetrapyrroles and derivatives
5	B	7	NICOTINE	147-85-346	C10H14N2	162.116	H2O	50-750	1.17	Organoheterocyclic compounds	Pyridines and derivatives
5	B	8	PREGNENOLONE SULFATE	1247-64-9	C21H32O5S	396.197	5 % MeOH	50-750		Lipids and lipid-like molecules	Steroids and steroid derivatives
5	B	9	KYNURENATE	492-27-3	C10H7NO3	189.043	5 % MeOH	50-750		Organoheterocyclic compounds	Quinolines and derivatives
5	B	10	ISOBUTYRATE	79-31-2	C4H8O2	88.052	H2O	50-750	0.94	Organic acids and derivatives	Carboxylic acids and derivatives
5	B	11	3-HYDROXY BENZYL ALCOHOL	620-24-6	C7H8O2	124.052	5 % MeOH	50-750		Benzenoids	Benzene and substituted derivatives
5	B	12	ANILINE	62-53-3	C6H7N	93.058	H2O	50-750	0.9	Benzenoids	Benzene and substituted derivatives
5	C	1	ACETOIN	513-86-0	C4H8O2	88.052	H2O	50-750		Organic oxygen compounds	Organooxygen compounds
5	C	2	3,5-DIIODO-L-TYROSINE	18835-59-1	C9H9I2NO3	432.867	H2O	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
5	C	3	MANDELATE	90-64-2	C8H8O3	152.047	H2O	50-750	0.62	Benzenoids	Benzene and substituted derivatives
5	C	4	TRYPTAMINE	61-54-1	C10H12N2	160.100	5 % MeOH	50-750	1.55	Organoheterocyclic compounds	Indoles and derivatives
5	C	5	4-AMINO BENZOATE	150-13-0	C7H7NO2	137.048	H2O	50-750	0.83	Benzenoids	Benzene and substituted derivatives
5	C	6	GLUTARATE	110-94-1	C5H8O4	132.042	H2O	50-750	-0.29	Organic acids and derivatives	Carboxylic acids and derivatives
5	C	7	5-VALERO LACTONE	542-28-9	C5H8O2	100.052	5 % MeOH	50-750			[NULL]
5	C	8	CAFFEATE	331-39-5	C9H8O4	180.042	5 % MeOH	50-750	1.15	Phenylpropanoids and polyketides	Cinnamic acids and derivatives

5	C	9	LUMICHROME	1086-80-2	C12H10N4O2	242.080	5 % MeOH	50-750			[NULL]
5	C	10	BETA-ALANINE	107-95-9	C3H7NO2	89.048	H2O	50-750	-3.05	Organic acids and derivatives	Carboxylic acids and derivatives
5	C	11	N-ACETYL PHENYLALANINE	2018-61-3	C11H13NO3	207.090	5 % MeOH	50-750	0.93	Organic acids and derivatives	Carboxylic acids and derivatives
5	C	12	N-ACETYLPROLINE	68-95-1	C7H11NO3	157.074	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
5	D	1	L-TRYPTOPHAN AMIDE	5022-65-1	C11H13N3O	203.106	5 % MeOH	70-1050		Organoheterocyclic compounds	Indoles and derivatives
5	D	2	PHENOL	108-95-2	C6H6O	94.042	H2O	70-1050	1.46	Benzenoids	Phenols
5	D	3	N-METHYL TRYPTAMINE	61-49-4	C11H14N2	174.116	5 % MeOH	70-1050		Organoheterocyclic compounds	Indoles and derivatives
5	D	4	OXALOACETATE	328-42-7	C4H4O5	132.006	H2O	70-1050		Organoheterocyclic compounds	Keto acids and derivatives
5	D	5	2,3-DIHYDROXYBENZOATE	303-38-8	C7H6O4	154.027	5 % MeOH	70-1050	1.2	Benzenoids	Benzene and substituted derivatives
5	D	6	2-PROPENOATE	79-10-7	C3H4O2	72.021	H2O	70-1050	0.35	Organic acids and derivatives	Carboxylic acids and derivatives
5	D	7	INDOLE-3-ETHANOL	526-55-6	C10H11NO	161.084	5 % MeOH	70-1050		Organoheterocyclic compounds	Indoles and derivatives
5	D	8	FERULATE	1135-24-6	C10H10O4	194.058	5 % MeOH	70-1050	1.51	Phenylpropanoids and polyketides	Cinnamic acids and derivatives
5	D	9	GLYCOCHOLATE	475-31-0	C26H43NO6	465.309	5 % MeOH	70-1050	1.65	Lipids and lipid-like molecules	Steroids and steroid derivatives
5	D	10	PHENYL ETHANOLAMINE	7568-93-6	C8H11NO	137.084	H2O	70-1050		Organic nitrogen compounds	Organonitrogen compounds
5	D	11	THIOPURINE S-METHYLETHER	50-66-8	C6H6N4S	166.031	5 % MeOH	70-1050		Organoheterocyclic compounds	Imidazo pyrimidines
5	D	12	2-HYDROXY-4-(METHYLTHIO) BUTANOATE	4857-44-7	C5H10O3S	150.035	5 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
5	E	1	GLYCOCHENODEOXYCHOLATE	16564-43-5	C26H43NO5	449.314	5 % MeOH	50-750	2.12	Lipids and lipid-like molecules	Steroids and steroid derivatives
5	E	2	BENZOATE	532-32-1	C7H6O2	122.037	H2O	50-750	1.87	Benzenoids	Benzene and substituted derivatives
5	E	3	3-AMINO-5-HYDROXY BENZOATE	76045-71-1	C7H7NO3	153.043	5 % MeOH	50-750			[NULL]
5	E	4	PYROCATECHOL	120-80-9	C6H6O2	110.037	H2O	50-750	0.88	Benzenoids	Phenols
5	E	5	3,4-DIHYDROXY BENZOATE	99-50-3	C7H6O4	154.027	H2O	50-750	0.86	Benzenoids	Benzene and substituted derivatives
5	E	6	CYCLOPENTANONE	120-92-3	C5H8O	84.058	5 % MeOH	50-750	0.38	Organic oxygen compounds	Organooxygen compounds
5	E	7	PANTOLACTONE	599-04-2	C6H10O3	130.063	5 % MeOH	50-750		Organoheterocyclic compounds	Lactones
5	E	8	GUAIACOL	90-05-1	C7H8O2	124.052	H2O	50-750	1.32	Benzenoids	Phenols
5	E	9	2- HYDROXY PHENYLACETATE	611-71-2	C8H8O3	152.047	H2O	50-750	0.66	Benzenoids	Benzene and substituted derivatives
5	E	10	10-HYDROXY DECANOATE	1679-53-4	C10H20O3	188.141	5 % MeOH	50-750			[NULL]
5	E	11	DIDECANOYL-GLYCEROPHOSPHOCHOLINE	3436-44-0	C28H56NO8P	565.374	5 % MeOH	50-750			[NULL]
5	E	12	2-HYDROXY PYRIDINE	142-08-5	C5H5NO	95.037	H2O	50-750		Organoheterocyclic compounds	Pyridines and derivatives
5	F	1	3,4-DIHYDROXY PHENYLACETATE	102-32-9	C8H8O4	168.042	H2O	70-1050	0.98	Benzenoids	Phenols
5	F	2	N6-(DELTA2-ISOPENTENYL)-ADENINE	2365-40-4	C10H13N5	203.117	5 % MeOH	70-1050			[NULL]
5	F	3	METHYL VANILLATE	3943-74-6	C9H10O4	182.058	5 % MeOH	70-1050		Benzenoids	Benzene and substituted derivatives
5	F	4	2-OXOBUTANOATE	600-18-0	C4H6O3	102.032	H2O	70-1050		Organoheterocyclic compounds	Keto acids and derivatives
5	F	5	LIPOAMIDE	940-69-2	C8H15NOS2	205.060	5 % MeOH	70-1050		Organoheterocyclic compounds	Dithiolanes

5	F	6	3-HYDROXY ANTHRANILATE	548-93-6	C7H7NO3	153.043	5 % MeOH	70-1050		Benzenoids	Benzene and substituted derivatives
5	F	7	3-(4-HYDROXYPHENYL) PYRUVATE	156-39-8	C9H8O4	180.042	5 % MeOH	70-1050		Benzenoids	Benzene and substituted derivatives
5	F	8	HEXANOATE	142-62-1	C6H12O2	116.084	H2O	70-1050	1.92	Lipids and lipid-like molecules	Fatty Acyls
5	F	9	METHYLMALONATE	516-05-2	C4H6O4	118.027	H2O	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
5	F	10	INDOLE-3-ACETATE	6505-45-9	C10H9NO2	175.063	H2O	70-1050	1.41	Organoheterocyclic compounds	Indoles and derivatives
5	F	11	CORTISOL 21-ACETATE	50-03-3	C23H32O6	404.220	5 % MeOH	70-1050			[NULL]
5	F	12	INDOLE-3-ACETAMIDE	879-37-8	C10H10N2O	174.079	5 % MeOH	70-1050		Organoheterocyclic compounds	Indoles and derivatives
5	G	1	HIPPURATE	495-69-2	C9H9NO3	179.058	H2O	50-750	0.31	Benzenoids	Benzene and substituted derivatives
5	G	2	ETHYLMALONATE	601-75-2	C5H8O4	132.042	H2O	50-750		Lipids and lipid-like molecules	Fatty Acyls
5	G	3	3,5-DIIODO-L-THYRONINE	534-51-0	C15H13I2NO4	524.893	5 % MeOH	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
5	G	4	FUMARATE	110-17-8	C4H4O4	116.011	H2O	50-750	0.46	Organic acids and derivatives	Carboxylic acids and derivatives
5	G	5	BENZALDEHYDE	100-52-7	C7H6O	106.042	H2O	50-750	1.48	Benzenoids	Benzene and substituted derivatives
5	G	6	4-HYDROXYBENZALDEHYDE	123-08-0	C7H6O2	122.037	H2O	50-750	1.35	Organic oxygen compounds	Organooxygen compounds
5	G	7	3-(2-HYDROXYPHENYL) PROPANOATE	495-78-3	C9H10O3	166.063	5 % MeOH	50-750		Phenylpropanoids and polyketides	Phenylpropanoic acids
5	G	8	3-METHOXY TYRAMINE	1477-68-5	C9H13NO2	167.095	5 % MeOH	50-750	-0.08	Benzenoids	Phenols
5	G	9	BENZYLAMINE	100-46-9	C7H9N	107.073	H2O	50-750	1.09	Benzenoids	Benzene and substituted derivatives
5	G	10	2-QUINOLINE CARBOXYLATE	93-10-7	C10H7NO2	173.048	H2O	50-750		Organoheterocyclic compounds	Quinolines and derivatives
5	G	11	SEROTONIN	153-98-0	C10H12N2O	176.095	H2O	50-750	0.21	Organoheterocyclic compounds	Indoles and derivatives
5	G	12	PTERIN	2236-60-4	C6H5N5O	163.049	H2O	50-750		Organoheterocyclic compounds	Pteridines and derivatives
5	H	1	BUTANOATE	107-92-6	C4H8O2	88.052	H2O	70-1050	0.79	Lipids and lipid-like molecules	Fatty Acyls
5	H	2	2-AMINOPHENOL	95-55-6	C6H7NO	109.053	5 % MeOH	70-1050			[NULL]
5	H	3	6-CARBOXY HEXANOATE	111-16-0	C7H12O4	160.074	H2O	70-1050	0.61	Lipids and lipid-like molecules	Fatty Acyls
5	H	4	INDOLE-3-PYRUVATE	392-12-1	C11H9NO3	203.058	5 % MeOH	70-1050		Organoheterocyclic compounds	Indoles and derivatives
5	H	5	DEHYDRO ASCORBATE	1571-72-8	C7H7NO3	153.043	5 % MeOH	70-1050		Organoheterocyclic compounds	Lactones
5	H	6	3-AMINO-4-HYDROXYBENZOATE	775-01-9	C8H8O5	184.037	5 % MeOH	70-1050			[NULL]
5	H	7	3,4 DIHYDROXY MANDELATE	6061-96-7	C7H710O7	206.043	5 % MeOH	70-1050		Benzenoids	Phenols
5	H	8	2-METHYLCITRATE	6779-87-9	C9H13N5O3	239.102	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
5	H	9	DIHYDRO BIOPTERIN	17181-54-3	C3H9O6P	172.014	5 % MeOH	70-1050		Organoheterocyclic compounds	Pteridines and derivatives
5	H	10	BETA-GLYCEROPHOSPHATE	59-56-3	C6H13O9P	260.030	5 % MeOH	70-1050		Lipids and lipid-like molecules	Glycerophospholipids
5	H	11	GLUCOSE 1-PHOSPHATE	4033-39-0	C3H8N2O2	104.059	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
5	H	12	2,3-DIAMINO PROPIONATE	490-83-5	C6H6O6	174.016	5 % MeOH	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
6	A	1	2,5-DIHYDROXY BENZOATE	490-79-9	C7H6O4	154.027	40 % MeOH	70-1050	1.74	Benzenoids	Benzene and substituted derivatives
6	A	2	4-QUINOLINE CARBOXYLATE	486-74-8	C10H7NO2	173.048	40 % MeOH	70-1050			[NULL]

6	A	3	HYDROQUINONE	123-31-9	C6H6O2	110.037	40 % MeOH	70-1050	0.59	Benzenoids	Phenols
6	A	4	DETHIOBIOTIN	533-48-2	C10H18N2O3	214.132	40 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
6	A	5	3-HYDROXY BENZOATE	99-06-9	C7H6O3	138.032	40 % MeOH	70-1050	1.5	Benzenoids	Benzene and substituted derivatives
6	A	6	2-METHYL BUTANAL	96-17-3	C5H10O	86.073	40 % MeOH	70-1050		Organic oxygen compounds	Organooxygen compounds
6	A	7	N-ACETYL SEROTONIN	1210-83-9	C12H14N2O2	218.106	40 % MeOH	70-1050		Organoheterocyclic compounds	Indoles and derivatives
6	A	8	HYDROPHENYL LACTIC ACID	306-23-0	C9H10O4	182.058	40 % MeOH	70-1050		Phenylpropanoids and polyketides	Phenylpropanoic acids
6	A	9	ITACONATE	97-65-4	C5H6O4	130.027	40 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
6	A	10	AZELATE	123-99-9	C9H16O4	188.105	40 % MeOH	70-1050	1.57	Lipids and lipid-like molecules	Fatty Acyls
6	A	11	OXOADIPATE	3184-35-8	C6H8O5	160.037	40 % MeOH	70-1050		Organoheterocyclic compounds	Keto acids and derivatives
6	A	12	2-METHYL GLUTARATE	18069-17-5	C6H10O4	146.058	40 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
6	B	1	PHENYL ACETALDEHYDE	122-78-1	C8H8O	120.058	40 % MeOH	70-1050	1.78	Benzenoids	Benzene and substituted derivatives
6	B	2	3-METHYL-2-OXOVALERATE	1460-34-0	C6H10O3	130.063	40 % MeOH	70-1050		Organoheterocyclic compounds	Keto acids and derivatives
6	B	3	PORPHOBILINOGEN	487-90-1	C10H14N2O4	226.095	40 % MeOH	70-1050		Organic nitrogen compounds	Organonitrogen compounds
6	B	4	DIACETYL	431-03-8	C4H6O2	86.037	40 % MeOH	70-1050	-1.34	Organic oxygen compounds	Organooxygen compounds
6	B	5	PYRUVATE	127-17-3	C3H4O3	88.016	40 % MeOH	70-1050		Organoheterocyclic compounds	Keto acids and derivatives
6	B	6	TRANS-CINNAM ALDEHYDE	14371-10-9	C9H8O	132.058	40 % MeOH	70-1050	1.9	Phenylpropanoids and polyketides	Cinnamaldehydes
6	B	7	2,6-DIHYDROXY PYRIDINE	10357-84-3	C5H5NO2	111.032	40 % MeOH	70-1050			[NULL]
6	B	8	VANILLIN	121-33-5	C8H8O3	152.047	40 % MeOH	70-1050	1.21	Benzenoids	Phenols
6	B	9	METHYL ACETOACETATE	105-45-3	C5H8O3	116.047	40 % MeOH	70-1050		Organoheterocyclic compounds	Keto acids and derivatives
6	B	10	SUBERATE	505-48-6	C8H14O4	174.089	40 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
6	B	11	ADIPATE	124-04-9	C6H10O4	146.058	40 % MeOH	70-1050	0.08	Lipids and lipid-like molecules	Fatty Acyls
6	B	12	GERANYL-PP	763-10-0 (free acid)	C10H20O7P2	314.068	40 % MeOH	70-1050		Lipids and lipid-like molecules	Prenol lipids
6	C	1	N-ACETYLLEUCINE	1188-21-2	C8H15NO3	173.105	40 % MeOH	70-1050	0.79	Organic acids and derivatives	Carboxylic acids and derivatives
6	C	2	2',4'-DIHYDROXY ACETOPHENONE	89-84-9	C8H8O3	152.047	40 % MeOH	70-1050		Organic oxygen compounds	Organooxygen compounds
6	C	3	BENZYL ALCOHOL	100-51-6	C7H8O	108.058	40 % MeOH	70-1050	1.1	Benzenoids	Benzene and substituted derivatives
6	C	4	MONOMETHYL GLUTARATE	1501-27-5	C6H10O4	146.058	40 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
6	C	5	INDOLE-3-METHYL ACETATE	1912-33-0	C11H11NO2	189.079	40 % MeOH	70-1050		Organoheterocyclic compounds	Indoles and derivatives
6	C	6	MEVALONATE	150-97-0 (free acid)	C6H12O4	148.074	40 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
6	C	7	3-METHOXY-4-HYDROXYMANDELATE	55-10-7	C9H10O5	198.053	40 % MeOH	70-1050		Benzenoids	Phenols
6	C	8	HOMOVANILLATE	306-08-1	C9H10O4	182.058	40 % MeOH	70-1050	0.33	Benzenoids	Phenols
6	C	9	2-METHYL MALEATE	498-23-7	C5H6O4	130.027	40 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
6	C	10	1-PHENYL ETHANOL	1445-91-6	C8H10O	122.073	40 % MeOH	70-1050		Benzenoids	Benzene and substituted derivatives
6	C	11	SALSOLINOL	59709-57-8	C10H13NO2	179.095	40 % MeOH	70-1050		Organoheterocyclic compounds	Tetrahydroisoquinolines

