INTEGRATED WASTEWATER MANAGEMENT FOR SUSTAINABLE AGRICULTURE OPERATIONS USING CYANOBACTERIA

by

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ABSTRACT

INTEGRATED WASTEWATER MANAGEMENT FOR SUSTAINABLE AGRICULTURE OPERATIONS USING CYANOBACTERIA

Continuous rise in world population causes higher demands for food and agricultural resources in an unsustainable way. To ensure growth and access to safe food now and meet demands in future, sustainable and economically feasible measures are needed urgently. One of the major problems in agricultural operations is management of wastewater. Despite the enriched nutrient and embedded energy contents of agricultural wastewater, environmentally sound and economically feasible methods to reuse these sources are still not at desired levels. In this thesis research, cyanobacteria *A. maxima* was cultivated in chitosan pretreated manure wastewater in custom-made photobioreactors. With pretreatment, total nitrogen, total phosphorus, and chemical oxygen demand were removed by 84.8%, 92.7%, 64.1%, respectively, and with cyanobacteria by 14.6%, 5.6%, 28.8%, respectively. Overall removal of 99.4% of total nitrogen, 98.1% of total phosphorus, and 92.9% of chemical oxygen demand were achieved. For comprehensive evaluation of biofertilizer use of biomass, protein, carbohydrate, lipid, fatty acid methyl esters, vitamins, amino acids, and elemental composition were analyzed. Biomass had protein content of 41.8%, total carbohydrate of 27.3% and total lipid of 24.1%. Biomass also had significantly higher amounts of B vitamins and considerable amounts of free and bound amino acids, some of which are key indicators of biostimulant presence. The study aimed to evaluate the use of cyanobacteria for wastewater treatment and harvested biomass as biofertilizer for farm applications. Overall aim was to suggest a sustainable livestock and farm management option that can manage its waste and resources in a sustainable and economically feasible way.

ÖZET

SÜRDÜRÜLEBİLİR HAYVANCILIK VE TARIM SİSTEMLERİ İÇİN SİYANOBAKTERİLER İLE ENTEGRE ATIK SU YÖNETİMİ

Dünya nüfusundaki sürekli artış, sürdürülebilir olmayan bir şekilde gıda ve tarımsal kaynaklara olan talebin artmasına neden olmaktadır. Büyümeyi ve güvenli gıdaya erişimi şimdi sağlamak ve gelecekteki talepleri karşılayabilmek için acilen sürdürülebilir ve ekonomik olarak uygulanabilir önlemlere ihtiyaç vardır. Tarımsal faaliyetlerdeki en büyük sorunlardan biri atık suların yönetimidir. Tarımsal atık suların zengin besin ve enerji içeriğine rağmen, bu kaynakları yeniden kullanmak için çevresel olarak sağlıklı ve ekonomik olarak uygulanabilir yöntemler hala istenilen seviyelerde değildir. Bu tez araştırmasında, siyanobakteri *A. maxima*, özel yapım fotobiyoreaktörlerde, kitosan ile ön arıtmaya tabi tutulmuş gübre atık suyunda yetiştirildi. Ön arıtma ile toplam nitrojen, toplam fosfor ve kimyasal oksijen ihtiyacı sırasıyla %84,8, %92,7, %64,1 ve siyanobakterilerle sırasıyla %14,6, %5,6, %28,8 oranında giderilmiştir. Toplam nitrojenin %99,4'ünün, toplam fosforun %98,1'inin ve kimyasal oksijen ihtiyacının %92,9'unun sonuç olarak uzaklaştırılması sağlandı. Biyokütlenin, proteini, karbonhidratı, lipidleri, yağ asidi metil esterleri, vitaminleri, amino asitleri ve element bileşimi biyogübre kullanımının kapsamlı değerlendirmesi için analiz edildi. Biyokütlenin toplam protein içeriği %41,8, toplam karbonhidrat içeriği %27,3 ve toplam lipid içeriği %24,1 olarak bulundu. Biyokütle ayrıca, önemli ölçüde daha yüksek miktarlarda B vitaminine ve önemli miktarlarda serbest ve bağlı amino asitlere sahipti. Bulunan bazı amino asitler biyouyarıcı varlığının temel göstergeleridir. Çalışma, atık su arıtımı için siyanobakterilerin kullanımını ve hasat edilen biyokütleyi çiftlik uygulamaları için biyogübre olarak değerlendirmeyi amaçladı. Genel amaç, atıklarını ve kaynaklarını sürdürülebilir ve ekonomik olarak uygulanabilir bir şekilde yönetebilen, sürdürülebilir bir hayvancılık ve çiftlik yönetimi seçeneği önermekti.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Unit
%	Percent	
λ	Lambda	nm
μ	Micro	
μL	Microliter	
μm	Micrometer	
μg	Microgram	
~	Approximately Equal	
°C	Degree Celsius	

Abbreviation	Explanation
ACN	Acetonitrile
BOD	Biological Oxygen Demand
BSA	Bovine Serum Albumin
C	Carbon
COD	Chemical Oxygen Demand
DIW	Deionized Water
DTT	Dithiothreitol
FA	Formic Acid
FAME	Fatty Acid Methyl Ester
FAO	Food and Agriculture Organization
FID	Flame Ionization Detector
FWW	Farm Wastewater
GC	Gas Chromatography
GHG	Greenhouse Gas
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
K	Potassium
LC-MS/MS	Liquid Chromatography-Mass spectrometry
M	Molarity
MBTH	3-methyl-2-benzothiazolinone hydrazine
MeOH	Methanol
N	Normality

N	Nitrogen
Na_2EDTA	Ethylenediaminetetraacetic Acid
NED	N-(1-naphtyl)-ethylenediamine dihydrochloride
nm	Nanometer
$N\text{-}NH_4^+$	Ammonia Nitrogen
N/A	Not Applicable
P	Phosphorus
PBR	Photobioreactor
PET	Polyethylene terephthalate
rpm	Revolutions Per Minute
SAG	The Sammlung von Algenkulturen der Universität Göttingen (Culture Collection of Algae at Göttingen University)
SIM	Stable Isotope Mix
TDS	Total Dissolved Solid
TN	Total Nitrogen
TP	Total Phosphorous
TS	Total Solid
TSS	Total Suspended Solid
UN	United Nations
UPW	Ultra-Pure Water
US	United States of America
UV	Ultraviolet
VIS	Visible
VS	Volatile Solid
WW	Wastewater

1. INTRODUCTION

At the beginning of the 19th century, the world human population was barely a billion. Yet, as of May 2022, it has doubled since the 1960s and reached almost 7.9 billion, according to the United States (US) Census Bureau (Gilland, 2002). Population is still increasing today, however, its growth rate is at its lowest since the 1950s (UN, 2019). There are several estimations for the growth for the century and the most possible one predicts that the population will reach almost 8.5 billion in 2030, and 9.7 billion in 2050, and 10.9 billion in 2100 (UN, 2019). According to the United Nation (UN) prospects, there is also a 27% possibility that the world's population could balance or begin to decrease sometimes before 2100 depending on different fertility scenarios (UN, 2019). Increased human population means increased amount of disturbance and damage on the planet and its resources, which are already overexploited. As the population continue to increase, limits of the land, water, energy, and environmental habitats are experienced (Janzen, 2011). Food supplies become less secure, clean energy reservoirs decline, freshwater resources diminish, atmospheric ability to absorb emissions weakens and habitable places for humans and other living organisms become scarce (Janzen, 2011).

Agriculture feeds billions of people, however, unsustainable food production as a result of increasing demand has very diverse and negative effects on the environment. It contributes to greenhouse gas (GHG) emissions, which in turn affects itself (FAO, 2021), pollutes the environment with nutrient run-off, causes water shortages due to over-consumption, leads to soil degradation and loss of biodiversity through land conversion and poor management, and ecosystem disruption due to the intensive harvesting of fish and other aquatic foods (Rayfuse & Weisfelt, 2012). It generates emissions of carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4), and is responsible for 10-12 % of the total GHGs (Friel et al., 2009; Smith et al., 2008). Almost half of these GHG emissions are generated during farming practices (Friel et al., 2009). Agriculture also consumes largest amount of water, accounting for 70% of total freshwater usage in the world (Cuellar-Bermudez et al., 2017; Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A. & Tempio, 2013).

One of the major competitors of agricultural land, energy and water is animal agriculture. Increased demand for meat and dairy products creates one of the greatest challenges for the food system (Gerber et al., 2013; Rayfuse & Weisfelt, 2012). Whilst the living standards continue to

improve in many parts of the world and increasing number of the middle class (Gerber et al., 2013), it seems unlikely that there will be a change in dietary choices from carnivorous to vegetarian or vegan (McAllister, Beauchemin, McGinn, Hao, & Robinson, 2011). Moreover, the past trends show the exact opposite is the case (McAllister et al., 2011). 27% of the calories are supplied by animal products in developed countries and 13% in developing countries. Animal products will continue to be a part of dietary choices for those who can afford it (Gilland, 2002). Over the past 50 years, it has already caused almost a 1.5 fold increase in the global numbers of cattle, sheep and goats, and an increase of almost 2.5 and 4.5 fold for pigs and chickens, respectively (FAO, 2009). On a global scale, the demand for livestock products is predicted to increase over the next 30 years, almost doubling the current numbers (Friel et al., 2009; McAllister et al., 2011).

Main objective of this thesis study is to eliminate some of these negative effects by offering a self-sustainable farm system that recovers and upcycles its resources and nutrients by utilizing a cyanobacterial growth system on floating reactors that can be placed on a nearby pond. Animal manure that is traditionally used as an organic fertilizer was converted into algae-based valuable products, i.e., bio-fertilizer, and wastewater was treated. The manure collected from livestock is separated into two phases that are liquid and solid, and liquid phase was used as a cultivation medium for cyanobacteria by filling it into custom made floating photobioreactors (PBR) in the lab that are simulated to be placed onto the surface of a pond in field. Following growth in liquid manure wastewater, cyanobacteria is harvested, and the biomass is evaluated for fertilization purposes of the farm. As a result of these processes, manure wastewater generated during the farm operations is treated and transformed into new value-added sources. Cyanobacteria utilize nutrients such as nitrogen (N) and phosphorus (P), readily present in manure wastewater that otherwise will be discharged either to some aquatic system nearby or diffused into the soil resulting from storage or soil applications and end up as agricultural run-off.

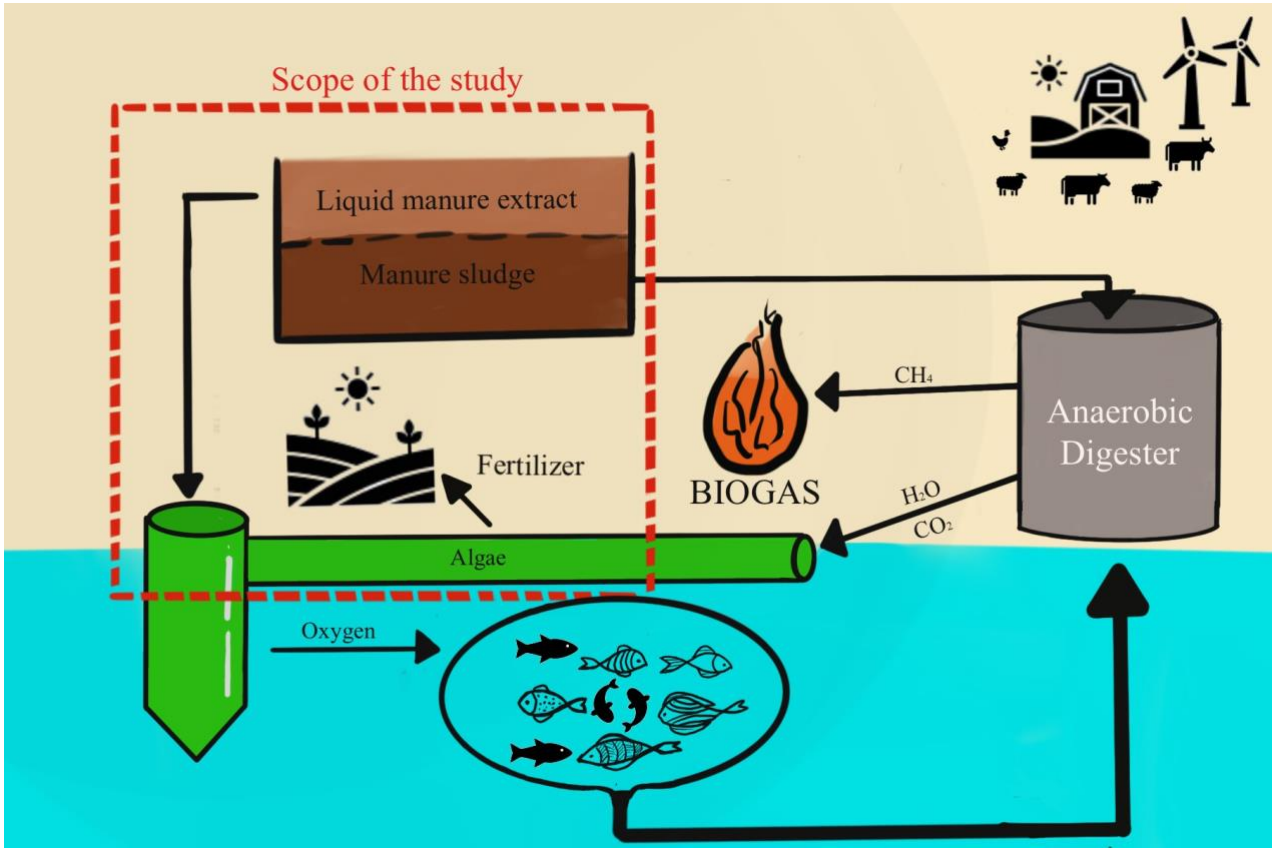


Figure 1.1. Suggested integrated waste and wastewater management system.

In this suggested comprehensive waste management system (Figure 1.1), remaining solid phase of the manure is directed to an anaerobic digester, where organic carbon is converted to CO_2 and CH_4. Produced CH_4 is used as a source of energy, that can generate electricity or heat, or it can replace any natural-gas-using process if upgraded to bio-methane. Generated heat can either be used in the farm itself or for sanitary purposes of the liquid phase of the wastewater. CO_2, that is also produced in addition to CH_4 in the digestion process, is directed into PBRs to enhance the growth of cyanobacteria. Any remaining biomass that cannot be utilized by means of fertilization is sent to the digestion facility as another carbon source in addition to the solid phase of manure. Water that is treated with the help of cyanobacteria is diffused into the pond system or used in the farm and agricultural applications. Additionally, pathogens and drug residues that might be present in the excreta is eliminated since manure is not applied onto soil, where proliferation and accumulation of these are possible. Water is recycled through purification and can help decrease the freshwater demand of the farm. In the end, there is almost no GHG emissions, no nutrient run-off due to excess use of fertilizers or manure soil applications, and no excessive water consumption. Every part of the farm provides for another part, creating a sustainable and circular system.

2. LITERATURE REVIEW

2.1. Agriculture and Livestock Production

One of the most delicate issues that arise with increased human numbers is agriculture. It feeds not only humans but also animals that are raised for human consumption. Today, agricultural lands occupy 37% of all land on the Earth (Smith et al., 2008), and at least for the next 40 years, food supply from these lands is expected to increase (Rayfuse & Weisfelt, 2012). In order to meet that demand, it is predicted that the agricultural food production needs to increase by almost 70% (Karunasagar & Karunasagar, 2016). In the past, the solution for such need of increase in production would be opening new lands for agriculture and exploiting new fish stocks (Rayfuse & Weisfelt, 2012). With current rates of population growth, to be able to maintain the same food consumption levels, agricultural lands would need an area equivalent to half to two-thirds of the current terrestrial land area by 2030 and 2070, respectively (Schneider et al., 2011). However, the most likely scenario is that the more food will need to be produced from the same or even less amount of area (Rayfuse & Weisfelt, 2012). One of the reasons for this situation is that the accelerating competition for land, water, and energy. The same amount of land is to be used for human settlements, industrial and agricultural purposes, sustaining the biodiversity and more. There is even competition amongst the agricultural land use for food production, animal agriculture, and energy crop agriculture. Another reason is that the current agricultural lands have been degraded by erosion, excessive disturbance due to fertilization, irrigation and pesticide applications, organic matter loss, salinization, acidification or other processes that diminishes the productivity (Smith et al., 2008). Even with improved seed quality, improved technology and/or fertilization, there are limits to what can be grown (Gilland, 2002).

The total use of fertilizers have increased 40% in 2019 when compared to 2000 (FAO, 2021). The number is 33% for N, 34% for P and 73% for potassium (K) (FAO, 2021). However, extensive usage of mineral fertilizers to replace the diminishing soil nutrients have adverse effects on both local and global environments (Basosi, Spinelli, Fierro, & Jez, 2014). It causes reduction in soil organic carbon content, resulting in degraded soil microbial community and in turn, higher emission of CO_2 and CH_4 (Basosi et al., 2014). Proliferated amounts of heavy metals such as arsenic, cadmium and lead are also linked with the excess fertilizer applications (Jiao, Chen, Chang, & Page, 2012). Additionally, production process of the fertilizers are responsible for 2-3% of total world energy consumption (Basosi et al., 2014).

Livestock sector provides jobs and creates livelihoods for nearly 1 billion people (FAO, 2021). Livestock products also provide almost one-third of humanity's protein intake (Gerber et al., 2013; Gilland, 2002) and are a potential remedy for undernourishment (Gerber et al., 2013). However, these benefits do not come without consequences. The sector is the sole largest anthropogenic land user; the overall area corresponds to 26% of the ice-free terrestrial surface of the planet, accounts for 70% of all agricultural land (Gerber et al., 2013; IPCC, 2019). It is responsible for the 18% of GHG emissions – a higher share then the transportation sector (Gerber et al., 2013; Smith et al., 2008). It accounts for 9% of human-induced CO_2 emissions – the biggest factor causing this trend comes from land-use changes, specifically deforestation caused by expansion of pastures and arable land for feed-crops (Gerber et al., 2013). CH_4 comes from livestock operations is due to enteric fermentation and manure, and accounts for almost 50% of global CH_4 emissions; and for N_2O emissions, most of it comes from soils that are applied N-fertilizers and manure storage, and accounts for 60-80% of the global N_2O emissions (Crosson et al., 2011; Gerber et al., 2013; Li, Zhang, Li, & Zhao, 2012; O'Mara, 2011; Smith et al., 2008). The water used by the livestock sector is almost over 8% of all freshwater use and mostly for irrigation of feed-crops, that accounts for 7% (Gerber et al., 2013). The sector is the biggest source of water pollution, causing eutrophication, dead zones in coastal areas, degradation of coral reefs, the emergence of antibiotic resistance, human health problems and many others (Gerber et al., 2013). The main sources of these pollutions are from animal manure, antibiotics and hormones, chemicals from tanneries, fertilizers, herbicides, and pesticides used for feed-crops and sediments from eroded pastures (Gerber et al., 2013). Moreover, the livestock sector may be the main reason behind the of loss of biodiversity since it is the major sector that causes land degradation and deforestation continuously (Gerber et al., 2013).

2.2. Wastewater, Manure and Manure Wastewater Treatment with Cyanobacteria

Wastewater (WW) is a medium that is rich in nutrients, water, and energy even though it is considered as waste (Drexler, Joustra, Prieto, Bair, & Yeh, 2014; Rawat, Bhola, Kumar, & Bux, 2013). Domestic WW contains high amounts of organic carbon, different forms of N and P (Selvaratnam et al., 2014) (Table 2.1). Discharging limits of these contaminants need to be met to prevent eutrophication and accumulation of other substances in receiving water bodies (Rawat et al., 2013; Selvaratnam et al., 2014).

Table 2.1. Typical composition of untreated domestic wastewater adopted from (Rawat, Ranjith Kumar, Mutanda, & Bux, 2011).

Contaminants	Unit	Concentration		
		Weak	Medium	Strong
Total Solids (TS)	mg/L	350	720	1200
Total dissolved solids (TDS)	mg/L	250	500	800
Total Suspended solids (TSS)	mg/L	100	220	350
Chemical Oxygen Demand (COD)	mg/L	250	500	1000
Total Nitrogen (TN)	mg/L	20	40	85
Total Phosphorus (TP)	mg/L	4	8	15

Current data suggest that on average 70% of high-income countries treat their WW while this percent is 38% for middle-income countries and only 8% for low-income countries (Sato, Qadir, Yamamoto, Endo, & Zahoor, 2013). Meanwhile, 2 million tons of human waste is predicted to be disposed to water bodies each day (Cuellar-Bermudez et al., 2017). Wastewater treatment is traditionally achieved by removing contaminants, namely solids, chemicals, microorganisms, and making the effluent safe and suitable for discharge (Rawat et al., 2011). Nitrogen is removed by bacterial utilization, released mostly as nitrogen gas (N_2), thus discarding its value as a fertilizer, and P is generally mitigated by physicochemical processes (Rawat et al., 2013; Selvaratnam et al., 2014). However, microalgae and cyanobacteria has recently drawn extensive attention as an environmentally and economically profitable possible option for these operations (Chang, Lee, & Den, 2013; Rawat et al., 2013; Zhou et al., 2012) even though its discussion as an alternative nutrient removal tool has been around since the 1950s (Oswald, Gotaas, Golueke, Kellen, & Gloyna, 1957).

Growing cyanobacteria in WW has tremendous benefits (Komolafe et al., 2014). Cyanobacteria can remove the excess nutrients such as N and P from the WW while sequestering CO_2 from the air (Chang et al., 2013; Selvaratnam et al., 2014) as well as decreasing COD (Komolafe et al., 2014) as the biomass increases (Rawat et al., 2011). This biological treatment will further increase the efficiency of metal and pathogen removal (Komolafe et al., 2014; Rawat et al., 2011). In this scheme, competition for water would not be present and there would be no excess nutrient addition to the environment. Instead, these constituents would be recycled and no additional waste would be generated in addition to almost no CO_2 emissions (Assemany, Calijuri, Do Couto, Santiago, & Dos Reis, 2015; Drexler et al., 2014; Komolafe et al., 2014; Rawat et al., 2011). In addition, overall costs could be lowered for both the WW treatment process and cultivation of algae (Komolafe et al., 2014; Lizzul et al., 2014; Rawat et al., 2011), which otherwise uses minerals and fertilizers, that accounts for 50% of cultivation costs (Rawat et al., 2013). Moreover, photosynthesis that would be done during the WW treatment would replace the need for mechanical aeration (Kotteswari, Murugesan, & R, 2012; Muñoz, Köllner, & Guieysse, 2009; Rawat et al., 2011), increase the efficiency of oxidation of

pollutants and lower the chances of a pollutant's evaporation due to mechanical aeration (Kotteswari et al., 2012; Muñoz et al., 2009; Rawat et al., 2011). Furthermore, the biomass that would be produced during the treatment can later be used for other valuable resource production such as biofuels, pigments, chemicals, fine chemicals, proteins, lipids, carbohydrates, solvents, pharmaceuticals, animal feed and biofertilizer (Rawat et al., 2013, 2011).

A broad range of cyanobacteria species, especially *Phormidium, Oscillatoria, Anabaena,* and *Spirulina (Arthrospira),* has been reported by many studies as effective to treat domestic WW (Chinnasamy, Bhatnagar, Hunt, & Das, 2010; Komolafe et al., 2014; Kong, Li, Martinez, Chen, & Ruan, 2010; Olguín, 2003; Wang et al., 2010). Particularly, nutrient and metal levels decreased rapidly after being exposed to cyanobacteria (Wang et al., 2010) and cell numbers of some species were doubled in 24 hours, and some doubled only in 4 hours (Komolafe et al., 2014). According to a research (Komolafe et al., 2014), cyanobacteria, *Oscillatoria* and *Arthrospira,* were the two indigenous dominant species in one of their reactors that contain domestic WW. They reduced TN by 55.4% and TP by 30.1%. Total coliforms were fully eliminated, as well. In another research (Zhai et al., 2017), *Spirulina platensis* was used to treat artificial domestic WW. Upon optimizing the growth conditions, its N and P removal efficiencies were 81.51% and 80.52% respectively. It is also mentioned both in this study and in several other studies that instead of using mono-cultures, mixed cultures could increase the removal efficiencies of WW constituents (Peccia, Haznedaroglu, Gutierrez, & Zimmerman, 2013).

Another study was done using anaerobically digested palm oil mill effluent (Zainal, Yaakob, Takriff, Rajkumar, & Ghani, 2012). *Spirulina platensis* was able to reduce the heavy metal amounts remarkably. Manganese was reduced by 84.9%; chromium by 83.8%; arsenic by 71.4%; nickel by 61.9%; zinc by 55%; copper by 52.8% and iron by 45.1%, showing that the Spirulina cultures can also be used to treat WWs that are contaminated by heavy metals.

Manure is another valuable source of nutrients, water, and energy. It is a good fertilizer for crops having nutrients primarily N, P and K (Lorimor, Powers, & Sutton, 2008) (Table 2.2). Especially filamentous cyanobacteria are great candidates for treatment process since they can grow in good amounts rapidly and due to the structure and size of them, they are relatively easy to harvest (Markou & Georgakakis, 2011).

A research was done using poultry litter that consisted of poultry manure, waste feed, bedding material, feathers and broken eggs (Markou & Georgakakis, 2011). After 9 days of cultivation in 400 mL bubble column reactors with several dilutions, *A. platensis* culture was able to remove ~99% of TP, and 38-40% of K, which presents a high load for poultry litter, in all dilutions. Protein removal rate was also investigated and was found to be 45% (Markou & Georgakakis, 2011). This suggests that the crude proteins in WW were utilized by the cyanobacteria.

Table 2.2. Several macro- and micro-nutrients contained in selected agro-industrial waste (W) & WW adopted from (Markou & Georgakakis, 2011).

W&WW	K	Na	Ca	Mg	Mn	Ni	Cu	Co	Fe	Zn	Ref.
Poultry manure	12.5-32.5 mg/g	2-7.4 mg/g	36.2-59.6 mg/g	1.8-6.6 mg/g	259-378 µg/g		38-68 µg/g		8-560 µg/g		(Edwards & Daniel, 1992)
Digested poultry manure	592 mg/L	214 mg/L	42 mg/L	54 mg/L	0.1 mg/L		0.04 mg/L	0.12 mg/L	2.5 mg/L	0.1 mg/L	(Belkin & Boussiba, 1991)
Digested cattle manure	116 mg/L	38 mg/L	171 mg/L	60 mg/L	0.12 mg/L		0.04 mg/L	0.02 mg/L	9 mg/L	0.44 mg/L	(Belkin & Boussiba, 1991)
Dairy wastewater	8.6-155.5 mg/L	263-1265 mg/L	1.4-58.5 mg/L	6.5-46.3 mg/L	<1-835 µg/L	2-71 µg/L	<1-30 µg/L	1-7 µg/L	39-4329 µg/L		(Danalewic, Papagiannis, Belyea, Tumbleson, & Raskin, 1998)

A study was done using *Spirulina maxima* with supernatant of aerated swine manure (Cañizares & Domínguez, 1993). *S. maxima* was able to grow at several dilutions, and even in the effluent without any dilutions, showing that its tolerance to inorganic contaminants. Yet, the best ammonia nitrogen ($N\text{-}NH_4^+$) and TP removal efficiency, 75% and 53% respectively, was achieved using 50% effluent. The same group did another research with the same set-up but this time immobilizing the cyanobacteria in K-carrageenan (Cañizares et al., 1994). This time removal efficiencies were increased notably to 80% and 90% respectively.

Spirulina platensis was used in another study with swine wastewater in several dilutions (Mezzomo et al., 2010). The maximum growth rate was achieved when the wastewater concentrations were 5% and 8.5%. On the other hand, the highest COD removal, that is 26.5%, was seen when the wastewater concentration was diluted to 30% (v/v). And the highest TP removal efficiency was seen in a medium that had 8.5% wastewater by 41.6%. In the same study, addition of sodium bicarbonate was also investigated and found to promote the maximum cell number that can be attained.

Table 2.3. Characteristics of several agro-industrial wastes and wastewaters adopted from (Markou & Georgakakis, 2011).

W &WW	COD	TS	VS	TN	TP	REF
Poultry manure				18.2 – 72 mg/g manure	13.5 – 34 mg/g manure	(Edwards & Daniel, 1992)
Swine manure	68 g/L	4.8 %				(de la Noüe & Bassères, 1989)
Anaerobically digested swine manure	7.7 g/L	0.89 %				(Pouliot, Buelna, Racin, & Noüe, 1989)
Swine liquid waste	19.7 – 21,2	18.7 – 19.7 g/L	15 – 15.4 g/L	974.8 – 1025.5 mg/L		(Hill & Bolte, 2000)
Anaerobically digested swine liquid waste	9.68 – 12.9 g/L	10.7 – 13.59 g/L	6.08 – 9.94 g/L	891.2 – 1015.2 mg/L		(Park, Jin, Lim, Park, & Lee, 2010)
Anaerobically stabilized swine wastewater	343.6 – 840 mg/L	1.48 – 1.53 mg/L			2.27 – 5.7 mg/L	(Cañizares-Villanueva, Domínguez, Cruz, & Ríos-Leal, 1995)
Sheep		28 %	23 %	11,3 %		(Benemann, 1997)
Cattle slurry		8 – 11 %	75 – 82 % TS	2,6 – 6,7 % TS		(Campbell & Smith, 1986)
Cheese - whey	61 – 68.8 g/L			940 – 1480 mg/L	379 – 510 mg/L	(Demirel, Yenigun, & Onay, 2005)

In another early research, *Spirulina* cultivated in cattle waste leachate supplemented Zarouk medium (Mitchell & Richmond, 1988). 83.7% Zarouk and 16.7% leachate containing medium had the highest cell density. Furthermore, the authors added that the higher production rates were seen when $NaHCO_3$, nitrate and phosphate were added into the medium. The results were higher 3 times, 100% and 50%, respectively.

2.3. Bioproducts of from Cyanobacteria Grown in Wastewater

As the increased population demands more and more food supply, conventional agriculture became heavily dependent on chemical fertilizers (Bhardwaj, Ansari, Sahoo, & Tuteja, 2014). And they are the reason for increased crop productivity and utilization of soils that are otherwise deficient

in nutrients (Gellings & Parmenter, 2004). However, producing N-based fertilizers is an energy demanding process and accounts for almost a third of US crop production (Gellings & Parmenter, 2004). Similarly, mined P prices are on the rise due to decreasing level of supply on earth (Rawat et al., 2013) and the current agricultural usage levels, P reserves are expected to last 50-100 years (Chowdhury, Viamajala, & Gerlach, 2012).

A more sustainable and safer alternative to chemical fertilizers is biofertilizer (Hanapi, Awad, & Aziz, 2012). Biofertilizer is a substance that is of usually live or harvested microorganisms or natural substances that supply or improve the availability of primary nutrients to the plants and improve soil fertility (Gharagozloo et al., 2014; Hanapi et al., 2012). It can contain not only cyanobacteria but also bacteria and fungi, either separately or as a combination, depending on the plant need (Hanapi et al., 2012). Biofertilizers help the soil to be rich in all kinds of micro- and macro-nutrients by nitrogen fixation, phosphate, and potassium solubilization or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Sinha, Valani, Chauhan, & Agarwal, 2010). When plants' inability to uptake all the nutrients that are supplied by the fertilizer is taken into consideration, these microbial inoculants have a predominant importance in nutrient management to sustain agricultural productivity and healthy environment (Adesemoye & Kloepper, 2009).

One of the most suitable candidates of biofertilizers is diazotrophic cyanobacteria. They can both fix atmospheric N_2, when applied live to paddy soils, generate bioactive substances that enhance the plant growth, repress pathogen-sourced diseases, in addition to organic acids that enhance the P uptake of the plant (Hanapi et al., 2012). However, cyanobacteria cultivation requires water and fertilizer, as well, increasing the overall costs. For instance, almost half of cost of *Spirulina* culturing is related to use of fertilizers (Markou & Georgakakis, 2011). Coupling WW treatment with cyanobacteria cultivation is a potent way of decreasing both the cultivation and treatment costs (Markou & Georgakakis, 2011). Additionally, bioproducts such as biostimulants, biopesticides produced by the cultivated algae would decrease the costs even further (Bhardwaj et al., 2014; Khan et al., 2019; Markou & Georgakakis, 2011). Moreover, using biofertilizers, biostimulants, biopesticides can boost seed germination, plant growth, flower set, and expand the responses to biotic and abiotic stresses (Ferreira et al., 2021).

Biostimulants are plant hormones that have many key regulatory functions in plants (Balcke et al., 2012). They affect the plant's physiology in different ways from seed germination to pathogen

response (Gharagozloo et al., 2014). They are a diverse class of organic and inorganic substances either produced by the plant or supplied externally (Chiaiese, Corrado, Colla, Kyriacou, & Rouphael, 2018). Today most of the biostimulants on the market and in use are extracted from red, green, and brown algae and use of microalgae and cyanobacteria for that matter is still very rare (Chiaiese et al., 2018). Phytohormones include auxins, gibberellins, cytokinins, ethylene, abscisic acid, salicylic acid, and brassinosteroids (Trapp, De Souza, Rodrigues-Filho, Boland, & Mithöfer, 2014; Viegas, Gouveia, & Gonçalves, 2021). Some of these hormones are found to be present in microalgae, as well, such as auxins, gibberellins, and ethylene (Gharagozloo et al., 2014).

A research is done by using *Spirulina platensis* that was grown in aquaculture WW (Wuang, Khin, Chua, & Luo, 2016). Its ability to enhance the growth of leafy vegetables such as Arugula (*Eruca sativa*), Bayam Red (*Ameranthus gangeticus*) and Pak Choy (*Brassica* rapa ssp. *chinensis*) was demonstrated. Also, Chinese Cabbage (*B. rapa* ssp. *chinensis*) and Kai Lan (*Brassica oleracea alboglabra*) germinations were improved in terms of seedlings' dry weight when the cyanobacteria were applied as fertilizer.

Another research is done by using *Nostoc* sp., *Anabaena doliolum*, *Calothrix sp*., *Westiellopsis sp*. and *Phormidium papyraceum*, which were indigenous species that were grown on the fly-ash dumping site (Tripathi et al., 2008). Growth performances and elemental analyses of rice plants that were amended with various combinations of bio-fertilizer, fly-ash and garden soil were done. Results indicate that the integrated use of bio-fertilizers and fly-ash will help the plant for improved growth, yield, and mineral composition besides reducing the high demand of nitrogen fertilizers.

A recent study was done with *Chlorella protothecoides* and *Tetradesmus obliquus* that were grown in cattle manure that was pretreated with bio-ash to remove the solids (Viegas et al., 2021). In addition to removal of TN, TP, and COD to almost full extend, they were also able to see that the watercress and wheat seeds were positively affected by the addition of microalgae. Wheat seeds' germination index was calculated to be 178% for *C. protothecoides*, and 82% for *T. obliquus*. And watercress seed' germination index was calculated to be 34% for *T. obliquus*. It is not as high as wheat seeds but still there is a considerable increase when compared to the control group which was germinated using only distilled water.

Recent another study was conducted with piggery wastewater (Ferreira et al., 2021). *Synechocystis sp*., *T. obliquus*, *C. protothecoides*, and *C. vulgaris* were grown in 1:20 diluted WW.