6	C	12	SALICYLAMIDE	65-45-2	C7H7NO2	137.048	40 % MeOH	70-1050		Benzenoids	Phenols
6	D	1	OXOGLUTARATE	22202-68-2	C5H6O5	146.022	40 % MeOH	70-1050		Organoheterocyclic compounds	Keto acids and derivatives
6	D	2	ETHYL 3-INDOLEACETATE	778-82-5	C12H13NO2	203.095	40 % MeOH	70-1050			[NULL]
6	D	3	3-ALPHA,11-BETA,17,21-TETRAHYDROXY-5-BETA-PREGNAN-20-ONE	53-02-1	C21H34O5	366.241	40 % MeOH	70-1050		Lipids and lipid-like molecules	Steroids and steroid derivatives
6	D	4	N,N-DIMETHYL-1,4-PHENYLENEDIAMINE	99-98-9	C8H12N2	136.100	40 % MeOH	70-1050			[NULL]
6	D	5	HOMOGENTISATE	451-13-8	C8H8O4	168.042	40 % MeOH	70-1050	0.86	Benzenoids	Benzene and substituted derivatives
6	D	6	INDOLEACETALDEHYDE	20095-27-6	C10H9NO	159.068	40 % MeOH	70-1050		Organoheterocyclic compounds	Indoles and derivatives
6	D	7	4-HYDROXY-3-METHOXYPHENYL GLYCOL	67423-45-4	C9H12O4	184.074	40 % MeOH	70-1050			[NULL]
6	D	8	3-HYDROXYPHENYL ACETATE	621-37-4	C8H8O3	152.047	40 % MeOH	70-1050	0.85	Benzenoids	Phenols
6	D	9	4-METHYL CATECHOL	452-86-8	C7H8O2	124.052	40 % MeOH	70-1050	1.37	Benzenoids	Phenols
6	D	10	PYRIDOXAL	65-22-5	C8H9NO3	167.058	40 % MeOH	70-1050		Organoheterocyclic compounds	Pyridines and derivatives
6	D	11	SALICYLATE	69-72-7	C7H6O3	138.032	40 % MeOH	70-1050	2.26	Benzenoids	Benzene and substituted derivatives
6	D	12	SEBACATE	111-20-6	C10H18O4	202.121	40 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
6	E	1	3-METHYL-2-OXINDOLE	1504-06-9	C9H9NO	147.068	40 % MeOH	70-1050			[NULL]
6	E	2	3-METHYL ADENINE	5142-23-4	C6H7N5	149.070	40 % MeOH	70-1050		Organoheterocyclic compounds	Imidazopyrimidines
6	E	3	HYDROXYPHENYL LACTATE	6482-98-0	C9H10O4	182.058	40 % MeOH	70-1050		Phenylpropanoids and polyketides	Phenylpropanoic acids
6	E	4	BIOTIN	58-85-5	C10H16N2O3S	244.088	40 % MeOH	70-1050		Organoheterocyclic compounds	Biotin and derivatives
6	E	5	MERCAPTO PYRUVATE	10255-67-1	C3H4O3S	119.988	40 % MeOH	70-1050		Organoheterocyclic compounds	Keto acids and derivatives
6	E	6	PYRUVIC ALDEHYDE	78-98-8	C3H4O2	72.021	40 % MeOH	70-1050		Organic oxygen compounds	Organooxygen compounds
6	E	7	PYRROLE-2-CARBOXYLATE	634-97-9	C5H5NO2	111.032	40 % MeOH	70-1050	0.85	Organoheterocyclic compounds	Pyrroles
6	E	8	5-HYDROXYINDOLE ACETATE	54-16-0	C10H9NO3	191.058	40 % MeOH	70-1050		Organoheterocyclic compounds	Indoles and derivatives
6	E	9	3-METHYL GLUTACONATE	5746-90-7	C6H8O4	144.042	40 % MeOH	70-1050		Lipids and lipid-like molecules	Fatty Acyls
6	E	10	RESORCINOL MONOACETATE	102-29-4	C8H8O3	152.047	40 % MeOH	70-1050			[NULL]
6	E	11	ACETOACETATE	3483-11-2	C4H6O3	102.032	40 % MeOH	70-1050		Organoheterocyclic compounds	Keto acids and derivatives
6	E	12	ACETYL PHOSPHATE	94249-01-1	C2H5O5P	139.987	40 % MeOH	70-1050		Organic acids and derivatives	Organic phosphoric acids and derivatives
6	F	1	SORBOSE	87-79-6	C6H12O6	180.063	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	F	2	XYLITOL	87-99-0	C5H12O5	152.068	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	F	3	RIBITOL	488-81-3	C5H12O5	152.068	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	F	4	MYOINOSITOL	87-89-8	C6H12O6	180.063	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	F	5	MANNOSE	3458-28-4	C6H12O6	180.063	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	F	6	XYLOSE	58-86-6	C5H10O5	150.053	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	F	7	SUCROSE	57-50-1	C12H22O11	342.116	H2O	70-1050	-3.7	Organic oxygen compounds	Organooxygen compounds

6	F	8	GALACTOSE	59-23-4	C6H12O6	180.063	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	F	9	ALPHA-D-GLUCOSE	492-62-6	C6H12O6	180.063	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	F	10	ALLOSE	2595-97-3	C6H12O6	180.063	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	F	11	MANNITOL	69-65-8	C6H14O6	182.079	H2O	70-1050	-3.1	Organic oxygen compounds	Organooxygen compounds
6	F	12	MELIBIOSE	585-99-9	C12H22O11	342.116	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	G	1	SORBITOL	50-70-4	C6H14O6	182.079	H2O	70-1050	-2.2	Organic oxygen compounds	Organooxygen compounds
6	G	2	MALTOSE	69-79-4	C12H22O11	342.116	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	G	3	TAGATOSE	87-81-0	C6H12O6	180.063	H2O	70-1050	-3.202	Organic oxygen compounds	Organooxygen compounds
6	G	4	L-GULONO LACTONE	6322-07-2	C6H10O6	178.048	H2O	70-1050	-2.571	Organoheterocyclic compounds	Lactones
6	G	5	ARABINOSE	10323-20-3	C5H10O5	150.053	H2O	70-1050			[NULL]
6	G	6	CELLOBIOSE	528-50-7	C12H22O11	342.116	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	G	7	PSICOSE	551-68-8	C6H12O6	180.063	H2O	70-1050			[NULL]
6	G	8	ARABITOL	7643-75-6	C5H12O5	152.068	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	G	9	LYXOSE	1114-34-7	C5H10O5	150.053	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	G	10	RIBOSE	50-69-1	C5H10O5	150.053	H2O	70-1050	-2.32	Organic oxygen compounds	Organooxygen compounds
6	G	11	PALATINOSE	343336-76-5	C12H22O11	342.116	H2O	70-1050			[NULL]
6	G	12	D-PINITOL	10284-63-6	C7H14O6	194.079	H2O	70-1050		Organic oxygen compounds	Organooxygen compounds
6	H	1	EMPTY								
6	H	2	EMPTY								
6	H	3	EMPTY								
6	H	4	EMPTY								
6	H	5	EMPTY								
6	H	6	EMPTY								
6	H	7	EMPTY								
6	H	8	EMPTY								
6	H	9	EMPTY								
6	H	10	EMPTY								
6	H	11	EMPTY								
6	H	12	EMPTY								
7	A	1	VITAMIN D2	50-14-6	C28H44O	396.339	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Steroids and steroid derivatives
7	A	2	SQUALENE	111-02-4	C30H50	410.391	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Prenol lipids
7	A	3	4-COUMARATE	501-98-4	C9H8O3	164.047	50 % ACN/IPA	50-750	1.79	Phenylpropanoids and polyketides	Cinnamic acids and derivatives
7	A	4	NONANOATE	112-05-0	C9H18O2	158.131	50 % ACN/IPA	50-750	3.42	Lipids and lipid-like molecules	Fatty Acyls

7	A	5	ESTRADIOL-17ALPHA	57-91-0	C18H24O2	272.178	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Steroids and steroid derivatives
7	A	6	CAPRYLATE	124-07-2	C8H16O2	144.115	50 % ACN/IPA	50-750	3.05	Lipids and lipid-like molecules	Fatty Acyls
7	A	7	URSODEOXY CHOLATE	128-13-2	C24H40O4	392.293	50 % ACN/IPA	50-750	3	Lipids and lipid-like molecules	Steroids and steroid derivatives
7	A	8	PETROSELINATE	593-39-5	C18H34O2	282.256	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Fatty Acyls
7	A	9	DIPALMITOYL GLYCEROL	30334-71-5	C35H68O5	568.507	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Glycerolipids
7	A	10	CORTICOSTERONE	50-22-6	C21H30O4	346.214	50 % ACN/IPA	50-750	1.94	Lipids and lipid-like molecules	Steroids and steroid derivatives
7	A	11	LITHOCHOLATE	434-13-9	C24H40O3	376.298	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Steroids and steroid derivatives
7	A	12	PROTOPORPHYRIN	553-12-8	C34H34N4O4	562.258	50 % ACN/IPA	50-750		Organoheterocyclic compounds	Tetrapyrroles and derivatives
7	B	1	HEPTANOATE	111-14-8	C7H14O2	130.099	50 % ACN/IPA	70-1050	2.42	Lipids and lipid-like molecules	Fatty Acyls
7	B	2	RETINOL	68-26-8	C20H30O	286.230	50 % ACN/IPA	70-1050	5.68	Lipids and lipid-like molecules	Prenol lipids
7	B	3	MENAQUINONE	11032-49-8	C41H56O2	580.428	50 % ACN/IPA	70-1050		Lipids and lipid-like molecules	Prenol lipids
7	B	4	ELAIDATE	112-79-8	C18H34O2	282.256	50 % ACN/IPA	70-1050	6.78	Lipids and lipid-like molecules	Fatty Acyls
7	B	5	CHENO DEOXYCHOLATE	474-25-9	C24H40O4	392.293	50 % ACN/IPA	70-1050	4.15	Lipids and lipid-like molecules	Steroids and steroid derivatives
7	B	6	MYRISTATE	544-63-8	C14H28O2	228.209	50 % ACN/IPA	70-1050	6.11	Lipids and lipid-like molecules	Fatty Acyls
7	B	7	CHOLESTERYL OLEATE	303-43-5	C45H78O2	650.600	50 % ACN/IPA	70-1050		Lipids and lipid-like molecules	Steroids and steroid derivatives
7	B	8	ROSMARINATE	20283-92-5	C18H16O8	360.085	50 % ACN/IPA	70-1050		Lipids and lipid-like molecules	Cinnamic acids and derivatives
7	B	9	GLYCERYL TRIPALMITATE	555-44-2	C51H98O6	806.736	50 % ACN/IPA	70-1050		Lipids and lipid-like molecules	Glycerolipids
7	B	10	CORTEXOLONE	152-58-9	C21H30O4	346.214	50 % ACN/IPA	70-1050	3.08	Lipids and lipid-like molecules	Steroids and steroid derivatives
7	B	11	LITHOCHOLYL TAURINE	6042-32-6	C26H45NO5S	483.302	50 % ACN/IPA	70-1050		Organoheterocyclic compounds	Steroids and steroid derivatives
7	B	12	PALMITOLEATE	373-49-9	C16H30O2	254.225	50 % ACN/IPA	70-1050		Lipids and lipid-like molecules	Fatty Acyls
7	C	1	PALMITATE	57-10-3	C16H32O2	256.240	50 % ACN/IPA	50-750	7.17	Lipids and lipid-like molecules	Fatty Acyls
7	C	2	LIOTHYRONINE	345957-19-9	C15H12I3NO4	650.790	50 % ACN/IPA	50-750		Organic acids and derivatives	Carboxylic acids and derivatives
7	C	3	SPHINGANINE	764-22-7	C18H39NO2	301.298	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Organonitrogen compounds
7	C	5	LAURATE	143-07-7	C12H24O2	200.178	50 % ACN/IPA	50-750	4.6	Lipids and lipid-like molecules	Prenol lipids
7	C	6	LANOSTEROL	79-63-0	C30H50O	426.386	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Fatty Acyls
7	C	6	ARACHIDATE	506-30-9	C20H40O2	312.303	50 % ACN/IPA	50-750	9.29	Lipids and lipid-like molecules	Fatty Acyls
7	C	7	ERUCATE	112-86-7	C22H42O2	338.318	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Fatty Acyls
7	C	8	DEOXYCHOLATE	302-95-4	C24H40O4	392.293	50 % ACN/IPA	50-750	3.5	Lipids and lipid-like molecules	Steroids and steroid derivatives
7	C	9	KETOLEUCINE	NONE	C6H10O3	130.063	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Keto acids and derivatives
7	C	10	EICOSA PENTAENOATE	10417-94-4	C20H30O2	302.225	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Fatty Acyls
7	C	11	HEPTADECANOATE	506-12-7	C17H34O2	270.256	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Fatty Acyls
7	C	12	GLYCERYL TRIMYRISTATE	555-45-3	C45H86O6	722.642	50 % ACN/IPA	50-750	16.26	Lipids and lipid-like molecules	Glycerolipids
7	D	1	LINOLEATE	60-33-3	C18H32O2	280.240	50 % ACN/IPA	70-1050	7.05	Lipids and lipid-like molecules	Fatty Acyls

7	D	2	SPHINGOMYELIN	85187-10-6	C41H83N2O6P	731.607	50 % ACN/IPA	70-1050		Lipids and lipid-like molecules	Sphingolipids
7	D	3	7-DEHYDRO CHOLESTEROL	434-16-2	C27H44O	384.339	50 % ACN/IPA	70-1050		Organic oxygen compounds	Steroids and steroid derivatives
7	D	4	THYROXINE	51-48-9	C15H11I4NO4	776.687	50 % ACN/IPA	70-1050		Organic acids and derivatives	Carboxylic acids and derivatives
7	D	5	BIS(2-ETHYLHEXYL) PHTHALATE	117-81-7	C24H38O4	390.277	50 % ACN/IPA	70-1050		Lipids and lipid-like molecules	[NULL]
7	D	6	GAMMA-LINOLENATE	506-26-3	C18H30O2	278.225	50 % ACN/IPA	70-1050		Lipids and lipid-like molecules	Fatty Acyls
7	D	7	OMEGA-HYDROXYDODECANOATE	505-95-3	C12H24O3	216.173	50 % ACN/IPA	70-1050		Organic acids and derivatives	Hydroxy acids and derivatives
7	D	8	METHYL JASMONATE	39924-52-2	C13H20O3	224.141	50 % ACN/IPA	70-1050		Lipids and lipid-like molecules	Fatty Acyls
7	D	9	DIPALMITOYL-PHOSPHATIDYLCHOLINE	8002-43-5	C40H80NO8P	733.562	50 % ACN/IPA	70-1050	-3.5	Lipids and lipid-like molecules	Glycerophospholipids
7	D	10	HEXADECANOL	36653-82-4	C16H34O	242.261	50 % ACN/IPA	70-1050		Lipids and lipid-like molecules	Fatty Acyls
7	D	11	5,6 DIMETHYL BENZIMIDAZOLE	582-60-5	C9H10N2	146.084	50 % ACN/IPA	70-1050	1.854	Organoheterocyclic compounds	Benzimidazoles
7	D	12	RETINOATE	302-79-4	C20H28O2	300.209	50 % ACN/IPA	70-1050	6.3	Lipids and lipid-like molecules	Prenol lipids
7	E	1	INDOLE	120-72-9	C8H7N	117.058	50 % ACN/IPA	50-750	2.14	Lipids and lipid-like molecules	Indoles and derivatives
7	E	2	CHOLATE	81-25-4	C24H40O5	408.288	50 % ACN/IPA	50-750	2.02	Lipids and lipid-like molecules	Steroids and steroid derivatives
7	E	3	PHYLLOQUINONE	84-80-0	C31H46O2	450.350	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Prenol lipids
7	E	4	CHOLESTERYL PALMITATE	601-34-3	C43H76O2	624.585	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Steroids and steroid derivatives
7	E	5	QUINOLINE	91-22-5	C9H7N	129.058	50 % ACN/IPA	50-750	2.03	Organoheterocyclic compounds	Quinolines and derivatives
7	E	6	DOCOSA HEXAENOATE	6217-54-5	C22H32O2	328.240	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Fatty Acyls
7	E	7	DIETHYL 2-METHYL-3-OXOSUCCINATE	759-65-9	C9H14O5	202.084	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Keto acids and derivatives
7	E	8	RETINYL PALMITATE	79-81-2	C36H60O2	524.459	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Fatty Acyls
7	E	9	2-UNDECANONE	121-12-9	C11H22O	170.167	50 % ACN/IPA	50-750	4.09	Lipids and lipid-like molecules	Organooxygen compounds
7	E	10	1-HYDROXY-2-NAPHTHOATE	86-48-6	C11H8O3	188.047	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	[NULL]
7	E	11	DIPALMITOYL-PHOSPHOETHANOLAMINE	5681-36-7	C37H74NO8P	691.515	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Glycerophospholipids
7	E	12	PHENYLPYRUVATE	114-76-1	C9H8O3	164.047	50 % ACN/IPA	50-750		Benzenoids	Benzene and substituted derivatives
7	F	1	TRANS-CINNAMATE	140-10-3	C9H8O2	148.052	50 % ACN/IPA	50-750	2.13	Lipids and lipid-like molecules	Cinnamic acids and derivatives
7	F	2	OLEATE	112-80-1	C18H34O2	282.256	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Fatty Acyls
7	F	3	STEARATE	57-11-4	C18H36O2	284.272	50 % ACN/IPA	50-750	8.23	Lipids and lipid-like molecules	Fatty Acyls
7	F	4	BETA-CAROTENE	7235-40-7	C40H56	536.438	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Prenol lipids
7	F	5	25-HYDROXY CHOLESTEROL	2140-46-7	C27H46O2	402.350	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Steroids and steroid derivatives
7	F	6	NERVONATE	506-37-6	C24H46O2	366.350	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Fatty Acyls
7	F	7	DESMOSTEROL	313-04-2	C27H44O	384.339	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Steroids and steroid derivatives
7	F	8	DEOXYCORTICOSTERONE ACETATE	56-47-3	C23H32O4	372.230	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	[NULL]
7	F	9	OLEOYL-GLYCEROL	111-03-5	C21H40O4	356.293	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Glycerolipids
7	F	10	ALPHA-TOCOPHEROL	59-02-9	C29H50O2	430.381	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Prenol lipids

7	F	11	GLYCEROL-MYRISTATE	589-68-4	C17H34O4	302.246	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	[NULL]
7	F	12	TRICOSANOATE	2433-96-7	C23H46O2	354.350	50 % ACN/IPA	50-750		Lipids and lipid-like molecules	Fatty Acyls
7	G	1	COENZYME Q10	303-98-0	C59H90O4	862.684	50 % ACN/IPA	70-1050		Lipids and lipid-like molecules	Prenol lipids
7	G	2	CORTISONE	53-06-5	C21H28O5	360.194	50 % ACN/IPA	70-1050	1.47	Lipids and lipid-like molecules	Steroids and steroid derivatives
7	G	3	DECANOATE	334-48-5	C10H20O2	172.146	50 % ACN/IPA	70-1050	4.09	Lipids and lipid-like molecules	Fatty Acyls

6.2 Experimental design

Table 6.2: *Metabolites from plate 6 rows F and G mixed and analyzed*

Mixture ID	*Metabolite standard*
6F_1	6F1, 6F2
6F_2	6F3, 6F4
6F_3	6F5, 6F6
6F_4	6F10, 6F11, 6F12
6G_1	6G1, 6G2
6G_2	6G3, 6G4, 6G5, 6G6
6G_3	6G7, 6G8, 6G9
6G_4	6G10, 6G11, 6G12

Table 6.3: *Total number of metabolites in plates 1-5 with a registered water solubility at HMDB or Sigma Aldrich and the number of those with a water solubility above 5 µg/100 µL*

	Registered at HMDB/ Sigma Aldrich	**> 5 µg /100 µL**
Water solubility	361	302

6.3 Computer software's outlook

6.3.1 MLSDiscovery

The "multiplex designer" tab:

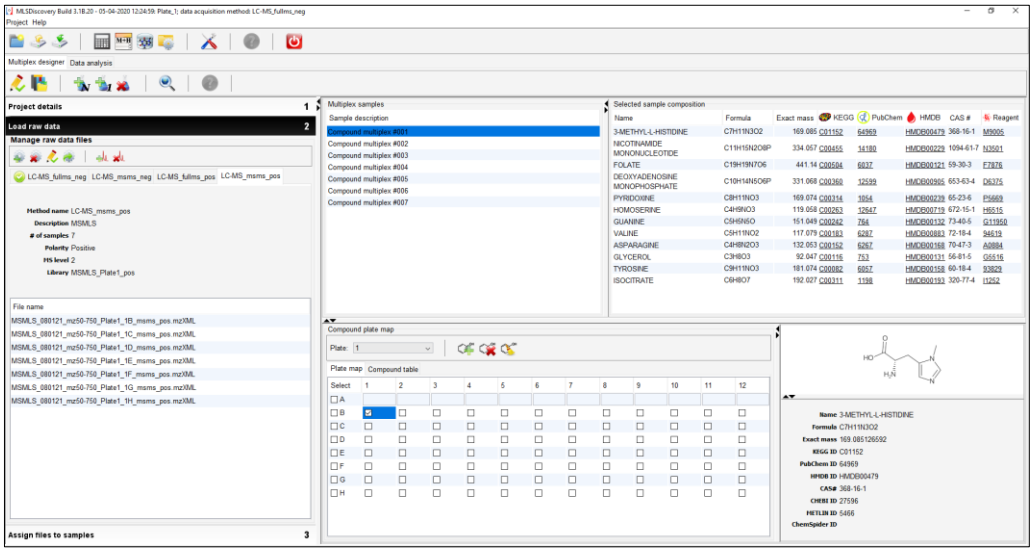

***Figure 6.1**: The "multiplex designer" tab on MLSDiscovery, where the method for data processing metabolite mixtures (multiplexed samples) is created before a data analysis is performed.*

The "data analysis" tab:

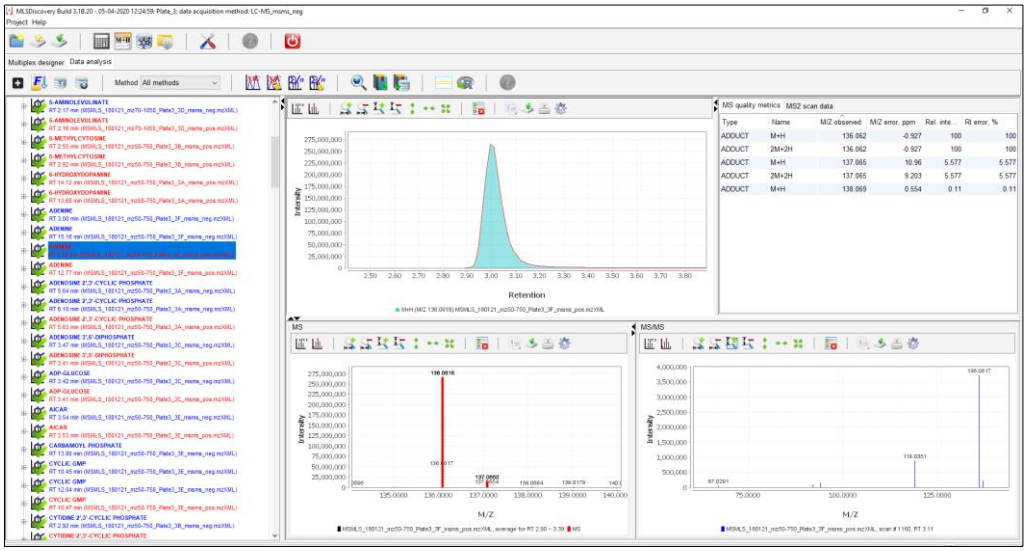

Figure 6.2: The "data analysis" tab on MLSDiscovery, where results from the extracting data mixtures raw files are shown, such as the peaks, MS^1 with a table showing possible molecular ions, and MS^2 for the molecular ion with highest relative intensity is shown

The built-in library database editor:

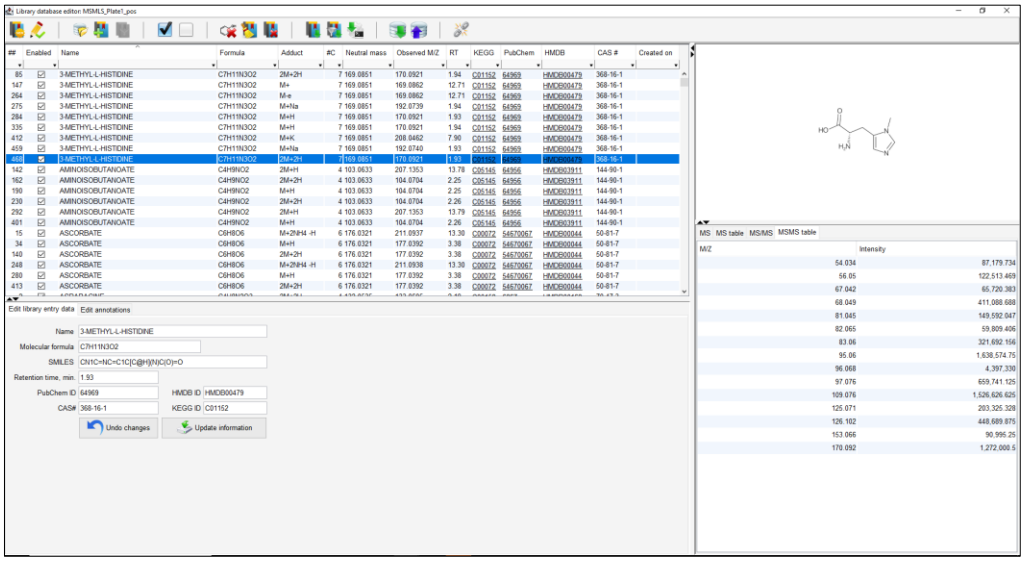

***Figure 6.3**: The library database editor, where extracted data may be imported prior to exporting them to a file for further transferring to other computer software*

6.3.2 Compound Discoverer, the untargeted metabolomics data processing workflow template, including established in-house library

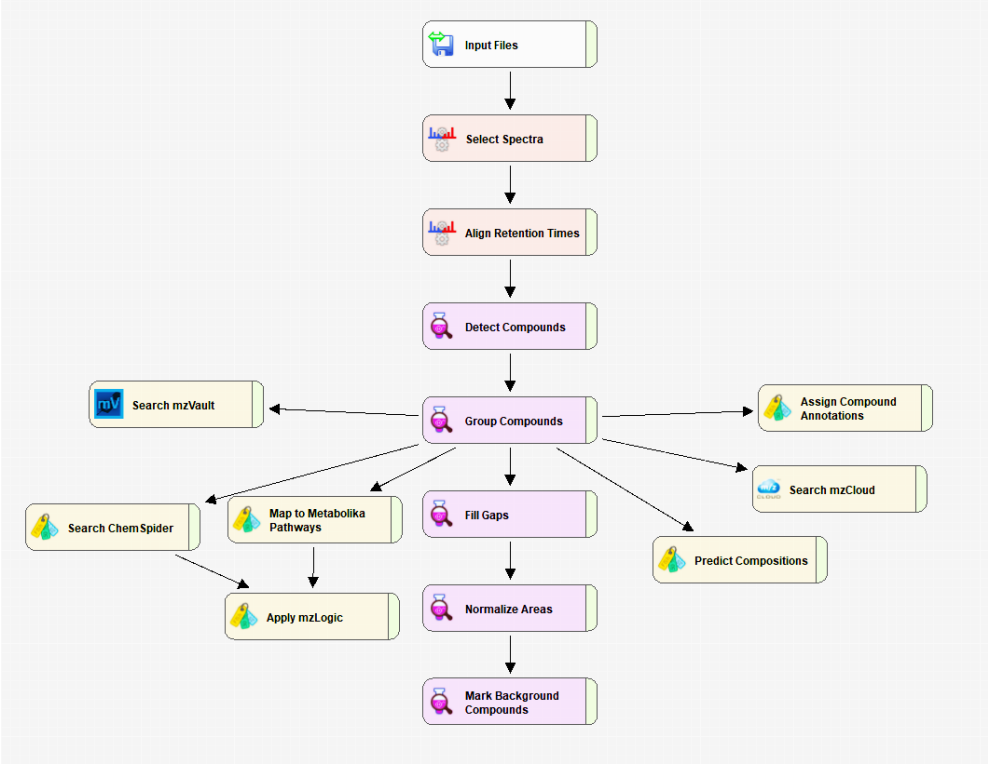

***Figure 6.4:** The untargeted metabolomics workflow with the in-house library (stored in mzVault) implemented*

mzVault parameters:

Table 6.4 shows the mzVault search settings applied in Compound Discoverer. Most of these settings were set to false. This is crucial to get a match from the mzVault files containing imported data from MLSDiscovery, due to these only containing m/z values, RT, and MS^2. Even though these were set to false, no matches from these mzVault files were obtained, which may be due to the missing integrated polarity in these files. The polarity may be added to the NIST MSP files exported from MLSDiscovery, before importing the file to mzVault, ensuring matches with the mzVault file when performing untargeted metabolomics at Compound Discoverer in future projects.

Table 6.4: *The mzVault search settings applied on Compound Discoverer*

Parameters	Search Settings
Compound classes	All
Match Ion Activation Type	False
Match Ion Activation Energy	Match with Tolerance
Ion Activation Energy Tolerance	20
Match Ionization Method	False
Apply Intensity Threshold	False
Precursor Mass Tolerance	10 ppm
Match Analyzer Type	False
Search Algorithm	HighChem High Res
Match Factor Threshold	50
RT Tolerance [min]	2
Use Retention Time	True

6.4 The molecular ions extracted for metabolites in plate 1-5 with MLSDiscovery

Table 6.5: *The molecular ions extracted for metabolites in plate 1-5, as well as how many times they different types molecular ions where extracted.*

Molecular ion	Extracted	Molecular ion	Extracted
M+H	279	M-3H+2Na	9
2M+2H	273	2M-2H+3Na	9
M-H	272	M-e	7
2M-2H	256	3M+H	7
M-H+HCOONa	177	M+	7
M+Na	152	M+NH4+H	7
M+e	149	M+2Na	6
2M-2H+Na	104	2M+CH3COO	6
2M-H	85	3M-3H+2Na	5
M-H + HCOOH	67	2M+3Na -4H	5
M+COOH	66	2M+4Na -3H	4
2M+Na	55	2M-H +2H2O	4
2M+H	52	2M+HCOO-H2O	4
M-2H+Na	50	3M-H	4
M-H -H2O	50	M-3H+Na	4
M+K	47	2M+COOH-H2O	4
2M+K	36	M+Na+H	4
M+Cl	36	3M+2Na -H	4
M+2Na -H	34	2M+NH4	3
2M+2Na -H	21	M-4H+3Na	3
2M-H -H2	19	M+3Na -2H	3
M+2H	18	M+Cl -H2O	2
M+CF3COO	18	M-3H+2K	2
M+COOH -H2O	17	M-2H+NH4	2
M+K+H	16	2M+Cl -H2O	2
M+CH3COO	16	M-2H	2
M+2NH4 -H	15	M-4H+2Na	2
M-2H+K	14	M-H+2K	2
2M-2H+K	13	M+2H+Na	2
2M+HCOO	12	M-H+OH	2
M+NH4	12	M+3H	2
M+H -CH2	11	M+H+2Na	2
M-H+H2O	10	M+3Na	1
M+OH	10	2M+Cl	1

6.5 Metabolites analyzed with their m/z on an inclusion list - results

Positive ionization

Table 6.6: The metabolites analyzed in positive ionization, with their molecular ion m/z value on an inclusion list. *The table consists of metabolites' plate, row and column number, their primary name, molecular formula, neutral monoisotopic molecular mass, observed ion mass, theoretical ion mass, the calculated mass resolution in ppm, RT observed from the first LC-MS analysis and if the peak observed in the first analysis and the MS2 spectra for the metabolite has been observed when doing an analysis including inclusion list.*

PLATE	NROW	NCOL	PRIMARY_NAME	MOLECULAR FORMULA	NEUTRAL MONOISOTOPIC MOLECULAR MASS	OBSERVED ION MASS	THEORETICAL ION MASS	M/Z ERROR [PPM]	RT OBSERVED	PEAK, MSMS
1	C	11	PHENYLALANINE	C9H11NO2	165,08	166,09	166,09	-1,56E+00	7,51	Not detected old peak or MSMS
1	F	11	ASCORBATE	C6H8O6	176,03	177,04	177,04	-9,49E-01	3,38	Detected old peak, and with MSMS
1	G	10	CYSTEINE	C3H7NO2S	121,02	122,03	122,03	-2,70E+00	2,13	Not detected old peak or MSMS
2	D	9	MELANIN	C18H10N2O4	318,06	319,07	319,07	-7,42E-01	5,49	Detected old peak, and with MSMS
2	G	1	NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE	C21H27N6O18P3	744,06	745,07	745,07	-1,07E-01	4,04	Noise
2	H	6	NICOTINAMIDE HYPOXANTHINE DINUCLEOTIDE	C21H26N6O15P2	664,09	665,1	665,1	-9,32E-01	6,29	Detected old peak, and with MSMS
3	C	7	FRUCTOSE 6-PHOSPHATE	C6H13O9P	260,03	283,02	283,02	-2,19E+00	2,66	Not detected old peak or MSMS
3	F	4	GUANOSINE DIPHOSPHATE	C10H15N5O11P2	443,02	444,03	444,03	-6,97E-01	3,59	Detected old peak, and with MSMS
4	B	10	RAFFINOSE	C18H32O16	504,17	527,16	527,16	-9,58E-01	2,5	Detected old peak, and with MSMS
4	C	4	LACTOSE	C12H22O11	342,12	343,12	343,12	-8,50E-01	2,45	Noise
4	E	6	ADENOSINE DIPHOSPHATE RIBOSE	C15H23N5O14P2	559,07	560,08	560,08	-9.88E-01	3,46	Detected old peak, and with MSMS
4	G	7	DEOXYADENOSINE TRIPHOSPHATE	C10H16N5O12P3	491	492,01	492,01	-2,14E-02	5,6	Noise
5	E	10	10-HYDROXYDECANOATE	C10H20O3	188,14	189,15	189,15	-6,61E-01	14,27	Noise
5	G	9	BENZYLAMINE	C7H9N	107,07	108,08	108,08	-7,34E-01	4,67	Not detected old peak or MSMS

Negative ionization

Table 6.7: The table shows the metabolites analyzed in negative ionization, with their molecular ion m/z value on an inclusion list. *The table consists of metabolites' plate, row and column number, their primary name, molecular formula, neutral monoisotopic molecular mass, observed ion mass, theoretical ion mass, the calculated mass resolution in ppm, RT observed from the first LC-MS analysis and if the peak observed in the first analysis and the MS2 spectra for the metabolite has been observed when doing an analysis including inclusion list.*

PLATE	NROW	NCOL	PRIMARY_NAME	MOLECULAR FORMULA	NEUTRAL MONOISOTOPIC MOLECULAR MASS	OBSERVED ION MASS	THEORETICAL ION MASS	M/Z ERROR [PPM]	RT OBSERVED	PEAK, MSMS
1	G	1	PYRAZOLE	C3H4N2	68,0	67,0	67,0	-1,01E+00	2,59	Not detected old peak or MSMS
2	F	12	ALPHA-HYDROXYISOBUTYRATE	C4H8O3	104,0	103,0	103,0	1,32E+00	2,73	Noise
2	G	12	DGTP	C10H16N5O13P3	507,0	506,0	506,0	2,66E-01	6,06	Noise
4	C	2	ADENOSINE TRIPHOSPHATE	C10H16N5O13P3	507,0	506,0	506,0	-7,22E-01	3,93	Noise
4	C	8	CYTIDINE TRIPHOSPHATE	C9H16N3O14P3	483,0	482,0	482,0	-6,59E-02	3,60	Noise
4	F	8	COENZYME A	C21H36N7O16P3S	767,1	766,1	766,1	4,79E+00	11,57	Noise
4	F	10	INOSINE TRIPHOSPHATE	C10H15N4O14P3	508,0	507,0	507,0	2,35E-01	4,57	Noise
5	H	1	BUTANOATE	C4H8O2	88,1	87,0	87,0	1,73E+00	9,30	Not detected old peak or MSMS
5	H	5	DEHYDROASCORBATE	C6H6O6	174,0	173,0	173,0	1,98E+00	2,70	Not detected old peak or MSMS
6	B	12	GERANYL-PP	C10H20O7P2	314,1	313,1	313,1	3,69E+00	6,28	Not detected

6.6 Metabolites re-analyzed with a concentration above 10 µM

Table 6.8: The metabolites from the MS-MLS kit having a concentration below 10 µM, when analyzed in mixtures created with the initially procedure for the MS-MLS sample preparation. *Their location in the kit, primary name, concentration in mixture and in pure stock solution, as well as if they are detected or not with a concentration below or above 10 µM are shown. The table continues to page 118.*