All species were able to remove ammonia above 79% and phosphate was almost completely removed. As for COD, all removal rates were above 60%. Harvested biomasses were used to see their fertilization and stimulation ability with cucumber (*Cucumis sativus*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), soybean (*Glycine max*), watercress (*Nasturium officinale*), and tomato (*Licopersicon esculentum*). Also antifungal potentials of the biomasses were tested against fungi *Fusarium oxysporum*. They were able to observe that all the plants that were supplemented with biomasses positively affected by the addition. For instance, germination index for plants that were supplemented with *T. obliquus* were all above the 100%, which was accepted as the base level that was calculated from the control group, which was only treated with distilled water. Also, for the biopesticide tests, all the fungi that were exposed to algae suspensions grew less than the control group, except for the culture that was against *Synechocystis sp*., which was reported almost ineffective.

3. MATERIALS AND METHODS

This chapter describes the methods for collection and preparation of wastewater, cultivation of *Arthrospira maxima*, chemical analyses of wastewater, chemical and biochemical analyses of harvested biomasses.

3.1. Model Organism Maintenance and Cultivation

Arthrospira maxima is a filamentous cyanobacteria that thrives in alkali freshwater bodies (Figure 3.1). Their filament size can reach up to two mm. Model organism, strain SAG 84.79, used in this study is obtained from the SAG Culture Collection of Algae (SAG, Germany). It is maintained at 25°C with a 1200 lux light intensity and under 12h:12h light:dark cycle in a growth chamber (Nüve GC401, Turkey). Liquid Spir culture medium, which is prepared by preparing and autoclaving two solutions, Spir 1 and Spir 2, separately and mixing after autoclave, was used to cultivate. Subculturing was done in every 15-20 days. Spir 1 and Spir 2 culture medium, P-IV metal solution and Chu Micronutrient solution recipes are given in Table 3.1, Table 3.2, Table 3.3, and Table 3.4, respectively.

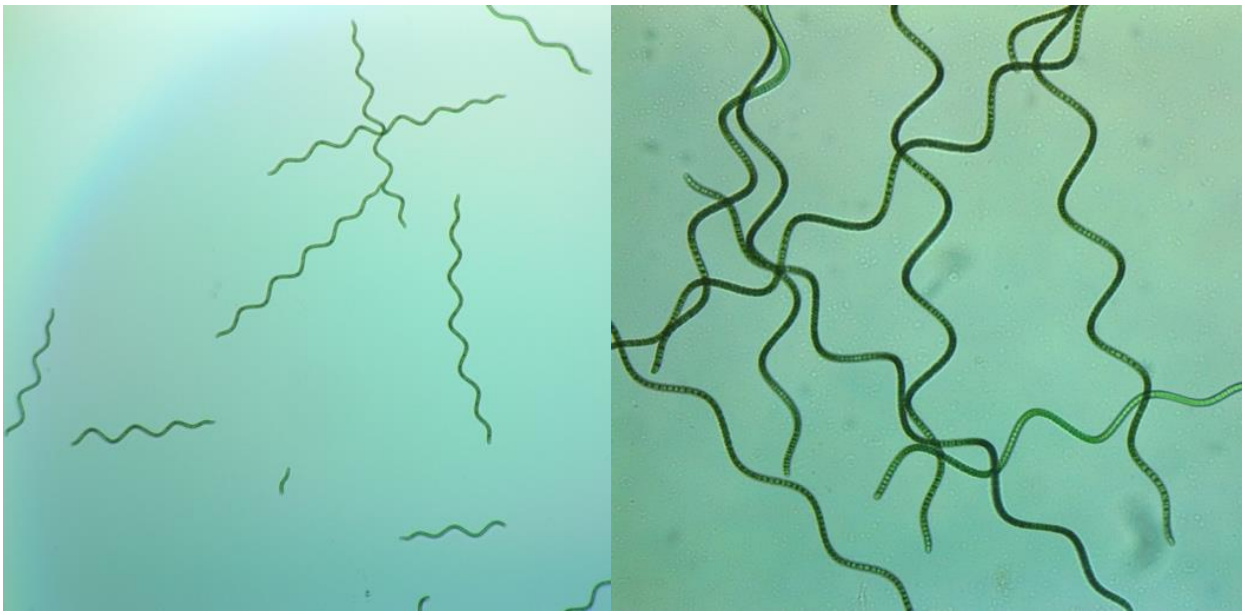


Figure 3.1. Light microscope image of *Arthrospira maxima*, strain SAG 84.79 taken at 4X (left) and 10X (right).

Table 3.1. Spir 1 culture medium ingredients.

#	Component	Amount (g/L ultra-pure water)	Final concentration (mM)
1	$NaHCO_3$	13.67	162
2	$NaCO_3$	4.03	38
3	K_2HPO_4	1	2.9

Table 3.2. Spir 2 culture medium ingredients.

#	Component	Amount	Final concentration (mM)
1	$NaNO_3$	2.5 (g/L)	29.4
2	K_2SO_4	1 (g/L)	5.74
3	NaCl	1 (g/L)	17.1
4	$MgSO_4.7H_2O$	0.2 (g/L)	0.81
5	CaCl2. $2H_2O$	0.04 (g/L)	0.27
6	P-IV Metal Solution	6 (mL/L)	
7	Chu Micronutrient Solution	1 (mL/L)	
8	Vitamin B_{12}	1 (mL/L)	

Table 3.3. P-IV metal solution ingredients.

#	Component	Amount (g/L)	Final concentration (mM)
1	$Na_2EDTA.2H_2O$	0.75	2
2	$FeCl_3.6H_2O$	0.097	0.36
3	$MnCl_2.4H_20$	0.041	0.21
4	$ZnCl_2$	0.005	0.037
5	$CoCl_2.6H_2O$	0.002	0.0084
6	$NaMoO_4.2H2O$	0.004	0.017

Table 3.4. Chu metal solution ingredients.

#	Component	Amount (g/L)	Final concentration (μM)
1	$CuSO_4.5H_2O$	0.02	0.08
2	$ZnSO_4.7H_2O$	0.044	0.15
3	$CoCl_2.6H_20$	0.02	0.084
4	$MnCl_2.4H_2O$	0.012	0.061
5	$NaMoO_4.2H_2O$	0.012	0.052
6	H_3BO_3	0.62	10
7	$Na_2EDTA.2H_20$	0.05	0.13

Prior to experimental procedures, maintenance cultures (Figure 3.2) were grown in custom-made Erlenmeyer flasks (Figure 3.2) to obtain seed cultures.

Figure 3.2. Representative maintenance cultures from laboratory (left), representative custom-made Erlenmeyer reactor (right).

3.2. Growth Curve Assay

To be able to see the growth profile of the cyanobacteria under the laboratory conditions, custom-made 1L Erlenmeyer flasks (Figure 3.3) that are the same as the ones that are used to obtain seed cultures, were used. Working volume of the reactors was 750 mL and Spir was used as the culturing medium. Each reactor was supplied with 0.5 L/min dry air passing through a 0.45-micron pore sized filter and diffusing through a 0.5 mm pore sized diffuser stone. Light intensity was kept at 3500 lux by using LED light under 12h:12h light:dark cycle. Additional mixing was provided with magnetic stirrers at approximately 150 revolutions per minute (rpm). Reactors were sealed with silicon caps and parafilm was used to cover the connection parts to ensure the culture sterility. All parts of the reactors were cleaned thoroughly and autoclaved prior to experiments. Sampling was done in every two days and the amounts that were sampled were replaced with fresh Spir medium in order to keep the culture volumes constant throughout the experiment. Optical density, dry weight, pH, and filament amounts were measured. Optical density measurement was done at 560 nm wavelength, which was determined by spectral scan of the individual culture (Figure 3.4). Experiment was conducted as biological triplicates.

Figure 3.3. 1L Erlenmeyer flasks used for growth curve assays.

Dry weight measurement was done according to (Zhu & Lee, 1997). 47 mm GF/F glass microfiber membrane filter was brought to constant weight by igniting in a muffle furnace (Daihan Scientific, South Korea) at 450°C for 2 hours and kept in a desiccator until use. After assembling the apparatus and the filter, first the filter was wet with 10 mL ultra-pure water (UPW) three times and all the water was vacuumed before filtering the culture. Then 10 mL of culture was filtered, and washing was done with same amount of UPW in order to eliminate any salt residue resulting from the culturing media. Filter paper with biomass was then dried for at least 2 hours at 105°C and brought to a constant weight.

Figure 3.4. Spectral scan of *A. maxima*, SAG 84.79.

3.3. Farm Wastewater Study

3.3.1. Collection of Farm Wastewater

Farm wastewater (FWW) was collected from a small dairy farm (Gündönümü Çiftliği, İstanbul, Turkey). The farm has their own simple wastewater collection system, that is apart from the municipal sewage system, and a primary separator. Manure, urine, and cleaning water that is used to clean the barns are collected through channels and directed to a concrete pit where they are mixed and pumped to the solid separator (Figure 3.5). Solid separation system is turned on when enough solids are accumulated to operate the system. Separated solids are used as fertilizers on the farm's fields that are used to grow the herd's feed.

FWW was collected at the effluent point of the separator by directly immersing the bottles into the stream. Collection was done by using 5 L polyethylene terephthalate (PET) bottles. After arrival to the laboratory, collected FWW was pooled in glass carboys, that were previously acid washed with 6 N HCl and autoclaved, for a more homogenous and representative sampling. Pooled wastewater was stored in 4°C cold room until the time of processing. TN, TP, COD, solids, and pH of the wastewater were determined immediately.

All the glassware that was further used in the experiments was washed with 6 N HCl and rinsed thoroughly with deionized water (DIW). Autoclave was used when sterile conditions needed.

Figure 3.5. Solid separator (top left), waste pit (top right), barn and field (bottom left), calf (bottom right).

3.3.2. Pretreatment of Farm Wastewater

Chitosan was used for the pretreatment of the farm wastewater as a coagulant as it is biobased. Its solution was prepared by first preparing a 2% acetic acid solution and then adding low molecular weight chitosan (Sigma Aldrich, St. Louis, MO, USA) to a final concentration of 0.45% (w/v) since chitosan requires acidic conditions to dissolve. Solution was left overnight on the stirrer for chitosan to dissolve completely. pH measurement was done before use.

A jar test was performed to determine the optimum amount of coagulant needed for complete removal of the solids and microorganisms (Figure 3.6). 50 mL of FWW was treated with varying amounts of chitosan solution from 8 mL to 28 mL with 2 mL increments. After addition of the solution, FWW was stirred at 200 rpm for 5 mins and then stirring was reduced to 80 rpm and continued for another 15-20 minutes. When stirring was turned off, FWW was left to settle for another 5 minutes. Coagulated solution was first filtered with a cheese cloth, that was placed over a beaker and fastened with a rubber band, so that the larger solids would be separated over a larger area more easily. Then the filtrate was passed through 4 metal sieves that have 850-, 250-,75- and 25-micron mesh sizes and were placed on top of each other from larger sized to lower sized. Optimum coagulant amount was decided by visually checking and comparing for solids and by microscopic control of the final filtrates.

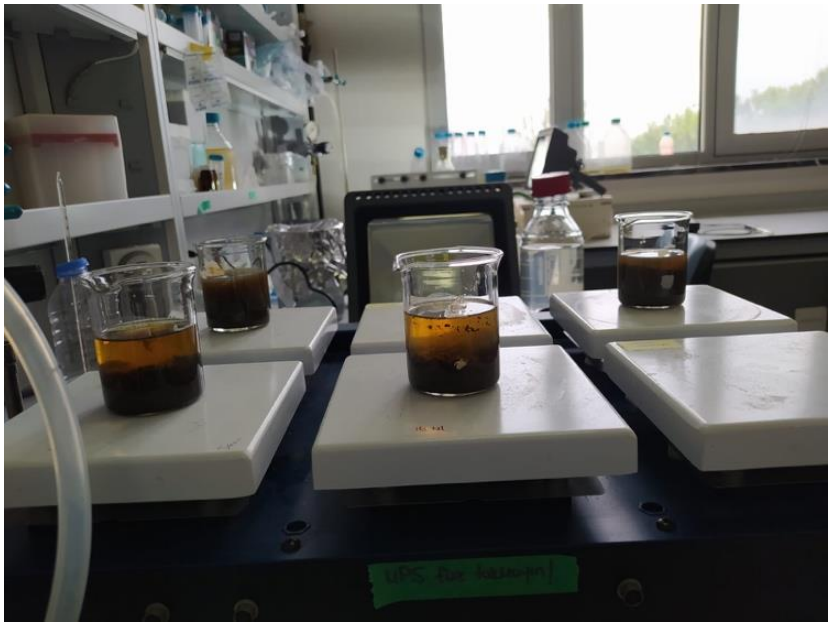
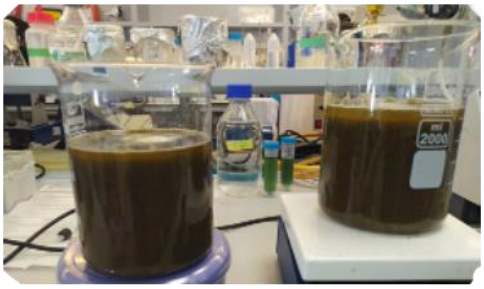


Figure 3.6. A section of the conducted jar test

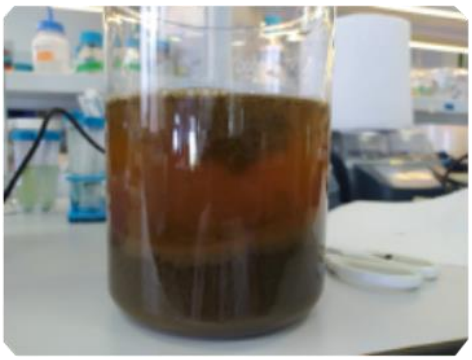
The rest of the FWW was treated accordingly after the optimum amount, which was 20 mL of chitosan solution per 50 mL of FWW, was determined (Figure 3.7). Decreased pH that was due to acidic chitosan solution treatment was corrected by using 2 M NaOH solution to the pH level of the culturing media, Spir, in order to support the growth of the cyanobacteria in further study. After pH adjustment, $NaHCO_3$ of 13.67 g/L was added to the final FWW and pasteurization was performed at 72°C for 30 minutes using a water bath (MaxTurdy-30, Daihan Scientific, Wonju, South Korea) to ensure a microorganism free medium. Pasteurized FWW was kept at 4°C until the time of use.

FWW was sampled into a 5 L glass bottle

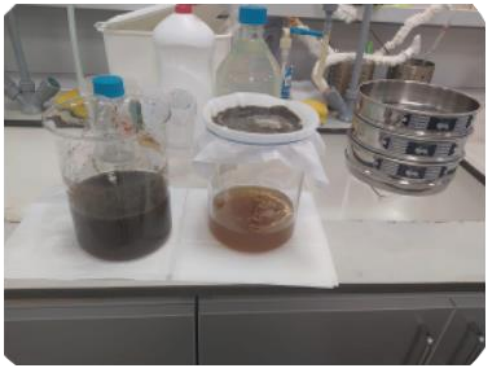
FWW was put into beakers and chitosan solution was added and mixed



FWW was mixed and settle for solids to aggregate



Aggregated solids were separeted by filtration



And a final clear solution was obtained

Figure 3.7. Pretreatment steps of FWW with chitosan solution.

3.3.3. Characterization of Farm Wastewater

Total N, total P, chemical oxygen demand and solids amounts were determined before and after pretreatment.

3.3.3.1. Total nitrogen determination. Total N is the sum of all the nitrogenous compounds in the medium, that are nitrate, nitrite, ammonium, dissolved and particulate organic nitrogen. Its determination was done by a spectrophotometric method that was developed and optimized by (Koistinen, Sjöblom, & Spilling, 2020). An optimized version of the common digestion method that is used to convert all the nitrogenous compounds to nitrate in an alkali medium while heating is followed by a reduction step of nitrates to nitrites by an acidic vanadium chloride reagent. Amines in the vanadium chloride reagent then form an azo dye that can be spectrophotometrically determined.

Oxidizing reagent was prepared by dissolving 10 g potassium peroxodisulfate and 6 g boric acid in 1 L 0.075 M sodium hydroxide solution. TN mix reagent was prepared by mixing solutions of sulfanilamide, N-(1-naphtyl)-ethylenediamine dihydrochloride (NED) and vanadium trichloride by using 40 mL, 40 mL, 200 mL of each respectively. Sulfanilamide solution was prepared by dissolving 1 g sulfanilamide in a solution of 85 mL UPW and 14.5 mL concentrated HCl. NED solution was prepared by dissolving 0.07 g NED in 100 mL UPW. Vanadium trichloride ($VnCl_3$) solution was prepared by dissolving 1.6 g $VnCl_3$ in a 170 mL UPW and 16.8 mL concentrated HCl mixture, and then completed to final volume of 200 mL. 5 mL of the sample and 5 mL of oxidizing reagent were added into a screw capped glass tubes and autoclaved for 30 minutes at 121°C. After the digestate cooled down to room temperature, 0.5 mL of digested sample and 3 mL of TN mix reagent were added into new tubes and incubated in a dry thermostat at 45°C for 1 hour. After cooling to room temperature, absorbance measurement was done at 545 nm wavelength by using a UV-Vis spectrophotometer (DR 3900, Hach, Colorado, USA). Calibration standards and blanks were treated same as the samples.

Total N standard of 140 μg/mL N (10 μmol/mL N) was prepared by dissolving 0.1862 g disodium ethylenediaminetetraacetic acid (Na_2-EDTA) in 100 mL UPW. Working solutions of concentrations 0.056, 0.112, 0.224, 0.336 and 0.560 mg/L were prepared fresh from TN standard on the day of experiments for the calibration curve (Figure 3.8). Equation 3.1 was used for sample total nitrogen calculations

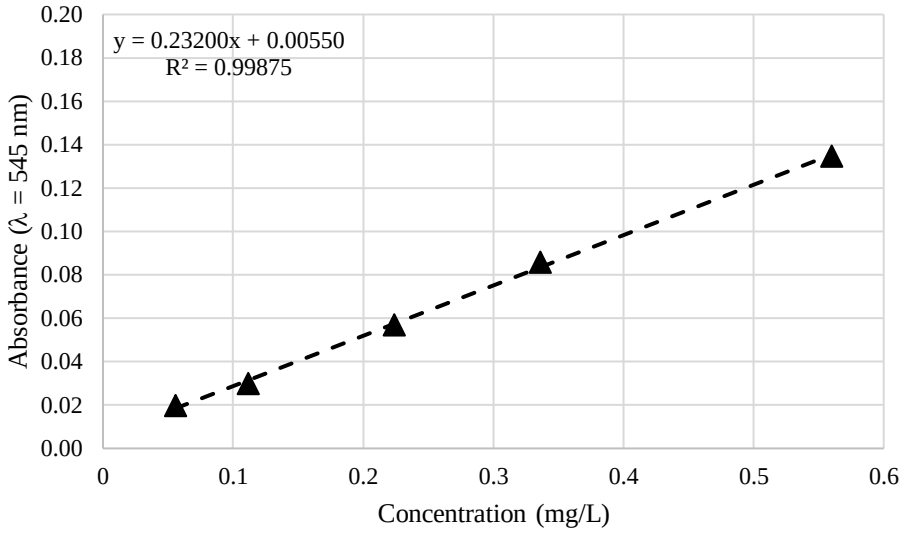


Figure 3.8. Calibration curve of Na_2EDTA.

$$y = 0.23200x + 0.00550 \quad (3.1)$$

where,

y = Absorbance of sample at 545 nm

x = Total nitrogen concentration of sample

3.3.3.2. Total phosphorus determination. International Standard that is developed for determination of different forms of P compounds present in ground, surface and wastewaters was used for determination of TP (International Organization for Standardization, 2004). All forms of P present in the media are converted to orthophosphate by oxidation with peroxodisulfate. Then orthophosphate ions, with an acid solution containing molybdate and antimony ions, form an antimony phosphomolybdate complex (International Organization for Standardization, 2004). Reduction of the complex with ascorbic acid to colored molybdenum blue complex allows the total phosphorus amount to be determined spectrophotometrically.

Appropriate amount of sample was added into a 100 mL Erlenmeyer flask with a screw cap and diluted to about 40 mL with UPW. 4 mL of 50 g/L peroxodisulfate solution was added into flasks and autoclaved at 121°C for 30 minutes. After the digestate cooled down to room temperature, pH was adjusted between 3-10 by using 2 M NaOH and 2 M sulfuric acid solution. Then the solution was poured into a 50 mL volumetric flask and while swirling 1 mL of 100 g/L ascorbic acid solution was added. After 30 seconds, 2 mL of acid molybdate solution was added into flask. Then volume was completed to 50 mL and flask was mixed thoroughly. Acid molybdate solution was prepared by

mixing 300 mL of 9 mol/L sulfuric acid, 100 mL of 130 g/L ammonium heptamolybdate tetrahydrate and 100 mL of 3.5 g/L antimony potassium tartrate hemihydrate solutions. Absorbance measurement was done within the next 10-30 minutes at 880 nm wavelength. Calibration standards and blanks were treated same as the samples.

Orthophosphate stock standard solution was prepared by dissolving 0.2197 g of KH_2PO_4, that was dried to constant mass at 105°C, in 800 mL UPW, adding 10 mL of 4.5 mol/L sulfuric acid and then completing to 1 L. Orthophosphate standard solution was prepared by diluting 20 mL of orthophosphate stock standard solution to 500 mL in a volumetric flask on the day of analyses. For calibration curve determination, from 1 mL to 10 mL of orthophosphate standard solution were used as representatives of concentrations from 0.04 mg/L to 0.4 mg/L (Figure 3.9). Orthophosphate concentrations of samples were calculated by using the Equation 3.2.

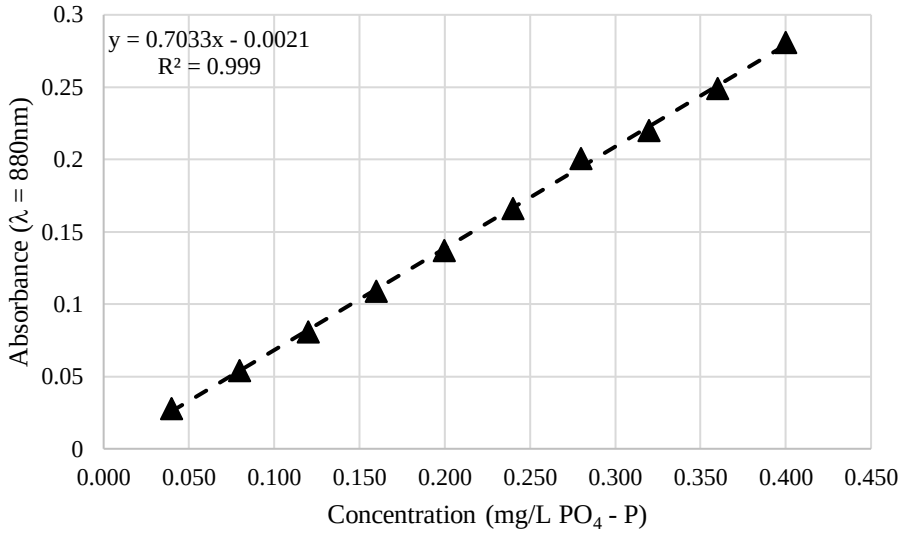


Figure 3.9. Calibration curve of KH_2PO_4.

$$y = 0.7033x - 0.0021 \quad (3.2)$$

where,

y = Absorbance of sample at 880 nm

x = Orthophosphate concentration in the sample

3.3.3.3. Chemical oxygen demand determination. COD is the measurement of the oxygen equivalent need of the specified oxidant to decompose the organic matter and oxidize the inorganic chemicals such as ammonia and nitrite. An optimized closed reflux method was used to determine the COD. In

principle, organic and inorganic matter are oxidized by $K_2Cr_2O_7$ in the presence of Ag_2SO_4 as catalyst in a strong acidic medium at elevated temperatures. $Cr_2O_7^{-2}$ ion is reduced to green Cr^{+3} ion in the process and the remaining yellow Cr^{+6} ion is measured spectrophotometrically at 620 nm wavelength.

3 mL COD solution, which was prepared by dissolving 1.63 g $K_2Cr_2O_7$, that was previously dried at 105°C for 2 hours, 6 g $HgSO_4$ and 6 g Ag_2SO_4 in 500 mL concentrated sulfuric acid, and 2 mL sample were added into a screw capped test tube. Then it was incubated in a dry thermostat (LT200, Hach, Colorado, USA) at 150°C for 2 hours. After cooling down to room temperature, spectrophotometric measurement was done at 620 nm wavelength. Calibration standards and blanks were treated same as the samples.

COD stock standard solution was prepared by dissolving 850 mg potassium hydrogen phthalate (KHP), that was previously dried overnight at 120°C, in 1000 mL UPW. Final concentration of the stock standard was 1000 mg/L COD. COD standard solutions of concentrations ranging from 300 mg/L to 900 mg/L were prepared on the day of analyses in order to determine the calibration curve (Figure 3.10). COD amount present in unknown samples were calculated using the Equation 3.3.

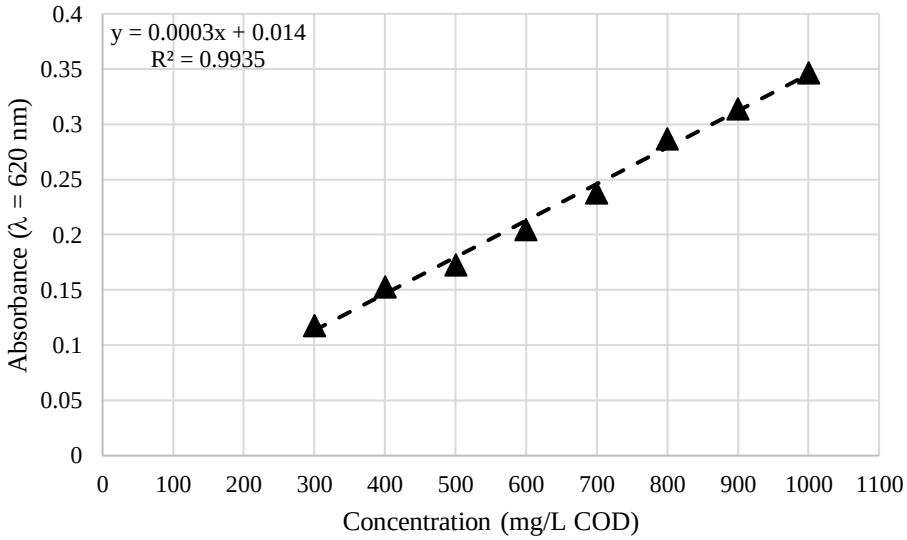


Figure 3.10. Calibration curve of KHP.

$$y = 0.0003x + 0.014 \quad (3.3)$$

where,

y = Absorbance of sample at 620 nm

x = COD concentration of sample

3.3.3.4. Solids determination. Solids of a wastewater or water is reported as the matter that is present in suspension or as dissolved form. Total solids (TS), total dissolved solids (TDS) and total suspended solids (TSS) measurements were done according to Standard Methods (APHA/AWWA/WEF, 2012). Briefly, TS and TDS were measured by using evaporating dishes that were previously brought to constant weight by igniting in a muffle furnace (Daihan Scientific, South Korea) at 550°C for 1 hour. For TS 10 mL of FWW was evaporated to dryness in a drying oven at 105°C. For TDS, 10 mL of FWW was filtered through a 47 mm GF/F glass microfiber membrane, that was brought to constant weight by the same procedure as the evaporating dishes prior to experiment. Filtrate was transferred to an evaporating dish and evaporated to dryness in an oven at 105°C. For TSS, glass microfiber membrane that was used to filter the FWW was evaporated to dryness in an oven at 105°C. All the samples were brought to constant weight by repeating the drying, cooling, desiccating, and weighing cycle. TS, TDS and TSS were calculated according to the Equation 3.4, Equation 3.5, and Equation 3.6, respectively.

$$\text{mg Total Solids/L} = \frac{(A-B)\times 1000}{\text{sample volume, mL}} \quad (3.4)$$

where,

A = weight of dried residue + dish, mg

B = weight of dish, mg.

$$\text{mg Total Dissolved Solids/L} = \frac{(A-B)\times 1000}{\text{sample volume, mL}} \quad (3.5)$$

where,

A = weight of dried residue + dish, mg

B = weight of dish, mg

$$\text{mg Total Suspended Solids/L} = \frac{(A-B)\times 1000}{\text{sample volume, mL}} \quad (3.6)$$

where,

A = weight of dried residue + filter, mg

B = weight of filter, mg

3.3.4. Multi Well Plate Study with Farm Wastewater

After characterization of FWW, a multi well plate study was conducted to determine the optimum amount of cyanobacteria culture to inoculate FWW in further plastic PBR study. 12-well plates were used (Figure 3.11) and in wells that have 2 mL working volumes, 4 different inoculation amounts were tried as $1/10^{th}$, $2/10^{th}$, $3/10^{th}$, and $4/10^{th}$ of the volume in 4 different well plates. In each plate, the same amount of culture was used to inoculate the Spir media for reference and comparison, as well. Culture used in the study was grown in Erlenmeyer flask as a seed culture. All plates also had a well with not inoculated Spir media as negative control and a well with UPW for blank. Daily absorbance measurements were taken to monitor the culture conditions by using a plate reader (SpectraMax i3, Molecular Devices, San Jose, CA, USA).

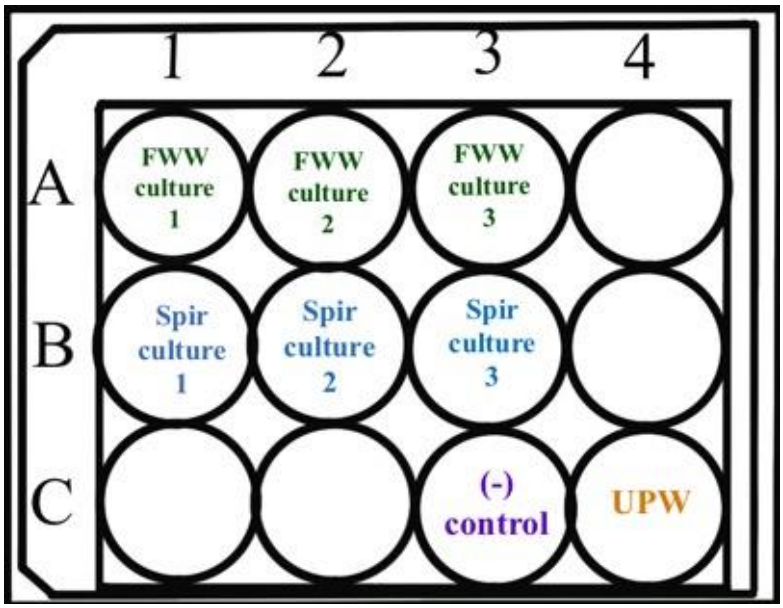


Figure 3.11. Representative multi well plate used in the experiment.

3.4. Plastic Photobioreactor Study

Arthrospira maxima, strain SAG 84.79, cultures were grown in custom-made plastic photobioreactors. A control study was conducted until the cultures reached the death phase of their growth. Optical density, dry weight, pH, and filament amount measurements were done daily. Same setup was used for a FWW study. Additional TN, TP and COD data were taken. TN and TP measurements were done on every other day, and COD data was taken on the first and last days of the culture. Harvested biomasses of both reactors were lyophilized and analyzed for their protein,

carbohydrate, lipid, amino acids, vitamins, fatty acid methyl esters, elemental profiles, and biostimulants for biofertilizer applications.

3.4.1. Design of Plastic Photobioreactors

Plastic PBRs were designed using low density polyethylene transparent hose that is originally made for agricultural irrigation. 1.30 m long fragments were cut from the hose that had 20 cm fixed width (Figure 3.12). Cut pieces were washed with hydrogen peroxide, rinsed with UPW and hanged for drying. Dried reactors' both ends were sealed with an impulse sealer (PCS-400, Brother, Texas, USA). Before loading the culturing media, Spir and FWW, and inoculating the reactors, they were sterilized for one hour with UVC. Then the holes for inlet, outlet, and sampling hoses, which were autoclaved prior to experiments, were pierced, and secured with a hot silicon gun. Culturing media was loaded with a glass funnel through one of the holes before attaching the hose. After filling the reactors, they were hanged on an aluminum skeleton setup and placed on an orbital shaker (3017, Lauda Gesellschaft für Labortechnik mbH, Germany) that would enable them to lie on their width and get the light from above. Inoculation was done through the sampling hoses after the reactors were hanged.

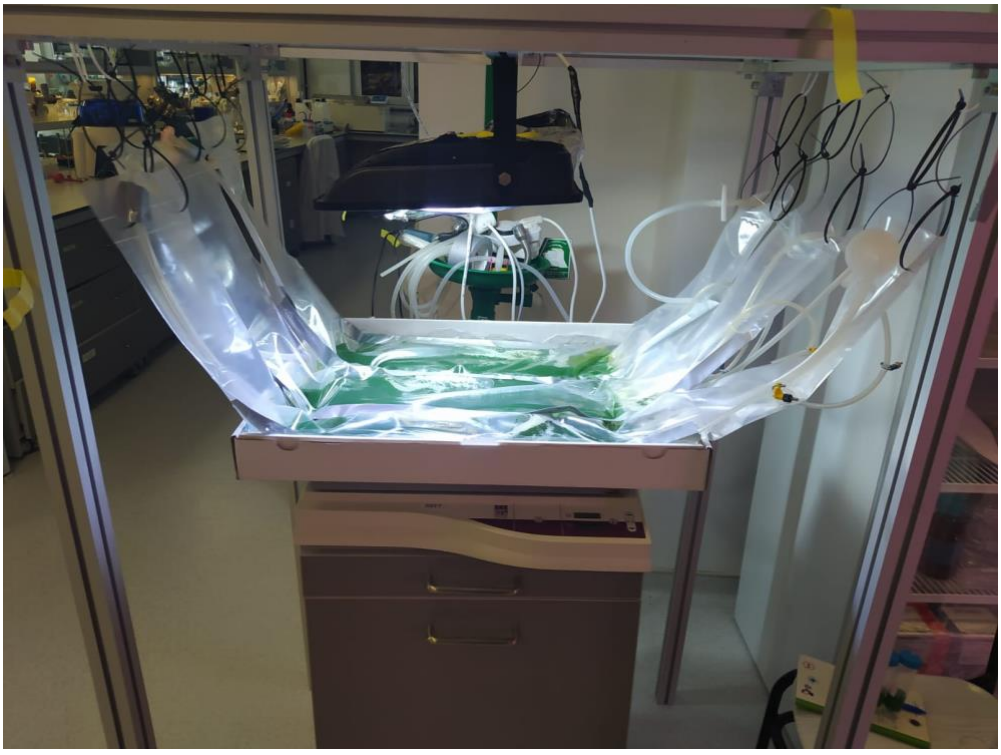


Figure 3.12. Control experiment in plastic PBRs.

3.4.2. Cultivation of Cyanobacteria in Plastic Photobioreactor

Reactors were loaded with 2 L Spir media for control study and 2 L pretreated FWW for WW study. Control reactors' inoculation was done with 200 mL culture and FWW reactors' inoculation was done with 800 mL culture (Figure 3.13). Both cultures that were used for inoculation were grown in a custom-made Erlenmeyer flask until their mid-exponential phase. Cultures were first centrifuged and then resuspended in their respective media and injected into the reactors. Reactors were supplied with an air flow of 0.5 L/min that was filtered through a 0.45-micron pore sized filter and diffused through a 0.5 mm pore sized diffusor stone. An illumination of 3500 lux with a 12h:12h light:dark cycle was provided from above using LED lights. Orbital shaker was operated at 60 rpm. Reactors were operated as a biological triplicate.