PLATE	NROW	NCOL	PRIMARY_NAME	C (In mixture, row) [µmol/L]	C(stock solution) [µmol/L]	ANALYZED IN MIXTURE C < 10 µM	ANALYZED WITH A C > 10 µm
1	B	3	FOLATE	9,45	113	Detected	
1	E	2	DIHYDROFOLATE	9,40	113	Not detected	Not detected
1	E	10	TETRAHYDROFOLATE	9,36	112	Not detected	Not detected
1	H	12	BETA-NICOTINAMIDE ADENINE DINUCLEOTIDE	6,28	75	Detected	
2	C	2	ADENOSINE 5'-DIPHOSPHATE	9,76	117	Not detected	Detected
2	C	11	3-HYDROXY-3-METHYLGLUTARYL-COA	4,57	55	Not detected	Not detected
2	E	1	CITICOLINE	8,54	102	Detected	
2	F	7	3-METHYLCROTONYL-COA	4,91	59	Not detected	Not detected
2	G	1	NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE	6,26	75	Unsure if detected	Analyzed with inclusion list. Not detected
2	G	5	NADP	5,60	67	Detected	
2	G	8	GUANOSINE TRIPHOSPHATE	7,97	96	Not detected	Not detected
2	G	9	DTDP-D-GLUCOSE	7,39	89	Detected	
2	G	12	DGTP	8,22	99	Not detected	Not detected
2	H	3	INOSINE 5'-DIPHOSPHATE	9,73	117	Not detected	Detected
2	H	6	NICOTINAMIDE HYPOXANTHINE DINUCLEOTIDE	6,27	75	Detected	
2	H	10	URIDINE TRIPHOSPHATE	8,61	103	Not detected	Not detected
3	A	7	DEOXYURIDINE TRIPHOSPHATE	8,90	107	Not detected	Not detected
3	A	12	DGDP	9,76	117	Unsure if detected	Detected
3	B	6	URIDINE DIPHOSPHATE GLUCOSE	7,36	88	Detected	
3	C	5	GUANOSINE DIPHOSPHATE MANNOSE	6,89	83	Unsure if detected	Detected
3	C	6	ADP-GLUCOSE	7,07	85	Detected	
3	C	8	ADENOSINE 3',5'-DIPHOSPHATE	9,76	117	Detected	
3	C	12	URIDINE DIPHOSPHATE GALACTOSE	7,36	88	Detected	
3	D	8	FAD	5,31	64	Not detected	Not detected
3	E	6	URIDINE DIPHOSPHATE-N-ACETYLGALACTOSAMINE	6,86	82	Detected	
3	F	3	URIDINE DIPHOSPHATE-N-ACETYLGLUCOSAMINE	6,86	82	Detected	
3	F	4	GUANOSINE DIPHOSPHATE	9,41	113	Unsure if detected	Detected. MS2 detected with inclusion list
3	F	6	URIDINE DIPHOSPHATE GLUCURONIC ACID	7,18	86	Unsure if detected	Not detected
3	G	2	PHYTATE	6,31	76	Not detected	Not detected
3	G	12	CYTOCHROME C	4,71	57	Not detected	Not detected
4	B	2	THIAMINE PYROPHOSPHATE	9,80	118	Detected	
4	B	10	RAFFINOSE	8,26	99	Unsure if detected	Detected. MS2 detected with inclusion list

4	C	2	ADENOSINE TRIPHOSPHATE	8,22	99	Not detected	Not detected
4	C	8	CYTIDINE TRIPHOSPHATE	8,63	104	Not detected	Not detected
4	D	2	NADPH	5,59	67	Not detected	Not detected
4	E	6	ADENOSINE DIPHOSPHATE RIBOSE	7,45	89	Detected. MS2 detected with inclusion	
4	E	10	BILIVERDIN	7,16	86	Not detected	Not detected
4	F	8	COENZYME A	5,43	65	Not detected	Not detected
4	F	10	INOSINE TRIPHOSPHATE	8,20	98	Not detected	Not detected
4	F	11	CDP-ETHANOLAMINE	9,34	112	Detected	
4	G	1	STACHYOSE	6,25	75	Detected	
4	G	7	DEOXYADENOSINE TRIPHOSPHATE	8,49	102	Not detected	Not detected
4	G	9	CYANOCOBALAMIN	3,07	37	Not detected	Detected
5	B	6	BILIRUBIN	7,13	86	Not detected	Not detected
5	C	2	3,5-DIIODO-L-TYROSINE	9,63	116	Detected	
5	D	9	GLYCOCHOLATE	8,95	107	Detected	
5	E	1	GLYCOCHENODEOXYCHOLATE	9,27	111	Detected	
5	E	11	DIDECANOYL-GLYCEROPHOSPHOCHOLINE	7,37	88	Not detected	Not detected
5	G	3	3,5-DIIODO-L-THYRONINE	7,94	95	Detected	
7	A	9	DIPALMITOYLGLYCEROL	9,16	110	Not detected	Not detected
7	A	12	PROTOPORPHYRIN	9,26	111	Not detected	Not detected
7	B	3	MENAQUINONE	8,97	108	Not detected	Not detected
7	B	7	CHOLESTERYL OLEATE	8,01	96	Not detected	Not detected
7	B	9	GLYCERYL TRIPALMITATE	6,46	77	Not detected	Not detected
7	C	2	LIOTHYRONINE	8,00	96	Detected	
7	C	12	GLYCERYL TRIMYRISTATE	7,21	86	Not detected	Not detected
7	D	2	SPHINGOMYELIN	7,12	85	Not detected	Not detected
7	D	4	THYROXINE	6,71	80	Detected	
7	D	9	DIPALMITOYL-PHOSPHATIDYLCHOLINE	7,10	85	Not detected	Not detected
7	E	4	CHOLESTERYL PALMITATE	8,34	100	Not detected	Not detected
7	E	8	RETINYL PALMITATE	9,93	119	Not detected	Not detected
7	E	11	DIPALMITOYL-PHOSPHOETHANOLAMINE	7,53	90	Not detected	Not detected
7	F	4	BETA-CAROTENE	9,71	117	Not detected	Not detected

6.7 Maps showing the data processing of metabolites in plates 6 and 7 to obtaining their MS2 and RT

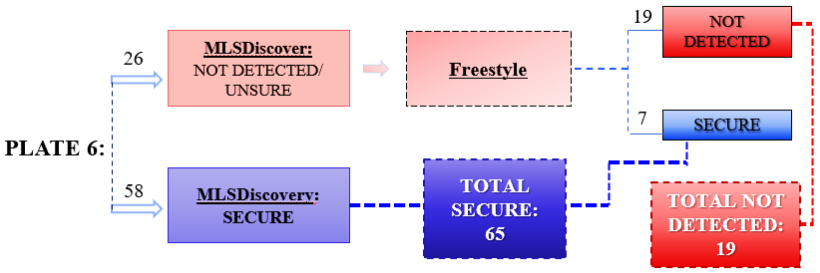

***Figure 6.5**: A map for the data preprocessing of metabolites in plate 6*

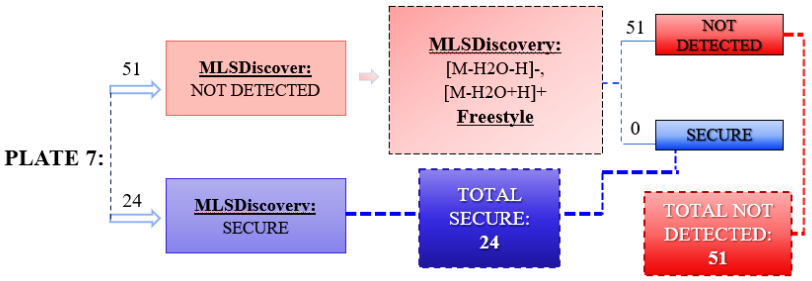

***Figure 6.6:** A map for the data preprocessing of metabolites in plate 7*

6.8 Overview of the classes of metabolites in MS-MLS, within each superclass

Table 6.9: *The classes withing each superclass which the MS-MLS kit contains.*

1. Benzenoids:
Benzene and substituted derivatives
Phenols
Anthracenes
2. Lipids and lipid-like molecules:
Fatty Acyls
Steroids and steroid derivatives
Prenol lipids
Glycerolipids
Glycerophospholipids
Sphingolipids
3. Nucleosides, nucleotides, and analogs:
Purine nucleotides
Pyrimidine nucleotides
Purine nucleosides
Pyrimidine nucleosides
(5'->5')-dinucleotides
5'-deoxyribonucleosides
Flavin nucleotides
Imidazole ribonucleosides and ribonucleotides
Pyridine nucleotides
4. Organic acids and derivatives:
Carboxylic acids and derivatives
Hydroxy acids and derivatives
Organic phosphoric acids and derivatives
Carboximidic acids and derivatives
Organic phosphonic acids and derivatives
Peptidomimetics
Organic carbonic acids and derivatives
Organic sulfonic acids and derivatives
Organic sulfuric acids and derivatives
Sulfinic acids and derivatives
Keto acids and derivatives

5. Organic nitrogen compounds:
Organonitrogen compounds
6. Organic oxygen compounds:
Organooxygen compounds
Organic oxoanionic compounds
7. Organoheterocyclic compounds:
Indoles and derivatives
Imidazopyrimidines
Pyridines and derivatives
Diazines
Lactones
Quinolines and derivatives
Tetrapyrroles and derivatives
Azoles
Pteridines and derivatives
Benzimidazoles
Biotin and derivatives
Dihydrofurans
Dithiolanes
Furofurans
Pyrroles
Tetrahydroisoquinolines
8. Organosulfur compounds
Thiols
Thioureas
9. Phenylpropanoids and polyketides:
Cinnamic acids and derivatives
Phenylpropanoid acids
Cinnamaldehydes

Plate 1-5:

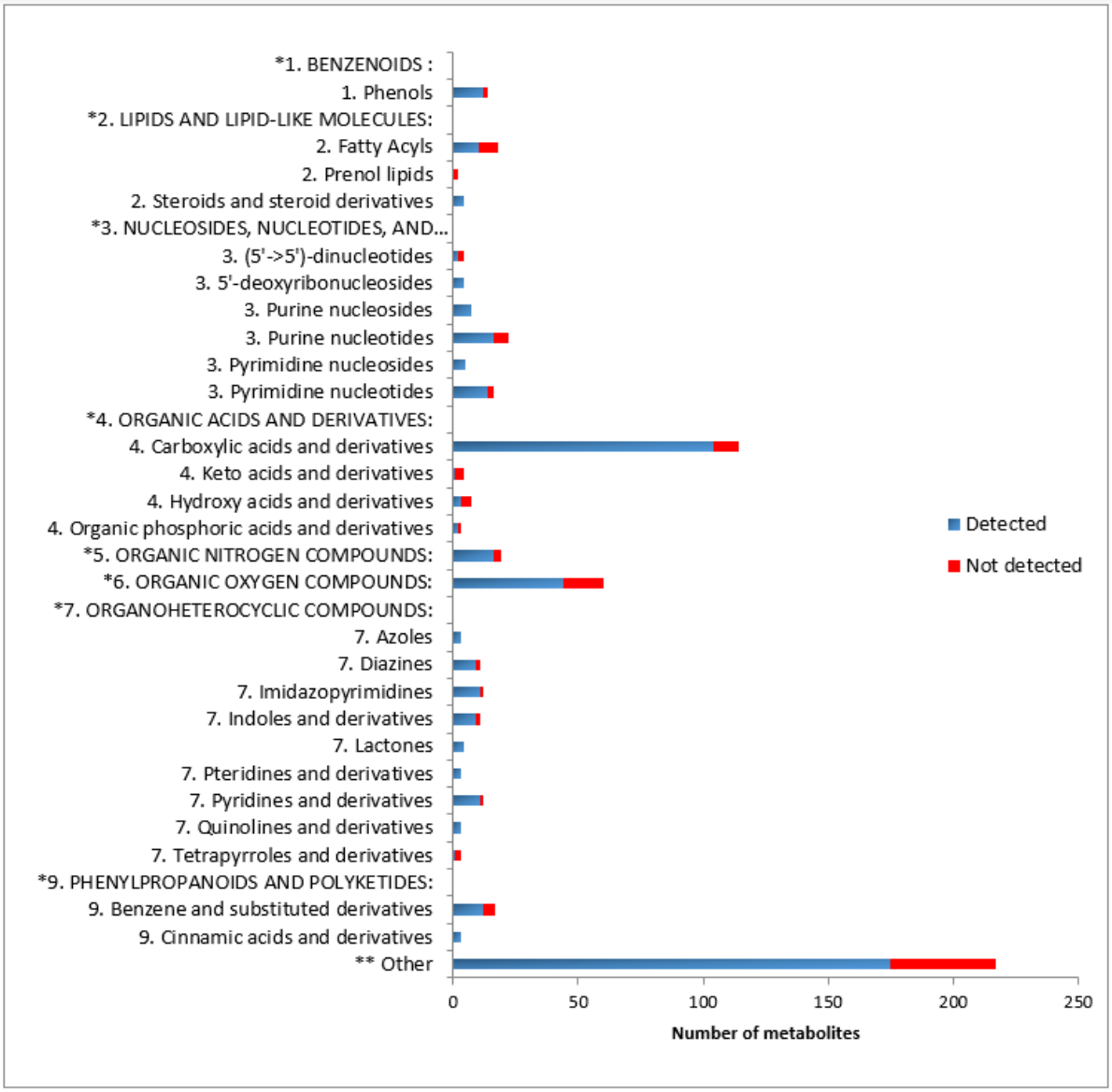

Figure 6.7: Metabolites from plates 1.5, categorized into their classes, showing the number of detected (blue) and not detected (red). *Those with a * are the superclasses, and those without * are the classes. The classes are extracted from HMDB. ** Other = Metabolites with no registered superclass on HMDB, and classes with a total of < 2 metabolites in plates 1-5, see table 6.10.*

Table 6.10: *The classes specified as others in figure 6.7.*

CLASSES	DETECTED	NOT DETECTED
1. Anthracenes	1	0
2. Glycerophospholipids	2	0
3. Flavin nucleotides	0	1
3. Imidazole ribonucleosides and ribonucleotides	1	0
3. Pyridine nucleotides	1	0
4. Carboximidic acids and derivatives	1	1
4. Organic carbonic acids and derivatives	1	0
4. Organic phosphonic acids and derivatives	2	0
4. Organic sulfonic acids and derivatives	1	0
4. Organic sulfuric acids and derivatives	1	0
4. Peptidomimetics	2	0
4. Sulfinic acids and derivatives	1	0
7. Dihydrofurans	1	0
7. Dithiolanes	1	0
7. Furofurans	1	0
8. Thiols	0	1
8. Thioureas	1	0
9 Phenylpropanoic acids	1	0
**Not specified*	24	13

6.9 Fatty acyls in plate 1-7

Table 6.11: Metabolites categorized as fatty acyls in HMDB. The plate number, their primary name, molecular formula, monoisotopic mass, solvent used, and experimental log P are included in the table. The table continues to page 124.

PLATE	PRIMARY NAME	MOLECULAR FORMULA	SOLVENT	EXPERIMENTAL LOG P (HMDB)	PREDICTED PKA VALUES (HMDB)
7	ARACHIDATE	C20H40O2	50 % ACN/IPA	9.29	4.95 (Strongest Acidic)
7	STEARATE	C18H36O2	50 % ACN/IPA	8.23	4.95 (Strongest Acidic)
7	PALMITATE	C16H32O2	50 % ACN/IPA	7.17	4.95 (Strongest Acidic)
7	LINOLEATE	C18H32O2	50 % ACN/IPA	7.05	4.99 (Strongest Acidic)
7	ELAIDATE	C18H34O2	50 % ACN/IPA	6.78	4.99 (Strongest Acidic)
7	MYRISTATE	C14H28O2	50 % ACN/IPA	6.11	4.95 (Strongest Acidic)
7	DECANOATE	C10H20O2	50 % ACN/IPA	4.09	4.95 (Strongest Acidic)
7	NONANOATE	C9H18O2	50 % ACN/IPA	3.42	5.23 (Strongest Acidic)
7	CAPRYLATE	C8H16O2	50 % ACN/IPA	3.05	5.19 (Strongest Acidic)
7	HEPTANOATE	C7H14O2	50 % ACN/IPA	2.42	5.15 (Strongest Acidic)
5	HEXANOATE	C6H12O2	H2O	1.92	5.09 (Strongest Acidic)
6	AZELATE	C9H16O4	40 % MeOH	1.57	4.14 (Strongest Acidic)
5	PENTANOATE	C5H10O2	H2O	1.39	5.01 (Strongest Acidic)
5	SORBATE	C6H8O2	H2O	1.33	5.01 (Strongest Acidic)
5	BUTANOATE	C4H8O2	H2O	0.79	4.91 (Strongest Acidic)
5	6-CARBOXYHEXANOATE	C7H12O4	H2O	0.61	4.05 (Strongest Acidic)
6	ADIPATE	C6H10O4	40 % MeOH	0.08	3.92 (Strongest Acidic)
7	PETROSELINATE	C18H34O2	50 % ACN/IPA	Not registered	4.89 (Strongest Acidic)
7	PALMITOLEATE	C16H30O2	50 % ACN/IPA	Not registered	4.99 (Strongest Acidic)
7	LANOSTEROL	C30H50O	50 % ACN/IPA	Not registered	19.55 (Strongest Acidic), 0.81 (Strongest Basic)
7	ERUCATE	C22H42O2	50 % ACN/IPA	Not registered	4.95 (Strongest Acidic)
7	EICOSAPENTAENOATE	C20H30O2	50 % ACN/IPA	Not registered	4.82 (Strongest Acidic)
7	HEPTADECANOATE	C17H34O2	50 % ACN/IPA	Not registered	4.95 (Strongest Acidic)
7	GAMMA-LINOLENATE	C18H30O2	50 % ACN/IPA	Not registered	4.92 (Strongest Acidic)
7	METHYL JASMONATE	C13H20O3	50 % ACN/IPA	Not registered	Not registered
7	HEXADECANOL	C16H34O	50 % ACN/IPA	Not registered	16.84 (Strongest Acidic) -2 (Strongest Basic)
7	DOCOSAHEXAENOATE	C22H32O2	50 % ACN/IPA	Not registered	4.89 (Strongest Acidic)
7	RETINYL PALMITATE	C36H60O2	50 % ACN/IPA	Not registered	-7 (Strongest Basic)
7	OLEATE	C18H34O2	50 % ACN/IPA	Not registered	4.99 (Strongest Acidic)
7	NERVONATE	C24H46O2	50 % ACN/IPA	Not registered	4.95 (Strongest Acidic)
7	TRICOSANOATE	C23H46O2	50 % ACN/IPA	Not registered	4.95 (Strongest Acidic)
6	MONOMETHYLGLUTARATE	C6H10O4	40 % MeOH	Not registered	4.22 (Strongest Acidic) -7 (Strongest Basic)
6	DETHIOBIOTIN	C10H18N2O3	40 % MeOH	Not registered	4.63 (Strongest Acidic) -1.8 (Strongest Basic)
6	ITACONATE	C5H6O4	40 % MeOH	Not registered	3.65 (Strongest Acidic)