Figure 3.13. FWW before inoculation (left), FWW after inoculation (right).

3.4.3. Nutrient Removal in Plastic Photobioreactors

Total N, Total P and COD measurements were done using the protocols that were previously described in detail in Section 3.3.3.

3.4.4. Chemical and Biochemical Characterization of Harvested Biomass

Harvested biomasses were analyzed in detail to evaluate their potential for biofertilizer applications. Total proteins, total carbohydrates, total lipids were determined in addition to fatty acid methyl esters (FAME), amino acids, vitamins, and elemental profiles of the biomass. Additionally, extracts from vitamins analysis were used for qualitative analysis for biostimulants. For all analyses, freeze-dried biomass was used.

3.4.4.1. Total protein analysis. For total protein analysis, a method that was developed by (Safi et al., 2014) was used for extraction and quantification was done by using Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). Lowry reagent A was prepared by dissolving 2 g anhydrous sodium carbonate in 100 mL of 0.1 N sodium hydroxide solution that was prepared with UPW. Lowry reagent B was prepared by dissolving sodium potassium tartrate tetrahydrate in 100 mL UPW. Lowry reagent C was prepared by dissolving 0.5 g $CuSO_4.5H_2O$ in 100 mL UPW. Lowry reagent D was prepared by mixing Lowry reagents A, B and C at a 48:1:1 ratio, respectively, on the day of analyses.

30 mg of freeze-dried biomass was weighed into 2 mL polypropylene microtubes (Sarstedt) and 1.5 mL UPW of pH 12, that was adjusted by using 2 M NaOH solution, was added. Then microtubes was incubated at 40°C for 1 hour in a shaking water bath (MaxTurdy-30, Daihan Scientific, South Korea) and vortexed in every 10 minutes. After cooling down to room temperature, tubes were centrifuged at 5000×g, 20 °C for 10 minutes. 200 µL of the supernatants was transferred into new microtubes, and 1 mL Lowry reagent D was added. Tubes were vortexed and incubated for 10 minutes. Then 100 µL 1 N Folin-Ciocalteu's phenol reagent was added, and the tubes were incubated for another 30 minutes. Absorbance measurements were done at 750 nm wavelength.

For the calibration curve, bovine serum albumin (BSA) (Sigma P536) of concentration 5 mg/mL was used. On the day of analysis, standards were prepared from the stock solution in the range of 0 – 3 mg/mL. Standard solutions were only subjected to the coloring process, not to the extraction process. Calibration curve is given in Figure 3.14. Unknown samples' protein amounts were calculated using the Equation 3.7 given below.

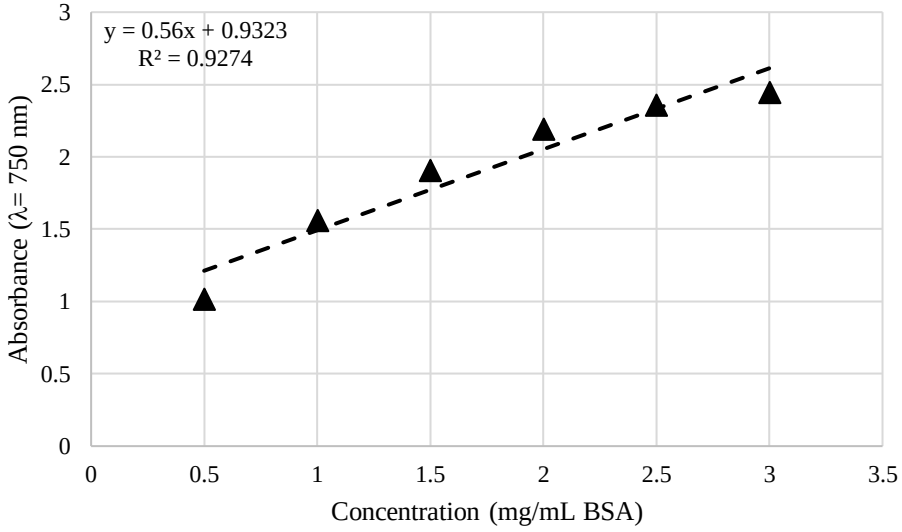


Figure 3.14. Calibration curve of BSA.

$$y = 0.56x + 0.9323 \quad (3.7)$$

where,

y = Absorbance of sample at 750 nm

x = Concentration of hydro-soluble protein in the solution.

3.4.4.2. Total carbohydrates analysis. Total carbohydrate analysis were done by using the method developed by (Van Wychen & Laurens, 2015). In principle, two-step sulfuric acid hydrolysis is followed by spectrophotometric quantification of released monosaccharides.

3-methyl-2-benzothiazolinone hydrazine (MBTH) solution was prepared at 3 mg/mL concentration. Dithiothreitol (DTT) solution was prepared at 1 mg/mL concentration. Ferric solution was prepared by dissolving 200 mg ferric ammonium sulfate dodecahydrate and 200 mg sulfamic acid in 40 mL 0.25 M HCl. MBTH working solution was prepared by mixing MBTH solution and DTT solution at 1:1 (v/v) ratio on the day of analyses.

Before the experiment, previously freeze-dried biomass was left in a drying oven at 60°C to ensure a moisture level that is below 10 %. 25 mg biomass was weighed into a previously weighed 10 mL screw capped glass tubes. 250 μL of 72 % (w/w) sulfuric acid was added and the tubes were vortexed carefully to ensure that all the biomass was mixed with the acid, did not clump, and stayed in the acid. Then tubes were placed in a water bath at 30°C for 1 hour and vortexed in every 10 minutes. After incubation, 7 mL UPW was added into tubes, which brought the sulfuric acid concentration to 4% (w/w), and tubes were autoclaved for 1 hour at 121°C. Once the tubes were at room temperature, they were filtered through a 0.22-micron pore sized nylon syringe filter and prepared for the spectrophotometric analysis. Filtered samples was diluted to 1:50 directly in the new reaction vials to a total volume of 500 μL. 500 μL 0.5 M NaOH solution and 500 μL MBTH working solution were added into tubes. After carefully vortexing, tubes were immediately placed in a dry thermostat at 80°C for 15 minutes. At the end of the 15 minutes, thermostat was turned off and 1 mL ferric solution was added into tubes while they were still in the thermostat. Once the solution was added, tubes were removed from the block, vortexed and left for 10-15 minutes. After they reached room temperature, 2.5 mL UPW was added and vortexed. Absorbance measurements were done at 620 nm wavelength within 1 hour.

For calibration curve, D (+) glucose stock solution that was prepared by dissolving 25 mg in 100 mL UPW was used. On the day of analyses, several concentrations from the stock solution were prepared in the 0 – 0.05 mg/mL range and the calibration curve given below is acquired (Figure 3.15). Unknown sample calculations were done by using the Equation 3.8.

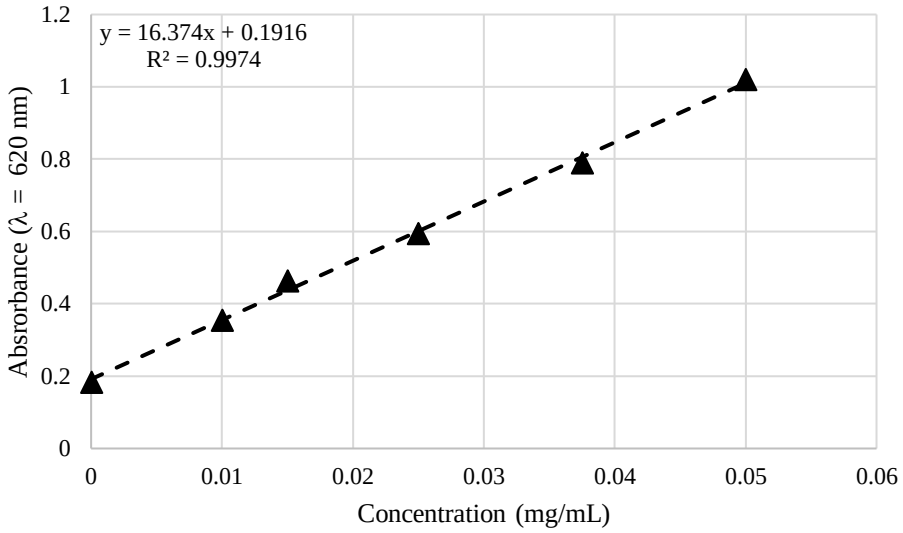


Figure 3.15. Calibration curve of glucose.

$$y = 16.374x + 0.1916 \quad (3.8)$$

where,

y = Absorbance of sample at 620 nm

x = Concentration of monomeric sugars in the solution.

3.4.4.3. Total lipid analysis. A gravimetric method was used for total lipid determination that is developed by (Breuer et al., 2013). Briefly, method disrupts the cells physically with beads and sonication, then extracted lipids are dissolved in organic solvent and recovered.

300 mg of 0.1 mm and 100 mg of 0.5 mm diameter glass beads, that were previously washed with 1 M nitric acid, rinsed with DI and dried in a drying oven at 105°C, weighed into polystyrene microtubes and autoclaved. Chloroform:methanol (MeOH) solution of 4:5 ratio were prepared and 50 mg/L nonadecanoic acid (C19:0) (Sigma N525) was dissolved in the solution as an internal standard. 50 mg biomass was added into tubes and mixed with 1 mL internal standard solution. Bead beating was done at 4500 rpm, 10°C for 60 seconds for 6 cycles with 15 seconds pause in between cycles in a homogenizer (Precellys Evolution with Cryolys Unit, Bertin Technologies, France).

Homogenized samples were transferred into 50 mL glass tubes with Teflon screw caps including the beads. Emptied microtubes was washed with 1 mL chloroform:methanol solution three times and washings was added into the glass tubes. Tubes was vortexed and placed in a sonicator (Sonorex Super RK 102 H, Bandelin, Germany) for 10 minutes. Then 2.5 mL of a solution of 50 mM 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris) and 1 M NaCl were added into the tubes. The pH of the solution was adjusted to 7 with 1 N HCl before adding. Tubes was vortexed, and sonicated additional 10 minutes. After sonication tubes was centrifuged at 1200×g, 10°C for 5 minutes. Multiple layers formed in the tubes upon centrifugation. Bottom layer, which consists of chloroform and lipids, was transferred to new glass tube that were previously weighed and labeled, using glass Pasteur pipettes carefully without pipetting beads or other layers. Extraction was repeated with the old tubes from the sonication part by adding 1 mL chloroform:methanol solution for several times to ensure no lipids were left in the separation process. Collected chloroform phases was pooled into same glass tubes and the chloroform was evaporated using N_2 gas flow. Weight of the dried phase that was left in the tubes gives the lipids amounts in the biomass. Calculation was done using the following Equation 3.9 as percent biomass. Extracts were stored at -20°C for further fatty acid methyl ester (FAME) analyses.

$$\% = \frac{L \times 100}{M} \tag{3.9}$$

where,

% = Percent lipid content in biomass

L = Mass of lipid extract, mg

M = Mass of biomass weighed, mg

3.4.4.4. Fatty acid methyl ester analysis. Esterification was performed in order to determine the fatty acid profile of the biomasses. 3 mL of methanol solution containing 5% (v/v) sulfuric acid was added to the tubes that contained the dried lipid extracts. Tubes were incubated in a water bath at 70°C for 3 hours and vortexed in every 30 minutes. After cooling down to room temperature, 3 mL UPW and 3 mL hexane were added to the tubes. Tubes were vortexed and placed on a rotator (Wisemix RT-10, Witeg, Wertheim, Germany) at 45 rpm for 15 mins. Then tubes were centrifuged at 1200×g for 5 minutes. After centrifugation, 2 mL of top layer that contained the hexane and the esterified lipids was collected into a new tube. 2 mL UPW was added to the collected hexane phase as a washing step. Tubes were vortexed and centrifuged at 1200×g for 5 minutes and the top layer was collected into GC vials for gas chromatography analyses.

Identification and quantification of the FAMEs were done by using a gas chromatography system (GC-2014, Shimadzu, Japan) with a flame ionization detector (FID). For the analyses SBL-IL11 polar ionic liquid column (Sigma) with 100 m length x 0.25 mm diameter x 0.20 µm film thickness was used. Oven temperature was initially set 140°C for 5 minutes, progressively increased with 8°C/min rate to 180°C, and then increased with 5°C/min rate to 260°C. Detector (FID) temperature was at 260°C, carrier gas (H_2) flow was set to 40 cm/sec, and 1 µL of injection was done with 100:1 split ratio. A certified FAME mix (CRM47885, Supelco, Inc., Bellefonte, Pennsylvania, United States) was used as a reference material (Figure 3.16).

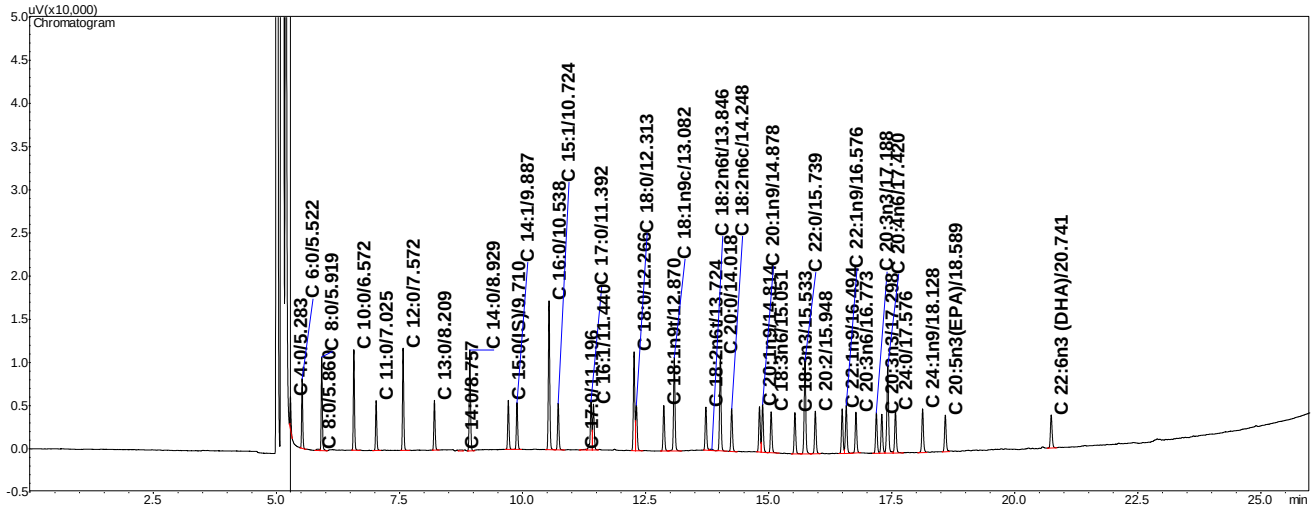


Figure 3.16. GC chromatogram of FAME mix.

3.4.4.5. Vitamins analysis. Water soluble B vitamins were analyzed by using a modified version of the method that was developed by (Lock, 2013; Lock & Noestheden, 2014). Cells were disrupted physically by bead beating and the vitamins were extracted by solvent.

125 mg biomass were weighed into microtubes, that were previously prepared by putting 150 mg of 0.1 mm and 50 mg of 0.5 mm diameter glass beads, which were washed with 1 M nitric acid, rinsed with DIW and dried in a drying oven at 105°C, and autoclaving. 1 mL UPW was added into tubes and bead beating was done at 4500 rpm, 10°C for 60 seconds for 6 cycles with 15 seconds pause in between cycles in a homogenizer (Precellys Evolution with Cryolys Unit, Bertin Technologies, France). Extracts were transferred into 15 mL plastic conical tubes including the beads. Microtubes were washed with 2 mL UPW, and 2 mL acetonitrile (ACN) and washings were added into the 15 mL tubes. 80 µL FA was added into tubes and after a brief vortex, tubes were placed onto a rotator at 45 rpm for 10 minutes. After rotating, tubes were centrifuged at 4500 rpm, 18°C for 5 minutes.

Supernatant was collected and filtered with a polypropylene 25 mm 0.45-micron pore sized syringe filters (729004, Macherey-Nagel, Düren, Germany). Concentrations of the standard solutions are given on Table 3.8 and calibration curves are shown on Figure 3.17-23.

Analysis was done using UHPLC-HRAM/MS and the conditions of the instrument are given below in Table 3.5 – 7.

Table 3.5. HPLC pump conditions of vitamin analysis.

#	Time	Flow (mL/min)	%B
1	-2	Equilibration	
2	0	Run	
3	0	0.4	2
4	5.5	0.4	50
5	8	0.4	80
6	8.1	0.4	98
7	8.1	0.6	98
8	11	0.6	98
9	11.1	0.6	2
10	14.4	0.6	2
11	14.5	0.4	2
12	15	Stop Run	

Table 3.6.MS analyzer conditions of vitamin analysis.

MS Analyzer	
Column	Thermo Scientific Accucore RP-MS, 150X2.1mm, 2.6um
Mobile Phase A	H20 (0.1 % FA
Mobile Phase B	MeOH (0.1% FA)
Oven Temperature	50
Polarity	(+)
Mode	PRM
Ion source	On
Properties of PRM	
Method duration	0 to 15 min
Resolution	350000
AGC Target	1e5
Maximum IT	100 ms
Isolation window	4.0 m/z
(N) CE / stepped nce	35

Table 3.7. MS ion source conditions of vitamin analysis.

Sheath gas flow rate (L/min)	50
Aux gas flow rate (L/min)	15
Sweep gas flow rate (L/min)	1
Spray voltage (kV)	3.00
Capillary temperature (°C)	380
S-lens RF level	50.0
Aux gas heater temperature (°C)	350

Table 3.8. Concentrations of calibration curve standards.

	Concentrations of standard levels (ng/mL)									
Vitamin	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
Thiamine B1	5	10	50	100	250	500	800	-	-	-
Riboflavin B2	1	2	5	10	20	50	100	250	500	-
Nicotinic acid B3	1	2,5	5	10	25	50	100	250	500	750
Pantothenic acid B5	1	2	5	10	50	100	250	500	-	-
Pyridoxine B6	1	2	5	10	50	100	250	800	-	-
Biotin B7	1	2	5	10	20	50	100	250	500	-
Folic acid B9	1	2	5	10	50	100	250	500	800	-

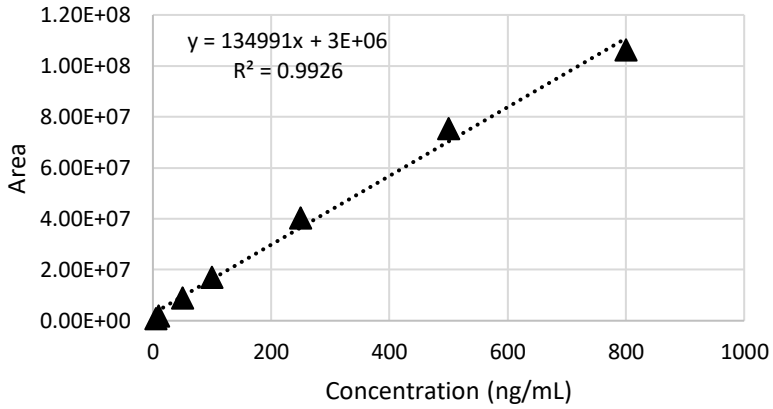


Figure 3.17. Calibration curve for Thiamine (B1).

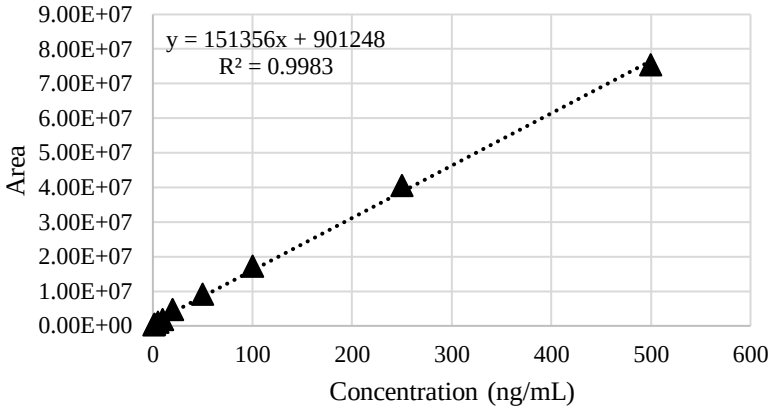


Figure 3.18. Calibration curve of Riboflavin (B2).

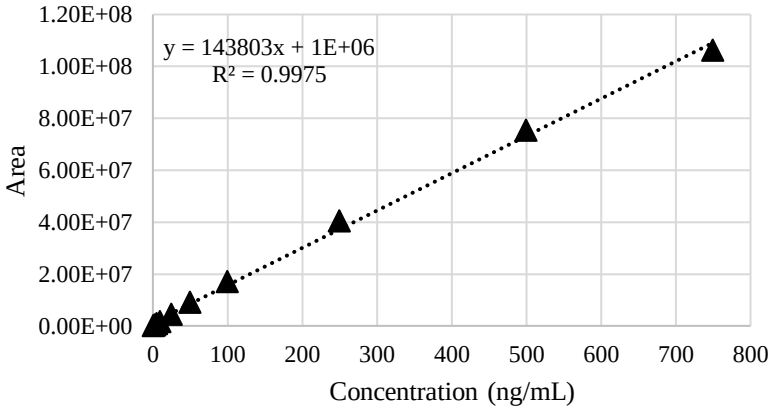


Figure 3.19. Calibration curve of Nicotinic acid (B3).

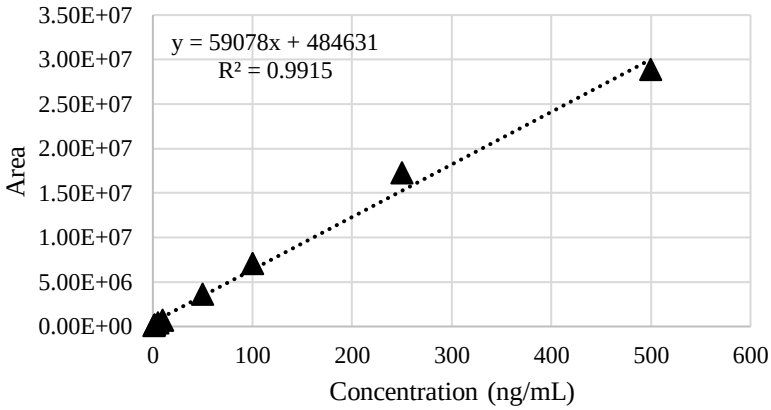


Figure 3.20. Calibration curve of D-Pantothenic acid (B5).

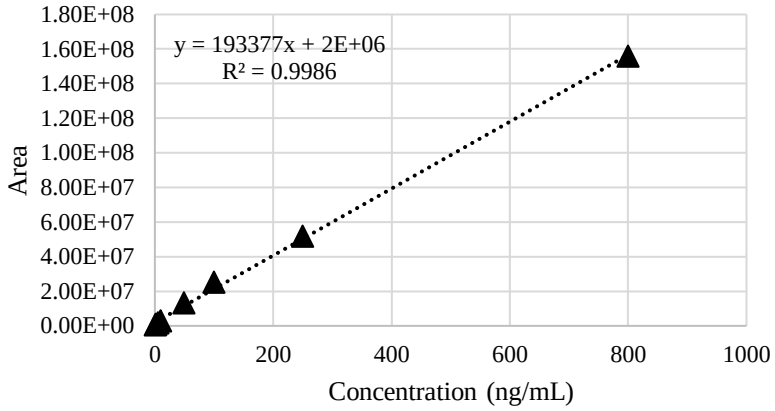


Figure 3.21. Calibration curve of Pyridoxine (B6).

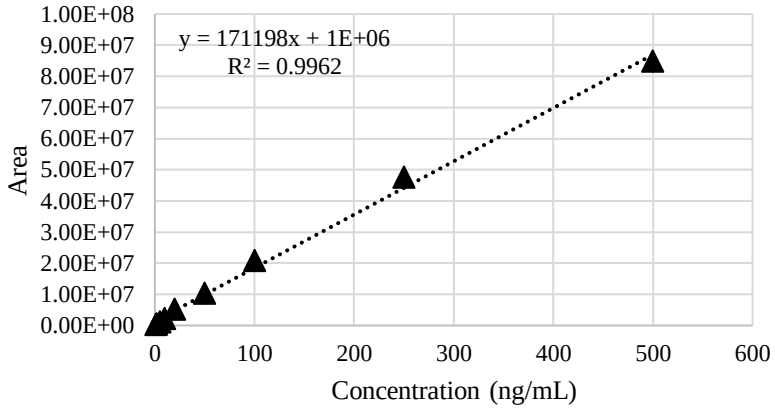


Figure 3.22. Calibration curve of Biotin (B7).

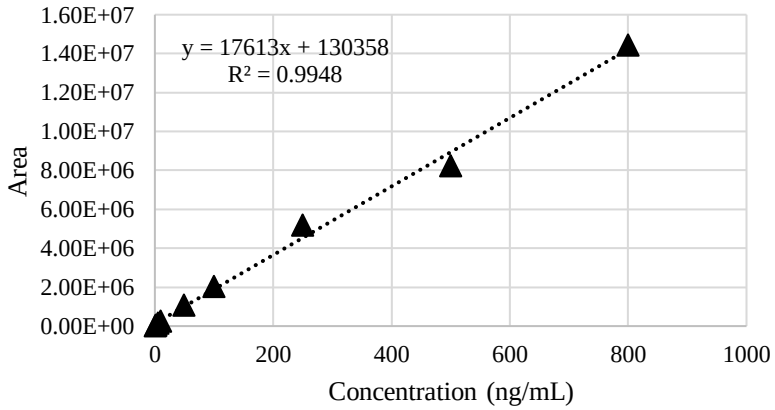


Figure 3.23. Calibration curve of Folic acid (B9).

3.4.4.6. Amino acid analysis. Similar method was used for amino acids analyses of the biomasses. Cells were disrupted physically by bead beating and the amino acids were extracted with an appropriate solvent.

120 mg of 0.1 mm and 40 mg of 0.5 mm diameter glass beads, that were previously washed with 1 M nitric acid, rinsed with DIW and dried in a drying oven at 105°C, weighed into polystyrene microtubes and autoclaved. 50 mg biomass was added into tubes with 1 mL UPW, and bead beating was done at 4500 rpm, 10°C for 60 seconds for 6 cycles with 15 seconds pause in between cycles in a homogenizer (Precellys Evolution with Cryolys Unit, Bertin Technologies, France). Extracts were transferred into 15 mL plastic conical tubes including the beads. Microtubes were washed with 3 mL UPW, and 4 mL ACN and washings were added into the 15 mL tubes. 80 μL FA was added into tubes and after a brief vortex, tubes were placed onto a rotator at 45 rpm for 10 minutes. After rotating, tubes were centrifuged at 4500 rpm, 18°C for 5 minutes. Supernatants were collected and filtered with a polypropylene 25 mm 0.22-micron pore sized syringe filters (729004, Macherey-Nagel, Düren, Germany). 960 μL 10% MeOH, 10 μL extract, and 30 μL 1/20 Stable Isotope Mix (SIM) (NSKA, Cambridge Isotope Laboratories, MA, United States), of which concentrations are shown at Table 3.9, as internal standards were added into chromatography vials. Concentrations of the standard solutions are given on Table 3.13 and calibration curves are shown on Figure 3.24-42.

Table 3.9. Concentrations of amino acid standards in SIM

Reference standard	Concentration (nmol/mL)
Glycine-2-13C,15N	2500
L-Alanine-2,3,3,3-d4	500
L-Arginine-5-13C,4,4,5,5-d41,3	500
L-Aspartic acid-2,3,3-d3	500
L-Leucine-5,5,5-d3	500
DL-Glutamic acid-2,4,4-d3	500
L-Methionine-(methyl-d3)	500
L-Ornithine:HCl (5,5-D2)1,3	500
Phenylalanine-[ring-13C6]	500
L-Tyrosine-[ring-13C6]	500
L-Valine-d8	500
L-Citrulline-5-13C,5,5-d2	500

Analysis was done using UHPLC-HRAM/MS and the conditions of the instrument are given below Table 3.10 – 12.

Table 3.10. HPLC pump conditions of amino acids analysis.

#	Time	Flow (mL/min)	%B
1	-1.5	Equilibration	Equilibration
2	0	Run	
3	0	0,6	12
4	1.5	0.6	12
5	5.5	0.6	50
6	6	0.6	50
7	6.1	0.6	95
8	6.5	0.6	95
9	6.6	0.8	95
10	8.5	0.8	95
11	8.6	0.8	12
12	11.5	0.8	12
13	11.6	0.6	12
14	Stop Run		

Table 3.11. MS analyzer conditions of the amino acids analysis.

MS Analyzer	
Column	Waters Corp. Nova-Pak C8, 150X3.9mm
Mobile Phase A	H_20 (0.1 % FA
Mobile Phase B	ACN (0.1% FA)
Oven temperature	35
polarity	(+)
Mode	ddMS2
Ion Source	On
Properties of Full MS/ddMS2	
General	
Run time:	1.8 to 6.5 min
Inclusion	On
Full MS	
Resolution	350000
AGC Target	1e6
Maximum IT	100 ms
Scan range	70 to 500 m/z
dd-MS2 / dd-SIM	
Resolution	17500
AGC Target	1e5
Maximum IT	50 ms
Loop count	5
Top N	5
Isolation window	8.0 m/z

(N) CE / stepped nce	25
dd settings	
Minimum AGC Target	1.00e4
Intensty threshold	2.0e5
Exlude isotope	On
Dynamic exlusion	10.0 s

Table 3.12. MS ion source conditions of the amino acid analysis.

Sheath gas flow rate (L/min)	50
Aux gas flow rate (L/min)	15
Sweep gas flow rate (L/min)	1
Spray voltage (kV)	3.00
Capillary temperature (°C)	380
S-lens RF level	50.0
Aux gas heater temperature (°C)	350

Table 3.13. Concentrations of calibration curve standards of amino acids.

Standard	Concentration of standard (ng/mL)						
Tag	L1	L2	L3	L4	L5	L6	L7
Alanine (ALA)	5	10	25	50	100	250	500
Arginine (ARG)	5	10	25	50	100	250	500
Aspartic acid (ASP)	5	10	25	50	100	250	500
Cysteine (CYS)	25	50	100	250	500	-	-
Glutamic acid (GLU)	10	25	50	100	250	500	-
Glycine (GLY)	25	50	100	250	500	-	-
Histidine (HIS)	5	10	25	50	100	250	500
Isoleucine (ILE)	5	10	25	50	100	250	500
Leucine (LEU)	5	10	25	50	100	250	500
Lysine (LYS)	5	10	25	50	100	250	500
Methionine (MET)	5	10	25	50	100	250	500
Phenylalanine (PHE)	5	10	25	50	100	250	500
Proline (PRO)	5	10	25	50	100	250	500
Serine (SER)	5	10	25	50	100	250	500
Taurine (TAU)	5	10	25	50	100	250	500
Threonine (THR)	5	10	25	50	100	250	500
Tryptophan (TRP)	5	10	25	50	100	250	500
Tyrosine (TYR)	5	10	25	50	100	250	500
Valine (VAL)	5	10	25	50	100	250	500

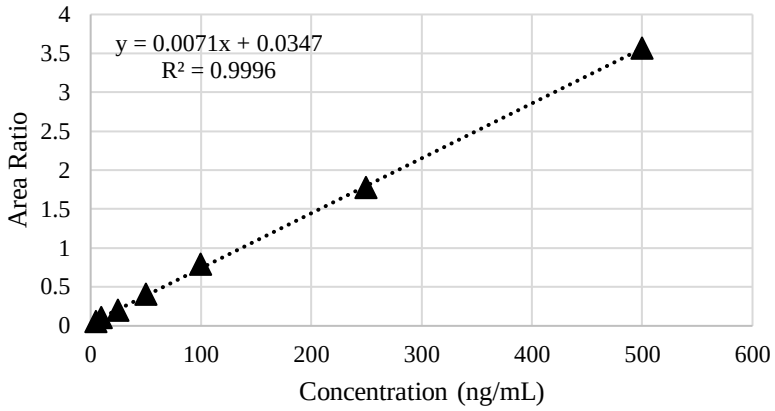


Figure 3.24. Calibration curve of ALA.

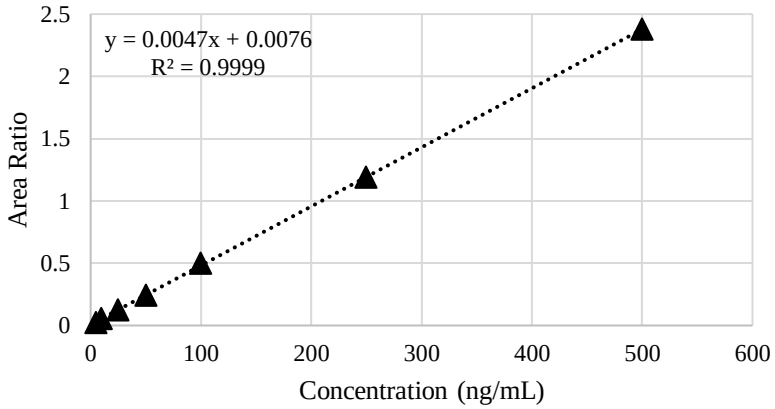


Figure 3.25. Calibration curve of ARG.

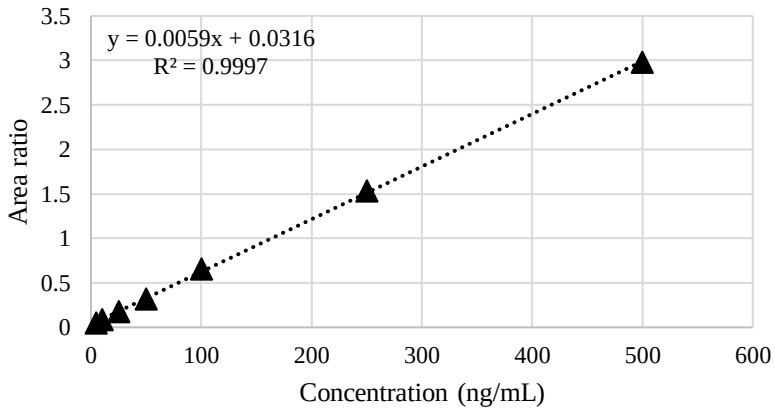


Figure 3.26. Calibration curve of ASP.

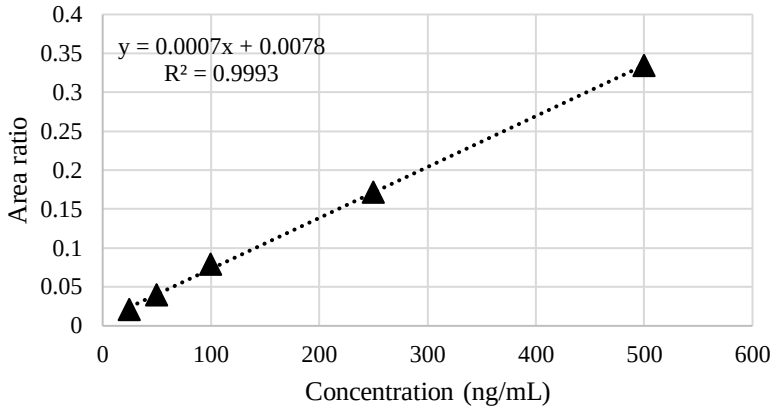


Figure 3.27. Calibration curve of CYS.

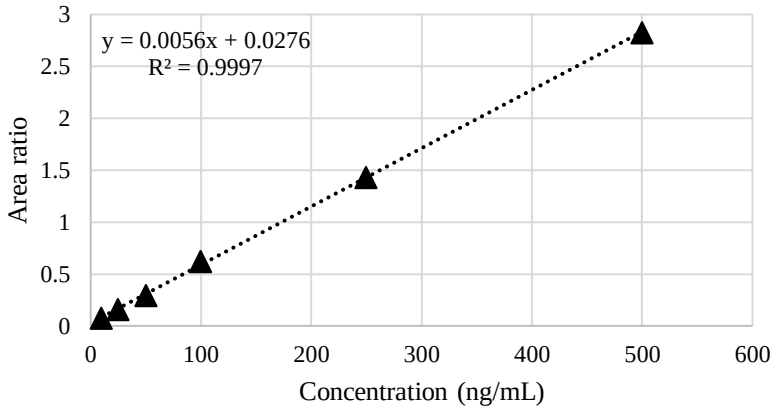


Figure 3.28. Calibration curve of GLU.

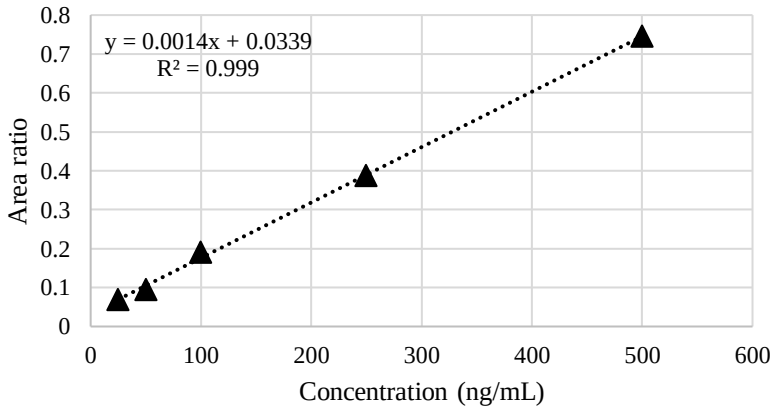


Figure 3.29. Calibration curve of GLY.

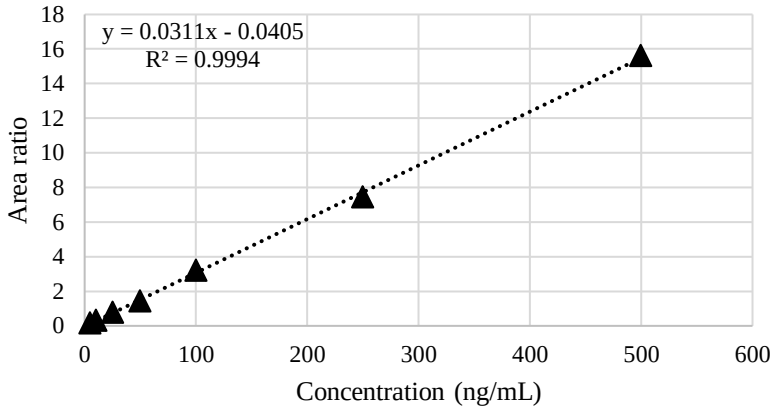


Figure 3.30. Calibration curve of HIS.

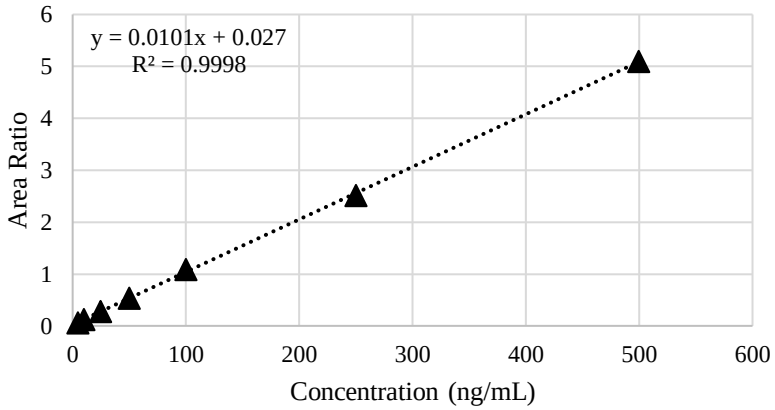


Figure 3.31. Calibration curve of ILE.

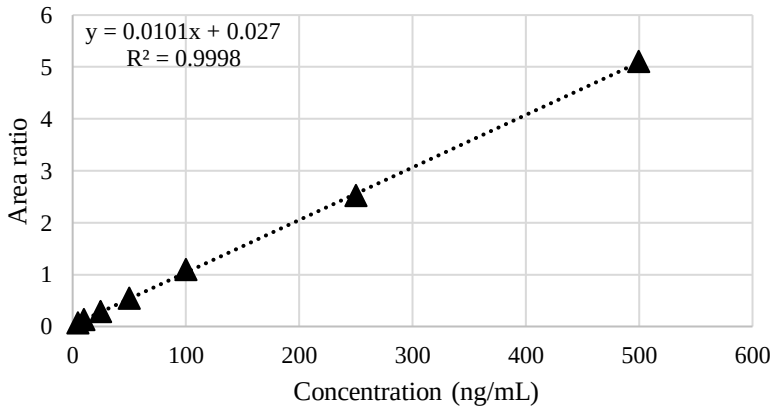


Figure 3.32. Calibration curve of LEU.

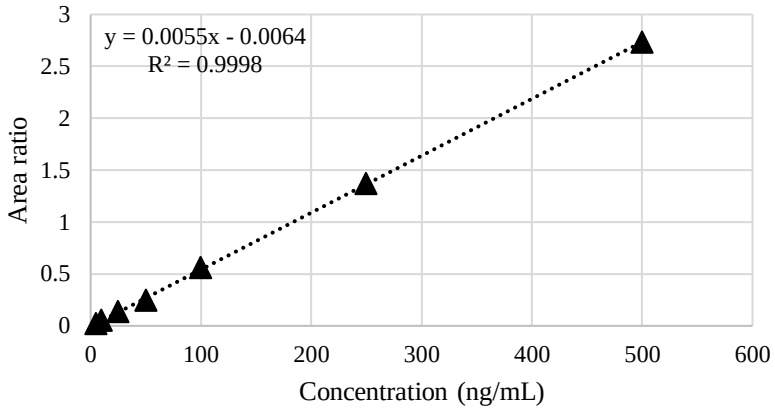


Figure 3.33. Calibration curve of LYS.

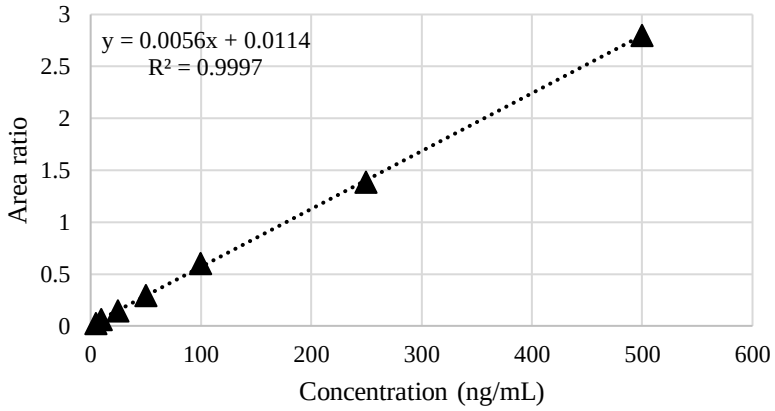


Figure 3.34. Calibration curve of MET.

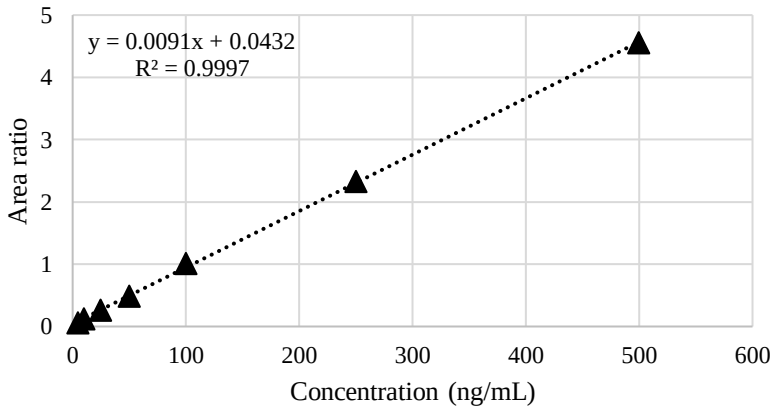


Figure 3.35. Calibration curve of PHE.

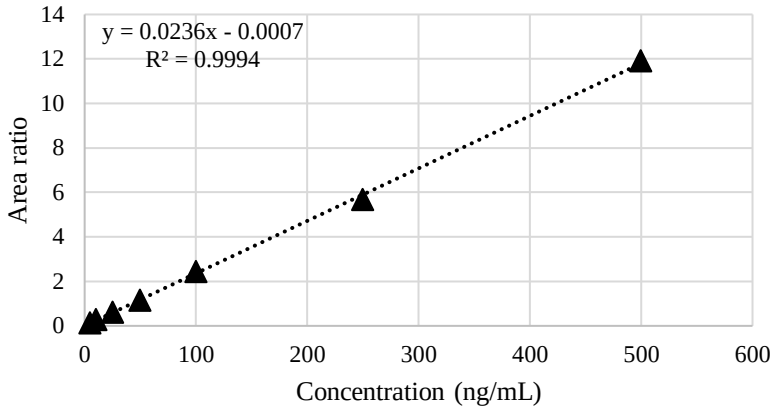


Figure 3.36. Calibration curve of PRO.

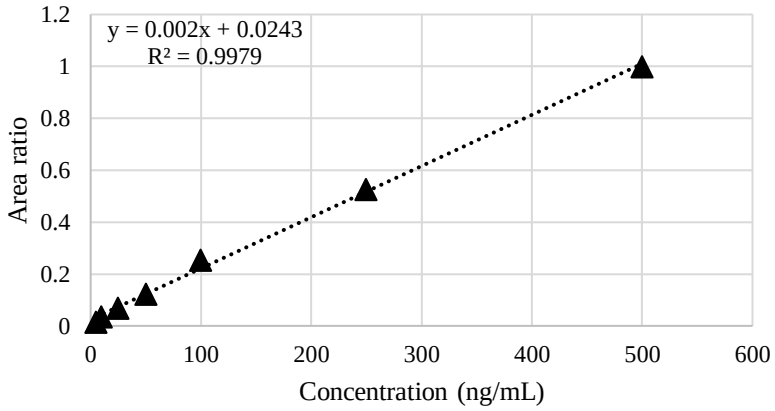


Figure 3.37. Calibration curve of SER.

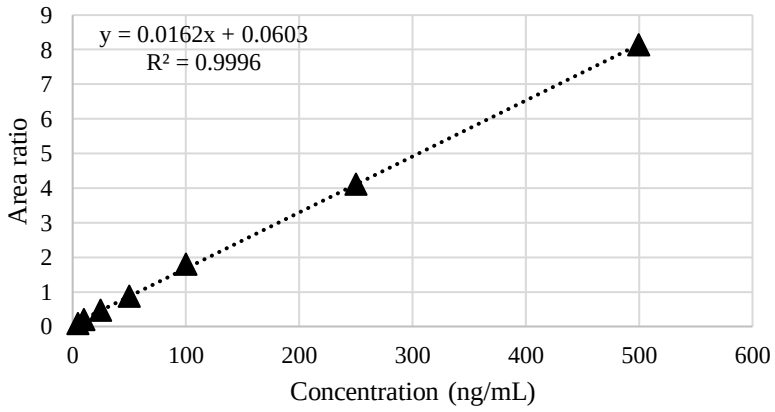


Figure 3.38. Calibration curve of TAU.

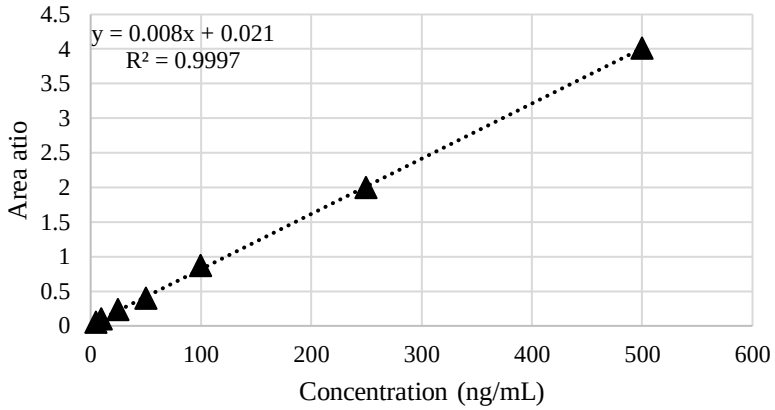


Figure 3.39. Calibration curve of THR.

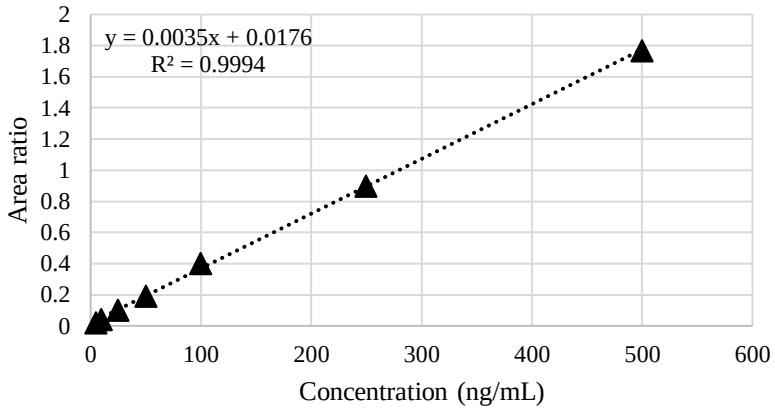


Figure 3.40. Calibration curve of TRP.

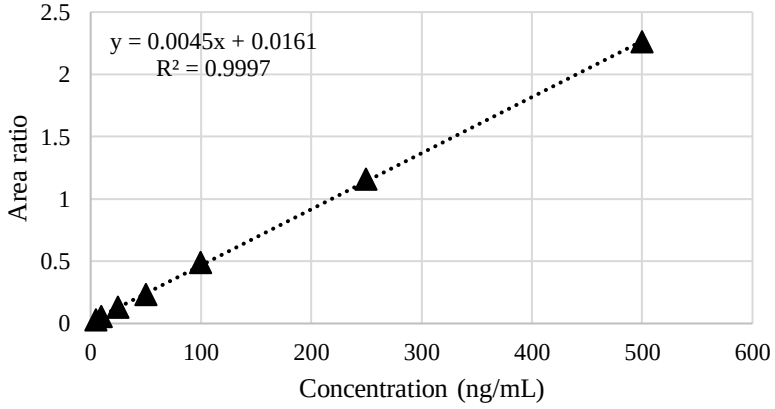


Figure 3.41. Calibration curve of TYR.

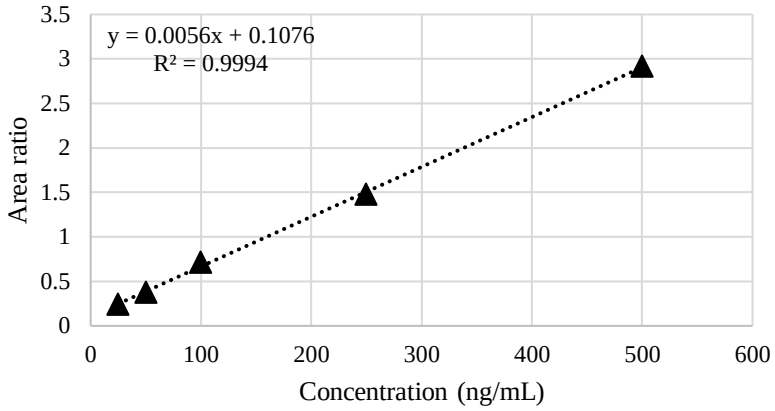


Figure 3.42. Calibration curve of VAL.

3.4.4.7. Elemental analysis. Elemental analyses were done using inductively coupled plasma-mass spectrometry ICP/MS (Thermo Scientific ICAP/RQ). Extraction method was adopted from (Boehnke & Delumyea, 2000). Briefly, biomass was digested with concentrated nitric acid while heating to extract and dissolve the metals. Extracted metals were analyzed using ICP/MS. Analyzed metals and their corresponding isotopes are given in Table 3.15. Metals' calibration curves were prepared from the stock solutions that were supplied with the instrument in range of 1-1000 μg/L (Figure 3.43-60).

50 mg biomass was weighed into 50 mL beakers that were previously washed with nitric acid and rinsed with DIW. 2 mL UPW and 2 mL ICP grade concentrated nitric acid were added into beakers and covered with watch glass, that were previously subjected to acid wash as well. Solutions were brought to a near boiling temperature for 30 minutes. Then, additional 2 mL UPW and 2 mL ICP grade concentrated nitric acid were added into beakers and brought to near boiling temperature for another 15 minutes. After cooling down to room temperature, extracts were transferred into 50 mL plastic tubes that were previously washed with nitric acid and rinsed with DIW. Watch glasses that were used to covering the beakers and beakers themselves were washed with UPW and washings were added onto extracts. Then extract volumes were brought to 50 mL with UPW. Extracts were filtered through 25 mm 0.22-micron pore sized polytetrafluoroethylene syringe filters before injecting into the system. Instrument conditions are given on Table 3.14.

Table 3.14. ICP/MS conditions for elemental analysis.

Dwell time (s)	0.01
Spacing (u)	0.01
Measurement mode	KED
Number of main runs	3
Time for main run (s)	1.8
Total time per run (s)	125
Cool gas flow (L/min)	14
Aux gas flow (L/min)	0.8
Nebulizer gas flow (L/min)	1.4
Plasma power (W)	1550

Table 3.15. Metals and corresponding isotopes analyzed.

Element	Isotope
Magnesium	24Mg
Aluminum	27Al
Potassium	39K
Calcium	44Ca
Vanadium	51V
Chromium	52Cr
Manganese	55Mn
Iron	57Fe
Cobalt	59Co
Nickel	60Ni
Copper	63Cu
Zinc	66Zn
Arsenic	75As
Selenium	77Se
Strontium	88Sr
Cadmium	111Cd
Barium	137Ba
Lead	208Pb

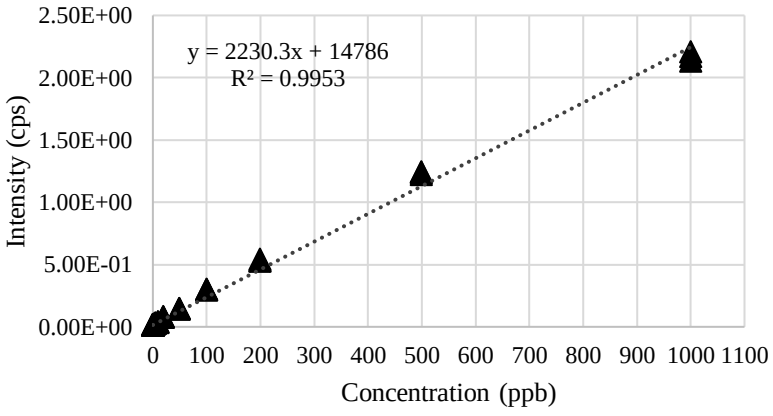


Figure 3.43. Calibration curve of 24Mg.

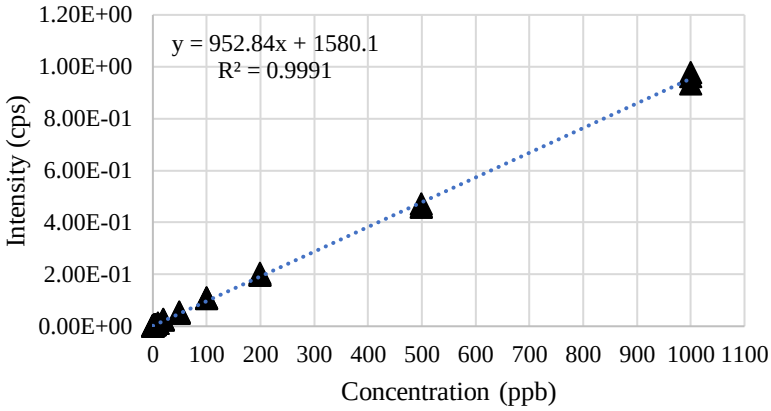


Figure 3.44. Calibration curve of 27Al.

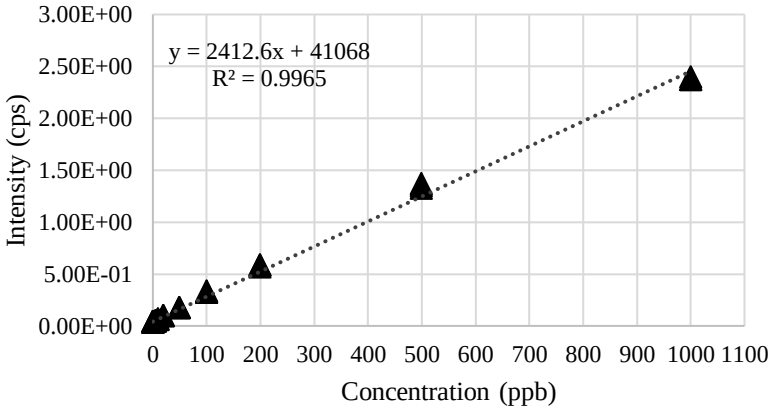


Figure 3.45. Calibration curve of 39K.

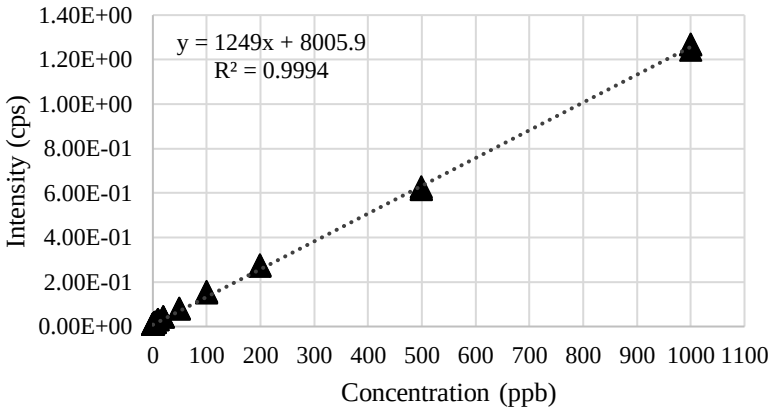


Figure 3.46. Calibration curve of 44Ca.

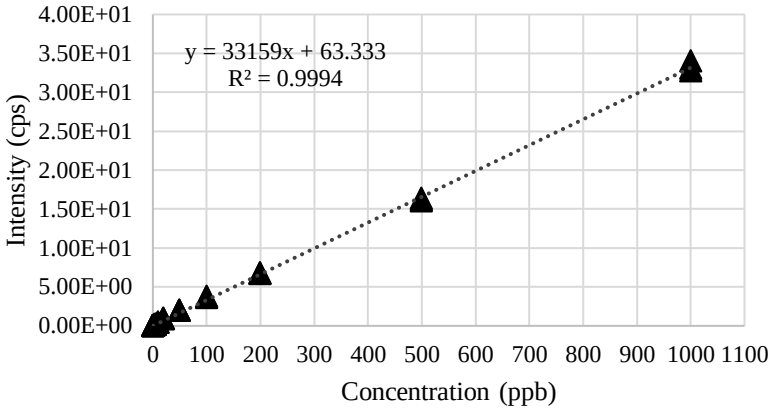


Figure 3.47. Calibration curve of 51V.

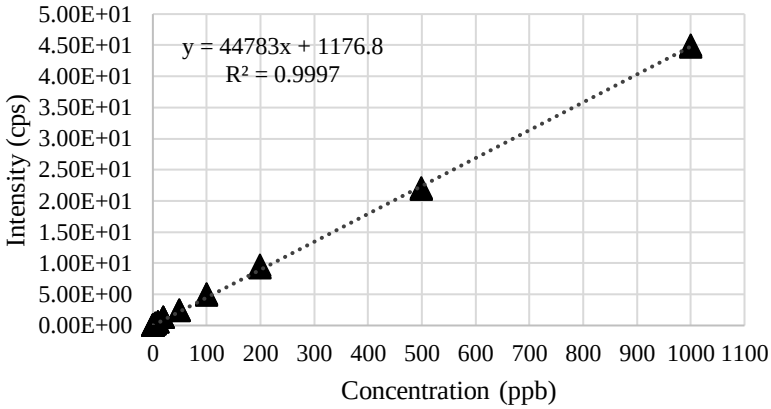


Figure 3.48. Calibration curve of 52Cr.

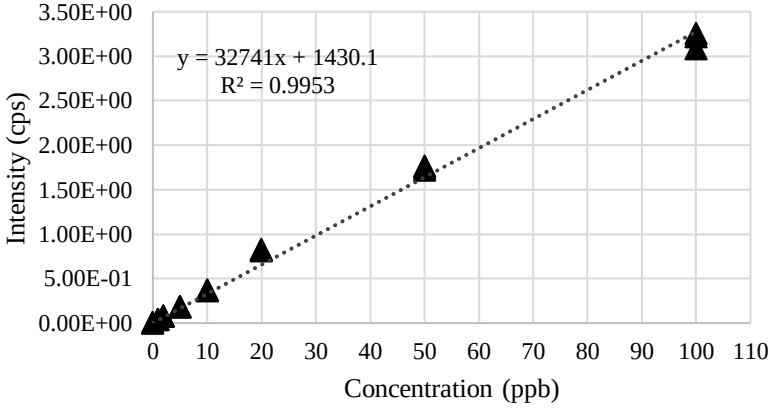


Figure 3.49. Calibration curve of 55Mn.

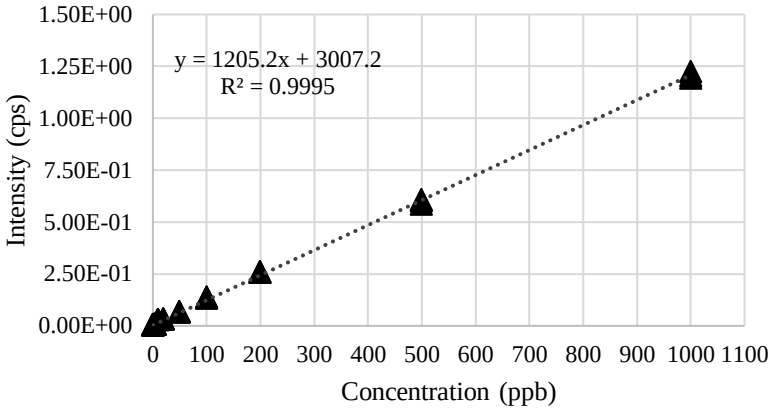


Figure 3.50. Calibration curve of 57Fe.

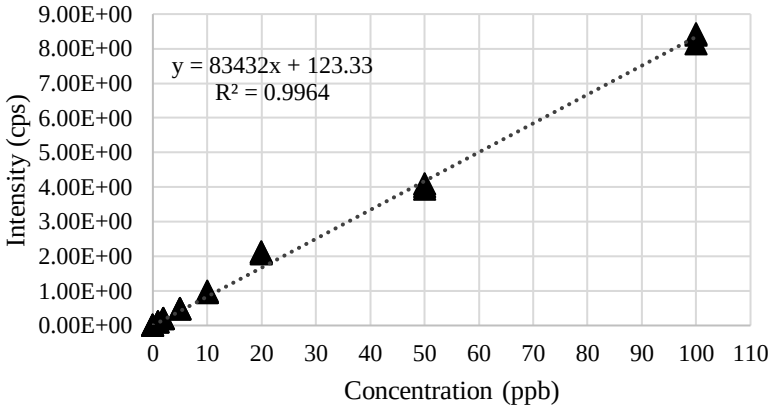


Figure 3.51. Calibration curve of 59Co.

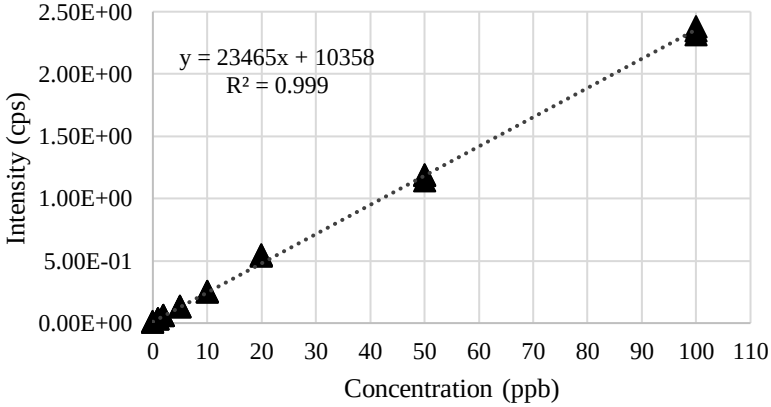


Figure 3.52. Calibration curve of 60Ni.

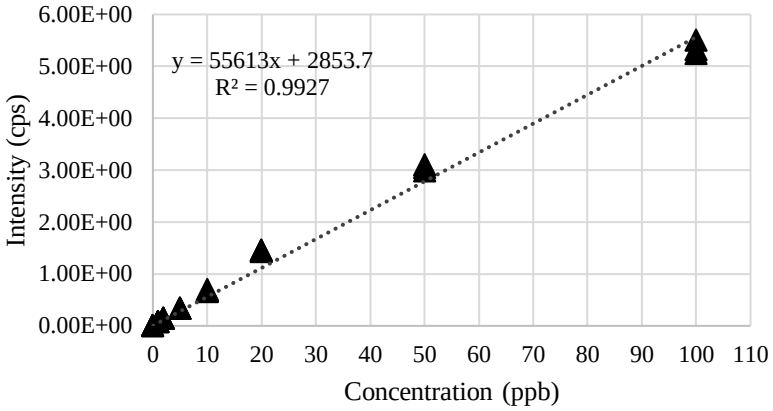


Figure 3.53. Calibration curve of 63Cu.

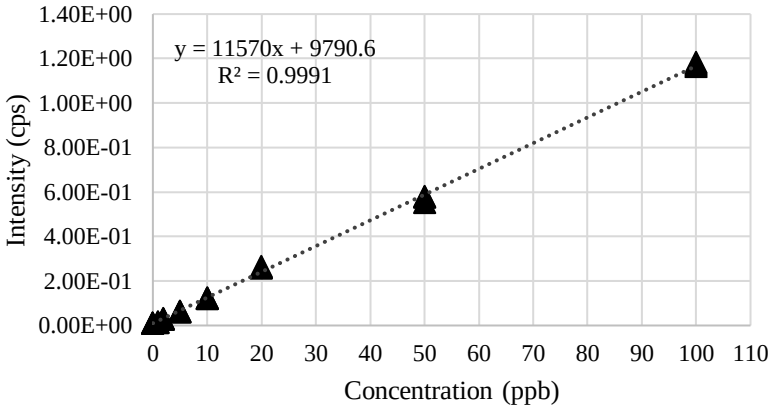


Figure 3.54. Calibration curve of 66Zn.

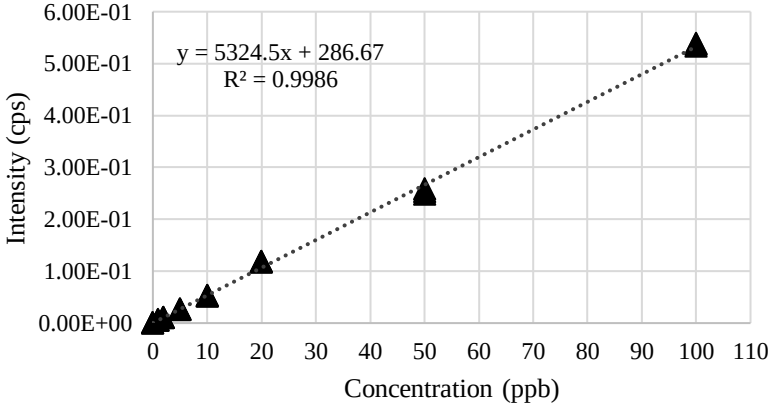


Figure 3.55. Calibration curve of 75As.

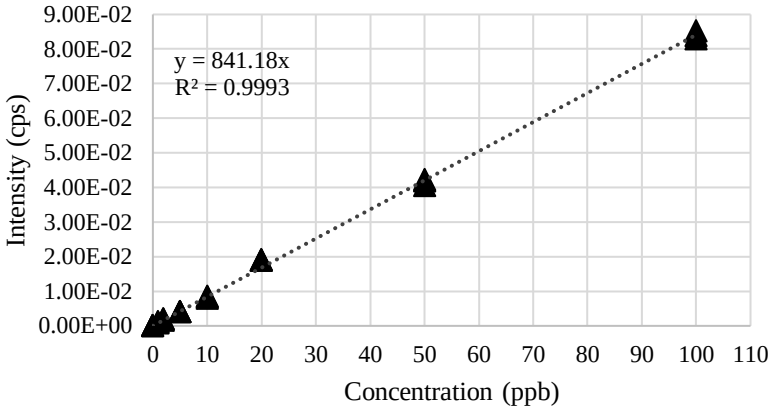


Figure 3.56. Calibration curve of 77Se.

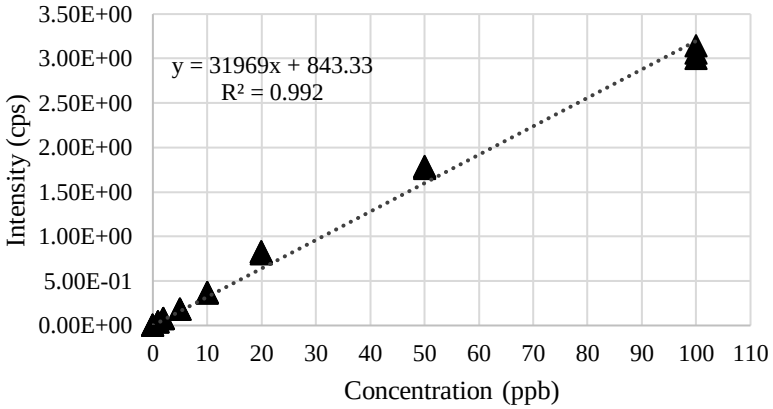


Figure 3.57. Calibration curve of 88Sr.

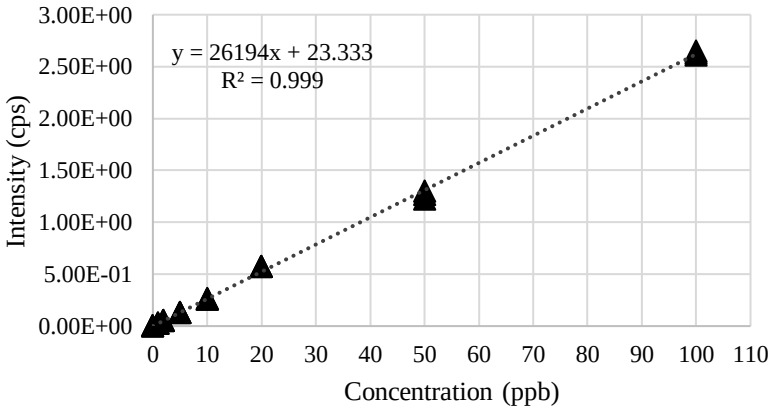


Figure 3.58. Calibration curve of 111Cd.