6	2-METHYLGLUTARATE	C6H10O4	40 % MeOH	Not registered	Not registered
6	SUBERATE	C8H14O4	40 % MeOH	0.687	4.15 (Strongest Acidic)
6	MEVALONATE	C6H12O4	40 % MeOH	Not registered	Not registered
6	2-METHYLMALEATE	C5H6O4	40 % MeOH	Not registered	2.5 (Strongest Acidic)
6	SEBACATE	C10H18O4	40 % MeOH	Not registered	4.72 (Strongest Acidic)
6	3-METHYLGLUTACONATE	C6H8O4	40 % MeOH	Not registered	3.85 (Strongest Acidic)
5	METHYGLUTARATE	C6H10O4	5 % MeOH	Not registered	3.91 (Strongest Acidic)
5	2-HYDROXY-4-(METHYLTHIO)BUTANOATE	C5H10O3S	5 % MeOH	Not registered	4.03 (Strongest Acidic) -3.8 (Strongest Basic)
5	ETHYLMALONATE	C5H8O4	H2O	Not registered	2.5 (Strongest Acidic)
4	CITRAMALATE	C5H8O5	5 % MeOH	Not registered	3.35 (Strongest Acidic) -4 (Strongest Basic)
3	DEOXYCARNITINE	C7H15NO2	5 % MeOH	Not registered	4.46 (Strongest Acidic)
3	O-ACETYLCARNITINE	C9H17NO4	5 % MeOH	Not registered	4.09 (Strongest Acidic) -7 (Strongest Basic)
3	LAUROYLCARNITINE	C19H37NO4	5 % MeOH	Not registered	4.22 (Strongest Acidic) -7.1 (Strongest Basic)
2	GLUTARYLCARNITINE	C12H21NO6	5 % MeOH	Not registered	3.68 (Strongest Acidic) -3 (Strongest Basic)
2	3-HYDROXYMETHYLGLUTARATE	C6H10O5	5 % MeOH	Not registered	3.33 (Strongest Acidic) -7.1 (Strongest Basic)
2	PALMITOYLCARNITINE	C23H45NO4	5 % MeOH	Not registered	4.22 (Strongest Acidic) -7.1 (Strongest Basic)
2	3-HYDROXY-3-METHYLGLUTARYL-COA	C27H44N7O20P3S	5 % MeOH	Not registered	Not registered
2	3-METHYLCROTONYL-COA	C26H42N7O17P3S	5 % MeOH	Not registered	Not registered
1	SUCCINATE SEMIALDEHYDE	C4H6O3	5 % MeOH	Not registered	4.13 (Strongest Acidic) -7 (Strongest Basic)

6.10 Metabolites in MS-MLS containing aldehyde(s)

Table 6.12: All metabolites in plate 1-7 with an aldehyde group, showing their primary name, molecular formula, monoisotopic mass, structure, if they are detected in this study (diphenyl), or the other MSMLS study (C18 and HILIC).

PRIMARY_NAME	MOLECULAR FORMULA	MONOISOTOPIC MASS	DIPHENYL (+/-)	C18 (+/-)	HILIC (+/-)
INDOLEACETALDEHYDE	C10H9NO	159,068	DETECTED (+) and (-)	NOT IN KIT	NOT IN KIT
4-HYDROXYBENZALDEHYDE	C7H6O2	122,037	NOT DETECTED	DETECTED (-)	DETECTED (+) and (-)
BENZALDEHYDE	C7H6O	106,042	NOT DETECTED	DETECTED (-)	NOT DETECTED
PHENYLACETALDEHYDE	C8H8O	120,058	NOT DETECTED	NOT DETECTED	NOT DETECTED
TRANS-CINNAMALDEHYDE	C9H8O	132,058	NOT DETECTED	NOT DETECTED	NOT DETECTED
SUCCINATE SEMIALDEHYDE	C4H6O3	102,032	NOT DETECTED	NOT DETECTED	NOT DETECTED
GLYCERALDEHYDE	C3H6O3	90,032	NOT DETECTED	NOT DETECTED	NOT DETECTED
GLYCOLALDEHYDE	C2H4O2	120,042	NOT DETECTED	NOT IN KIT	NOT IN KIT
GLYCERALDEHYDE 3-PHOSPHATE	C3H7O6P	169,998	NOT DETECTED	NOT IN KIT	NOT IN KIT
PYRUVIC ALDEHYDE	C3H4O2	72,021	NOT DETECTED	NOT DETECTED	NOT DETECTED
3-HYDROXYBENZALDEHYDE	C7H6O2	122,037	NOT DETECTED	NOT DETECTED	DETECTED (-)

6.11 Overview subclasses with low or high possibility to be detected with the LC-HR-MS method used

Table 6.13: Subclasses with a low or high possibility of being detected with the initial LC-HR-MS method used for this thesis. The table continues to page 127.

Subclass	Total	% detected	Class	Superclass
Amino acids, peptides, and analogues	99	96 %	Carboxylic acids and derivatives	Organic acids and derivatives
Carbohydrates and carbohydrate conjugates	64	88 %	Organooxygen compounds	Organic oxygen compounds
Amines	15	80 %	Organonitrogen compounds	Organic nitrogen compounds
Purines and purine derivatives	13	92 %	Imidazopyrimidines	Organoheterocyclic compounds
Benzoic acids and derivatives	12	83 %	Benzene and substituted derivatives	Benzenoids
Purine ribonucleotides	12	67 %	Purine nucleotides	Nucleosides, nucleotides, and analogues
Carbonyl compounds	12	17 %	Organooxygen compounds	Organic oxygen compounds
Benzenediols	11	82 %	Phenols	Benzenoids
Pyrimidines and pyrimidine derivatives	10	90 %	Diazines	Organoheterocyclic compounds
Bile acids, alcohols, and derivatives	9	89 %	Steroids and steroid derivatives	Lipids and lipid-like molecules
Dicarboxylic acids and derivatives	9	78 %	Carboxylic acids and derivatives	Organic acids and derivatives
Pyrimidine ribonucleotides	7	86 %	Pyrimidine nucleotides	Nucleosides, nucleotides, and analogues
Pyridinecarboxylic acids and derivatives	7	86 %	Pyridines and derivatives	Organoheterocyclic compounds
Methoxyphenols	6	100 %	Phenols	Benzenoids
Pyrimidine nucleotide sugars	5	100 %	Pyrimidine nucleotides	Nucleosides, nucleotides, and analogues
Tryptamines and derivatives	5	100 %	Indoles and derivatives	Organoheterocyclic compounds
Hydroxycinnamic acids and derivatives	5	100 %	Cinnamic acids and derivatives	Phenylpropanoids and polyketides
Hydroxysteroids	4	100 %	Steroids and steroid derivatives	Lipids and lipid-like molecules
Tricarboxylic acids and derivatives	4	100 %	Carboxylic acids and derivatives	Organic acids and derivatives
Quaternary ammonium salts	4	100 %	Organonitrogen compounds	Organic nitrogen compounds
Pyrimidine deoxyribonucleotides	4	75 %	Pyrimidine nucleotides	Nucleosides, nucleotides, and analogues
Gamma butyrolactones	4	75 %	Lactones	Organoheterocyclic compounds
Quinone and hydroquinone lipids	4	25 %	Prenol lipids	Lipids and lipid-like molecules
Alpha hydroxy acids and derivatives	4	25 %	Hydroxy acids and derivatives	Organic acids and derivatives
5'-deoxy-5'-thionucleosides	3	100 %	5'-deoxyribonucleosides	Nucleosides, nucleotides, and analogues
Cyclic purine nucleotides	3	100 %	Purine nucleotides	Nucleosides, nucleotides, and analogues
Purine nucleotide sugars	3	100 %	Purine nucleotides	Nucleosides, nucleotides, and analogues
Pyrimidine 2'-deoxyribonucleosides	3	100 %	Pyrimidine nucleosides	Nucleosides, nucleotides, and analogues
Imidazoles	3	100 %	Azoles	Organoheterocyclic compounds
Quinoline carboxylic acids	3	100 %	Quinolines and derivatives	Organoheterocyclic compounds
Isoprenoid phosphates	3	0 %	Prenol lipids	Lipids and lipid-like molecules
Lineolic acids and derivatives	3	0 %	Fatty Acyls	Lipids and lipid-like molecules
Carboxylic acids	3	0 %	Carboxylic acids and derivatives	Organic acids and derivatives
Benzyl alcohols	2	100 %	Benzene and substituted derivatives	Benzenoids
Glycerophosphates	2	100 %	Glycerophospholipids	Lipids and lipid-like molecules

Purine 2'-deoxyribonucleosides	2	100 %	Purine nucleosides	Nucleosides, nucleotides, and analogues
Organic phosphonic acids	2	100 %	Organic phosphonic acids and derivatives	Organic acids and derivatives
Hybrid peptides	2	100 %	Peptidomimetics	Organic acids and derivatives
Guanidines	2	100 %	Organonitrogen compounds	Organic nitrogen compounds
Medium-chain keto acids and derivatives	2	100 %	Keto acids and derivatives	Organoheterocyclic compounds
Pterins and derivatives	2	100 %	Pteridines and derivatives	Organoheterocyclic compounds
Pyridine carboxaldehydes	2	100 %	Pyridines and derivatives	Organoheterocyclic compounds
Fatty acyl thioesters	2	0 %	Fatty Acyls	Lipids and lipid-like molecules
Triradylcglycerols	2	0 %	Glycerolipids	Lipids and lipid-like molecules
Triterpenoids	2	0 %	Prenol lipids	Lipids and lipid-like molecules
Steroid esters	2	0 %	Steroids and steroid derivatives	Lipids and lipid-like molecules
Alpha-keto acids and derivatives	2	0 %	Keto acids and derivatives	Organoheterocyclic compounds
Beta-keto acids and derivatives	2	0 %	Keto acids and derivatives	Organoheterocyclic compounds

6.12 Overview of the RTs and molecular ions detected for metabolites from the MS-MLS kit

Table 6.14: *The types of molecular ion (adduct(s)) detected for metabolites in the MS-MLS kit, their RT, and if their data were extracted from MLSDiscovery or Freestyle. Metabolites with columns marked with green were detected and red were not detected. The table continues to page 142.*

PLATE	ROW	COLUMN	PRIMARY_NAME	ADDUCT(S) [POSITIVE]		RT (POSITIVE)	RT (NEGATIVE)	ADDUCT(S) [NEGATIVE]		MLSDISCOVERY	FREESTYLE
1	A	1	EMPTY								
1	A	2	EMPTY								
1	A	3	EMPTY								
1	A	4	EMPTY								
1	A	5	EMPTY								
1	A	6	EMPTY								
1	A	7	EMPTY								
1	A	8	EMPTY								
1	A	9	EMPTY								
1	A	10	EMPTY								
1	A	11	EMPTY								
1	A	12	EMPTY								
1	B	1	3-METHYL-L-HISTIDINE	[M+H]+		1.93	1.92	[M-H]-		✓	
1	B	2	NICOTINAMIDE MONONUCLEOTIDE	[M+H]+		3.20	3.19	[M+COOH]-		✓	
1	B	3	FOLATE				12.24	[M-H]-		✓	
1	B	4	DEOXYADENOSINE MONOPHOSPHATE	[M+H]+		4.91	4.88	[M-H]-		✓	
1	B	5	PYRIDOXINE	[M+H]+		3.67	3.60	[M-H]-		✓	
1	B	6	HOMOSERINE	[M+H]+		2.24	2.23	[M-H]-		✓	
1	B	7	GUANINE	[M+H]+		3.16	3.14	[M-H]-		✓	
1	B	8	VALINE	[M+H]+		2.76				✓	
1	B	9	ASPARAGINE	[M+H]+		2.21	2.20	[M-H]-		✓	
1	B	10	GLYCEROL								
1	B	11	TYROSINE	[M+H]+		4.68	4.63	[M-H]-		✓	
1	B	12	ISOCITRATE				3.27	[M-H]-		✓	
1	C	1	MALATE				3.16	[M-H]-		✓	
1	C	2	DIHYDROURACIL	[M+H]+		4.40				✓	
1	C	3	GUANOSINE	[M+H]+		7.13	7.07	[M-H]-		✓	
1	C	4	L-DOPA	[M+H]+		3.72	3.67	[M-H]-		✓	

1	C	5	CREATINE	[M+H]+		2.54	2.53	[M-H]-		✓	
1	C	6	HYPOXANTHINE	[M+H]+		4.75	4.75	[M-H]-		✓	
1	C	7	DEOXYCYTIDINE MONOPHOSPHATE	[M+H]+		3.25	3.24	[M-H]-		✓	
1	C	8	ASPARTATE	[M+H]+		2.25	2.25	[M-H]-		✓	
1	C	9	THIOUREA	[M+H]+		2.87	2.85	[M+COOH]-		✓	
1	C	10	URACIL	[M+H]+		3.94	3.94	[M-H]-		✓	
1	C	11	PHENYLALANINE								
1	C	12	SUCCINATE				5.39	[M-H]-		✓	
1	D	1	SHIKIMATE				3.18	[M-H]-		✓	
1	D	2	NICOTINAMIDE	[M+H]+		4.13				✓	
1	D	3	CARNOSINE	[M+2H]2+		1.87	1.85	[M-H]-		✓	
1	D	4	FRUCTOSE BISPHOSPHATE								
1	D	5	URIDINE	[M+H]+		4.98	4.94	[M-H]-		✓	
1	D	6	LACTATE								
1	D	7	SUCCINATE SEMIALDEHYDE								
1	D	8	THYMINE	[M+H]+		6.70	6.69	[M-H]-		✓	
1	D	9	PROLINE	[M+H]+		2.67				✓	
1	D	10	URIDINE MONOPHOSPHATE	[M+H]+		3.69	3.68	[M-H]-		✓	
1	D	11	DIETHANOLAMINE	[M+H]+		2.01				✓	
1	D	12	L-ALANINE	[M+H]+		2.19				✓	
1	E	1	CYSTINE	[M+H]+		2.13	2.14	[M-H]-		✓	
1	E	2	DIHYDROFOLATE								
1	E	3	XANTHINE	[M+H]+		5.32	5.32	[M-H]-		✓	
1	E	4	GULOSE	[M+Na]+		2.39	2.39	[M-H]-		✓	
1	E	5	AMINOISOBUTANOATE	[M+H]+		2.26				✓	
1	E	6	CYS-GLY								
1	E	7	THYMIDINE	[M+Na]+		11.00	11.01	[M+COOH]-		✓	
1	E	8	METHYLTHIOADENOSINE				11.72	[M+COOH]-		✓	
1	E	9	2-PHOSPHOGLYCERATE								
1	E	10	TETRAHYDROFOLATE								
1	E	11	METHIONINE	[M+H]+		3.30	3.31	[M-H]-		✓	
1	E	12	GLYCINE	[M+H]+		2.15				✓	
1	F	1	FORMAMIDE								
1	F	2	GUANIDINOACETATE	[M+H]+		2.22	2.24	[M-H]-		✓	
1	F	3	MALONATE	[M+H]+		3.48	3.47	[M-H]-		✓	
1	F	4	DIHYDROOROTATE	[M+H]+		3.76	3.77	[M-H]-		✓	
1	F	5	QUINATE				2.76	[M-H]-		✓	
1	F	6	CREATININE	[M+H]+		2.39	2.39	[M-H]-		✓	
1	F	7	SARCOSINE	[M+H]+		2.27				✓	
1	F	8	GLYCOLATE								
1	F	9	N-ACETYLGLUCOSAMINE	[M+H]+		2.60	2.70	[M+COOH]-	[M-H]-	✓	✓
1	F	10	P-HYDROXYPHENYLACETATE			12.42	12.40	[M-H]-			✓
1	F	11	ASCORBATE	[M+H]+		2.98					✓
1	F	12	GLUTAMATE	[M+H]+		2.30	2.31	[M-H]-		✓	
1	G	1	PYRAZOLE								
1	G	2	ISOLEUCINE	[M+H]+		3.68	3.67	[M-H]-		✓	