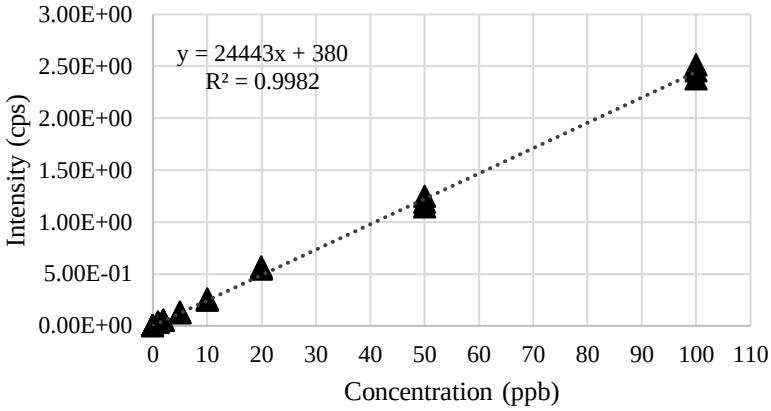


Figure 3.59. Calibration curve of 137Ba.

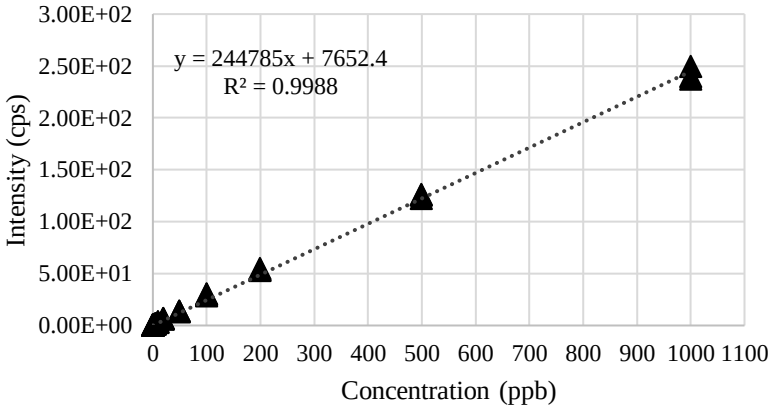


Figure 3.60. Calibration curve of 208Pb.

3.4.4.8. Biostimulant analysis. Qualitative biostimulant analysis were done using the extracts from vitamin analyses by using UHPLC-HRAM/MS. Compounds to be analyzed are given on Table 3.16. These compounds were chosen because of the extensive research and data available of them (Balcke et al., 2012; Han et al., 2012; Jon et al., 2020; Müller & Munné-Bosch, 2011; Trapp et al., 2014; Zhao et al., 2013) that could be implemented with this study.

Table 3.16. Biostimulants to be analyzed qualitatively.

#	Compound name
1	12-hydroxyjasmonic acid
2	(+)-7-iso-Jasmonic acid
3	(+)-7-iso-jasmonoyl isoleucine
4	Salicylic acid
5	Abscisic acid
6	cis-12-oxophytodienoic acid
7	Indole-3-acetic acid
8	Gibberellin A1
9	Gibberellin A3 (gibberellic acid)
10	ent-Gibberellane
11	ent-Kaurene
12	Gibberellin A19
13	Gibberellin A24
14	Gibberellin A9
15	Gibberellin A4
16	1-Aminocyclopropane-1-carboxylic acid
17	t-Zeatin
18	Dihydrozeatin
19	Zeatin riboside
20	Dihydrozeatin riboside
21	Kinetin
22	Isopentenyladenine
23	Isopentenyladenosine (Riboprine)

4. RESULTS AND DISCUSSION

This chapter contains growth profiles of culture grown in Erlenmeyer flasks and plastic PBRs and the detailed chemical and biochemical analyses of biomasses. Acquired data are evaluated and discussed.

4.1. Growth Profiles

To determine the growth profile of the biomass and the mid-exponential day of the growth process, Erlenmeyer flasks, that were explained in detail in Chapter 3, were used. Optical density versus time, pH versus time, filaments amount versus time, and dry weight versus time graphs are shown below at Figure 4.1, Figure 4.2, Figure 4.3, and Figure 4.4, respectively.

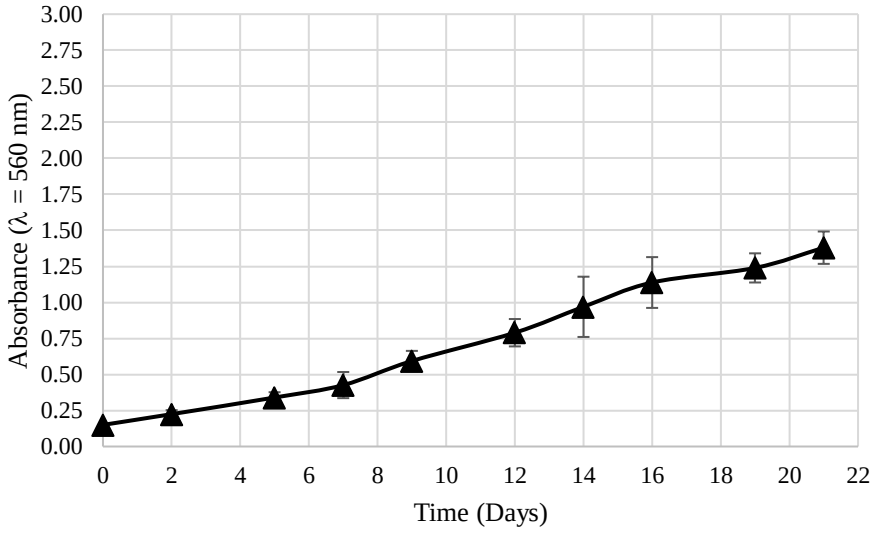


Figure 4.1. Optical density over time graph of *A. maxima*, strain SAG 84.79.

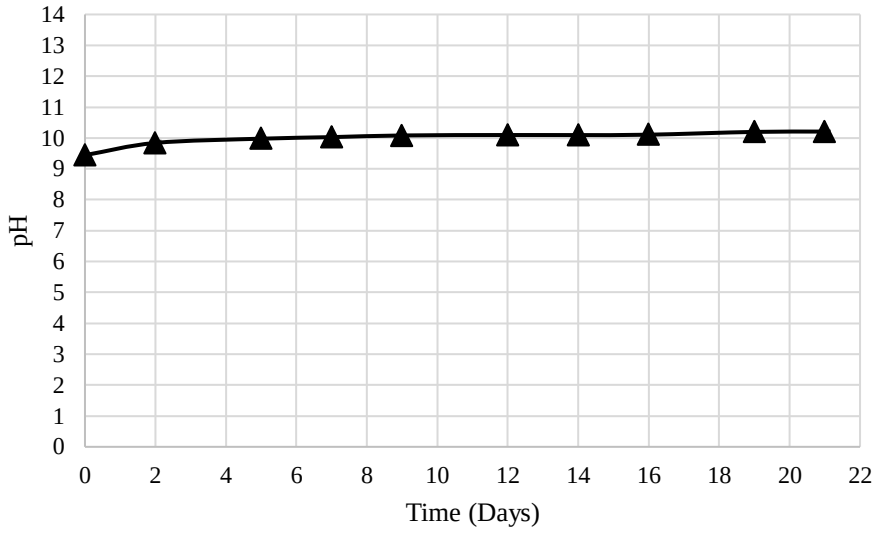


Figure 4.2. pH over time graph of *A. maxima*, strain SAG 84.79

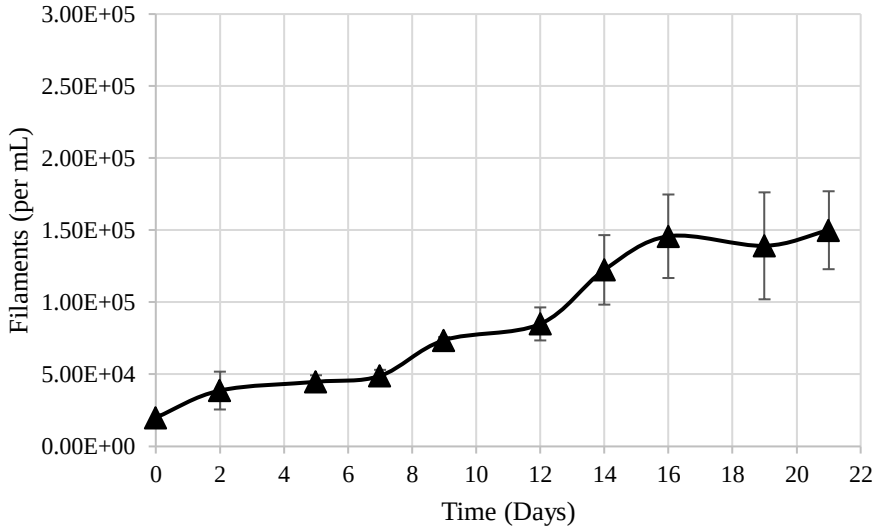


Figure 4.3. Filament number over time graph of *A. maxima*, strain SAG 84.79.

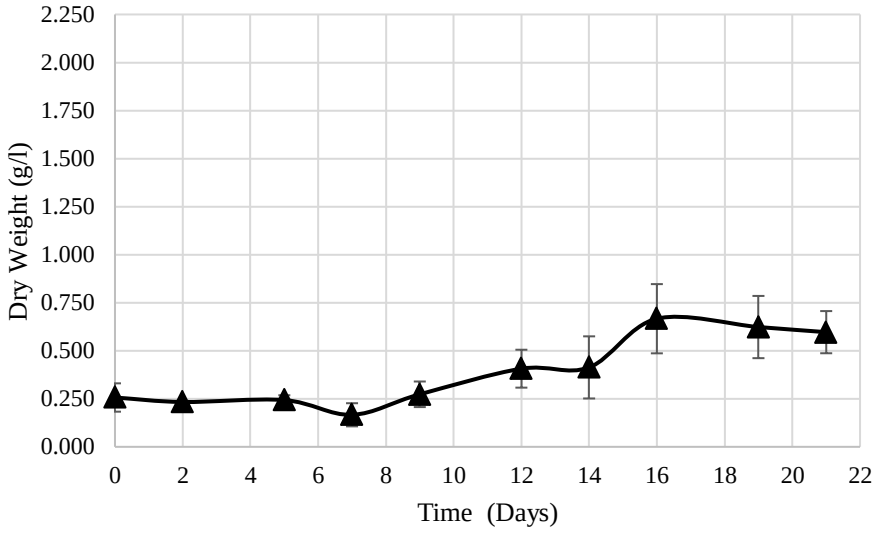


Figure 4.4. Dry weight over time graph of *A. maxima*, strain SAG 84.79.

4.2. Farm Wastewater Study

Total N, total P and chemical oxygen demand were determined before and after the pretreatment step with chitosan solution.

4.2.1. Characteristics of Farm Wastewater

Total nitrogen level that was 1376.7 mg/L before pretreatment was reduced to 209.8 mg/L, total phosphorus was reduced to 17.6 mg/L from 242.5 mg/L, and COD of 37.1 g/L became 13.3 mg/L after chitosan application (Table 4.1).

Table 4.1. TN, TP, COD, and solids results of FWW before and after pretreatment.

	Before Pretreatment	**After Pretreatment**	**% Removal**
Total Nitrogen (mg/L TN)	1376.70	209.77	81.84
Total Phosphorous (mg/L TP)	242.50	17.58	88.44
COD (g/L COD)	37.1	13.3	64.12

4.2.2. Multi Well Plate Study with Farm Wastewater

As a result of the conducted multi well plate study with several inoculation amounts, the best amount to inoculate FWW was found to be 4/10th of the total volume of the reactor. Cultures showed better growth as the culture amount used for inoculation was increased (Figure 4.5-6).

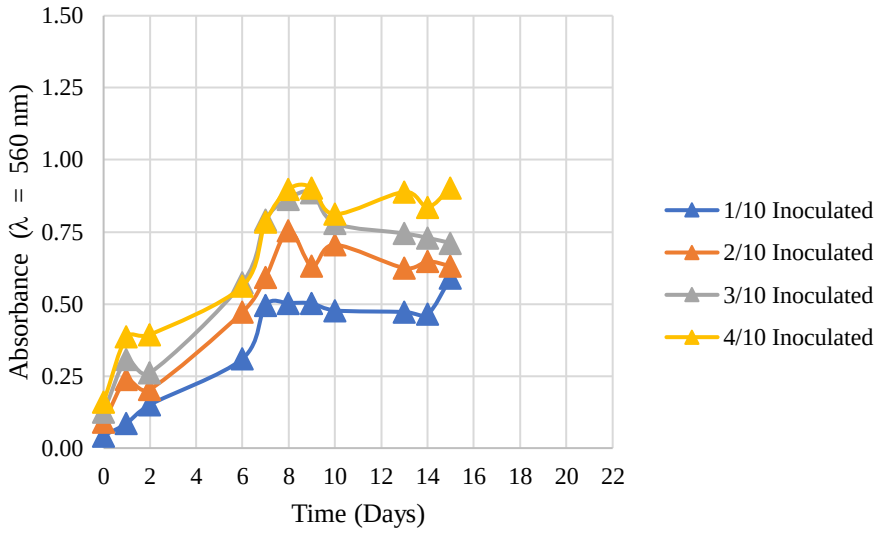


Figure 4.5. Optical density over time graph of cultures grown in Spir media in multi well plates.

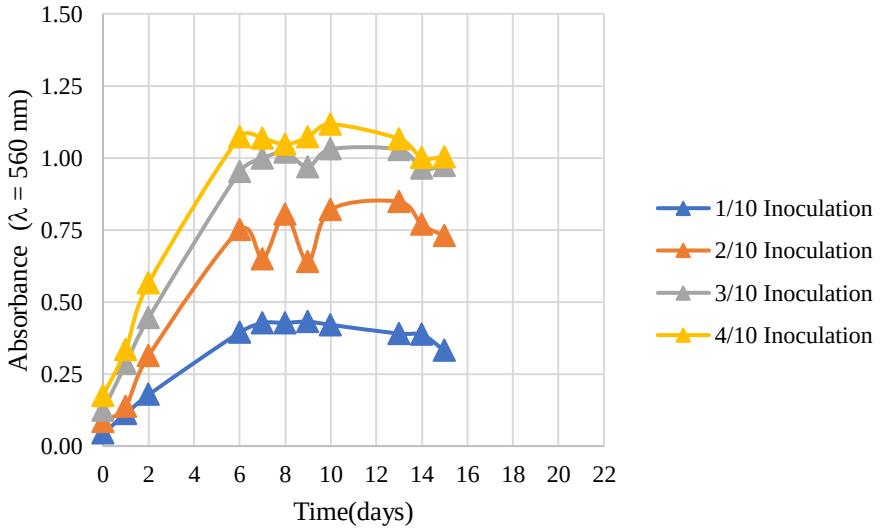


Figure 4.6. Optical density over time graph of cultures grown in FWW in multi well plates.

4.3. Plastic Photobioreactor Study

Plastic PBRs were used after the growth profile determination in Erlenmeyer flasks. A control study was conducted with Spir culturing media to see how *A. maxima* culture reacts to a changed culturing set up when compared to Erlenmeyer flasks. And after that, culture was grown in FWW using the same set up. Cultures were grown in PBRs as biological triplicates until they reached the death phase. Optical density, pH, dry weight, and filaments measurements were done daily.

4.3.1. Plastic Photobioreactor Growth Profile of Control Culture

Even though the chances of contamination were higher for plastic PBRs than glass Erlenmeyer PBRs, *A. maxima* culture showed a better growth in plastic PBRs (Figure 4.7-10.). There might be several contributing factors for this better growth. The area of the culture that faces the light at a given time is larger than that is of Erlenmeyer flasks. Increased surface area and exposure to less shading for any individual filament apparently affected the culture positively. Additionally, orbitally shaking the culture might have increased the nutrient availability without disturbing the filaments' dignity, which might be threatened when using a magnetic stirrer that might cause a high shear stress.

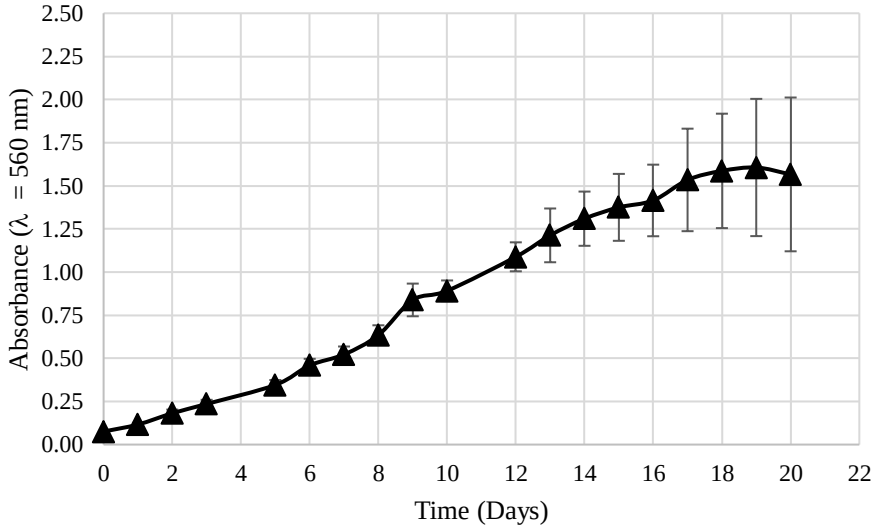


Figure 4.7. Optical density over time graph of *A. maxima*, strain SAG 84.79 grown in Spir Media.

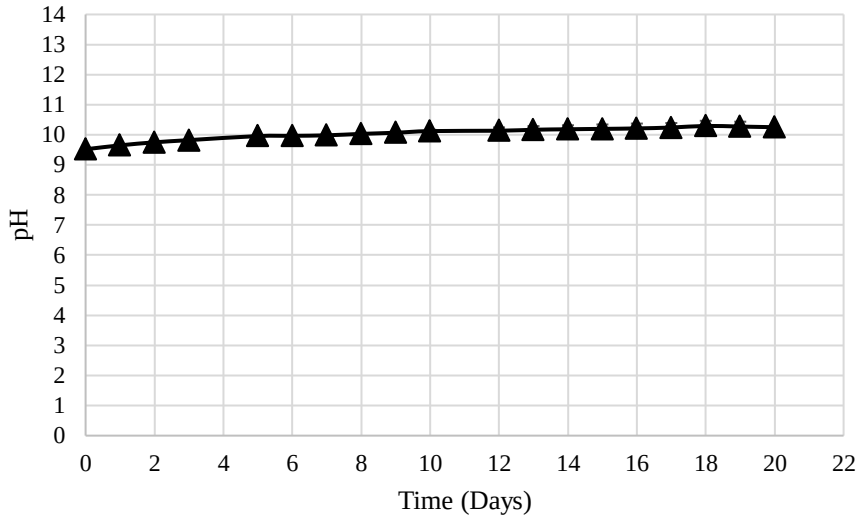


Figure 4.8. pH over time graph of *A. maxima*, strain SAG 84.79 grown in Spir Media.

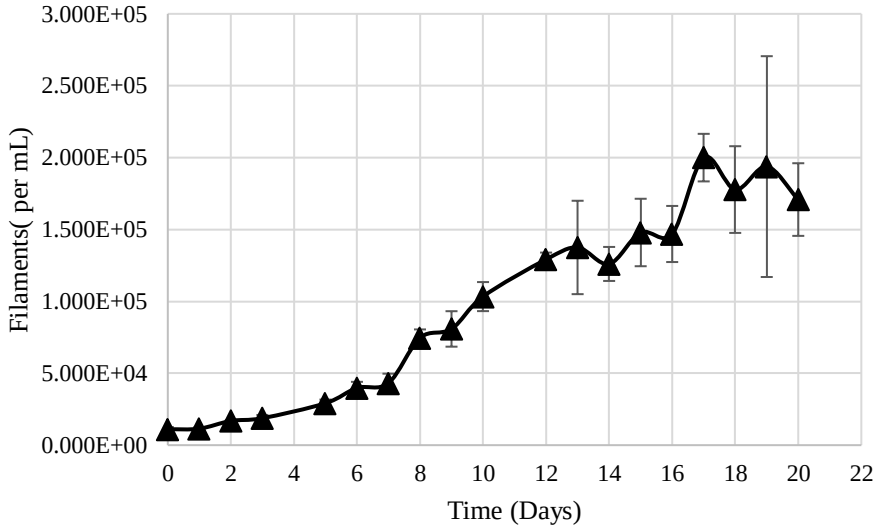


Figure 4.9. Filament number over time graph of *A. maxima*, strain SAG 84.79 grown in Spir Media.

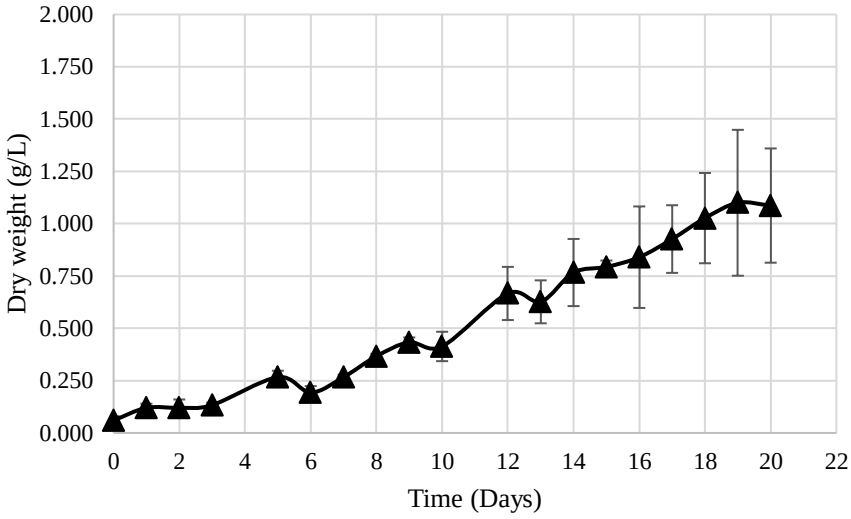


Figure 4.10. Dry weight over time graph of *A. maxima*, strain SAG 84.79 grown in Spir Media.

4.3.2. Plastic Photobioreactor Growth Profile of Farm Wastewater Culture

Culture grown in FWW showed an apparent worse growth than the culture that was grown in the Spir media (Figure 4.11-14). Even though the dry weight results and final harvested biomass amounts were higher and might be considered as promising, filament numbers did not increase notably whereas in the control study, the culture showed growth until the 12th day. There might be several reasons for this situation. The most important reason is that the nutrient amounts in the FWW were significantly lower than they are in Spir media. Particularly phosphorus, which is the main growth limiting factor in nature, that was available to the culture after chitosan pretreatment was lower than the 1/4th of the culture need. Nitrogen level was half of the culture's usual media, as well. Additionally, in the presence of an organically rich FWW, bacteria culture, that has a symbiotic relationship with the cyanobacteria culture, thrived. And this might have caused the nutrient scarcity even further. This might also explain the optical density values' sharp increase and the stable trend over the time.

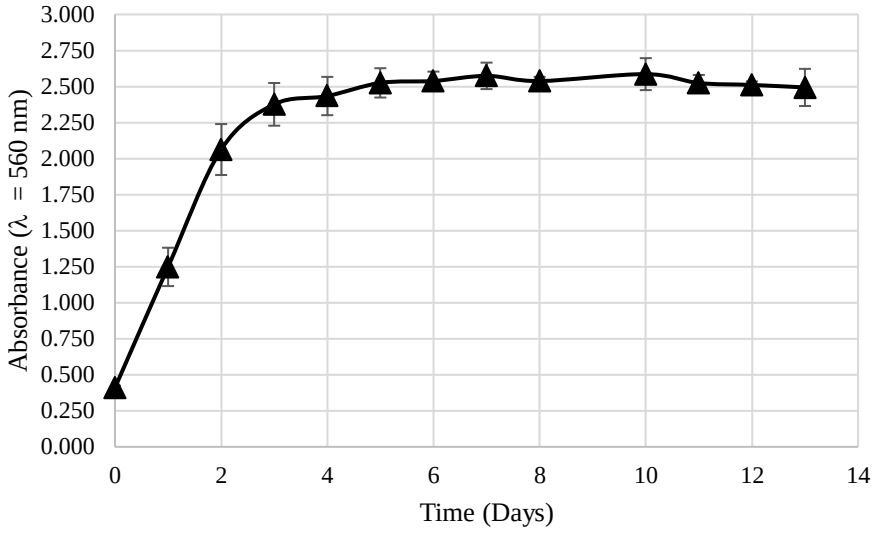


Figure 4.11. Optical density over time graph of *A. maxima*, strain SAG 84.79 grown in FWW.

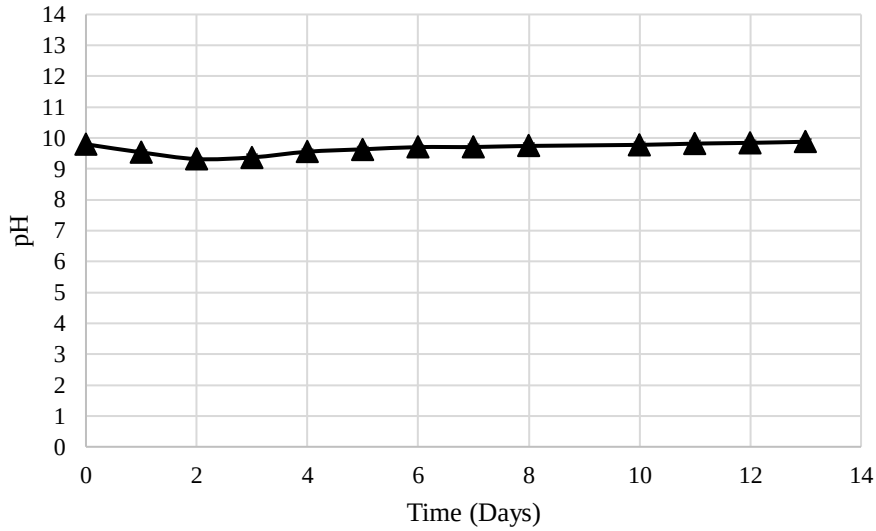


Figure 4.12. pH over time graph of *A. maxima*, strain SAG 84.79 grown in FWW.

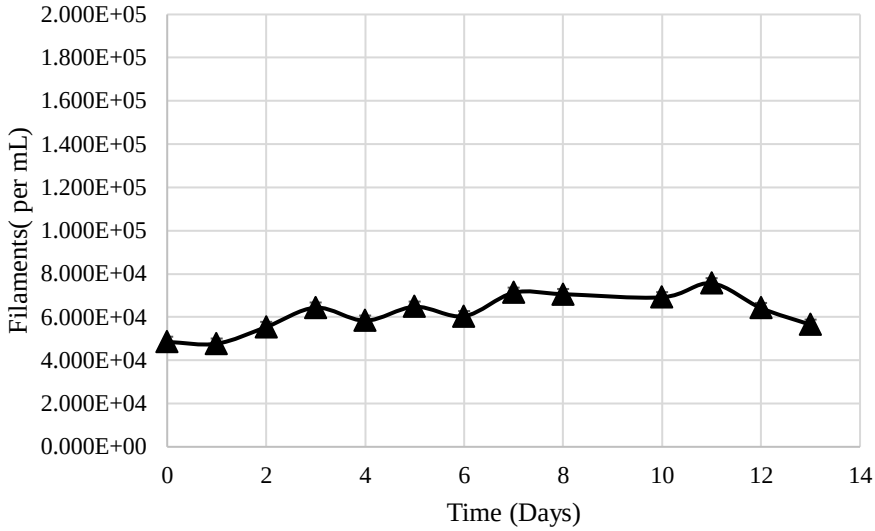


Figure 4.13. Filament number over time graph of *A. maxima*, strain SAG 84.79 grown in FWW.

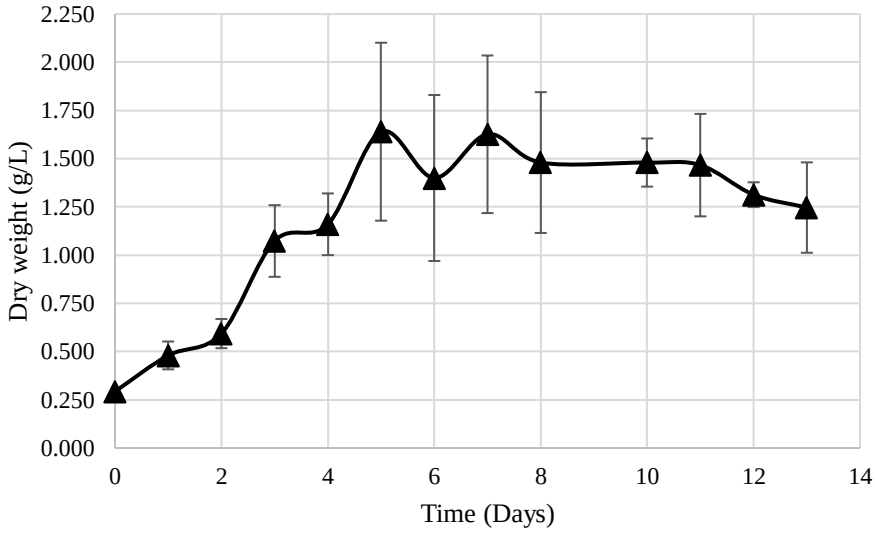


Figure 4.14. Dry weight over time graph of *A. maxima*, strain SAG 84.79 grown in FWW.

4.3.3. Nutrient Removal Profiles

TN and TP measurements were taken for the FWW reactors in every two days and removal profiles were obtained (Figure 4.15 and 4.16). COD and TS data are only taken from the influent and effluent WW. An average removal of 99.4%, 98.1%, 92.9% and 92.6% were achieved for TN, TP, COD and for solids, respectively (Table 4.2, 4.3, 4.4 and 4.5). TN and TP removal graphs show that in the first 2 days of the culture, most of the nitrogen and phosphorus were taken up and then the removal trends slow. These might also account for the lesser growth of the culture in FWW than in Spir media. Culture used most of the already limited nutrients in the first days. There is also a slight increase in phosphorus amounts towards the end of the culture after the lowest amount was reached.

This might be due to dead cells and cell debris that could not be filtered. Thus, during the digestion process of the phosphorus measurement, they might have been digested as well.

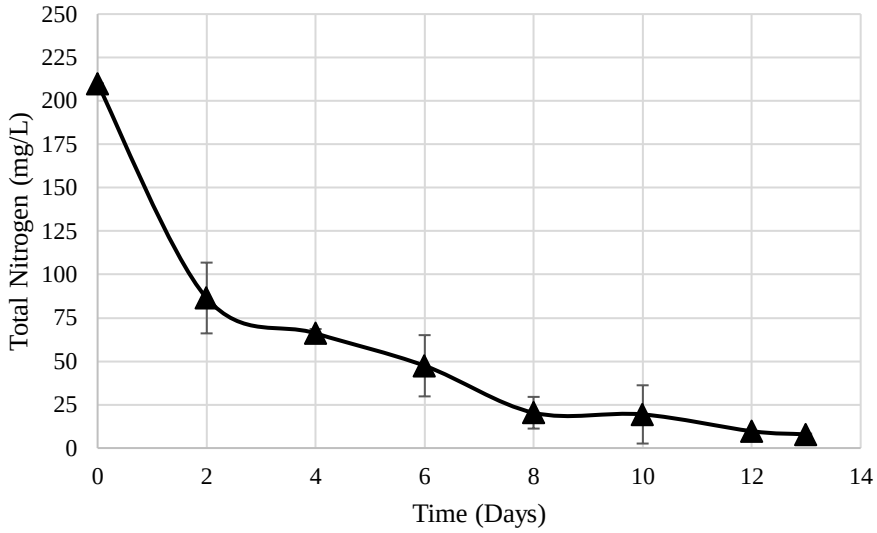


Figure 4.15. Total nitrogen over time profile of culture grown in FWW.

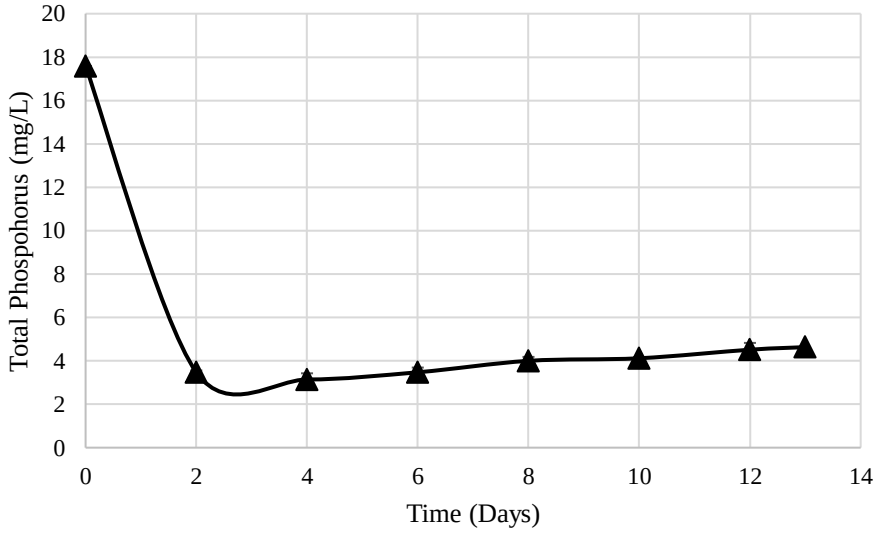


Figure 4.16. Total phosphorus over time profile of culture grown in FWW.

Table 4.2. Total nitrogen removal of FWW PBRs.

FWW PBR	TN of raw FWW (mg/L)	TN of FWW after chitosan treatment (mg/L)	TN of FWW after phycoremediation (mg/L)	% Removal of phycoremediation	Mean % removal of phycoremediation	Overall % removal
1	1376.7	209.8	7.5	14.7		
2	1376.7	209.8	8.4	14.6	14.7	99.4
3	1376.7	209.8	7.7	14.7		

Table 4.3. Total phosphorus removal of FWW PBRs.

FWW PBR	TP of raw FWW (mg/L)	TP of FWW after chitosan treatment (mg/L)	TP of FWW after phycoremediation (mg/L)	% Removal of phycoremediation	Mean % removal of phycoremediation	Overall % removal
1	242.5	17.9	4.9	5.2		
2	242.5	17.9	4.8	5.3	5.3	98.1
3	242.5	17.9	4.2	5.5		

Table 4.4. COD removal of FWW PBRs.

FWW PBR	COD of raw FWW (g/L)	COD of FWW after chitosan treatment (g/L)	COD of FWW after phycoremediation (g/L)	% Removal of phycoremediation	Mean % removal of phycoremediation	Overall % removal
1	37.1	13.3	3.0	27.8		
2	37.1	13.3	2.4	29.5	28.8	92.9
3	37.1	13.3	2.5	29.0		

Table 4.5. Solids removal of FWW PBRs.

Solid Type	Raw FWW (g/L)	After phycoremediation (g/L)	Overall % removal
TS	21.7	1.6	
TDS	11.5	0.8	92.6
TSS	10.2	0.7	

4.3.4. Chemical and Biochemical Profile of Biomass

In this section, vitamin analyses, amino acids analyses, FAME analyses and elemental analyses of harvested biomasses from control and FWW PBRs are presented in addition to total hydro-soluble proteins, total carbohydrates, and total lipids, and compared in detail. Also, preliminary data of the

plant biostimulants analysis is discussed. Potential for a biofertilizer application of the culture grown in FWW is evaluated.

Culture grown in FWW showed a significant lower total hydro-soluble protein content and slightly lower carbohydrate content (Table 4.6 and 4.7). However, it still might be considered as a high protein content when compared to the other cyanobacteria and algae species. Lipid portion of the culture that was grown in control PBR was very small whereas the culture grown in FWW showed considerably higher amount of lipids (Table 4.8). These findings are also in agreement with the present literature data. When the available nutrient levels are low, lipid amounts in algae and cyanobacteria tend to increase. Although all the conditions were identical, differences could be seen between the biological triplicates both in the control culture and FWW culture. For example, the 3rd PBR of the control culture's carbohydrate level is lower, which is compensated by the higher level of lipids content, when compared to the other two reactors of the same set. However, the FAME variability was decreased even though the total lipids that the biomass accumulated increased (Table 4.9) (Appendix A). Expected higher amount of lipids that were found in gravimetric total lipids measurement cannot be seen on the FAME amounts table (Table 4.10). Culture might have stored the lipids in other forms that we cannot and/or did not detect and measure. All the B vitamins are found to be in higher amounts in the biomass that was grown FWW (Table 4.11 and 4.12) (Appendix B). Moreover, some of the vitamins were not detected in the biomass grown in Spir media whereas the biomass that was grown in FWW was found to have them such as pyridoxine (Vitamin B6) and biotin (Vitamin B7). Constituents of the FWW might have promoted the vitamin production even though the nutrients were scarce. The same argument is not valid for the amino acids content of the biomass. Some of them are higher in the biomass that was grown in Spir media, and the others are higher in the biomass grown in FWW (Table 4.13, 14) (Appendix C). But in some of them, a huge difference can be seen between the biomasses. For example, MET, PHE, TYR, and VAL amounts in FWW biomass is very high whereas Spir biomass content of these are very low and almost negligible in some of them. The difference between couple of their amounts are 10 to 17 folds. This clearly suggests that the FWW promotes certain amino acids production to a great extent. However, most of the metal amounts are lower in the biomass grown in FWW (Table 4.15). This might be due to the pretreatment step. Chitosan might have removed the metals as it is an excellent adsorbent and used extensively for metal removal purpose from various wastewater types due to this characteristic. However, biomass potential as a biofertilizer remains depending on the plant that it might be used to fertilize. Additionally, some of the heavy metal amounts are found to be higher in the biomass grown in FWW such as As, Cr, and Ba, as cyanobacteria are used for their heavy metal removal abilities.

Further studies should be conducted to determine sources causing this observation because there might be several reasons. One of them might be the fact that the farm where FWW was collected is very close to a very busy main transition road between Europe and Turkey and a newly built highway. As a consequence, the farm's fields might have deposited these heavy metals, resulting in contaminated feed for the animals. Another reason might be the water that is used either for irrigation or for drinking water for animals. Also some of the most used pesticides are As based as it is highly toxic (Punshon et al., 2017) and $BaCO_3$ was one of the most used rodenticide until recently (Peana et al., 2021). Even though the farm is operated organically today, previous chemical-based operations might have caused a soil deposition. For qualitative biostimulant analysis, water soluble vitamins extracts were used, and 23 compounds were chosen to be analyzed. (+)-7-isojasmonic acid, 12-hydroxyjasmonic acid and Gibberellin A1 were found to be present in FWW PBR (Table 4.16). Their respective chromatograms are given in Appendix F. 1-aminocyclopropane-1-carboxylic acid is the immediate ethylene precursor (de Poel & Van Der Straeten, 2014). Jasmonic acids are responsible for responses to environmental stresses such as pathogens, herbivores, UV radiation, drought, and wounding (Balcke et al., 2012). Gibberellins are a diverse class of hormones and they are mainly responsible for the plant growth and development (Gao, Zhang, He, & Fu, 2017). Their presence in FWW biomass clearly suggest that FWW promoted their production, and these findings agree with present literature data that was mentioned in Section 2.3. Additionally, the higher amount of amino acids TRP and ARG in FWW biomass might suggest that biostimulants that were not detected or not captured might be present below detection levels as well since these amino acids are also precursors of some key phytohormones (Chiaiese et al., 2018).

Table 4.6. Total hydro-soluble protein content of the harvested biomasses from PBRs.

PBRs	Hydro-soluble protein in dry biomass (mg/g)	Hydro-soluble protein % in dry weight	Average %	STD Dev.
Control PBR 1	715.5	71.5		
Control PBR 2	751.0	75.1	72.4	2.4
Control PBR 3	704.8	70.5		
FWW PBR 1	389.1	38.9		
FWW PBR 2	380.7	38.1	41.3	4.8
FWW PBR 3	468.2	46.8		

Table 4.7. Total carbohydrates content of the harvested biomasses from PBRs.

PBRs	Total carbohydrates in dry biomass (mg/g)	Total carbohydrates % in dry weight	Average %	STD Dev
Control PBR 1	490.0	49.0		
Control PBR 2	441.0	44.1	39.8	11.9
Control PBR 3	263.0	26.3		
FWW PBR 1	249.0	24.9		
FWW PBR 2	310.3	31.0	27.3	3.2
FWW PBR 3	261.1	26.1		

Table 4.8. Total lipids content of the harvested biomasses from PBRs.

PBRs	Total lipids in dry biomass (mg/g)	Total lipids % in dry weight	Average %	STD Dev.
Control PBR 1	98.7	9.9		
Control PBR 2	93.2	9.3	12.7	5.3
Control PBR 3	188.3	18.8		
FWW PBR 1	244.0	24.3		
FWW PBR 2	144.0	14.4	24.1	9.5
FWW PBR 3	334.2	33.4		

Table 4.9. Concentrations of FAMEs in measured samples.

	Concentration (μg/mL hexane)					
PBRs	C16:0	C16:1	C18:0	C18:1n9c	C18:2n6c	C18:3n6
Control PBR 1	112.5	10.8	22.3	12.5	35.0	54.6
Control PBR 2	109.6	9.2	26.7	13.2	30.9	50.6
Control PBR 3	146.7	13.1	31.9	12.7	45.4	70.5
FWW PBR 1	87.7	10.1	28.9	-	-	-
FWW PBR 2	91.4	11.6	31.5	-	-	-
FWW PBR 3	94.1	11.8	33.4	-	-	-

Table 4.10. FAME content of harvested biomasses from PBRs.

FAME	PBRs	FAME amount in dry biomass (mg/g)	FAME % in dry biomass	Average %	STD Dev.
C16:0	Control PBR 1	6.67	0.67	0.73	0.12
	Control PBR 2	6.48	0.65		
	Control PBR 3	8.70	0.87		
	FWW PBR 1	5.17	0.52	0.54	0.02
	FWW PBR 2	5.43	0.54		
	FWW PBR 3	5.58	0.56		
C 16:1	Control PBR 1	0.64	0.06	0.07	0.01
	Control PBR 2	0.54	0.05		
	Control PBR 3	0.78	0.08		
	FWW PBR 1	0.60	0.06	0.07	0.01
	FWW PBR 2	0.69	0.07		
	FWW PBR 3	0.70	0.07		
C 18:0	Control PBR 1	1.32	0.13	0.16	0.03
	Control PBR 2	1.58	0.16		
	Control PBR 3	1.89	0.19		
	FWW PBR 1	1.70	0.17	0.18	0.01
	FWW PBR 2	1.87	0.19		
	FWW PBR 3	1.98	0.20		
C 18:1n9c	Control PBR 1	0.74	0.07	0.08	0.00
	Control PBR 2	0.78	0.08		
	Control PBR 3	0.81	0.08		
	FWW PBR 1				
	FWW PBR 2				
	FWW PBR 3				
C 18:2n6c	Control PBR 1	2.08	0.21	0.22	0.04
	Control PBR 2	1.83	0.18		
	Control PBR 3	2.69	0.27		
	FWW PBR 1				
	FWW PBR 2				
	FWW PBR 3				
C 18:3n6	Control PBR 1	3.25	0.32	0.35	0,06
	Control PBR 2	2.99	0.30		
	Control PBR 3	4.18	0.42		
	FWW PBR 1				
	FWW PBR 2				
	FWW PBR 3				

Table 4.11. Concentrations of B vitamins in measured samples.

Vitamins	Concentration in sample (ng/mL)							
	Control PBR 1	Control PBR 2	Control PBR 3	Average	FWW PBR 1	FWW PBR 2	FWW PBR 3	Average
Thiamin (B1)	0.7	2.4	Below LOQ	1.5	9.5	5.0	12.5	9.0
Riboflavin (B2)	22.3	13.3	22.7	19.4	19.3	23.8	25.2	22.7
Nicotinic acid (B3)	55.7	48.9	29.0	44.5	118.0	82.4	94.3	98.2
Pantothenic acid (B5)	6.5	7.5	14.4	9.45	32.8	25.3	38.0	32.0
Pyridoxine (B6)	Below LOQ	Below LOQ	Below LOQ		1.3	0.6	2.4	1.4
Biotin (B7)	N/F	N/F	N/F		4.8	4.4	3.8	4.3
Folic acid (B9)	N/F	N/F	N/F		N/F	N/F	N/F	

Table 4.12. B vitamins content of harvested biomasses from PBRs.

Vitamin	PBRs	Vitamin in dry biomass (mg/kg)	Average (mg/kg)	STD Dev.
Thiamine B1	Control PBR 1	0.03	0.06	0.05
	Control PBR 2	0.09		
	Control PBR 3	Below LOQ		
	FWW PBR 1	0.38	0.36	0.15
	FWW PBR 2	0.20		
	FWW PBR 3	0.50		
Riboflavin B2	Control PBR 1	0.89	0.77	0.21
	Control PBR 2	0.53		
	Control PBR 3	0.90		
	FWW PBR 1	0.77	0.91	0.12
	FWW PBR 2	0.95		
	FWW PBR 3	1.00		
Nicotinic acid B3	Control PBR 1	2.22	1.77	0.55
	Control PBR 2	1.95		
	Control PBR 3	1.15		
	FWW PBR 1	4.69	3.91	0.72
	FWW PBR 2	3.29		
	FWW PBR 3	3.76		
Pantothenic acid B5	Control PBR 1	0.26	0.30	0.03
	Control PBR 2	0.30		
	Control PBR 3	0.33		
	FWW PBR 1	1.30	1.28	0.25
	FWW PBR 2	1.01		
	FWW PBR 3	1.51		
Pyridoxine B6	Control PBR 1	Below LOQ		
	Control PBR 2	Below LOQ		

	Control PBR 3	Below LOQ		
	FWW PBR 1	0.05		
	FWW PBR 2	0.02	0.06	0.04
	FWW PBR 3	0.09		
Biotin B7	Control PBR 1	Below LOQ		
	Control PBR 2	Below LOQ		
	Control PBR 3	Below LOQ		
	FWW PBR 1	0.19		
	FWW PBR 2	0.17	0.17	0.02
	FWW PBR 3	0.15		

Table 4.13. Amino acids concentrations in measured samples.

	Concentration in sample (ng/mL)							
Amino acid	**Control PBR 1**	**Control PBR 2**	**Control PBR 3**	**Average**	**FWW PBR 1**	**FWW PBR 2**	**FWW PBR 3**	**Average**
ALA	72.7	106.2	155.8	111.6	75.5	52.0	74.7	67.4
ARG	16.5	25.7	12.1	18.1	34.9	29.6	36.6	33.7
ASP	70.7	80.6	70.1	73.8	24.7	18.1	37.4	26.7
CYS	Below LOQ	Below LOQ	Below LOQ		Below LOQ	Below LOQ	Below LOQ	
GLU	2263.6	2472.4	1391.7	2042.5	1199.1	1154.1	1207.3	1186.8
GLY	Below LOQ	Below LOQ	Below LOQ		Below LOQ	Below LOQ	Below LOQ	
HIS	3.0	4.3	2.9	3.4	9.9	7.7	11.7	9.8
ILE + LEU	7.9	8.3	3.3	6.5	47.8	36.7	54.2	46.2
LYS	20.5	21.8	15.3	19.2	40.7	34.3	32.9	36.0
MET	4.1	3.0	1.1	2.7	27.1	20.9	29.3	25.8
PHE	3.0	1.8	Below LOQ	2.4	44.6	33.5	47.7	41.9
PRO	17.2	29.8	3.2	16.8	3.5	2.2	4.5	3.4
SER	21.7	22.2	27.7	23.9	18.6	8.0	17.1	14.6
TAU	ND	ND	ND		ND	ND	ND	
THR	21.6	18.9	15.9	18.8	36.1	26.1	33.0	31.8
TRP	Below LOQ	Below LOQ	Below LOQ		4.2	1.8	6.9	4.3
TYR	2.9	3.3	3.78	3.3	15.4	11.6	21.3	16.1
VAL	63.5	71.7	82.3	72.5	1163.4	1135.3	1324.7	1207.8

Table 4.14. Amino acids content of harvested biomass from PBRs.

Amino acids	PBRs	Amino acid in dry biomass (mg/kg)	Average (mg/kg)	STD Dev.
ALA	Control PBR 1	1361.6	1834.5	564.2
	Control PBR 2	1682.8		
	Control PBR 3	2459.1		
	FWW PBR 1	1196.8	1066.1	208.5
	FWW PBR 2	825.6		
	FWW PBR 3	1175.9		
ARG	Control PBR 1	259.8	285.8	110.7
	Control PBR 2	407.2		
	Control PBR 3	190.5		
	FWW PBR 1	552.9	533.2	55.9
	FWW PBR 2	470.0		
	FWW PBR 3	576.5		
ASP	Control PBR 1	1115.0	1165.9	96.1
	Control PBR 2	1276.8		
	Control PBR 3	1106.0		
	FWW PBR 1	391.2	422.2	153.9
	FWW PBR 2	286.1		
	FWW PBR 3	589.2		
GLU	Control PBR 1	35686.5	32278.6	9105.7
	Control PBR 2	39188.8		
	Control PBR 3	21960.6		
	FWW PBR 1	19013.6	18746.5	450.9
	FWW PBR 2	18225.9		
	FWW PBR 3	19000.0		
GLY	Control PBR 1	Below LOQ		
	Control PBR 2	Below LOQ		
	Control PBR 3	Below LOQ		
	FWW PBR 1	Below LOQ		
	FWW PBR 2	Below LOQ		

Amino acids	PBRs	Amino acid in dry biomass (mg/kg)	Average (mg/kg)	STD Dev.
	FWW PBR 3	Below LOQ		
HIS	Control PBR 1	46.8	53.6	12.4
	Control PBR 2	67.9		
	Control PBR 3	46.1		
	FWW PBR 1	157.3	154.7	31.2
	FWW PBR 2	122.2		
	FWW PBR 3	184.5		
ILE + LEU	Control PBR 1	118.0	100.4	42.4
	Control PBR 2	131.2		
	Control PBR 3	52.1		
	FWW PBR 1	757.8	731.2	137.5
	FWW PBR 2	582.31		
	FWW PBR 3	853.5		
LYS	Control PBR 1	322.7	303.4	54.6
	Control PBR 2	345.7		
	Control PBR 3	241.8		
	FWW PBR 1	644.4	568.8	66.7
	FWW PBR 2	544.4		
	FWW PBR 3	517.8		
MET	Control PBR 1	64.1	43.0	23.5
	Control PBR 2	47.1		
	Control PBR 3	17.7		
	FWW PBR 1	429,9	407,5	67,7
	FWW PBR 2	331,4		
	FWW PBR 3	461,3		
PHE	Control PBR 1	46.7	37.7	12.7
	Control PBR 2	28.7		
	Control PBR 3	Below LOQ		
	FWW PBR 1	707.5	663,2	116,6

Amino acids	PBRs	Amino acid in dry biomass (mg/kg)	Average (mg/kg)	STD Dev.
	FWW PBR 2	531.0		
	FWW PBR 3	751.2		
PRO	Control PBR 1	272.0	265.3	210.8
	Control PBR 2	472.6		
	Control PBR 3	51.2		
	FWW PBR 1	55.8		
	FWW PBR 2	34.2	53.4	18.2
	FWW PBR 3	70.3		
SER	Control PBR 1	341.9	377.1	53.0
	Control PBR 2	351.4		
	Control PBR 3	438.0		
	FWW PBR 1	294.5		
	FWW PBR 2	127.4	230.3	90.0
	FWW PBR 3	269.0		
TAU	Control PBR 1	Below LOQ		
	Control PBR 2	Below LOQ		
	Control PBR 3	Below LOQ		
	FWW PBR 1	Below LOQ		
	FWW PBR 2	Below LOQ		
	FWW PBR 3	Below LOQ		
THR	Control PBR 1	361.4	317.9	44.9
	Control PBR 2	320.7		
	Control PBR 3	271.7		
	FWW PBR 1	592.1		
	FWW PBR 2	435.0	522.1	79.9
	FWW PBR 3	539.1		
TRP	Control PBR 1	Below LOQ		
	Control PBR 2	Below LOQ		
	Control PBR 3	Below LOQ		
	FWW PBR 1	0.06	0.07	0.04

Amino acids	PBRs	Amino acid in dry biomass (mg/kg)	Average (mg/kg)	STD Dev.
	FWW PBR 2	0.03		
	FWW PBR 3	0.10		
TYR	Control PBR 1	60.1	57.6	3.8
	Control PBR 2	53.2		
	Control PBR 3	59.4		
	FWW PBR 1	243.7	254.8	76.0
	FWW PBR 2	185.0		
	FWW PBR 3	335.8		
VAL	Control PBR 1	1042.4	1185.2	147.6
	Control PBR 2	1176.0		
	Control PBR 3	1337.1		
	FWW PBR 1	18335.12	18957.3	1545.7
	FWW PBR 2	17819.5		
	FWW PBR 3	20717.0		

Table 4.15. Metal content of harvested biomass from PBRs

Metal isotope	PBRSs	Metal in dry biomass (mg/kg)	Average	STD
24Mg	Control PBR 1	2096.8	2116.1	169.3
	Control PBR 2	1957.2		
	Control PBR 3	2294.2		
	FWW PBR 1	1007.9	1026.6	310.8
	FWW PBR 2	725.5		
	FWW PBR 3	1346.4		
27Al	Control PBR 1	77.0	75.5	18.5
	Control PBR 2	56.3		
	Control PBR 3	93.3		
	FWW PBR 1	58.3	55.3	14.9
	FWW PBR 2	39.1		
	FWW PBR 3	68.4		
39K	Control PBR 1	25165.0	25469.4	296.4
	Control PBR 2	25485.9		
	Control PBR 3	25757.2		
	FWW PBR 1	14029.8	14292.6	259.9
	FWW PBR 2	14298.9		
	FWW PBR 3	14549.2		
44Ca	Control PBR 1	56.3	59.8	3.0
	Control PBR 2	61.4		

Metal isotope	PBRSs	Metal in dry biomass (mg/kg)	Average	STD
	Control PBR 3	61.7		
	FWW PBR 1	64.3		
	FWW PBR 2	64.8	60.6	6.9
	FWW PBR 3	52.7		
51V	Control PBR 1	0.05		
	Control PBR 2	0.03	0.03	0.01
	Control PBR 3	0.03		
	FWW PBR 1	0.06		
	FWW PBR 2	0.04	0.06	0.02
	FWW PBR 3	0.09		
52Cr	Control PBR 1	1,7		
	Control PBR 2	1,34	1,4	0,3
	Control PBR 3	1.1		
	FWW PBR 1	2.8		
	FWW PBR 2	2.7	3.1	0.6
	FWW PBR 3	3.7		
55Mn	Control PBR 1	23.3		
	Control PBR 2	22.6	24.8	3.2
	Control PBR 3	28.4		
	FWW PBR 1	6.1		
	FWW PBR 2	7.6	6.9	0.7
	FWW PBR 3	6.9		
57Fe	Control PBR 1	119.2		
	Control PBR 2	105.82	150.0	65.2
	Control PBR 3	224.9		
	FWW PBR 1	40.2		
	FWW PBR 2	71.3	53.8	15.9
	FWW PBR 3	49.8		
59Co	Control PBR 1	0.66		
	Control PBR 2	0.73	0.70	0.04
	Control PBR 3	0.71		
	FWW PBR 1	1.0		
	FWW PBR 2	0.6	0.9	0.2
	FWW PBR 3	0.9		
69Ni	Control PBR 1	11.2		
	Control PBR 2	4.6	6.3	4.3
	Control PBR 3	3.1		
	FWW PBR 1	3.3		
	FWW PBR 2	5.0	4.1	0.9
	FWW PBR 3	3.9		
63Cu	Control PBR 1	2.8		
	Control PBR 2	3.7	3,6	0,8
	Control PBR 3	4.4		
	FWW PBR 1	4.3	3.7	0.6
	FWW PBR 2	3.0		

Metal isotope	PBRSs	Metal in dry biomass (mg/kg)	Average	STD
	FWW PBR 3	3.9		
66Zn	Control PBR 1	20.2		
	Control PBR 2	14.4	16.8	3.0
	Control PBR 3	15.6		
	FWW PBR 1	28.8		
	FWW PBR 2	30.5	30.1	1.1
	FWW PBR 3	30.9		
75As	Control PBR 1	0.04		
	Control PBR 2	0.03	0.04	0.01
	Control PBR 3	0.04		
	FWW PBR 1	0.5		
	FWW PBR 2	0.4	0.4	0.1
	FWW PBR 3	0.4		
77Se	Control PBR 1	0.01		
	Control PBR 2	0.01	0.01	0.01
	Control PBR 3	0.02		
	FWW PBR 1	0.02		
	FWW PBR 2	0.03	0.03	0.01
	FWW PBR 3	0.03		
88Sr	Control PBR 1	2.1		
	Control PBR 2	2.4	2.6	0.7
	Control PBR 3	3.4		
	FWW PBR 1	2.4		
	FWW PBR 2	1.9	2.1	0.3
	FWW PBR 3	2.0		
111Cd	Control PBR 1	0.05		
	Control PBR 2	0.02	0.03	0.01
	Control PBR 3	0.04		
	FWW PBR 1	0.05		
	FWW PBR 2	0.05	0.08	0.05
	FWW PBR 3	0.14		
137Ba	Control PBR 1	0.5		
	Control PBR 2	0.4	0.5	0.1
	Control PBR 3	0.5		
	FWW PBR 1	1.0		
	FWW PBR 2	0.7	0.9	0.1
	FWW PBR 3	0.8		
208Pb	Control PBR 1	1.2		
	Control PBR 2	1.5	1.4	0.2
	Control PBR 3	1.5		
	FWW PBR 1	0.80		
	FWW PBR 2	0.85	0.86	0.06
	FWW PBR 3	0.92		

Table 4.16. Qualitative biostimulants results of biomass grown in FWW.

Compound	FWW PBRs
12-hydroxyjasmonic acid	+
(+)-7-isojasmonic acid	+
Gibberellin A1	+
1-aminocyclopropane-1-carboxylic acid	+

5. CONCLUSION

Cyanobacterial species have promising potential for wastewater treatment. Their resilience and fast adapting nature make them excellent candidates for various types of wastewaters. In this study, *A. maxima*, SAG 84.79, was evaluated as a biological nutrient removal option for farm wastewater coupled with potential use of biofertilizer. In summary, FWW was pretreated with chitosan and resulting liquid portion was inoculated with cyanobacteria using a custom designed plastic PBR. Harvested biomass was analyzed for its potential for biofertilizer use as part of a sustainable and circular farm operation.

This thesis study showed that with chitosan pretreatment, total nitrogen, total phosphorus, and chemical oxygen demand were removed by 84.8%, 92.7%, 64.1%, respectively, and with *A. maxima* by 14.6%, 5.6%, 28.8% from FWW, respectively. Overall removal of 99.4% of total nitrogen, 98.1% of total phosphorus, 92.9% of chemical oxygen demand, and 92.6% of total solids were achieved. Resulting biomass had a protein content of 41.27%, which is lower than the control group but still higher than most of the species, total carbohydrate of 27.35% and total lipid of 24.08%, which is higher than the control group. Biomass from FWW treatment reactors also had significantly higher amounts of B vitamins and considerable amounts of free and bound amino acids, some of which are key indicators of biostimulant presence. This observation was also proved by a preliminary qualitative analysis conducted by mass spectrometric scan. Some of the detected hormones, such as Jasmonic acids and Gibberellins, play crucial roles in plant growth and development, defense against pathogens and stress responses. In addition to high protein contents, vitamins and necessary macro and micro elements, these hormones could increase the positive effect of the FWW treated biomass when applied as a biofertilizer. Optimization of the treatment process could even further advance the resulting biomass quality and actual effects on crops.

Overall, this thesis study concluded that *A. maxima* grown in FWW has great potential both as a nutrient capture and to be used as biofertilizer with sufficient constituents to support plant crop. As such, it could help reduce the need of chemical fertilizers and pesticides in addition to circulating the nutrients available in the farm waste. It would be safe to say that proposed sustainable and circular farm system might be possible to achieve.

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APPENDIX A

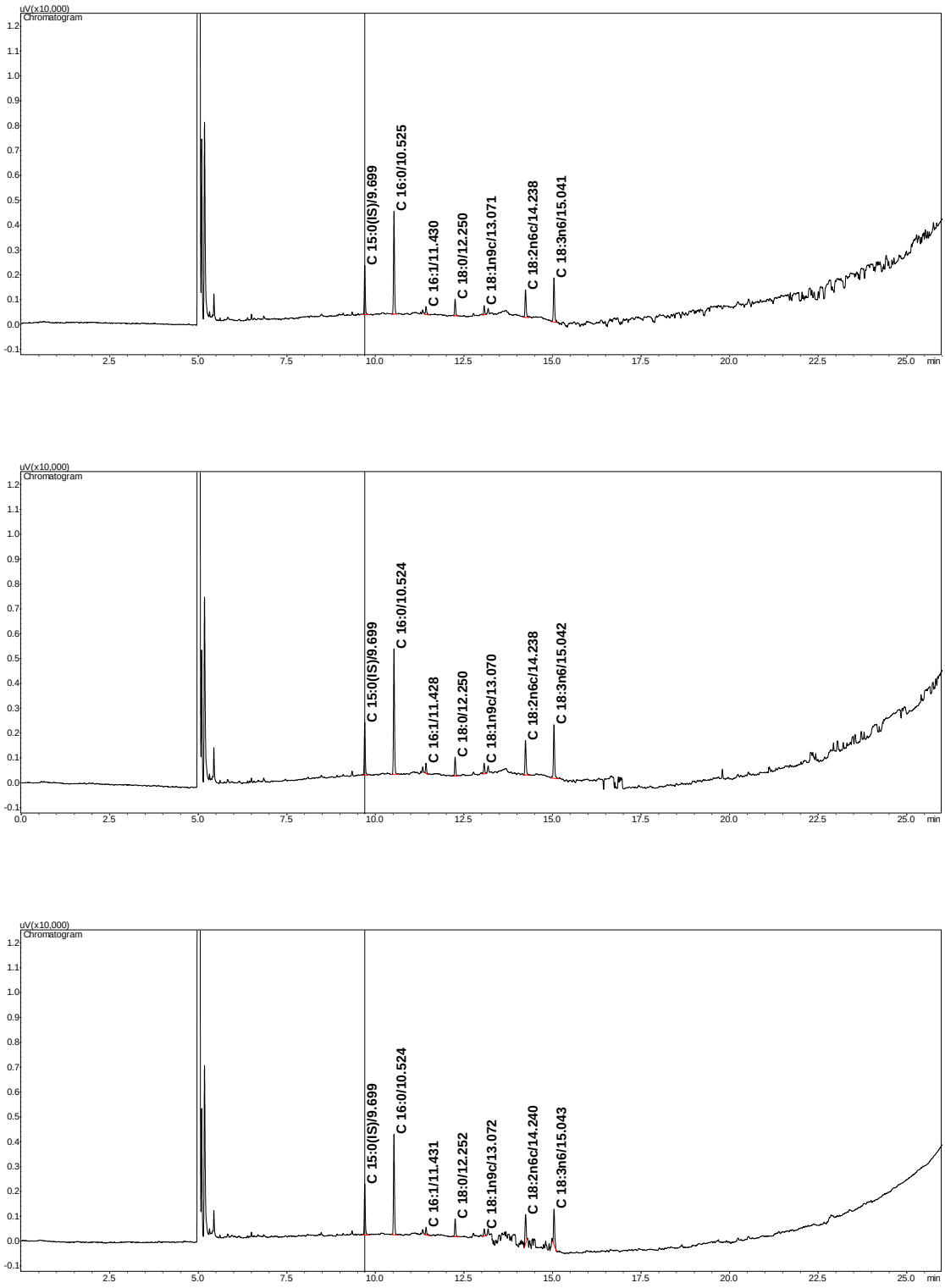


Figure A.1. GC chromatograms of triplicate analysis of Control PBR 1.

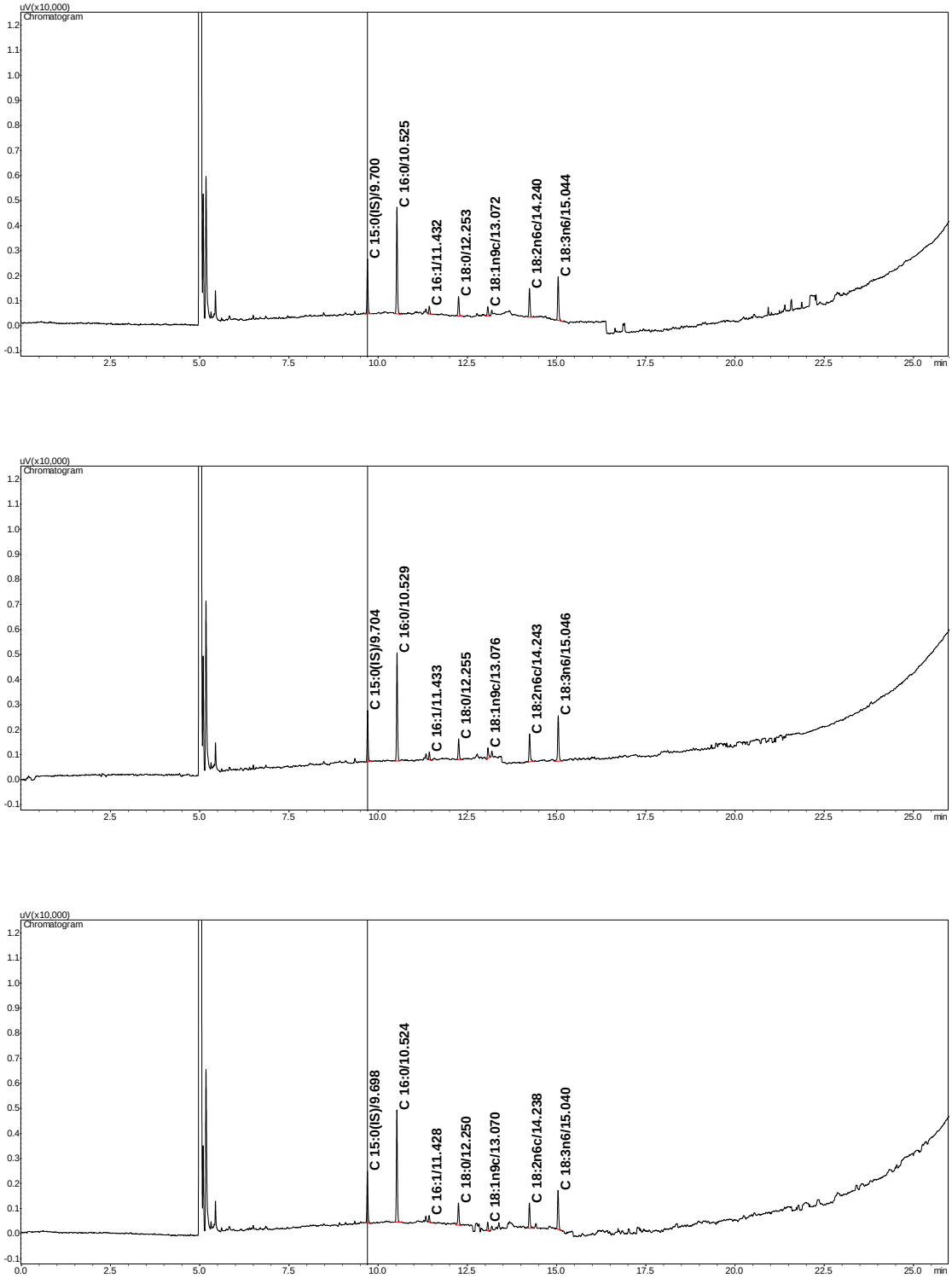


Figure A.2. GC chromatograms of triplicate analysis of Control PBR 2.

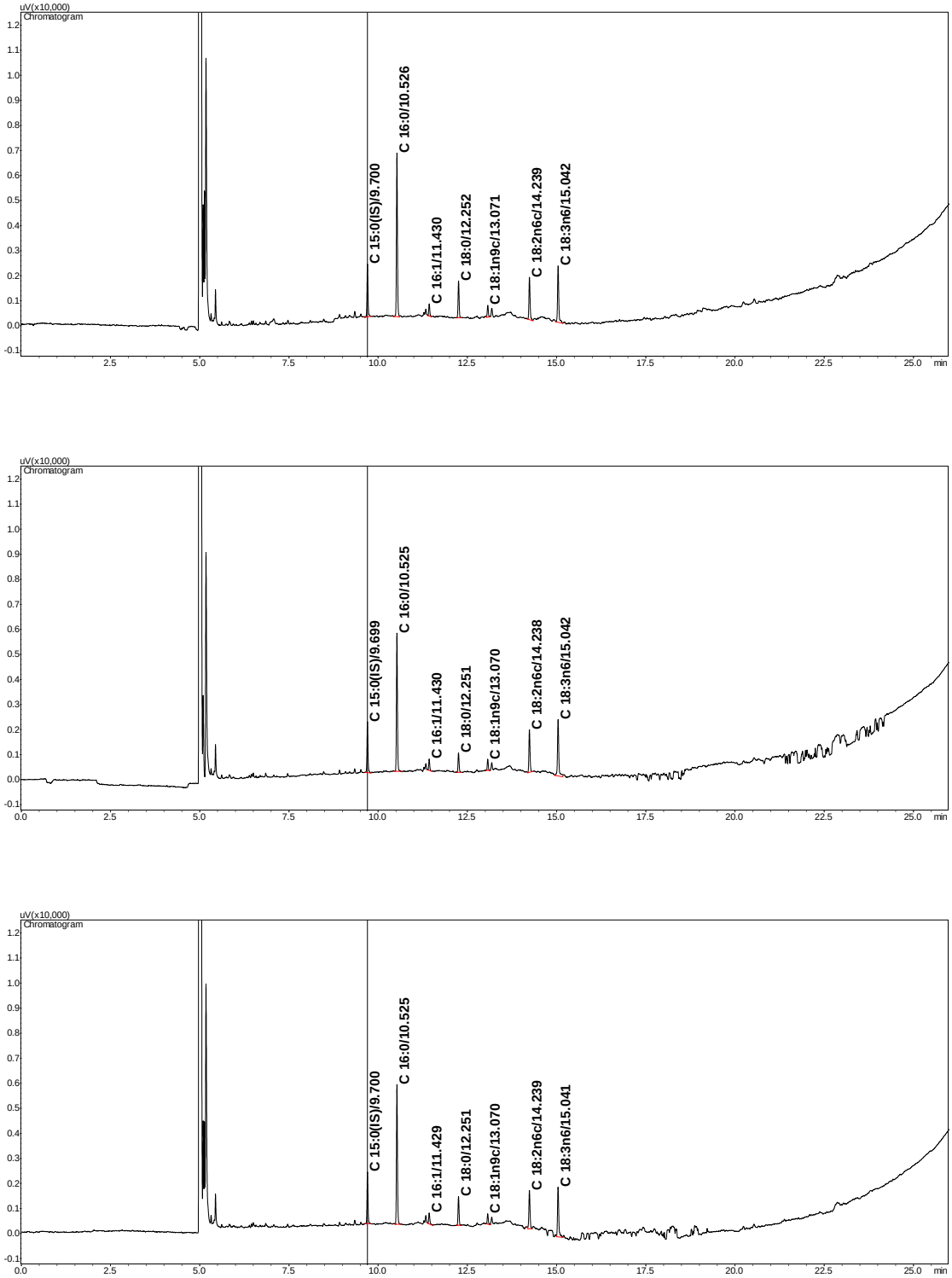


Figure A.3. GC chromatograms of triplicate analysis of Control PBR 3.