1	G	3	CYTOSINE	[M+H]+		2.19				✓	
1	G	4	GAMMA-AMINOBUTYRATE	[M+H]+		2.10				✓	
1	G	5	INOSINE	[M+H]+		7.30	7.28	[M-H]-		✓	
1	G	6	NICOTINATE	[M+H]+		4.05	4.05	[M-H]-		✓	
1	G	7	N-ACETYLTRYPTOPHAN	[M+H]+		12.99	13.00	[M-H]-		✓	
1	G	8	TAURINE	[M+H]+		2.27	2.30	[M-H]-		✓	
1	G	9	CITRULLINE	[M+H]+		2.35	2.37	[M-H]-		✓	
1	G	10	CYSTEINE								
1	G	11	SERINE	[M+H]+		2.16	2.18	[M-H]-		✓	
1	G	12	CYTIDINE	[M+H]+		2.61	2.61	[M-H]-		✓	
1	H	1	URATE	[M+H]+		4.07	4.06	[M-H]-		✓	
1	H	2	TRANS-ACONITATE				5.96	[M-H]-		✓	
1	H	3	PYRIMIDINE								
1	H	4	N-ACETYLMANNOSAMINE	[M+Na]+		2.71	2.68	[M-H]-			✓
1	H	5	N-ACETYLNEURAMINATE	[M+H]+		2.71	2.79	[M-H]-		✓	✓
1	H	6	PURINE	[M+H]+		6.00	6.01	[M-H]-		✓	
1	H	7	THREONINE	[M+H]+		2.25	2.25	[M-H]-		✓	
1	H	8	CITRATE				4.30	[M-H]-		✓	
1	H	9	N-METYL-ALANINE	[M+H]+		2.40				✓	
1	H	10	HYPOTAURINE	[M+H]+		2.31	2.30	[M-H]-		✓	
1	H	11	GLUTAMINE	[M+H]+		2.25	2.25	[M-H]-		✓	
1	H	12	BETA-NICOTINAMIDE ADENINE DINUCLEOTIDE	[M+2H]2+		4.84	4.83	[M-H]-		✓	
2	A	1	DIAMINOPIMELATE	[M+H]+		2.04	2.04	[M-H]-		✓	
2	A	2	AMINOADIPATE	[M+H]+		2.52	2.53	[M-H]-		✓	
2	A	3	DEOXYCYTIDINE	[M+H]+		3.11	3.09	[M+COOH]-		✓	
2	A	4	NORADRENALINE				2.26	[M-H]-		✓	
2	A	5	GLUCOSAMINE 6-PHOSPHATE	[M+H]+		2.16	2.15	[M-H]-		✓	
2	A	6	TARTRATE				2.72	[M-H]-		✓	
2	A	7	3-DEHYDROSHIKIMATE				3.74	[M-H]-		✓	
2	A	8	CAFFEINE	[M+H]+		12.72				✓	
2	A	9	HOMOCYSTEINE	[M+H]+		2.68				✓	
2	A	10	THEOPHYLLINE	[M+H]+		12.20	12.21	[M-H]-		✓	
2	A	11	LEUCINE	[M+H]+		3.75	3.72	[M-H]-		✓	
2	A	12	TREHALOSE				2.49	[M+COOH]-		✓	
2	B	1	BETAINE	[M+H]+		2.83	2.84	[M+COOH]-		✓	✓
2	B	2	TRYPTOPHAN	[M+H]+		11.52	11.50	[M-H]-		✓	
2	B	3	3-SULFINOALANINE	[M+H]+		2.56	2.56	[M-H]-		✓	
2	B	4	O-SUCCINYL-HOMOSERINE	[M+H]+		3.74	3.73	[M-H]-		✓	
2	B	5	ALLANTOIN	[M+H]+		2.64	2.63	[M-H]-		✓	
2	B	6	GLYCERALDEHYDE								
2	B	7	D-GLUCURONOLACTONE	[M+Na]+		2.93	2.92	[M-H]-		✓	
2	B	8	(2-AMINOETHYL)PHOSPHONATE	[M+H]+		2.20	2.19	[M-H]-		✓	
2	B	9	2.5-DIHYDROBENZOIC ACID								
2	B	10	MALEIMIDE				3.94	[M+OH]-			✓
2	B	11	THREITOL	[M+Na]+		2.47	2.48	[M+COOH]-		✓	
2	B	12	GLUCOSAMINE	[M+H]+		1.93	1.92	[M+COOH]-		✓	

2	C	1	PARAXANTHINE	[M+H]+		12.23				✓	
2	C	2	ADENOSINE 5'-DIPHOSPHATE	[M+H]+		3.29					✓
2	C	3	2-DEOXY-D-GLUCOSE	[M+H]+		2.38	2.37	[M+OH]-		✓	
2	C	4	1-METHYL-L-HISTIDINE	[M+H]+		1.99				✓	
2	C	5	GALACTITOL	[M+Na]+		2.36	2.37	[M-H]-		✓	
2	C	6	OXOPROLINE	[M+H]+		5.49	5.48	[M-H]-		✓	
2	C	7	4-PYRIDOXATE	[M+H]+		7.78	7.77	[M-H]-		✓	
2	C	8	QUINOLINATE				4.68	[M-H]-			✓
2	C	9	METHYLGUANIDINE	[M+H]+		2.20				✓	
2	C	10	DEOXYGUANOSINE-MONOPHOSPHATE	[M+H]+		6.24	6.18	[M-H]-		✓	
2	C	11	3-HYDROXY-3-METHYLGLUTARYL-COA								
2	C	12	GLUCURONATE				2.42	[M-H]-		✓	
2	D	1	1-METHYLADENOSINE	[M+H]+		10.18	10.12	[M+COOH]-		✓	
2	D	2	DEOXYURIDINE	[M+Na]+		7.25	7.21	[M+COOH]-		✓	
2	D	3	GLUCONATE				2.45	[M-H]-		✓	
2	D	4	UROCANATE	[M+H]+		3.11	3.14	[M-H]-		✓	✓
2	D	5	KYNURENINE	[M+H]+		8.33	8.28	[M-H]-		✓	
2	D	6	PYROGLUTAMATE	[M+H]+		5.49	5.36	[M-H]-		✓	✓
2	D	7	4-ACETAMIDOBUTANOATE	[M+H]+		7.52	7.49	[M-H]-		✓	
2	D	8	TRANS-1.2-CYCLOHEXANEDIOL								
2	D	9	MELANIN	[M+H]+		4.56					✓
2	D	10	DOPAMINE	[M+H]+		3.43	3.40	[M-H]-		✓	
2	D	11	ADENOSINE-MONOPHOSPHATE	[M+H]+		3.45	3.44	[M-H]-		✓	
2	D	12	LYSINE	[M+H]+		1.83	1.83	[M-H]-		✓	
2	E	1	CITICOLINE	[M+2H]2+	[M+H]+	2.74	2.75	[M+COOH]-		✓	✓
2	E	2	1.3-DIAMINOPROPANE	[M+H]+		1.63					✓
2	E	3	PHOSPHOSERINE	[M+H]+		2.48	2.48	[M-H]-		✓	
2	E	4	1-AMINOCYCLOPROPANECARBOXYLATE	[M+H]+		2.25				✓	
2	E	5	GLUTARYLCARNITINE	[M+H]+		7.61	7.54	[M-H]-		✓	
2	E	6	CYSTATHIONINE	[M+H]+		2.11	2.10	[M-H]-		✓	
2	E	7	NORVALINE	[M+H]+		2.76				✓	
2	E	8	3-HYDROXYMETHYLGLUTARATE	[M+Na]+		5.91	5.96	[M-H]-		✓	
2	E	9	PHOSPHONOACETATE	[M+H]+		2.83	2.80	[M-H]-		✓	
2	E	10	PICOLINATE								
2	E	11	ETHANOLAMINE								
2	E	12	ARGININE	[M+H]+		1.92	1.91	[M-H]-		✓	
2	F	1	TRANS-4-HYDROXY-L-PROLINE	[M+H]+		2.36	2.36	[M-H]-		✓	
2	F	2	FUCOSE	[M+Na]+		2.88	2.88	[M+COOH]-		✓	
2	F	3	HOMOCYSTINE	[M+H]+		2.61	2.60	[M-H]-		✓	
2	F	4	N-METHYLGLUTAMATE				2.52	[M-H]-		✓	
2	F	5	D-ORNITHINE	[M+H]+		1.82	1.82	[M-H]-		✓	
2	F	6	XANTHOSINE	[M+H]+		9.53	9.54	[M-H]-		✓	✓
2	F	7	3-METHYLCROTONYL-COA								
2	F	8	THYROTROPIN RELEASING HORMONE	[M+H]+		7.10	7.01	[M-H]-		✓	
2	F	9	CYSTEATE	[M+H]+		2.55	2.55	[M-H]-		✓	
2	F	10	N-METHYLASPARTATE	[M+H]+		2.42	2.44	[M-H]-		✓	

2	F	11	GALACTARATE				2.44	[M-H]-		✓	
2	F	12	ALPHA-HYDROXYISOBUTYRATE								
2	G	1	NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE								
2	G	2	N-ACETYLASPARAGINE	[M+H]+		3.28	3.28	[M-H]-		✓	
2	G	3	PIPECOLATE	[M+H]+		3.20					✓
2	G	4	GLUCOSE 6-PHOSPHATE				2.59	[M-H]-		✓	
2	G	5	NADP	[M+2H]2+		4.38	4.39	[M-H]-		✓	
2	G	6	6-PHOSPHOGLUCONATE				2.80	[M-H]-			✓
2	G	7	ISOPENTENYL PYROPHOSPHATE								
2	G	8	GUANOSINE TRIPHOSPHATE								
2	G	9	DTDP-D-GLUCOSE				5.51	[M-H]-		✓	
2	G	10	AGMATINE SULFATE	[M+H]+		1.70				✓	
2	G	11	GLYCOLALDEHYDE								
2	G	12	DGTP								
2	H	1	N-ACETYLGLYCINE	[M+H]+		3.64	3.64	[M-H]-		✓	
2	H	2	N-ACETYLASPARTATE	[M+H]+		4.06	4.05	[M-H]-		✓	
2	H	3	INOSINE 5'-DIPHOSPHATE				3.89	[M-H]-			✓
2	H	4	PALMITOYLCARNITINE	[M+H]+		14.34				✓	
2	H	5	NORSPERMIDINE	[M+H]+		1.47				✓	
2	H	6	NICOTINAMIDE HYPOXANTHINE DINUCLEOTIDE	[M+H]+		6.30	6.22	[M-H]-		✓	✓
2	H	7	S-ADENOSYLMETHIONINE	[M+2H]2+		1.98				✓	
2	H	8	ERYTHRITOL	[M+Na]+		2.45	2.46	[M+COOH]-		✓	
2	H	9	GLUCOSAMINATE				2.17	[M-H]-		✓	
2	H	10	URIDINE TRIPHOSPHATE								
2	H	11	2-KETO-3-DEOXY-D-GLUCONIC ACID				2.84	[M-H]-		✓	
2	H	12	D-SEDOHEPTULOSE	[M+Na]+		2.42	2.41	[M-H]-		✓	
3	A	1	1.4-DIAMINOBUTANE DIHYDROCLORIDE	[M+H]+		1.63				✓	
3	A	2	DEOXYCARNITINE	[M+H]+		2.99	2.98	[M+COOH]-		✓	
3	A	3	ADENOSINE 2'.3'-CYCLIC PHOSPHATE	[M+H]+		5.63	5.64	[M-H]-		✓	
3	A	4	MEVALOLACTONE	[M+H]+		7.41				✓	
3	A	5	GALACTOSE 1-PHOSPHATE	[M+H]+		2.68	2.65	[M-H]-		✓	
3	A	6	GAMMA.GAMMA-DIMETHYLALLYL PYROPHOSPHATE								
3	A	7	DEOXYURIDINE TRIPHOSPHATE								
3	A	8	PHOSPHORYLCHOLINE	[M+Na]+		2.53	2.52	[M+COOH]-		✓	
3	A	9	O-ACETYLCARNITINE	[M+H]+		3.61	3.62	[M+COOH]-		✓	
3	A	10	6-HYDROXYDOPAMINE								
3	A	11	THIAMINE	M+		2.03				✓	
3	A	12	DGDP				4.87	[M-H]-			✓
3	B	1	5-METHYLCYTOSINE	[M+H]+		2.55				✓	
3	B	2	GLYCERATE	[M+Na]+		2.72	2.72	[M-H]-		✓	
3	B	3	CYTIDINE 2'.3'-CYCLIC PHOSPHATE	[M+H]+		2.92	2.92	[M-H]-		✓	
3	B	4	N.N.N-TRIMETHYLLYSINE	[M+H]+		2.02				✓	
3	B	5	RIBOFLAVIN	[M+H]+		12.58	12.58	[M+COOH]-		✓	
3	B	6	URIDINE DIPHOSPHATE GLUCOSE			4.59	3.53	[M-H]-		✓	
3	B	7	METHYL GALACTOSIDE	[M+Na]+		2.78	2.84				✓
3	B	8	PYRIDOXAL-PHOSPHATE	[M+H]+		4.35	4.34	[M-H]-		✓	

3	B	9	DIHYDROXYACETONE PHOSPHATE				2.75	[M-H]-			✓
3	B	10	PHOSPHOENOLPYRUVATE				2.84	[M+OH]-		✓	
3	B	11	MANNOSE 6-PHOSPHATE	[M+H]+		2.69	2.68	[M-H]-		✓	
3	B	12	3-PHOSPHOGLYCERATE	[M+H]+		2.83	2.84	[M-H]-		✓	
3	C	1	L-CARNITINE	[M+H]+		2.42	2.42	[M+COOH]-		✓	
3	C	2	O-PHOSPHOETHANOLAMINE	[M+H]+		2.23	2.22	[M-H]-		✓	
3	C	3	O-ACETYLSERINE	[M+H]+		2.50				✓	
3	C	4	CYTIDINE MONOPHOSPHATE	[M+H]+		2.71	2.71	[M-H]-		✓	
3	C	5	GUANOSINE DIPHOSPHATE MANNOSE				3.77	[M-H]-		✓	
3	C	6	ADP-GLUCOSE	[M+H]+		3.41	3.42	[M-H]-		✓	
3	C	7	FRUCTOSE 6-PHOSPHATE				2.69	[M-H]-		✓	
3	C	8	ADENOSINE 3'.5'-DIPHOSPHATE	[M+H]+		3.41	3.47	[M-H]-		✓	
3	C	9	3-NITRO-L-TYROSINE	[M+H]+		8.52	8.52	[M-H]-		✓	
3	C	10	P-OCTOPAMINE	[M+H]+		2.52				✓	
3	C	11	N-ALPHA-ACETYLLYSINE	[M+H]+		2.42	2.43	[M-H]-		✓	
3	C	12	URIDINE DIPHOSPHATEGALACTOSE				3.51	[M-H]-		✓	
3	D	1	DIHYDROXYFUMARATE								
3	D	2	PYRIDOXAMINE	[M+H]+		1.88	1.87	[M-H]-		✓	
3	D	3	5-AMINOLEVULINATE	[M+H]+		2.16	2.17	[M-H]-		✓	
3	D	4	DEOXYURIDINE-MONOPHOSPHATE	[M+H]+		4.94	4.92	[M-H]-		✓	
3	D	5	5'-DEOXYADENOSINE	[M+H]+		7.85	7.86	[M+COOH]-	[M-H]-	✓	✓
3	D	6	RIBOSE 1.5-BISPHOSPHATE								
3	D	7	XANTHOSINE-MONOPHOSPHATE	[M+H]+		4.80	4.77	[M-H]-		✓	
3	D	8	FAD								
3	D	9	DEOXYGUANOSINE	[M+H]+		9.19	9.18	[M-H]-		✓	
3	D	10	OROTATE	[M+H]+		3.75	3.75	[M-H]-		✓	
3	D	11	LAUROYLCARNITINE	[M+H]+		13.03	13.01	[M+COOH]-		✓	
3	D	12	1-METHYLNICOTINAMIDE	M+		2.51	2.51	[M+COOH]-		✓	
3	E	1	SPERMINE	[M+H]+		1.42				✓	
3	E	2	N-ACETYLMETHIONINE	[M+Na]+		12.03	12.02	[M-H]-		✓	
3	E	3	CARBAMOYL PHOSPHATE								
3	E	4	PHOSPHORIBOSYL PYROPHOSPHATE								
3	E	5	AICAR	[M+H]+		3.53	3.54	[M-H]-		✓	
3	E	6	URIDINE DIPHOSPHATE-N-ACETYLGALACTOSAMINE	[M+H]+		3.70	3.71	[M-H]-		✓	
3	E	7	GLYCERALDEHYDE 3-PHOSPHATE								
3	E	8	CYCLIC GMP	[M+H]+		10.47	10.45	[M-H]-		✓	
3	E	9	HOMOCYSTEINE THIOLACTONE	[M+H]+		2.38				✓	
3	E	10	O-PHOSPHOSERINE	[M+H]+		2.49	2.49	[M-H]-		✓	
3	E	11	S-ADENOSYLHOMOCYSTEINE	[M+2H]2+		4.40	4.40	[M-H]-		✓	
3	E	12	L-ORNITHINE	[M+H]+		1.82	1.84	[M-H]-		✓	
3	F	1	ADENINE	[M+H]+		3.00	3.00	[M-H]-		✓	
3	F	2	NORMETANEPHRINE	[M+H]+		2.95				✓	
3	F	3	URIDINE DIPHOSPHATE-N-ACETYLGLUCOSAMINE	[M+H]+		3.71	3.71	[M-H]-		✓	
3	F	4	GUANOSINE DIPHOSPHATE	[M+H]+		3.78					✓
3	F	5	GLUTATHIONE REDUCED	[M+H]+		3.65				✓	
3	F	6	URIDINE DIPHOSPHATE GLUCURONIC ACID								

3	F	7	N.N-DIMETHYLARGININE	[M+H]+		2.28	2.27	[M-H]-		✓	
3	F	8	CYTIDINE DIPHOSPHATE								
3	F	9	SELENOCYSTAMINE	[M+H]+		1.71					✓
3	F	10	HISTAMINE	[M+H]+		1.65				✓	
3	F	11	INDOXYL SULFATE				13.79	[M-H]-		✓	
3	F	12	ETHYL 3-UREIDOPROPIONATE								
3	G	1	DEOXYRIBOSE								
3	G	2	PHYTATE								
3	G	3	THIAMINE MONOPHOSPHATE	[M+2H]2+		2.24				✓	
3	G	4	URACIL 5-CARBOXYLATE	[M+H]+		4.59	4.58	[M-H]-		✓	
3	G	5	S-HEXYL-GLUTATHIONE								
3	G	6	GLYOXYLATE								
3	G	7	GUANOSINE MONOPHOSPHATE	[M+H]+		4.15	4.13	[M-H]-		✓	
3	G	8	N-ACETYLALANINE	[M+H]+		5.11	5.10	[M-H]-		✓	
3	G	9	4-GUANIDINOBUTANOATE	[M+H]+		2.86	2.86	[M-H]-		✓	
3	G	10	HYDROXYPYRUVATE								
3	G	11	D-MANNOSAMINE	[M+H]+		1.94	1.94	[M+COOH]-	[M-H]-	✓	✓
3	G	12	CYTOCHROME C								
3	H	1	EMPTY								
3	H	2	EMPTY								
3	H	3	EMPTY								
3	H	4	EMPTY								
3	H	5	EMPTY								
3	H	6	EMPTY								
3	H	7	EMPTY								
3	H	8	EMPTY								
3	H	9	EMPTY								
3	H	10	EMPTY								
3	H	11	EMPTY								
3	H	12	EMPTY								
4	A	1	DEOXYADENOSINE	[M+H]+		7.35	7.34	[M+COOH]-		✓	
4	A	2	N-ACETYLPUTRESCINE	[M+H]+		2.38					✓
4	A	3	N-ACETYLGALACTOSAMINE	[M+Na]+	[M+H]+	2.70	2.53	[M-H]-		✓	✓
4	A	4	N-ACETYLGLUTAMATE	[M+H]+		5.15	5.15	[M-H]-		✓	
4	A	5	2.4-DIHYDROXYPTERIDINE	[M+H]+		6.92	6.93	[M-H]-		✓	
4	A	6	6-HYDROXYNICOTINATE	[M+H]+		7.62	7.60	[M-H]-		✓	
4	A	7	N-ACETYLCYSTEINE	[M+Na]+	[M+H]+	7.20				✓	✓
4	A	8	INOSINE-MONOPHOSPHATE	[M+H]+		4.59	4.57	[M-H]-		✓	
4	A	9	PANTOTHENATE	[M+H]+		10.79	10.78	[M-H]-		✓	
4	A	10	2-AMINOISOBUTYRATE	[M+H]+		2.35				✓	
4	A	11	ANILINE-2-SULFONATE	[M+H]+		6.95	6.91	[M-H]-		✓	
4	A	12	S-CARBOXYMETHYLCYSTEINE	[M+H]+		2.80	2.69	[M-H]-		✓	✓
4	B	1	RHAMNOSE	[M+Na]+		2.63	2.63	[M-H]-		✓	
4	B	2	THIAMINE PYROPHOSPHATE	[M+2H]2+	[M+H]+	2.64	2.80	[M-H]-		✓	✓
4	B	3	HISTIDINOL	[M+H]+		1.65	1.65	[M-H]-		✓	
4	B	4	THYMIDINE-MONOPHOSPHATE	[M+H]+		6.80	6.81	[M-H]-		✓	✓