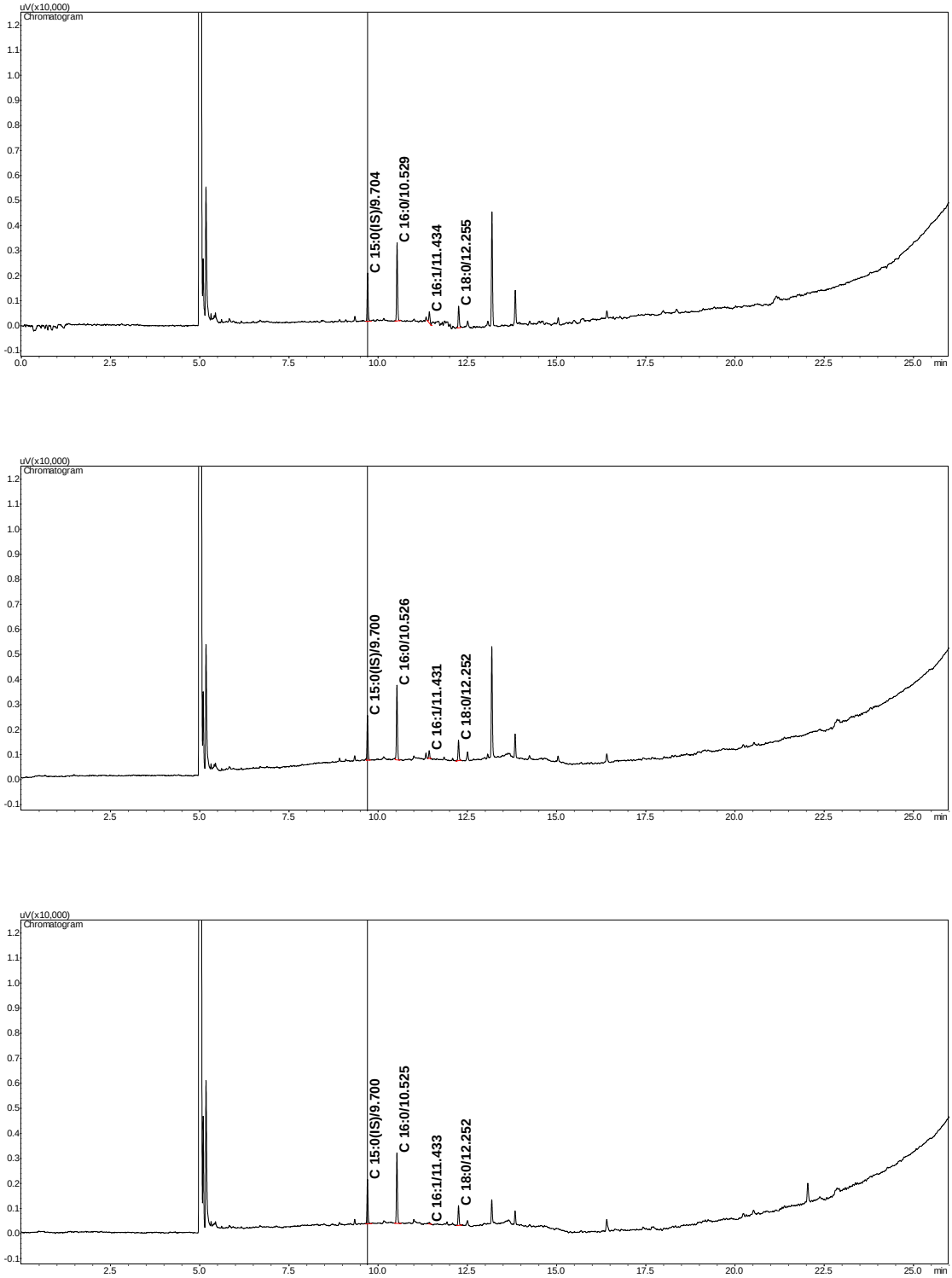


Figure A.4. GC chromatograms of triplicate analysis of FWW PBR 1.

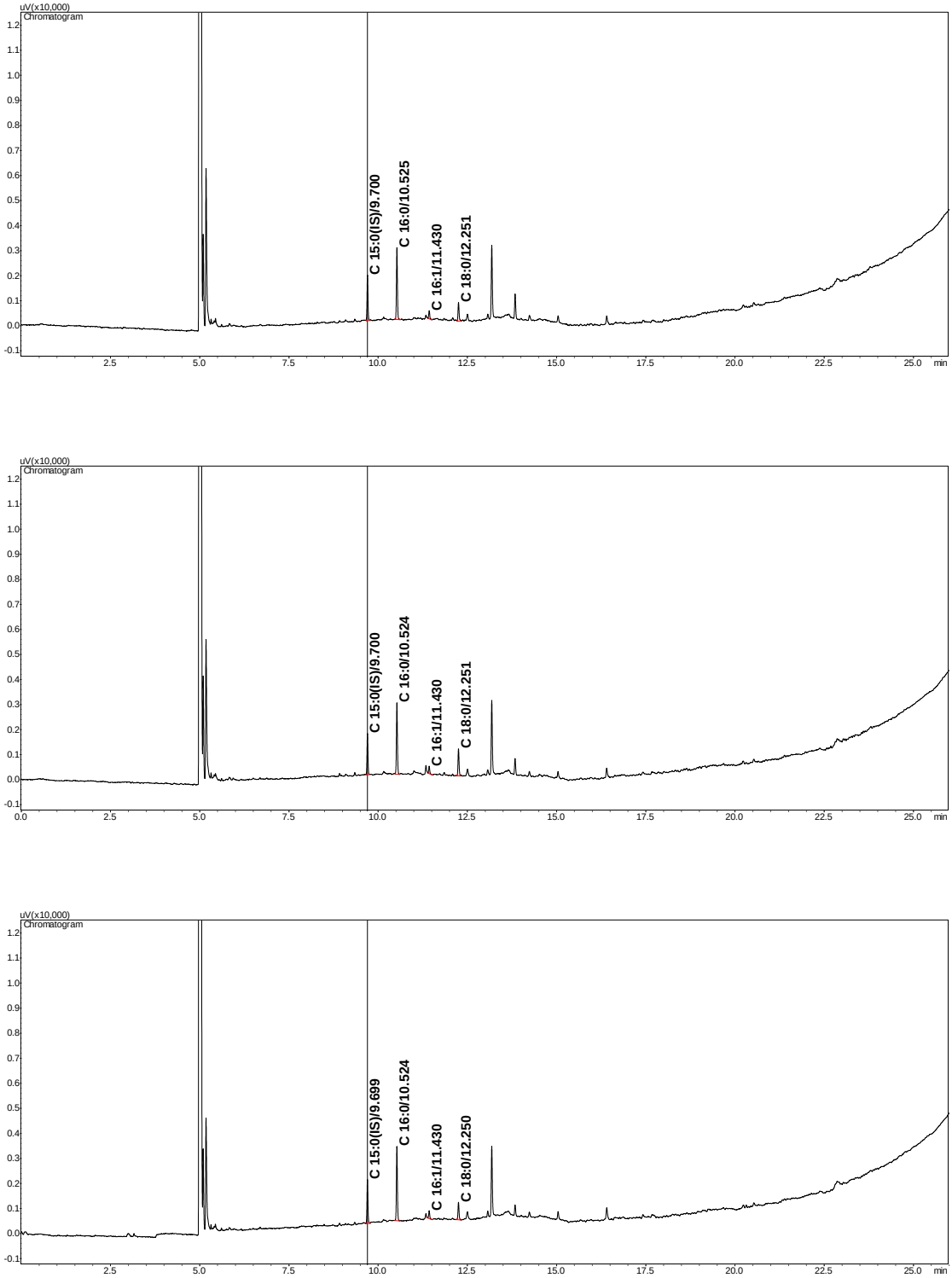


Figure A.5. GC chromatograms of triplicate analysis of FWW PBR 2.

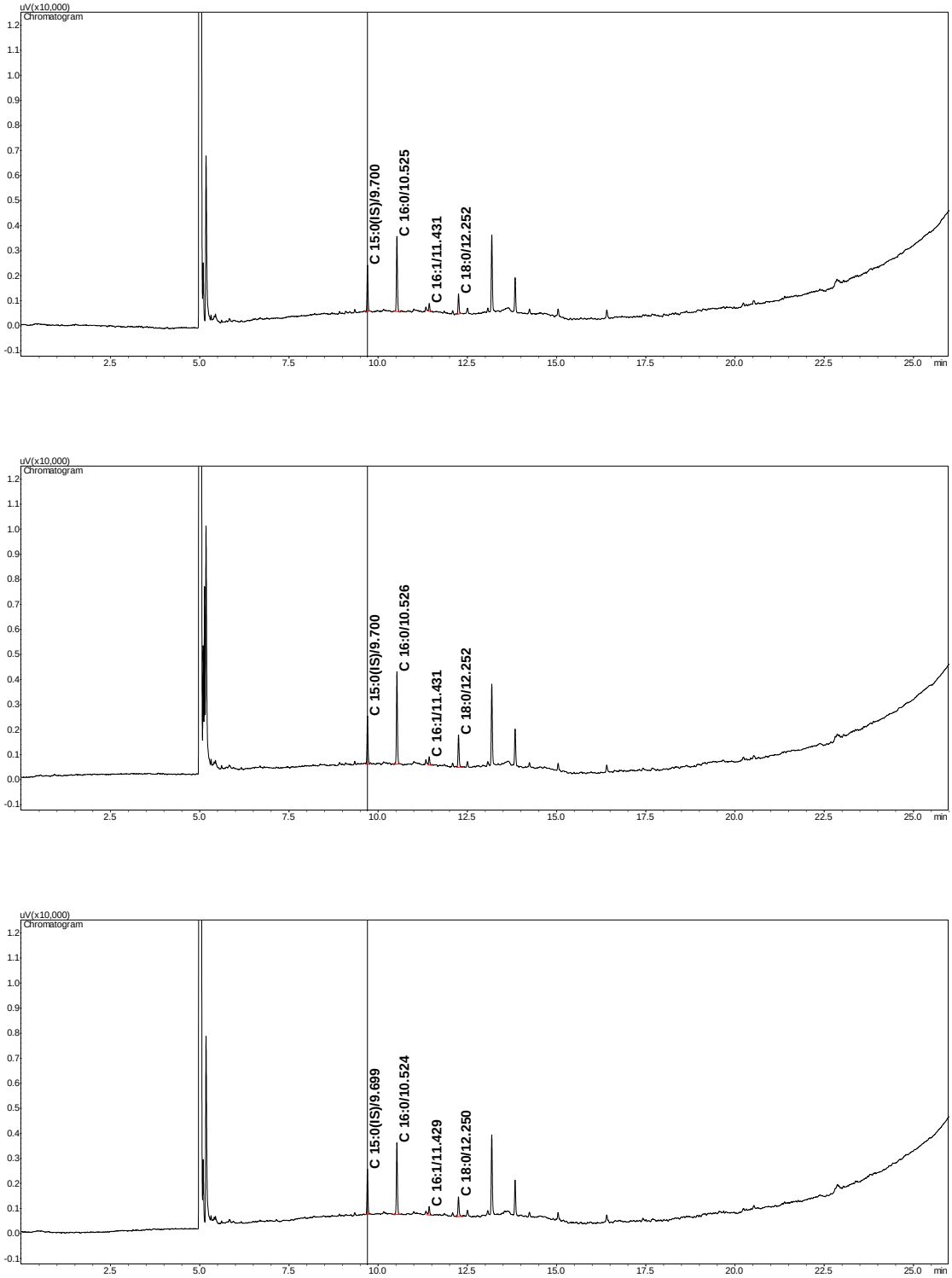


Figure A.6. GC chromatograms of triplicate analysis of FWW PBR 3.

APPENDIX B

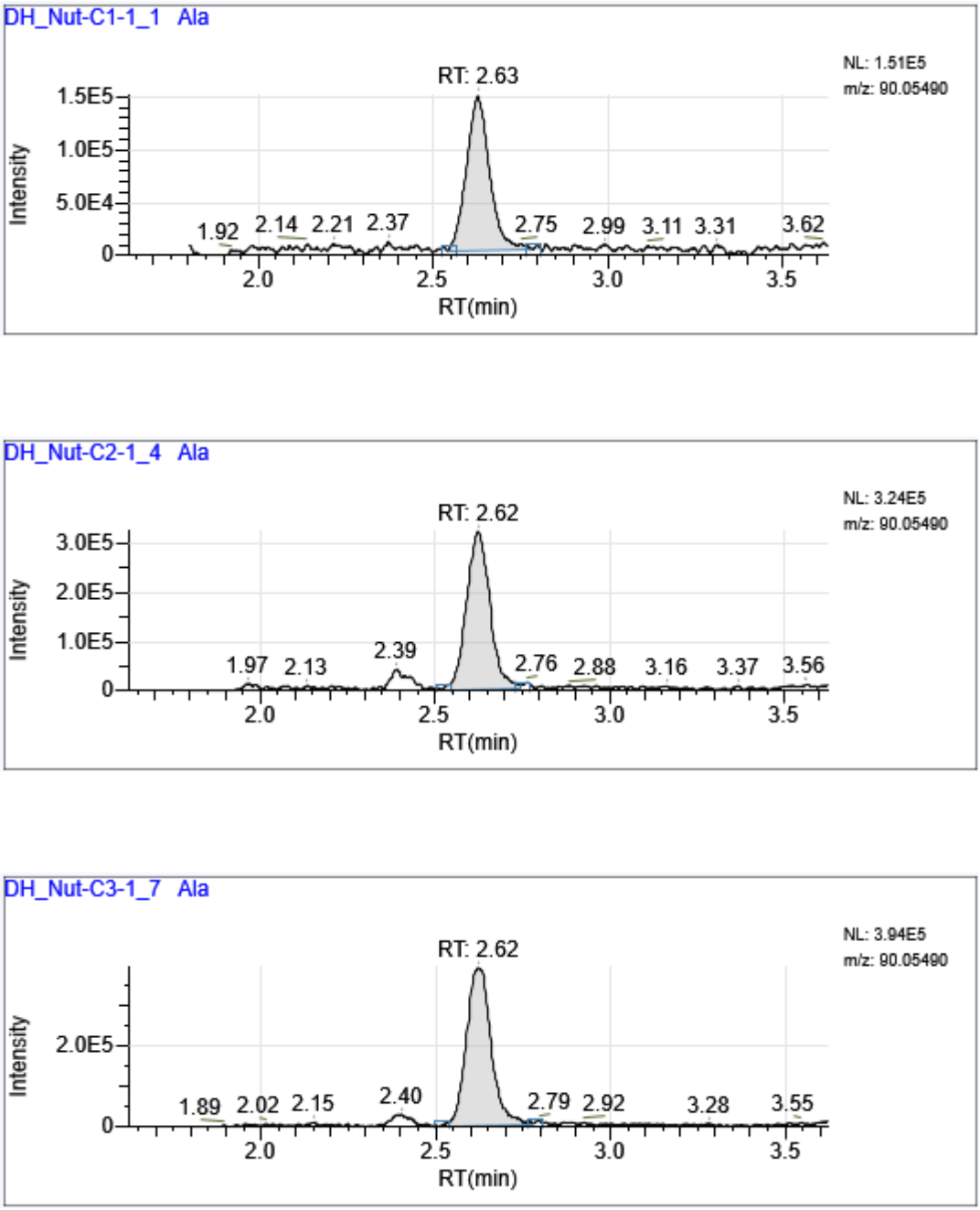
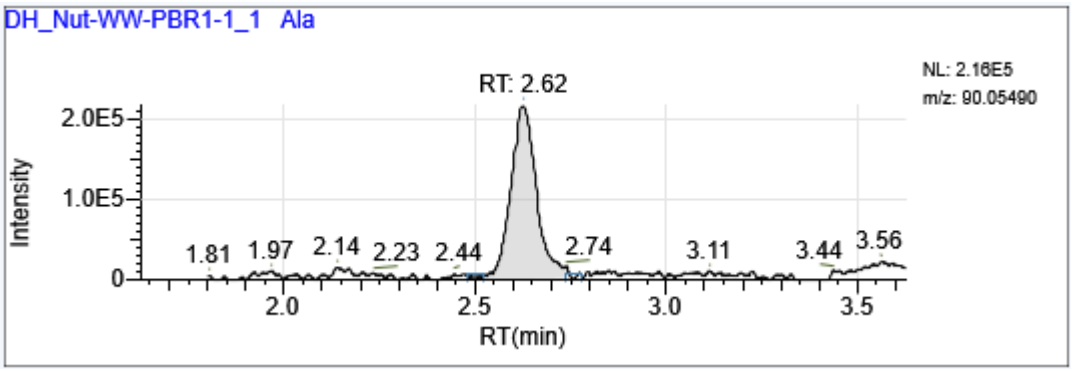
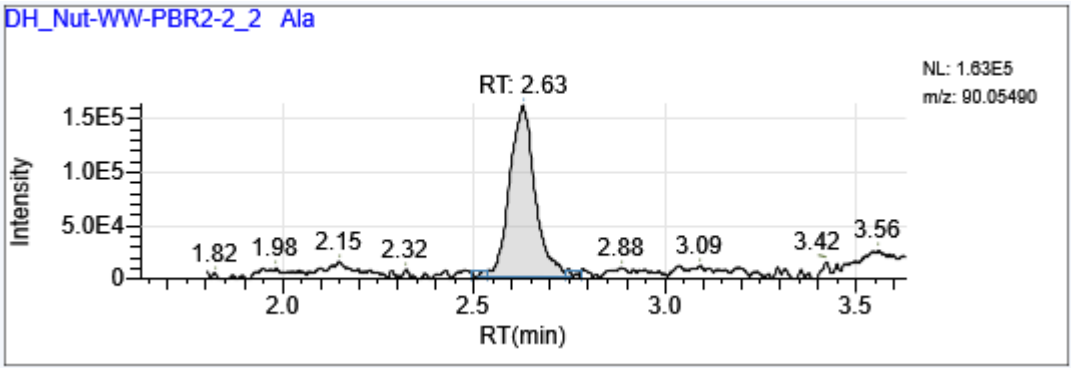


Figure B.1. LC-MS chromatograms of ALA of Control PBRs 1-2-3.