4	B	5	UREIDOPROPIONATE				3.62	[M-H]-		✓	
4	B	6	5-AMINOPENTANOATE	[M+H]+		2.39				✓	
4	B	7	NORLEUCINE	[M+H]+		3.78	3.78	[M-H]-		✓	
4	B	8	N-FORMYLGLYCINE	[M+H]+		3.12	3.13	[M-H]-		✓	
4	B	9	ADENOSINE	[M+H]+		5.58	5.55	[M+COOH]-		✓	
4	B	10	RAFFINOSE	[M+Na]+		2.63					✓
4	B	11	MESO-TARTRATE				2.55	[M-H]-			✓
4	B	12	2-ACETAMIDO-2-DEOXY-BETA-D-GLUCOSYLAMINE								
4	C	1	SACCHARATE				2.51	[M-H]-		✓	
4	C	2	ADENOSINE TRIPHOSPHATE								
4	C	3	3-METHOXYTYROSINE	[M+H]+		6.53	6.52	[M-H]-		✓	
4	C	4	LACTOSE				2.38	[M-H]-			✓
4	C	5	3-HYDROXYBUTANOATE				12.28	[M-H]-			✓
4	C	6	4-IMIDAZOLEACETATE	[M+H]+		2.40	2.41	[M-H]-		✓	
4	C	7	GALACTURONATE	[M+Na]+		2.50	2.49	[M-H]-		✓	
4	C	8	CYTIDINE TRIPHOSPHATE								
4	C	9	CYCLIC AMP	[M+H]+		8.05	8.04	[M-H]-		✓	
4	C	10	METHIONINE SULFOXIMINE	[M+H]+		2.18	2.18	[M-H]-		✓	
4	C	11	CIS-4-HYDROXY-D-PROLINE	[M+H]+		2.32	2.33	[M-H]-		✓	
4	C	12	N1-ACETYLSPERMINE	[M+H]+		1.54				✓	
4	D	1	GLUCOSAMINE 6-SULFATE	[M+H]+		2.26	2.27	[M-H]-		✓	
4	D	2	NADPH								
4	D	3	3-METHYLHISTAMINE								
4	D	4	MALEAMATE								
4	D	5	CHOLINE	M+		2.33				✓	
4	D	6	METHYL 4-AMINOBUTYRATE	[M+H]+		2.70				✓	
4	D	7	N-FORMYL-L-METHIONINE	[M+Na]+		11.69	11.71	[M-H]-		✓	
4	D	8	ACETYLCHOLINE	M+		3.30				✓	
4	D	9	OXALATE								
4	D	10	5-HYDROXY-L-TRYPTOPHAN	[M+H]+		7.67	7.59	[M-H]-		✓	✓
4	D	11	D-ALANINE	[M+H]+		2.20				✓	
4	D	12	THEOBROMINE	[M+H]+		12.02				✓	
4	E	1	GUANIDINOSUCCINATE	[M+H]+		2.37	2.38	[M-H]-		✓	
4	E	2	HISTIDINE	[M+H]+		1.89	1.89	[M-H]-		✓	
4	E	3	ALLOTHREONINE	[M+H]+		2.26	2.25	[M-H]-		✓	
4	E	4	PHOSPHOCREATINE	[M+H]+		2.83					✓
4	E	5	SPERMIDINE	[M+H]+		1.49				✓	
4	E	6	ADENOSINE DIPHOSPHATE RIBOSE	[M+H]+		3.13	3.42	[M-H]-		✓	✓
4	E	7	2-METHOXYETHANOL								
4	E	8	CITRAMALATE	[M+Na]+		4.68	4.69	[M-H]-		✓	
4	E	9	ANSERINE	[M+2H]2+		1.97	1.96	[M-H]-		✓	
4	E	10	BILIVERDIN								
4	E	11	5-HYDROXYLYSINE	[M+H]+		1.81	1.81	[M-H]-		✓	
4	E	12	CYSTEAMINE								
4	F	1	OPHTHALMATE	[M+H]+		3.67	3.68	[M-H]-		✓	
4	F	2	MESOXALATE				2.71	[M-H]-		✓	

4	F	3	TRIGONELLINE	[M+H]+		3.55	3.55	[M+COOH]-		✓	
4	F	4	EPINEPHRINE	[M+H]+		2.74	2.74	[M-H]-		✓	
4	F	5	3.4-DIHYDROXYPHENYLGLYCOL				4.82	[M-H-H2O]-		✓	
4	F	6	CADAVERINE	[M+H]+		1.64				✓	
4	F	7	2-HYDROXYBUTYRATE				5.13	[M-H]-		✓	
4	F	8	COENZYME A								
4	F	9	OXALOMALATE								
4	F	10	INOSINE TRIPHOSPHATE								
4	F	11	CDP-ETHANOLAMINE	[M+H]+		2.40	2.44	[M-H]-		✓	✓
4	F	12	2.5-DIMETHYLPYRAZINE								
4	G	1	STACHYOSE				2.52	[M+COOH]-		✓	
4	G	2	DEOXYCYTIDINE-DIPHOSPHATE				3.33	[M-H]-		✓	
4	G	3	2.3-BUTANEDIOL								
4	G	4	D-RIBOSE 5-PHOSPHATE				2.71	[M-H]-		✓	
4	G	5	HYDROXYKYNURENINE								
4	G	6	GALACTOSAMINE	[M+Na]+	[M+H]+	1.88	1.92				✓
4	G	7	DEOXYADENOSINE TRIPHOSPHATE								
4	G	8	GLYCEROL 3-PHOSPHATE	[M+H]+		2.72	2.78	[M-H]-		✓	
4	G	9	CYANOCOBALAMIN				12.57	[M+COOH]-			✓
4	G	10	4-HYDROXY-L-PHENYLGLYCINE	[M+H]+		2.52	2.52	[M-H]-		✓	
4	G	11	N-ACETYLSERINE	[M+H]+		3.27	3.28	[M-H]-		✓	
4	G	12	URIDINE 5'-DIPHOSPHATE								
4	H	1	EMPTY								
4	H	2	EMPTY								
4	H	3	EMPTY								
4	H	4	EMPTY								
4	H	5	EMPTY								
4	H	6	EMPTY								
4	H	7	EMPTY								
4	H	8	EMPTY								
4	H	9	EMPTY								
4	H	10	EMPTY								
4	H	11	EMPTY								
4	H	12	EMPTY								
5	A	1	METHYGLUTARATE	[M+Na]+		12.19	11.96	[M-H]-		✓	
5	A	2	SORBATE								
5	A	3	MONOETHYLMALONATE								
5	A	4	GLUCONOLACTONE	[M+H]+		2.60	2.49	[M+OH]-		✓	
5	A	5	4-HYDROXYBENZOATE				12.66	[M-H]-		✓	
5	A	6	TYRAMINE	[M+H]+		4.38				✓	
5	A	7	CORTISOL	[M+Na]+		14.97	14.70	[M+COOH]-		✓	
5	A	8	PRENOL								
5	A	9	3-HYDROXYBENZALDEHYDE								
5	A	10	XANTHURENATE	[M+H]+		13.35	13.17	[M-H]-		✓	
5	A	11	2-METHYLPROPANAL								
5	A	12	INDOXYL β-GLUCOSIDE				12.55	[M+COOH]-		✓	

5	B	1	TRIMETHYLAMINE	[M+H]+		2.45						✓
5	B	2	MELATONIN	[M+H]+		13.78	12.62	[M-H]-		✓		
5	B	3	MALEATE				5.15	[M-H]-		✓		
5	B	4	PENTANOATE									
5	B	5	PROPANOATE									
5	B	6	BILIRUBIN									
5	B	7	NICOTINE	[M+H]+		4.71						✓
5	B	8	PREGNENOLONE SULFATE				28.57	[M-H]-		✓		
5	B	9	KYNURENATE	[M+H]+		13.54	13.42	[M-H]-		✓		
5	B	10	ISOBUTYRATE									
5	B	11	3-HYDROXYBENZYL ALCOHOL				12.05	[M-H]-		✓		
5	B	12	ANILINE									
5	C	1	ACETOIN				8.14	[M-H]-		✓		
5	C	2	3.5-DIIODO-L-TYROSINE	[M+H]+		12.77	12.60	[M-H]-		✓		
5	C	3	MANDELATE				12.44	[M-H]-		✓		
5	C	4	TRYPTAMINE	[M+H]+		11.66				✓		
5	C	5	4-AMINOBENZOATE	[M+H]+		12.25				✓		
5	C	6	GLUTARATE	[M+Na]+		8.35	8.14	[M-H]-		✓		
5	C	7	5-VALEROLACTONE									
5	C	8	CAFFEATE	[M+H]+		12.97	12.78	[M-H]-		✓		
5	C	9	LUMICHROME	[M+Na]+		14.14	13.93	[M-H]-		✓		
5	C	10	BETA-ALANINE	[M+H]+		2.10				✓		
5	C	11	N-ACETYLPHENYLALANINE	[M+Na]+		13.53	13.34	[M-H]-		✓		
5	C	12	N-ACETYLPROLINE	[M+Na]+		12.22	12.02	[M-H]-		✓		
5	D	1	L-TRYPTOPHANAMIDE	[M+H]+		9.05	8.81	[M-H]-		✓		
5	D	2	PHENOL									
5	D	3	N-METHYLTRYPTAMINE	[M+H]+		12.01				✓		
5	D	4	OXALOACETATE									
5	D	5	2.3-DIHYDROXYBENZOATE				13.01	[M-H]-		✓		
5	D	6	2-PROPENOATE									
5	D	7	INDOLE-3-ETHANOL	[M+H]+		13.71				✓		
5	D	8	FERULATE	[M+Na]+		13.63	13.35	[M-H]-		✓		
5	D	9	GLYCOCHOLATE	[M+H]+		16.35	16.10	[M-H]-		✓		
5	D	10	PHENYLETHANOLAMINE									
5	D	11	THIOPURINE S-METHYLETHER	[M+H]+		13.19	13.04	[M-H]-		✓		
5	D	12	2-HYDROXY-4-(METHYLTHIO)BUTANOATE	[M+Na]+		11.89	11.65	[M-H]-		✓		
5	E	1	GLYCOCHENODEOXYCHOLATE	[M+Na]+		17.11	16.78	[M-H]-		✓		
5	E	2	BENZOATE									
5	E	3	3-AMINO-5-HYDROXYBENZOATE	[M+H]+		4.26	4.02	[M-H]-		✓		✓
5	E	4	PYROCATECHOL				11.75	[M-H]-				✓
5	E	5	3.4-DIHYDROXYBENZOATE	[M+H]+		12.06	11.81	[M-H]-		✓		
5	E	6	CYCLOPENTANONE									
5	E	7	PANTOLACTONE	[M+H]+		13.23				✓		
5	E	8	GUAIACOL				12.06	[M-H]-		✓		
5	E	9	2-HYDROXYPHENYLACETATE	[M+Na]+		12.68	12.43	[M-H]-		✓		
5	E	10	10-HYDROXYDECANOATE				14.12	[M-H]-				✓

5	E	11	DIDECANOYL-GLYCEROPHOSPHOCHOLINE										
5	E	12	2-HYDROXYPYRIDINE	[M+H]+		7.46					✓		
5	F	1	3.4-DIHYDROXYPHENYLACETATE	[M+Na]+		12.27					✓		
5	F	2	N6-(DELTA2-ISOPENTENYL)-ADENINE										
5	F	3	METHYL VANILLATE				12.93	[M-H]-			✓		
5	F	4	2-OXOBUTANOATE										
5	F	5	LIPOAMIDE	M+	[M+H]+	13.89					✓		
5	F	6	3-HYDROXYANTHRANILATE	[M+H]+		11.57	11.47	[M-H]-			✓		
5	F	7	3-(4-HYDROXYPHENYL)PYRUVATE				11.90	[M-H]-			✓		
5	F	8	HEXANOATE										
5	F	9	METHYLMALONATE				5.48	[M-H]-			✓		
5	F	10	INDOLE-3-ACETATE	[M+H]+		13.76	13.64	[M-H]-			✓		
5	F	11	CORTISOL 21-ACETATE	[M+Na]+	[M+H]+	15.70							✓
5	F	12	INDOLE-3-ACETAMIDE										
5	G	1	HIPPURATE	[M+Na]+		12.91	12.95	[M-H]-			✓		
5	G	2	ETHYLMALONATE	[M+Na]+		9.27	9.32	[M-H]-			✓		
5	G	3	3.5-DIIODO-L-THYRONINE	[M+H]+		13.54	13.59	[M-H]-			✓		
5	G	4	FUMARATE				4.82	[M-H]-			✓		
5	G	5	BENZALDEHYDE										
5	G	6	4-HYDROXYBENZALDEHYDE										
5	G	7	3-(2-HYDROXYPHENYL)PROPANOATE	[M+Na]+		13.51	13.55	[M-H]-			✓		
5	G	8	3-METHOXYTYRAMINE	[M+H]+		6.26					✓		
5	G	9	BENZYLAMINE										
5	G	10	2-QUINOLINECARBOXYLATE	[M+H]+		13.25					✓		
5	G	11	SEROTONIN	[M+H]+		7.10					✓		
5	G	12	PTERIN	[M+H]+		6.42	6.48	[M-H]-			✓		
5	H	1	BUTANOATE										
5	H	2	2-AMINOPHENOL	[M+H]+		3.16	3.55	[M-H]-			✓		
5	H	3	6-CARBOXYHEXANOATE				13.02	[M-H]-			✓		
5	H	4	INDOLE-3-PYRUVATE										
5	H	5	DEHYDROASCORBATE				2.71	[M+OH]-			✓		
5	H	6	3-AMINO-4-HYDROXYBENZOATE	[M+H]+		3.51	3.55	[M-H]-			✓		
5	H	7	3.4 DIHYDROXYMANDELATE				4.65	[M-H]-			✓		
5	H	8	2-METHYLCITRATE				6.97	[M-H]-					✓
5	H	9	DIHYDROBIOPTERIN	[M+H]+		5.93	5.90	[M-H]-			✓		✓
5	H	10	BETA-GLYCEROPHOSPHATE	[M+H]+		2.91	2.95	[M-H]-			✓		
5	H	11	GLUCOSE 1-PHOSPHATE	[M+H]+		2.78	2.81	[M-H]-			✓		
5	H	12	2.3-DIAMINOPROPIONATE	[M+H]+		1.92					✓		
6	A	1	2.5-DIHYDROXYBENZOATE	[M+H]+		12.39	12.32	[M-H]-			✓		
6	A	2	4-QUINOLINECARBOXYLATE	[M+H]+		7.83	7.72	[M-H]-			✓		
6	A	3	HYDROQUINONE										
6	A	4	DETHIOBIOTIN	[M+H]+		13.21	13.16	[M-H]-			✓		
6	A	5	3-HYDROXYBENZOATE				12.67	[M-H]-			✓		
6	A	6	2-METHYLBUTANAL										
6	A	7	N-ACETYLSEROTONIN				12.75	[M-H]-			✓		
6	A	8	HYDROPHENYLLACTIC ACID	[M+Na]+		11.94	11.87	[M-H]-			✓		

6	A	9	ITACONATE	[M+Na]+		7.57	7.47	[M-H]-		✓	
6	A	10	AZELATE								
6	A	11	OXOADIPATE				4.73	[M-H]-		✓	
6	A	12	2-METHYLGLUTARATE	[M+Na]+		11.62	11.54	[M-H]-		✓	
6	B	1	PHENYLACETALDEHYDE								
6	B	2	3-METHYL-2-OXOVALERATE								
6	B	3	PORPHOBILINOGEN				6.27	[M-H]-		✓	
6	B	4	DIACETYL								
6	B	5	PYRUVATE								
6	B	6	TRANS-CINNAMALDEHYDE								
6	B	7	2.6-DIHYDROXYPYRIDINE	[M+H]+		5.28	5.28	[M-H]-		✓	
6	B	8	VANILLIN				13.35	[M-H]-		✓	
6	B	9	METHYL ACETOACETATE								
6	B	10	SUBERATE	[M+H]+			13.12	[M-H]-		✓	
6	B	11	ADIPATE	[M+Na]+		11.56	11.55	[M-H]-		✓	
6	B	12	GERANYL-PP								
6	C	1	N-ACETYLLEUCINE	[M+Na]+		12.77	12.71	[M-H]-		✓	
6	C	2	2'.4'-DIHYDROXYACETOPHENONE								
6	C	3	BENZYL ALCOHOL				12.64	[M-H]-		✓	
6	C	4	MONOMETHYLGLUTARATE								
6	C	5	INDOLE-3-METHYL ACETATE								
6	C	6	MEVALONATE	[M+Na]+		4.57	4.48	[M-H]-		✓	
6	C	7	3-METHOXY-4-HYDROXYMANDELATE				7.94	[M-H]-		✓	
6	C	8	HOMOVANILLATE	[M+Na]+		12.88	12.82	[M-H]-		✓	
6	C	9	2-METHYLMALEATE				6.01	[M-H]-		✓	
6	C	10	1-PHENYLETHANOL								
6	C	11	SALSOLINOL	[M+H]+		4.28 and 6.7	4.21 and 6.62	[M-H]-		✓	
6	C	12	SALICYLAMIDE	[M+H]+		12.83	12.77	[M-H]-		✓	
6	D	1	OXOGLUTARATE				3.68	[M-H]-		✓	
6	D	2	ETHYL 3-INDOLEACETATE	[M+Na]+		14.57				✓	
6	D	3	3-ALPHA.11-BETA.17.21-TETRAHYDROXY- 5-BETA-PREGNAN-20-ONE								
6	D	4	N.N-DIMETHYL-1.4-PHENYLENEDIAMINE	[M+H]+		4.31				✓	
6	D	5	HOMOGENTISATE	[M+H]+		8.29	8.27	[M-H]-		✓	
6	D	6	INDOLEACETALDEHYDE	[M+H]+		12.40	12.42	[M-H]-		✓	
6	D	7	4-HYDROXY-3-METHOXYPHENYLGLYCOL	[M+Na]+		8.56				✓	
6	D	8	3-HYDROXYPHENYLACETATE								
6	D	9	4-METHYLCATECHOL				8.26	[M-H]-		✓	
6	D	10	PYRIDOXAL	[M+H]+		3.16	3.17	[M-H]-		✓	
6	D	11	SALICYLATE				8.25	[M-H]-		✓	
6	D	12	SEBACATE				13.84	[M-H]-		✓	
6	E	1	3-METHYL-2-OXINDOLE	[M+H]+		13.59	12.48	[M-H]-		✓	
6	E	2	3-METHYLADENINE	[M+H]+		4.23				✓	
6	E	3	HYDROXYPHENYLLACTATE	[M+Na]+		11.88	11.86	[M-H]-		✓	
6	E	4	BIOTIN	[M+H]+		13.08	13.07	[M-H]-		✓	
6	E	5	MERCAPTOPYRUVATE								
6	E	6	PYRUVIC ALDEHYDE								

6	E	7	PYRROLE-2-CARBOXYLATE	[M+H]+		10.85	10.82	[M-H]-		✓	
6	E	8	5-HYDROXYINDOLEACETATE	[M+H]+		12.49	12.48	[M-H]-		✓	
6	E	9	3-METHYLGLUTACONATE	[M+Na]+		11.08				✓	
6	E	10	RESORCINOL MONOACETATE				13.35	[M-H]-			✓
6	E	11	ACETOACETATE	[M+Na]+		4.49	4.48	[M-H]-		✓	
6	E	12	ACETYLPHOSPHATE								
6	F	1	SORBOSE				2.34	[M-H]-			✓
6	F	2	XYLITOL	[M+H]+		2.41	2.54	[M-H]-			✓
6	F	3	RIBITOL	[M+Na]+	[M+H]+	2.42	2.42	[M-H]-		✓	✓
6	F	4	MYOINOSITOL	[M+H]+		2.26	2.23	[M-H]-			✓
6	F	5	MANNOSE				2.39	[M-H]-		✓	
6	F	6	XYLOSE				2.55	[M-H]-			✓
6	F	7	SUCROSE	[M+Na]+		2.56	2.67	[M-H]-		✓	✓
6	F	8	GALACTOSE				2.39	[M-H]-		✓	
6	F	9	ALPHA-D-GLUCOSE	[M+Na]+		2.52	2.39	[M-H]-		✓	✓
6	F	10	ALLOSE				2.41	[M-H]-		✓	
6	F	11	MANNITOL	[M+Na]+		2.40	2.40	[M-H]-		✓	✓
6	F	12	MELIBIOSE				2.51	[M-H]-			✓
6	G	1	SORBITOL				2.40	[M-H]-		✓	
6	G	2	MALTOSE	[M+Na]+		2.50	2.45	[M-H]-		✓	✓
6	G	3	TAGATOSE	[M+Na]+		2.38	2.39	[M-H]-		✓	
6	G	4	L-GULONOLACTONE	[M+Na]+		2.56	2.57	[M-H]-		✓	
6	G	5	ARABINOSE				2.48	[M+COOH]-	[M-H]-		✓
6	G	6	CELLOBIOSE	[M+Na]+		2.43	2.60	[M-H]-			✓
6	G	7	PSICOSE				2.63	[M-H]-			✓
6	G	8	ARABITOL	[M+H]+		2.41	2.42	[M-H]-		✓	✓
6	G	9	LYXOSE				2.43	[M-H]-			✓
6	G	10	RIBOSE				2.51	[M+COOH]-	[M-H]-	✓	✓
6	G	11	PALATINOSE				2.51	[M-H]-		✓	
6	G	12	D-PINITOL	[M+Na]+		2.36	2.40	[M+COOH]-	[M-H]-		✓
6	H	1	EMPTY								
6	H	2	EMPTY								
6	H	3	EMPTY								
6	H	4	EMPTY								
6	H	5	EMPTY								
6	H	6	EMPTY								
6	H	7	EMPTY								
6	H	8	EMPTY								
6	H	9	EMPTY								
6	H	10	EMPTY								
6	H	11	EMPTY								
6	H	12	EMPTY								
7	A	1	VITAMIN D2								
7	A	2	SQUALENE								
7	A	3	4-COUMARATE	[M+H]+		12.96	12.92	[M-H]-		✓	
7	A	4	NONANOATE								

7	A	5	ESTRADIOL-17ALPHA										
7	A	6	CAPRYLATE										
7	A	7	URSODEOXYCHOLATE				15.62	[M+COOH]-		✓			
7	A	8	PETROSELINATE										
7	A	9	DIPALMITOYLGLYCEROL										
7	A	10	CORTICOSTERONE	[M+H]+		15.06				✓			
7	A	11	LITHOCHOLATE				18.54	[M+COOH]-		✓			
7	A	12	PROTOPORPHYRIN										
7	B	1	HEPTANOATE										
7	B	2	RETINOL										
7	B	3	MENAQUINONE										
7	B	4	ELAIDATE										
7	B	5	CHENODEOXYCHOLATE	[M+Na]+		17.32	17.20	[M+COOH]-		✓			
7	B	6	MYRISTATE										
7	B	7	CHOLESTERYL OLEATE										
7	B	8	ROSMARINATE	[M+Na]+		13.22	13.24	[M-H]-		✓			
7	B	9	GLYCERYL TRIPALMITATE										
7	B	10	CORTEXOLONE	[M+H]+		15.05	15.03	[M+COOH]-		✓			
7	B	11	LITHOCHOLYLTAURINE				23.90	[M-H]-				✓	
7	B	12	PALMITOLEATE										
7	C	1	PALMITATE										
7	C	2	LIOTHYRONINE	[M+H]+		13.65	13.67	[M-H]-		✓			
7	C	3	SPHINGANINE	[M+H]+		13.91				✓			
7	C	5	LAURATE										
7	C	6	ARACHIDATE										
7	C	6	LANOSTEROL										
7	C	7	ERUCATE										
7	C	8	DEOXYCHOLATE	[M+Na]+		17.35	17.32	[M-H]-		✓			
7	C	9	KETOLEUCINE				11.65	[M-H]-		✓			
7	C	10	EICOSAPENTAENOATE										
7	C	11	HEPTADECANOATE										
7	C	12	GLYCERYL TRIMYRISTATE										
7	D	1	LINOLEATE										
7	D	2	SPHINGOMYELIN										
7	D	3	7-DEHYDROCHOLESTEROL										
7	D	4	THYROXINE	[M+H]+		14.18	14.20	[M-H]-		✓			
7	D	5	BIS(2-ETHYLHEXYL)PHTHALATE										
7	D	6	GAMMA-LINOLENATE										
7	D	7	OMEGA-HYDROXYDODECANOATE	[M+Na]+		14.52	14.51	[M-H]-		✓			
7	D	8	METHYL JASMONATE										
7	D	9	DIPALMITOYL-PHOSPHATIDYLCHOLINE										
7	D	10	HEXADECANOL										
7	D	11	5.6 DIMETHYLBENZIMIDAZOLE	[M+H]+		11.95	11.94	[M-H]-		✓			
7	D	12	RETINOATE										
7	E	1	INDOLE										
7	E	2	CHOLATE				16.22	[M+COOH]-		✓			

7	E	3	PHYLLOQUINONE	[M+Na]+		19.10				✓	
7	E	4	CHOLESTERYL PALMITATE								
7	E	5	QUINOLINE	[M+H]+		11.27				✓	
7	E	6	DOCOSAHEXAENOATE								
7	E	7	DIETHYL 2-METHYL-3-OXOSUCCINATE								
7	E	8	RETINYL PALMITATE								
7	E	9	2-UNDECANONE								
7	E	10	1-HYDROXY-2-NAPHTHOATE			14.95	14.95	[M-H]-		✓	
7	E	11	DIPALMITOYL-PHOSPHOETHANOLAMINE								
7	E	12	PHENYLPYRUVATE								
7	F	1	TRANS-CINNAMATE								
7	F	2	OLEATE								
7	F	3	STEARATE								
7	F	4	BETA-CAROTENE								
7	F	5	25-HYDROXYCHOLESTEROL								
7	F	6	NERVONATE								
7	F	7	DESMOSTEROL				20.00	[M-H-H2O]-	[M-H]-	✓	
7	F	8	DEOXYCORTICOSTERONE ACETATE	[M+H]+		17.52				✓	
7	F	9	OLEOYL-GLYCEROL	[M+Na]+		19.05				✓	
7	F	10	ALPHA-TOCOPHEROL								
7	F	11	GLYCEROL-MYRISTATE	[M+Na]+		17.03				✓	
7	F	12	TRICOSANOATE								
7	G	1	COENZYME Q10								
7	G	2	CORTISONE	[M+H]+		14.38	14.37	[M+COOH]-		✓	
7	G	3	DECANOATE								

6.13 Regional Committee for Medical and Health Research Ethics informed consent form

To investigate the normal metabolome, an application to the Regional Committee for Medical and Health Research Ethics was applied. The participant was given the consent form described below.

Oslo universitetssykehus

FORESPØRSEL OM Å AVGI BIOLOGISK MATERIALE TIL

KARTLEGGING AV NORMALMETABOLOMET

BAKGRUNN OG HENSIKT

Formålet med biobanken er å undersøke sammensetningen av stoffene som kan påvises i kroppsvæsker i en normalbefolkning. Metabolismen er summen av alle kjemiske reaksjoner i kroppen og innebærer nedbrytning og oppbygging av ulike stoffer som inngår i disse prosessene. Stoffene som deltar i metabolismen kalles metabolitter, og sammensetningen av metabolitter utgjør det som kalles metabolomet. Metabolismen er et dynamisk system, det vil si det er i endring hele tiden, og påvirkes av en rekke naturlige biologiske faktorer som alder, kjønn, tid på døgnet m.m., og ytre påvirkninger som matinntak/faste, fysisk aktivitet, sykdom, inntak av legemidler m.m. For å kunne skille variasjoner i metabolomet som skyldes normale prosesser fra avvik som skyldes sykdom er det nødvendig å undersøke metabolomet fra et stort antall friske personer under ulike betingelser.

Forskningsansvarlig er Oslo Universitetssykehus, Avdeling for medisinsk biokjemi ved avdelingsleder. Prosjektleder er Katja B. Prestø Elgstøen ved samme avdeling.

Det biologiske materialet blir oppbevart på ubestemt tid så lenge det er godkjent av regional komité for medisinsk og helsefaglig forskningsetikk og Personvernombudet ved Oslo universitetssykehus og skal brukes i forskning som har til hensikt å kartlegge hvordan metabolomet hos friske personer påvirkes under ulike betingelser (tid på døgnet, fødeinntak, fysisk aktivitet, m.m.).

HVILKET BIOLOGISK MATERIALE SKAL INNSAMLES?

Vi undersøker i hovedsak blod og/eller urin. I sjeldne tilfeller kan det være aktuelt med hår, spytt, tårevæske eller vev eller annet materiale fra deg. Det vil bli et stikk (blodprøve fra armen og/eller stikk i fingeren) i forbindelse med blodprøvetaking. Dersom du gir fibroblaster (hudprøve), vil du få lokalbedøvelse før vi tar en knappenålshode stor prøve fra huden din. Dersom du gir vev, vil dette være i forbindelse med en annen undersøkelse eller operasjon og du vil ikke oppleve ekstra ubehag pga dette. Det vil aldri tas prøver av deg uten at du er informert om dette og har gitt ditt skriftlige, informerte samtykke.

BREDT SAMTYKKE

Når du avgir biologisk materiale til denne forskningsbiobanken avgir du også et bredt samtykke til at materiale og relevante helseopplysninger skal brukes til fremtidig forskning som har til hensikt å kartlegge normalmetabolomet, og som normalkontroller i studie av ulike sykdommer. Materiale og helseopplysninger

brukes kun i forskning og studier som er godkjent av regional komité for medisinsk og helsefaglig forskningsetikk, personvernombud og andre relevante instanser.

INNSAMLING OG BRUK AV HELSEOPPLYSNINGER

Biobanken vil inneholde noen opplysninger om deg (navn, kjønn og alder). Navnet ditt er kun tilgjengelig gjennom en koblingsnøkkel som skal beskytte din identitet, men samtidig gjøre det mulig å knytte opplysningene om deg til ditt materiale gjennom en kodeliste. Virksomheten er ansvarlig for at koblingsnøkkel oppbevares og forvaltes forsvarlig. Materiale og opplysningene om deg lagres permanent og vil analyseres i forbindelse med godkjente, spesifiserte forskningsprosjekter.

Vi vil innhente og registrere følgende opplysninger om deg: kjønn og alder. Det kan også være ønskelig å registrere annen relevant informasjon (kosthold, medisinbruk, kjent sykdom eller liknende). Du står i så fall fritt til å oppgi dette eller ikke.

Det kan være aktuelt å gjøre relevante undersøkelser på gennivå for å bidra til bedre forståelse av variasjoner i metabolomet (se under, «Genetiske undersøkelser»).

SAMMENSTILLING AV DATA FRA BIOBANKEN MED ANDRE OPPLYSNINGER

I enkelte forskningsprosjekter kan det være aktuelt å sammenstille informasjon fra biobanken med opplysninger fra pasientjournal, helseundersøkelser, helseregistre eller offentlige administrative registre. Dette vil i så fall kun skje når dette er godkjent av regional komité for medisinsk og helsefaglig forskningsetikk, personvernombud og andre relevante instanser.

GENETISKE UNDERSØKELSER

Det kan være aktuelt å gjøre genetiske analyser på det materialet som er samlet inn for å se på sammenhengen mellom metabolomet og underliggende gener og genvarianter (sekvensering av enkeltgen, grupper av gener eller hele genomet). Genomsekvensen til hvert enkelt menneske er unik, så ingen prøver som inneholder DNA (i praksis vil det si alle prøver som tas av en person) kan i teorien være anonyme. I praksis blir imidlertid alle prøver avidentifiserte og ingen (verken forskere eller personer som deltar i biobanken) informeres om resultater og funn hos enkeltpersoner.

Det er en viss risiko for at det oppdages noe i prøvene som kan indikere sykdom eller risiko for å utvikle sykdom, såkalt utilsiktede funn. Slike funn er informasjon som dels kan være noe usikker og uansett bare utgjør biter av et helhetsbilde. Med mindre noe annet er spesifikt avtalt med forsøksdeltakerne, vil slik informasjon ikke bli formidlet tilbake til forsøkspersonene.

Alle fremtidige studier der det er aktuelt å bruke det biologiske materialet eller data fra materialet skal godkjennes av regional komité for medisinsk og helsefaglig forskningsetikk, personvernombud og andre relevante instanser. Dersom noen av disse instansene mener det skal innhentes nytt samtykke for genetiske eller andre undersøkelser, vil det bli gjort.

GODKJENNING AV FREMTIDIGE FORSKNINGSPROSJEKTER

Alle fremtidige forskningsprosjekter som benytter materialet fra deg skal forhåndsgodkjennes av en regional komité for medisinsk og helsefaglig forskningsetikk, men du vil kun unntaksvis bli spurt på nytt om slik bruk.

INFORMASJON OM FREMTIDIGE PROSJEKTER

Informasjon om forskningsbiobanken *Kartlegging av normalmetabolomet* finnes på Biobankportalen til Oslo Universitetssykehus: https://oslo-universitetssykehus.no/forskningsbiobanker-ved-oslo-universitetssykehus

På denne nettsiden er det lenke videre til forskningsprosjektets nettside hvor informasjon om pågående og framtidige prosjekter er beskrevet.

UTLEVERING AV PRØVEMATERIALE

Det kan være aktuelt at biologisk materiale utleveres til forskningsinstitusjoner i inn- og utland (innad i EU og USA) som et ledd i internasjonalt samarbeid. Materialet vil i så fall kun utleveres uten navn, fødselsnummer eller andre personidentifiserbare opplysninger. Data som overføres til USA behandles i henhold til EUs personverndirektiv.

DET ER FRIVILLIG Å DELTA

Å avgi biologisk materiale til Kartlegging av normalmetabolomet er frivillig og krever samtykke. Det vil ikke ha noen betydning for deg dersom du velger å ikke avgi prøve, eller dersom du senere ønsker å trekke deg.

Dersom du ønsker å delta og godkjenner at prøvene dine lagres for eventuell bruk i fremtidige forskningsprosjekter, undertegner du samtykkeerklæringen på siste side. Der vil det bli spurt spesifikt om studier av normalmetabolomet, bruk av prøvene som normalkontroll i andre forskningsprosjekter, og om det kan utføres relevante analyser på gennivå knyttet til kartlegging av normalmetabolomet.

MULIGHET FOR Å TREKKE SITT SAMTYKKE, INNSYNSRETT, ENDRING OG SLETTING AV OPPLYSNINGER

Du kan til enhver tid få innsyn i hvilket materiale som er lagret fra deg. Du kan når som helst kreve at materialet blir destruert, uten at du må oppgi noen grunn. Destruksjon av materialet vil imidlertid ikke innebære sletting av utledete opplysninger som har inngått i sammenstilling eller analyser eller er brukt i vitenskapelige publikasjoner.

KONTAKT

Kontaktperson/Ansvarlig for biobanken:

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SAMTYKKE TIL LAGRING AV BIOLOGISK MATERIALE

Jeg avgir herved bredt samtykke til at mitt biologiske materiale kan oppbevares varig i biobanken *Kartlegging av normalmetabolomet* og brukes til ulike studier som omhandler kartlegging av normalmetabolomet, som normalkontroll i andre forskningsprosjekter, og at det kan utføres relevante analyser av mine prøver på gennivå knyttet til kartlegging av normalmetabolomet. Alle forskningsprosjekter hvor mitt biologiske materiale benyttes skal være godkjent av regional komité for medisinsk og helsefaglig forskningsetikk, personvernombud og andre relevante instanser.

Sted og dato	Deltakers signatur	
	Deltakers navn med trykte bokstaver	