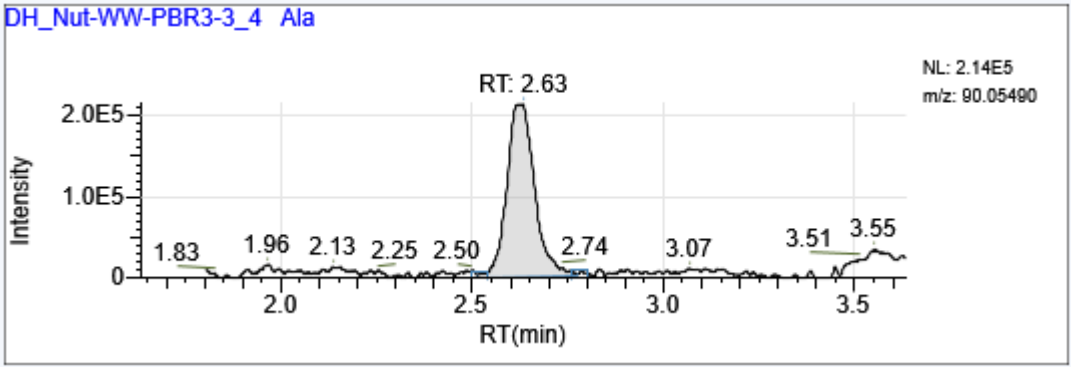


Figure B.2. LC-MS chromatograms of ALA of FWW PBRs 1-2-3

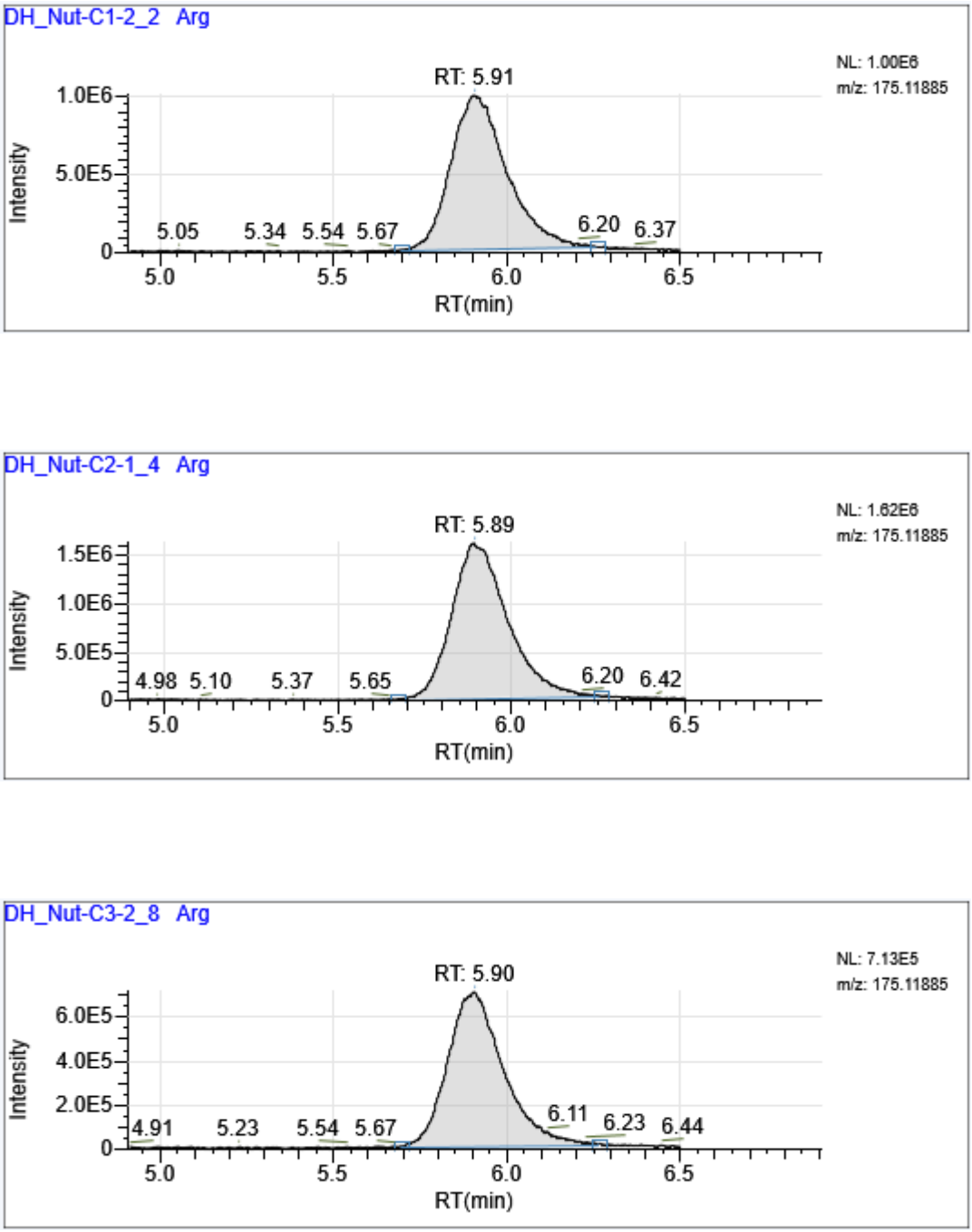


Figure B.3. LC-MS chromatograms of ARG of Control PBRs 1-2-3

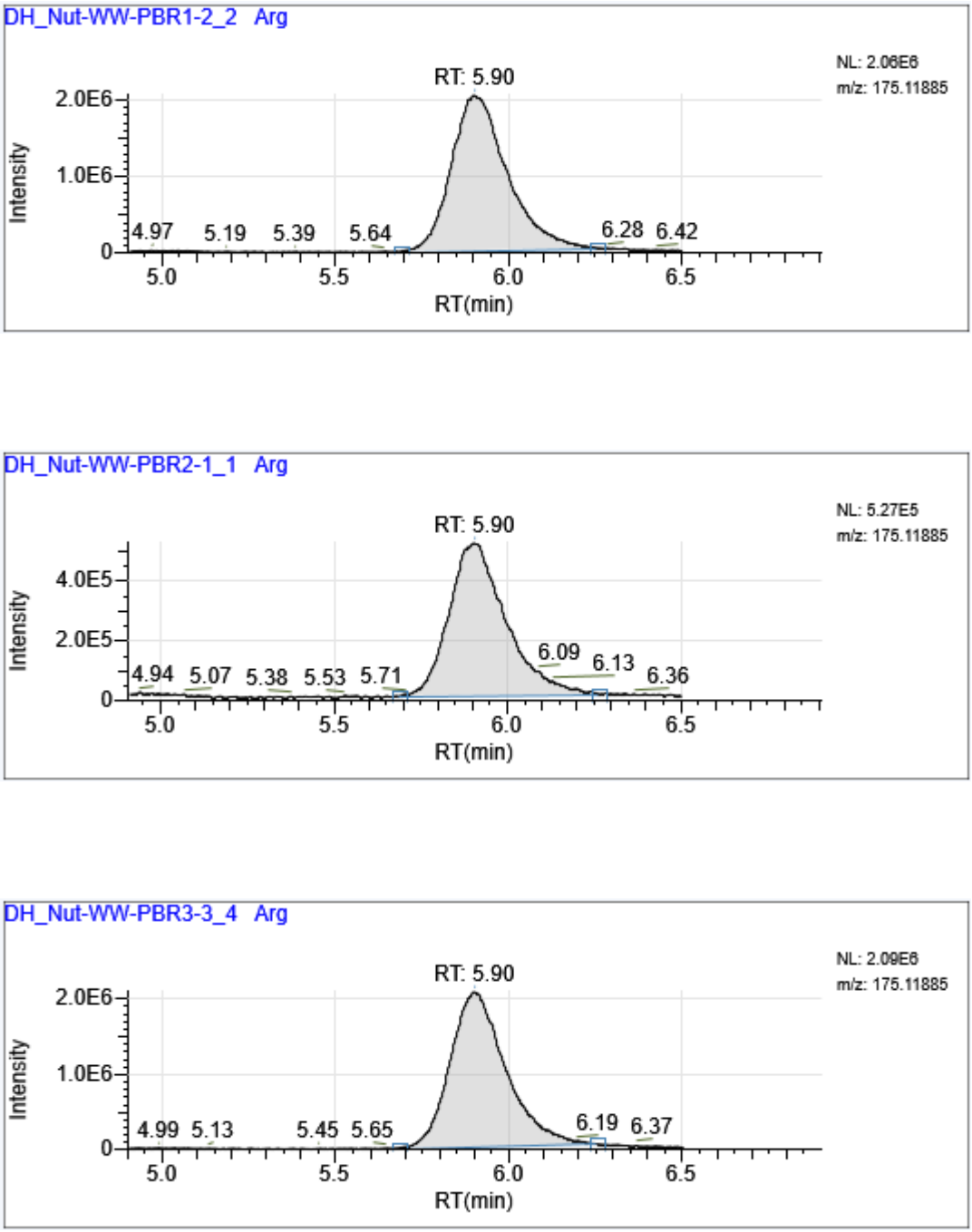


Figure B.4. LC-MS chromatograms of ARG of FWW PBRs 1-2-3

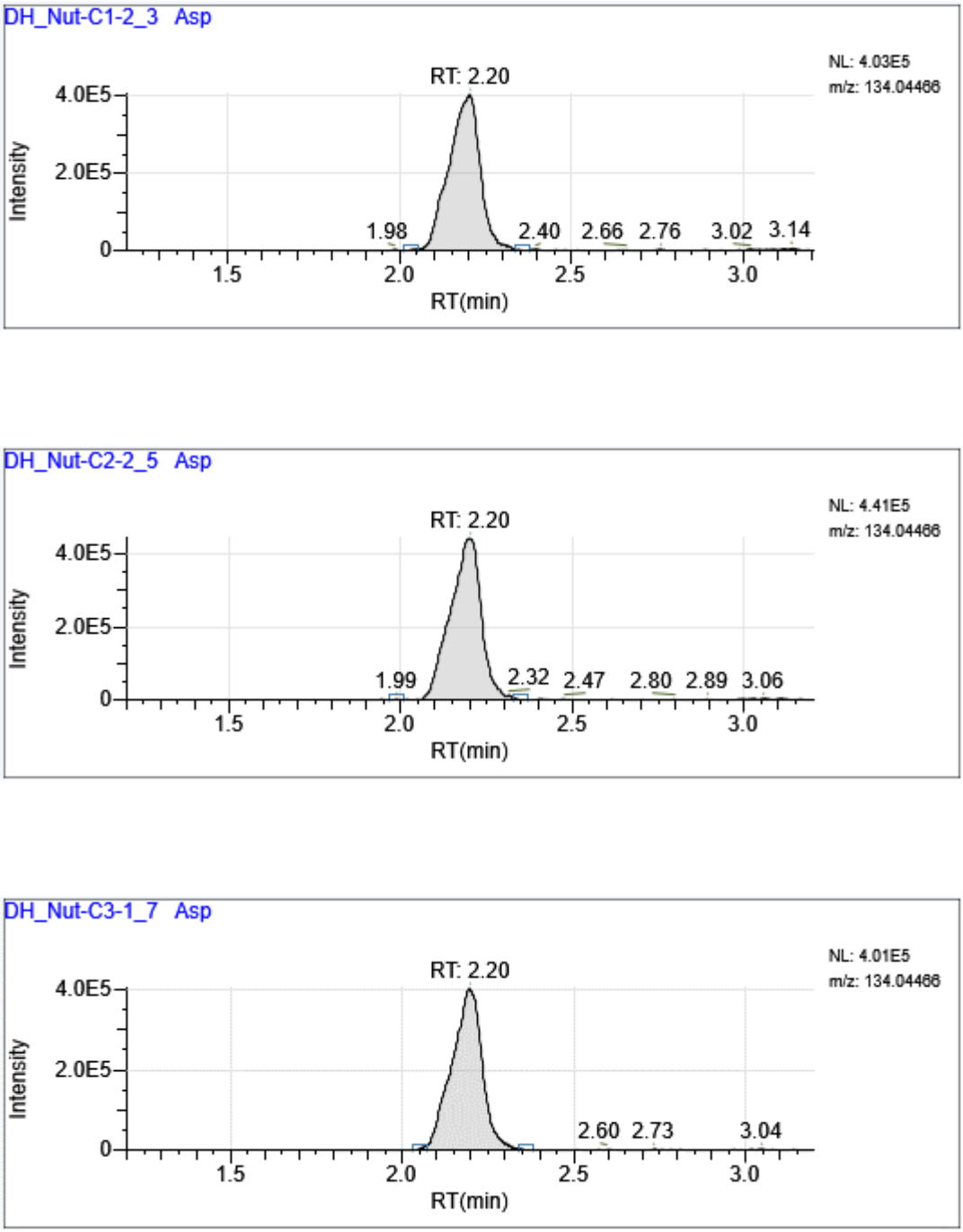


Figure B.5. LC-MS chromatograms of ASP of Control PBRs 1-2-3

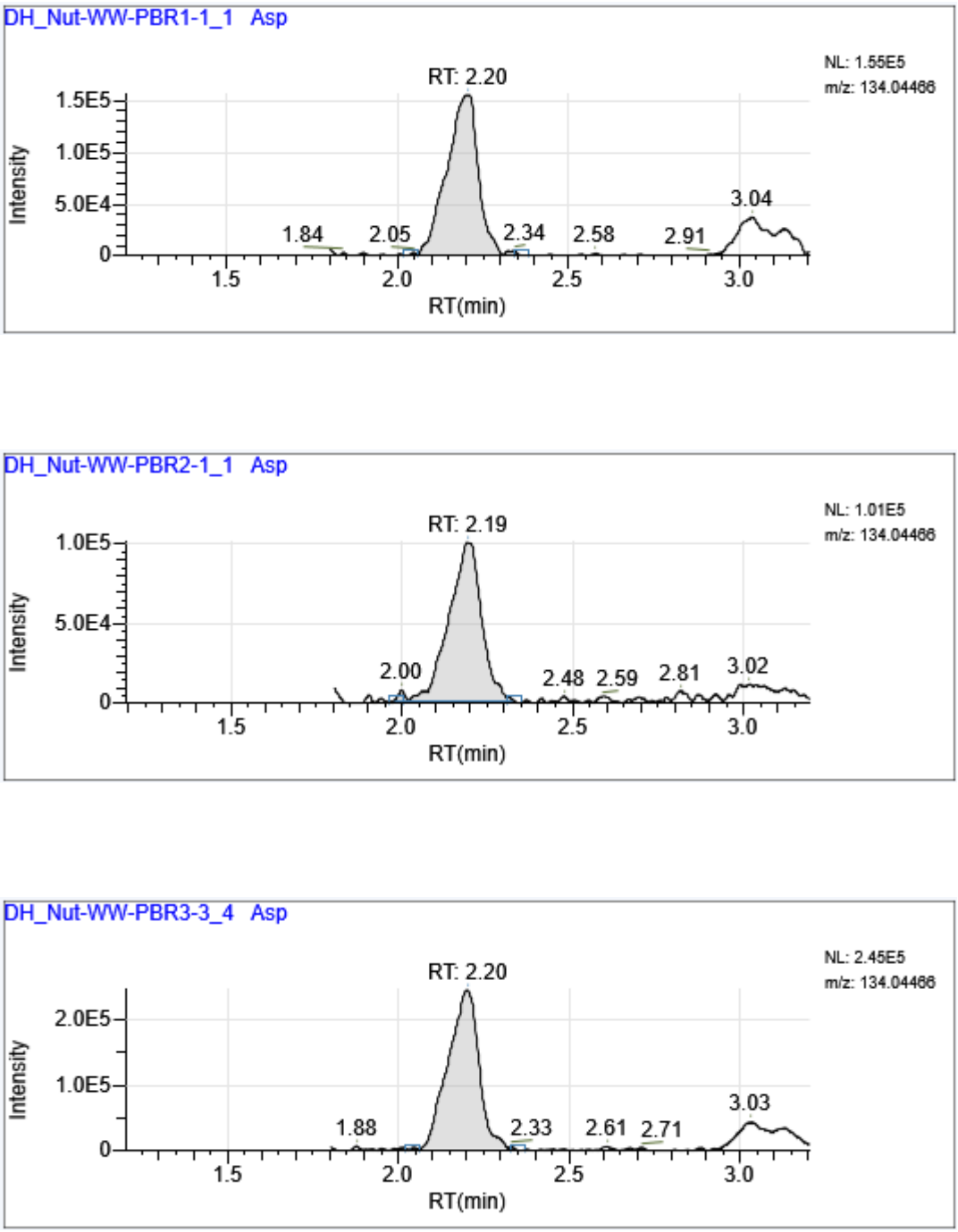


Figure B.6. LC-MS chromatograms of ASP of FWW PBRs 1-2-3

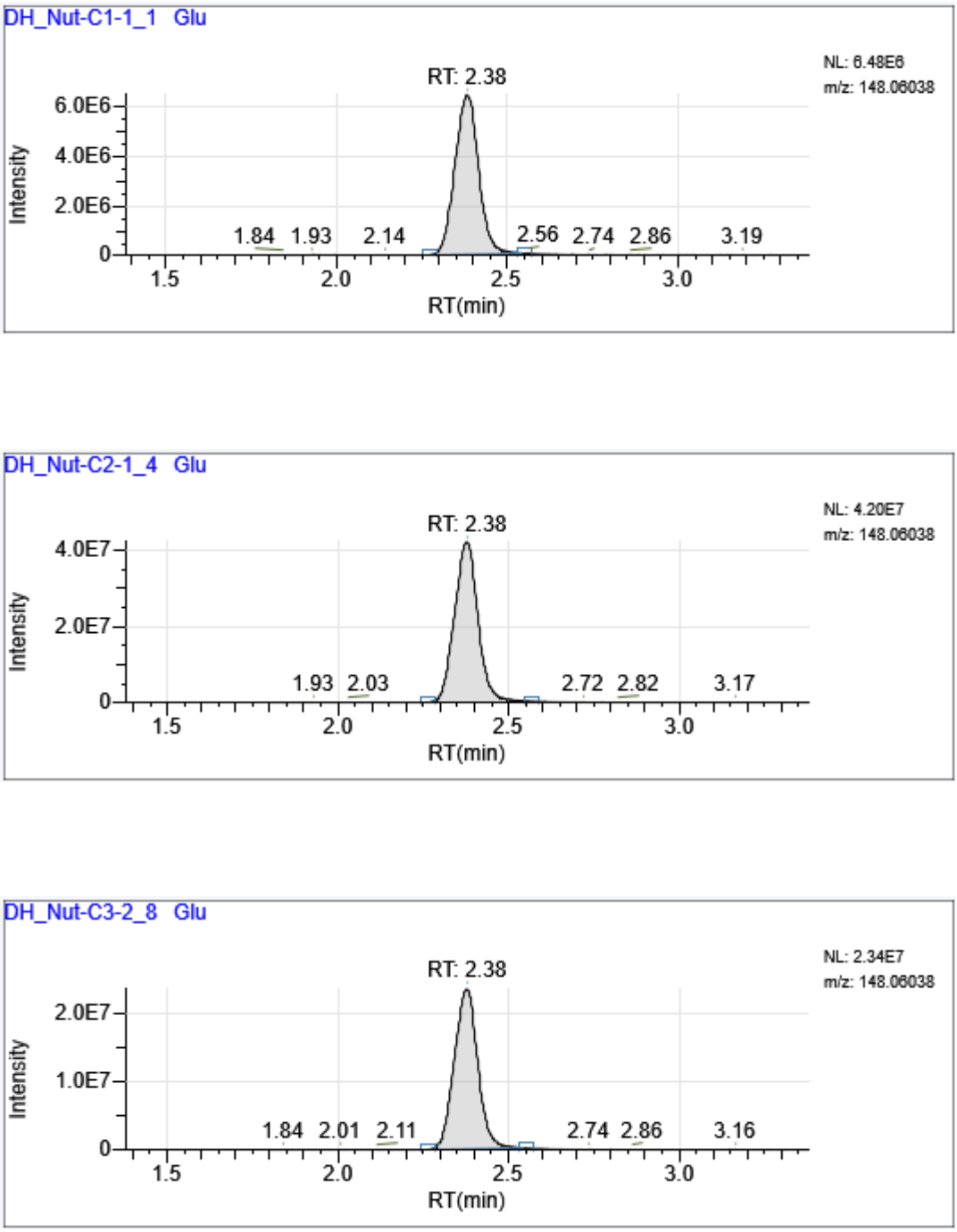
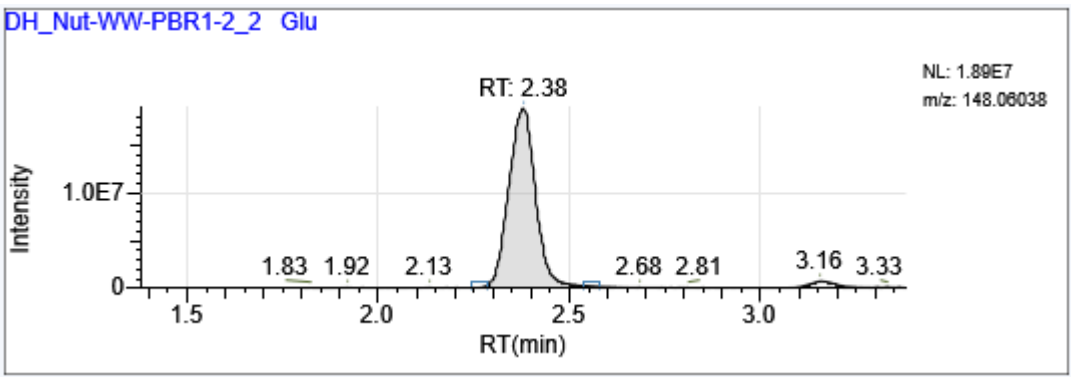


Figure B.7. LC-MS chromatograms of GLU of Control PBRs 1-2-3



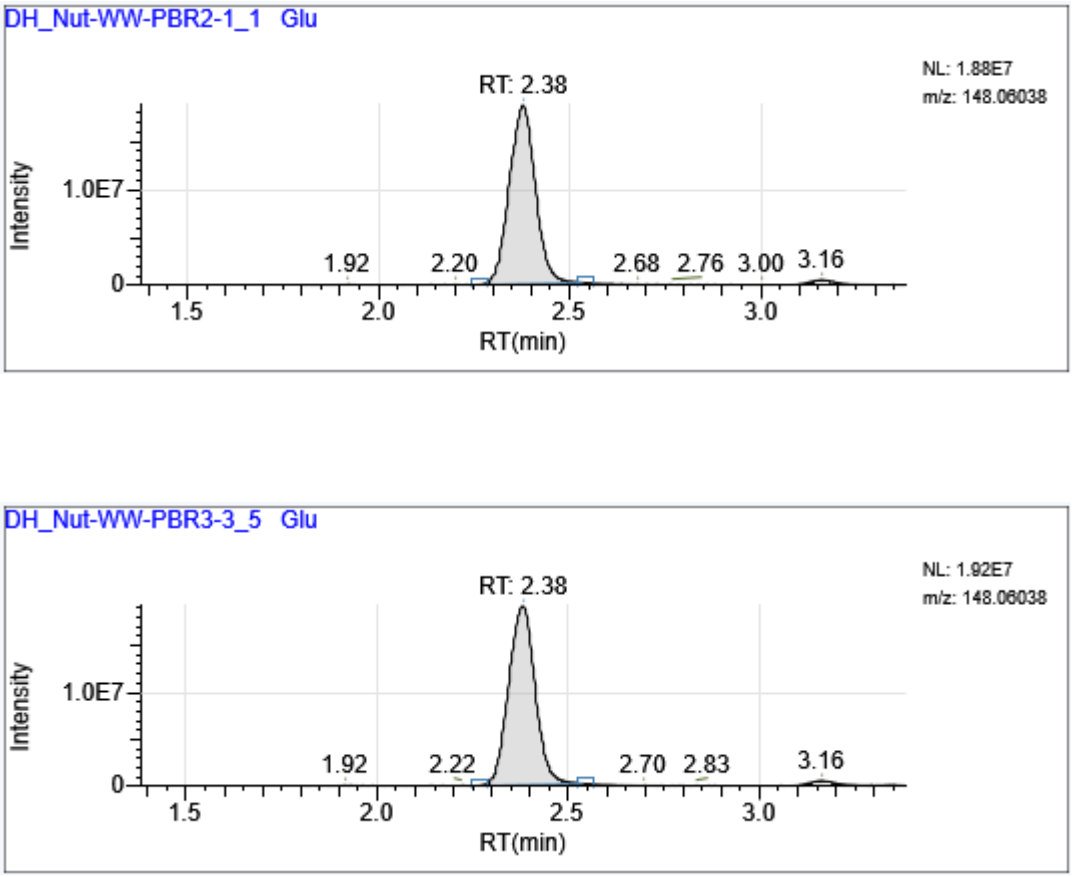


Figure B.8. LC-MS chromatograms of GLU of FWW PBRs 1-2-3

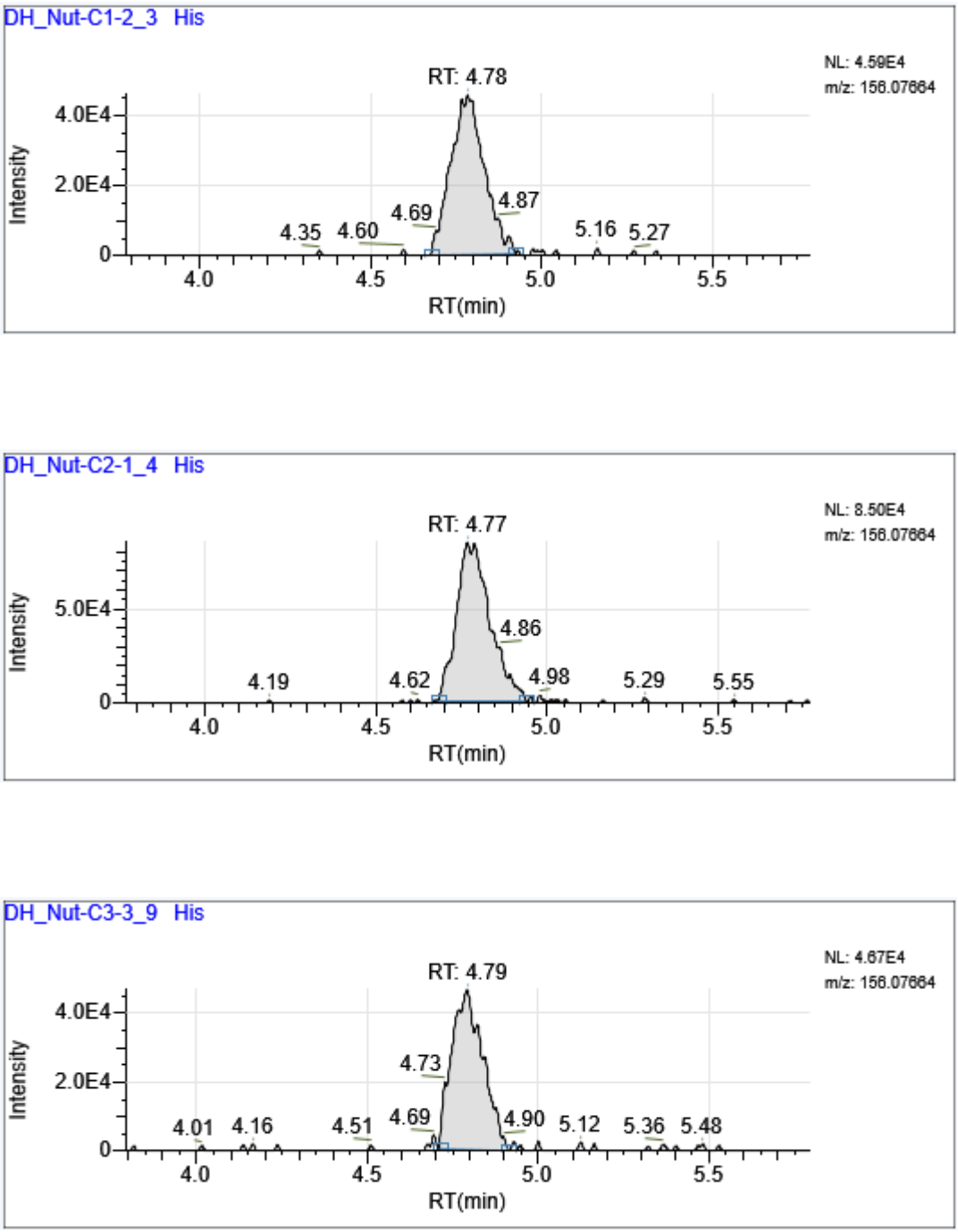


Figure B.9. LC-MS chromatograms of HIS of Control PBRs 1-2-3.

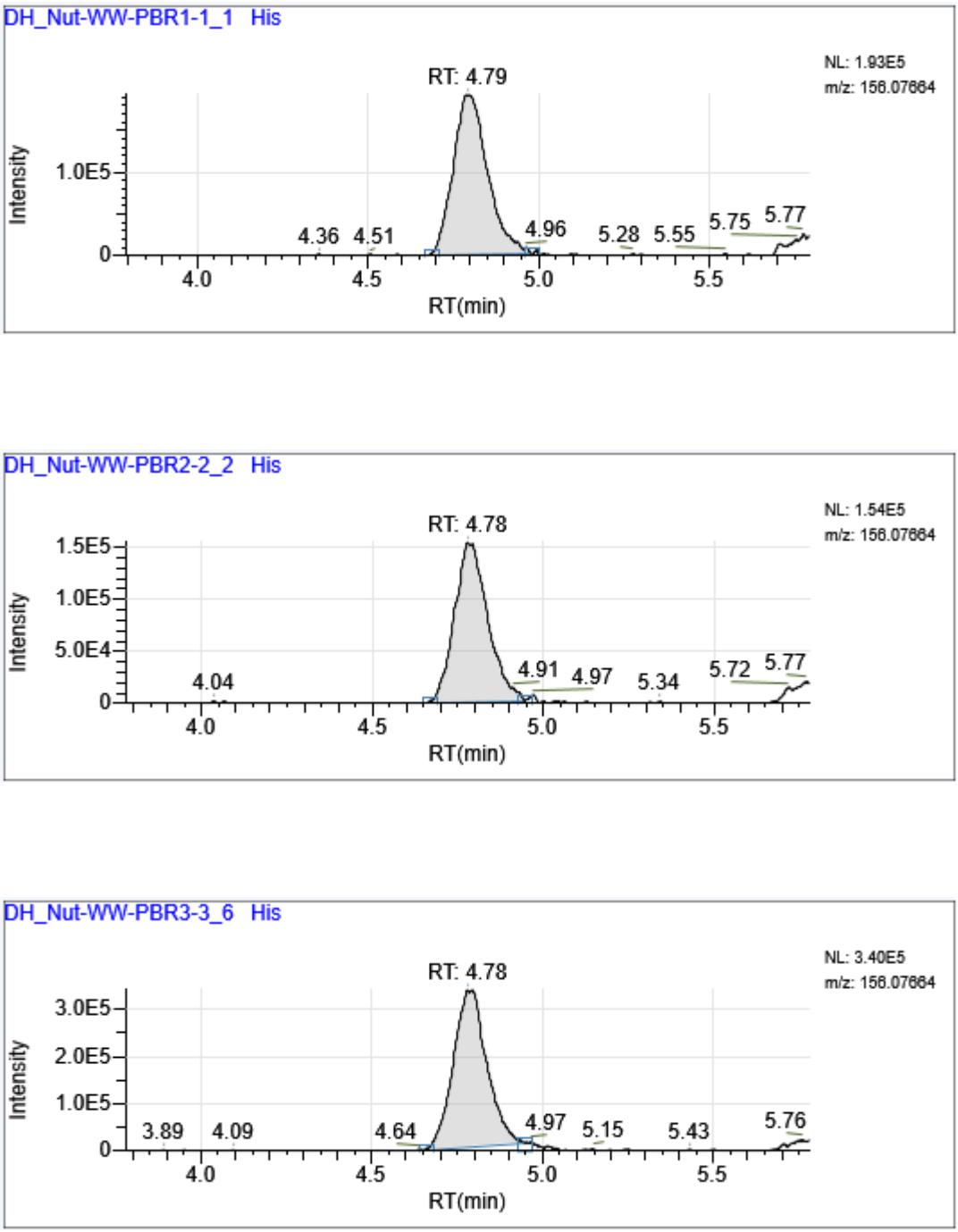


Figure B.10. LC-MS chromatograms of HIS of FWW PBRs 1-2-3.

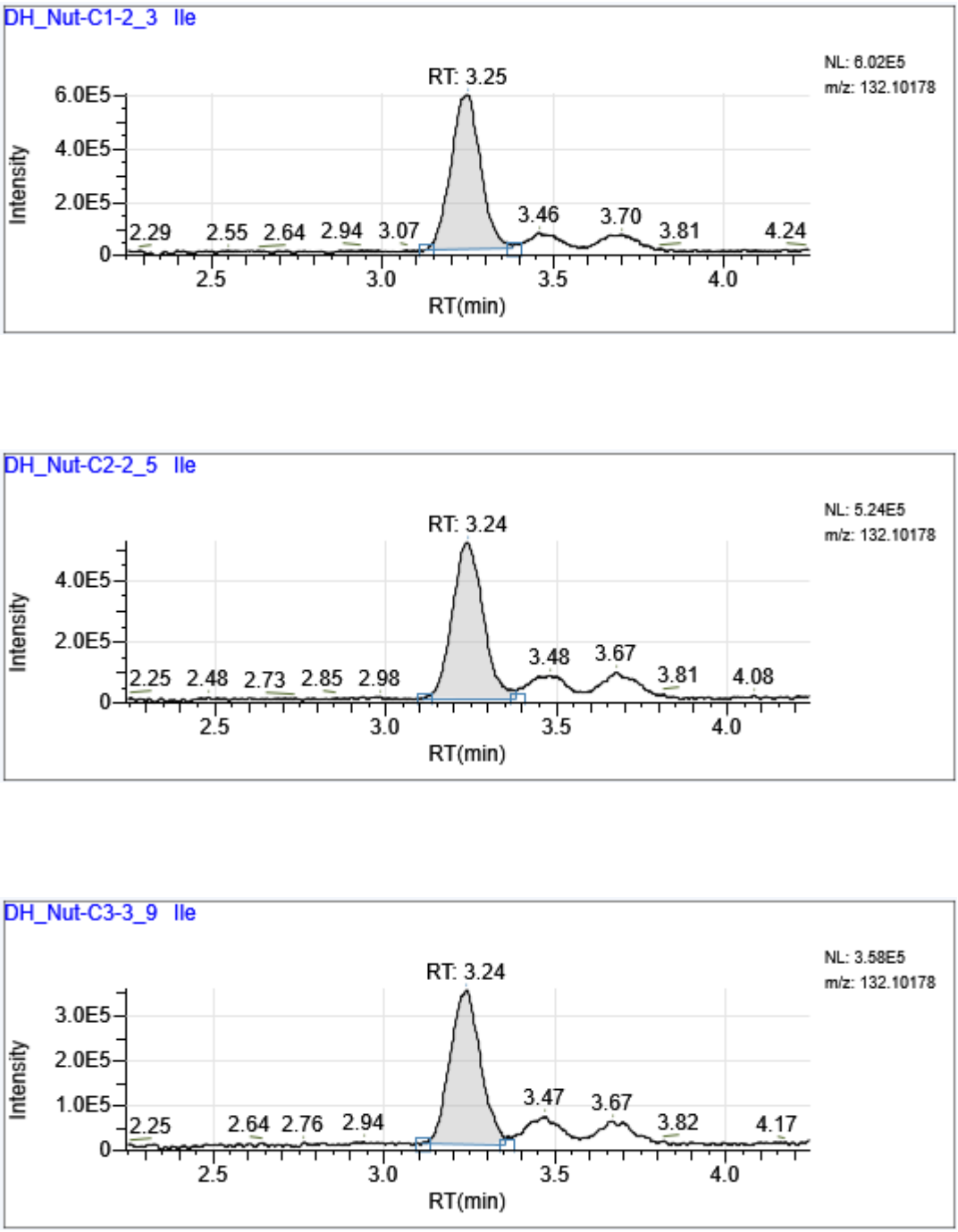


Figure B.11. LC-MS chromatograms of ILE + LEU of Control PBRs 1-2-3.

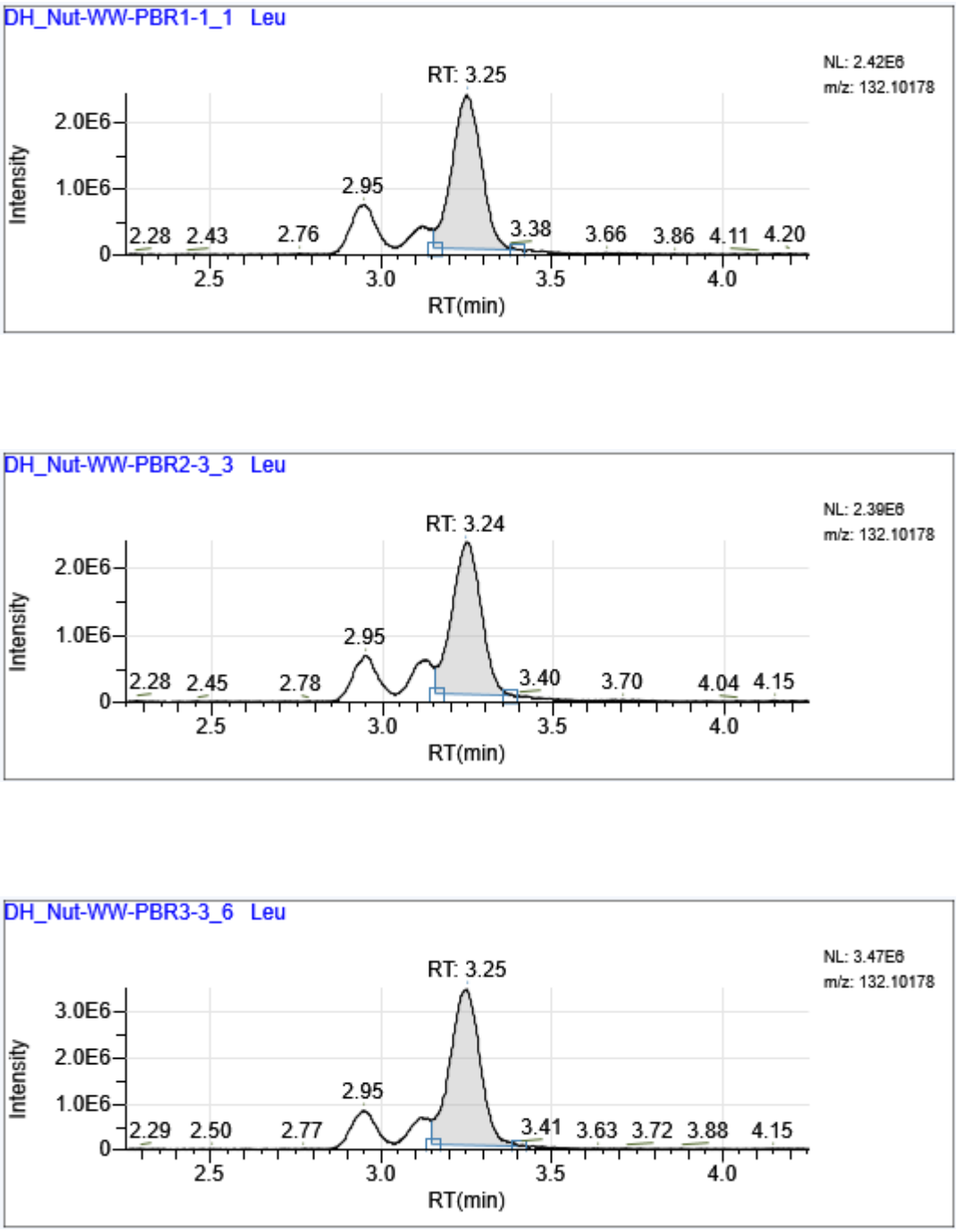


Figure B.12. LC-MS chromatograms of ILE + LEU of FWW PBRs 1-2-3.

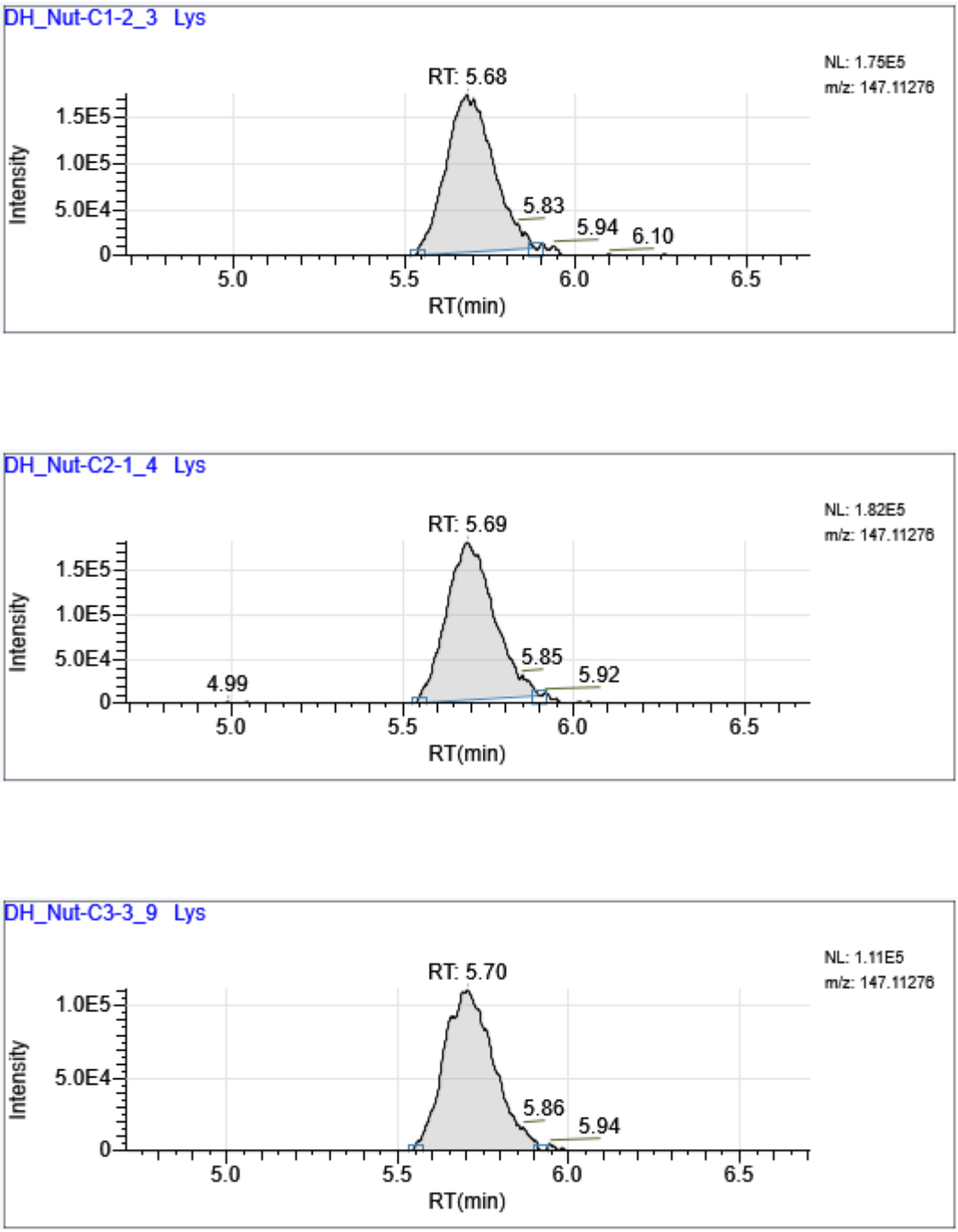


Figure B.13. LC-MS chromatograms of LYS of Control PBRs 1-2-3.

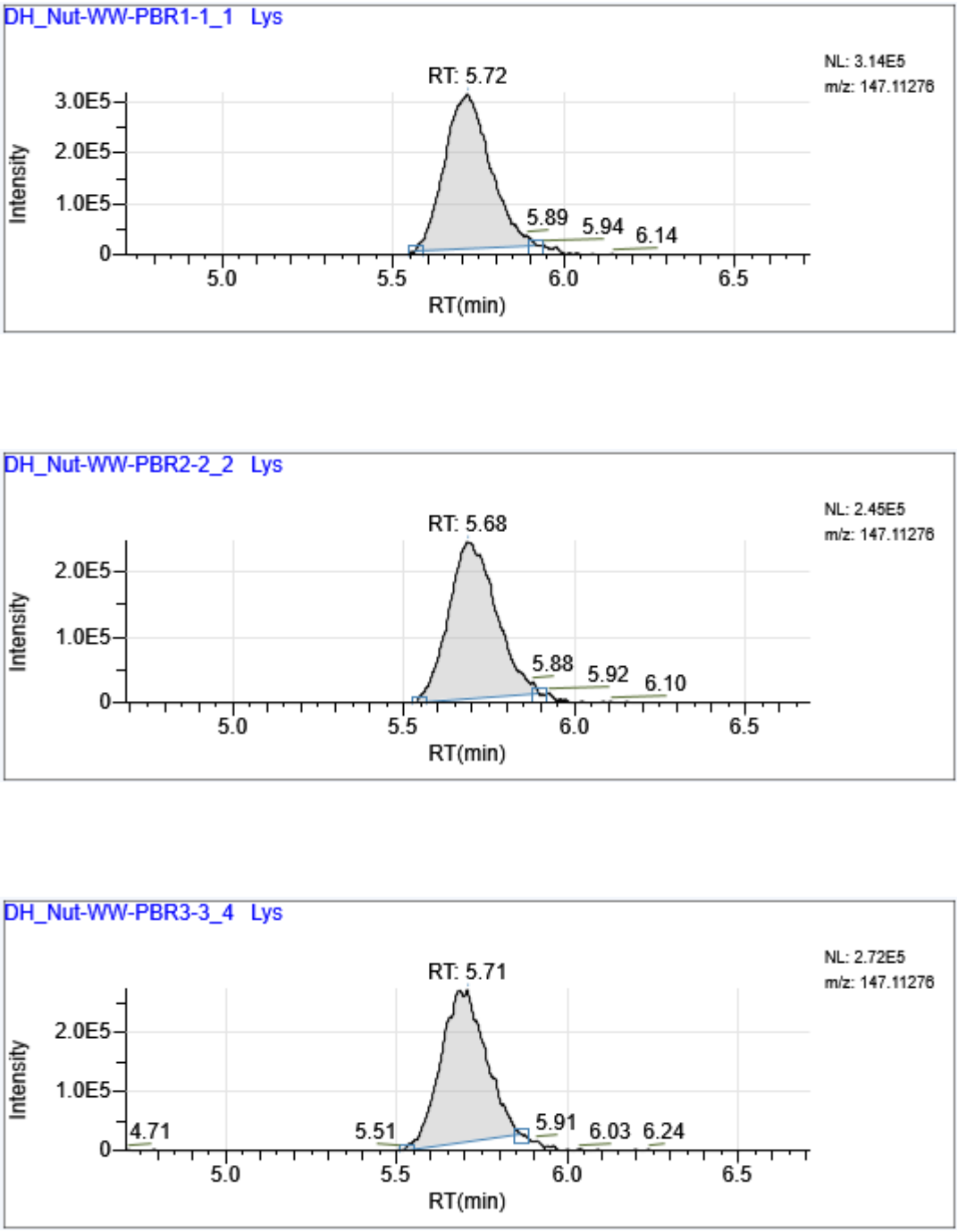


Figure B.14. LC-MS chromatograms of LYS of FWW PBRs 1-2-3.

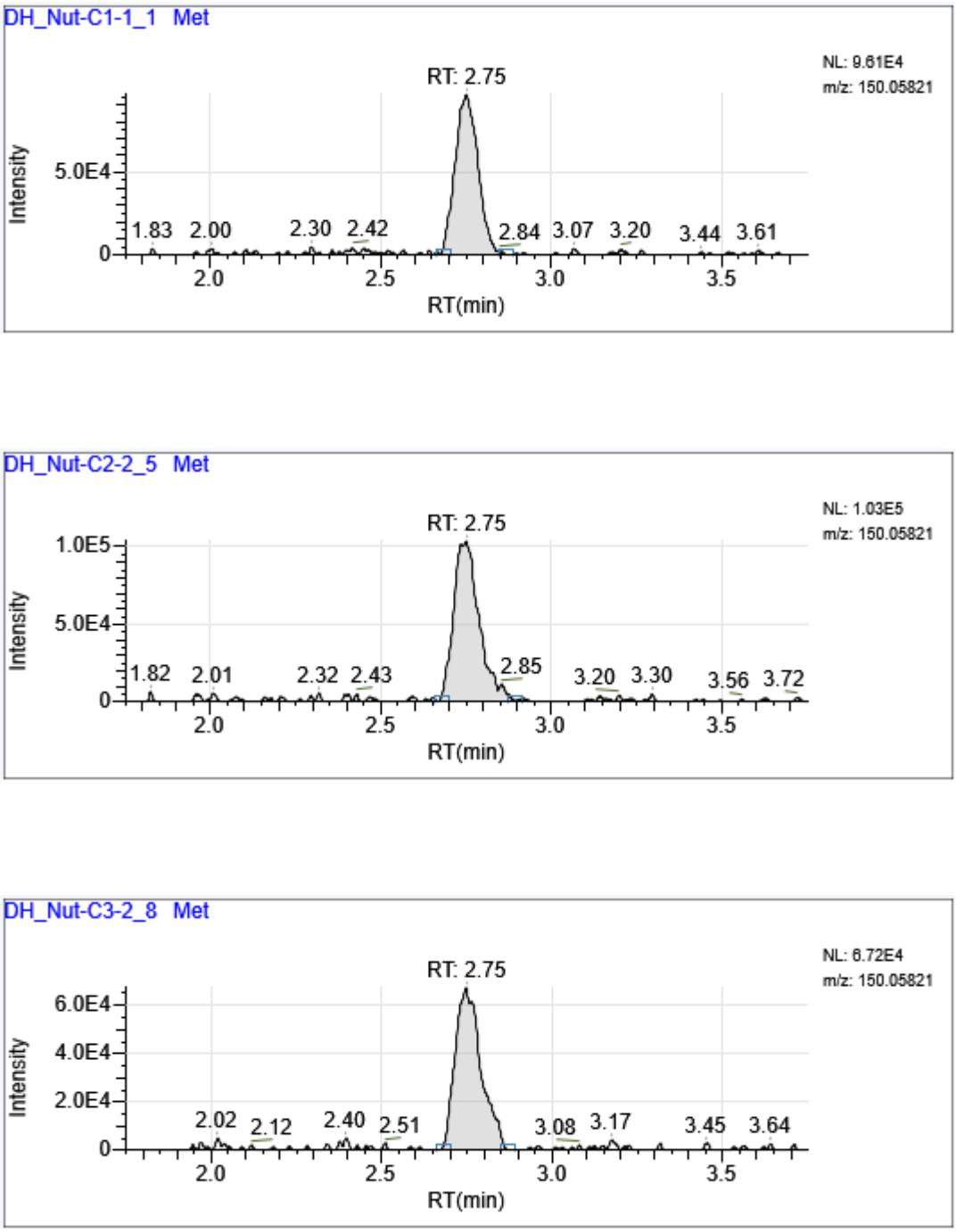


Figure B.15. LC-MS chromatograms of MET of Control PBRs 1-2-3.

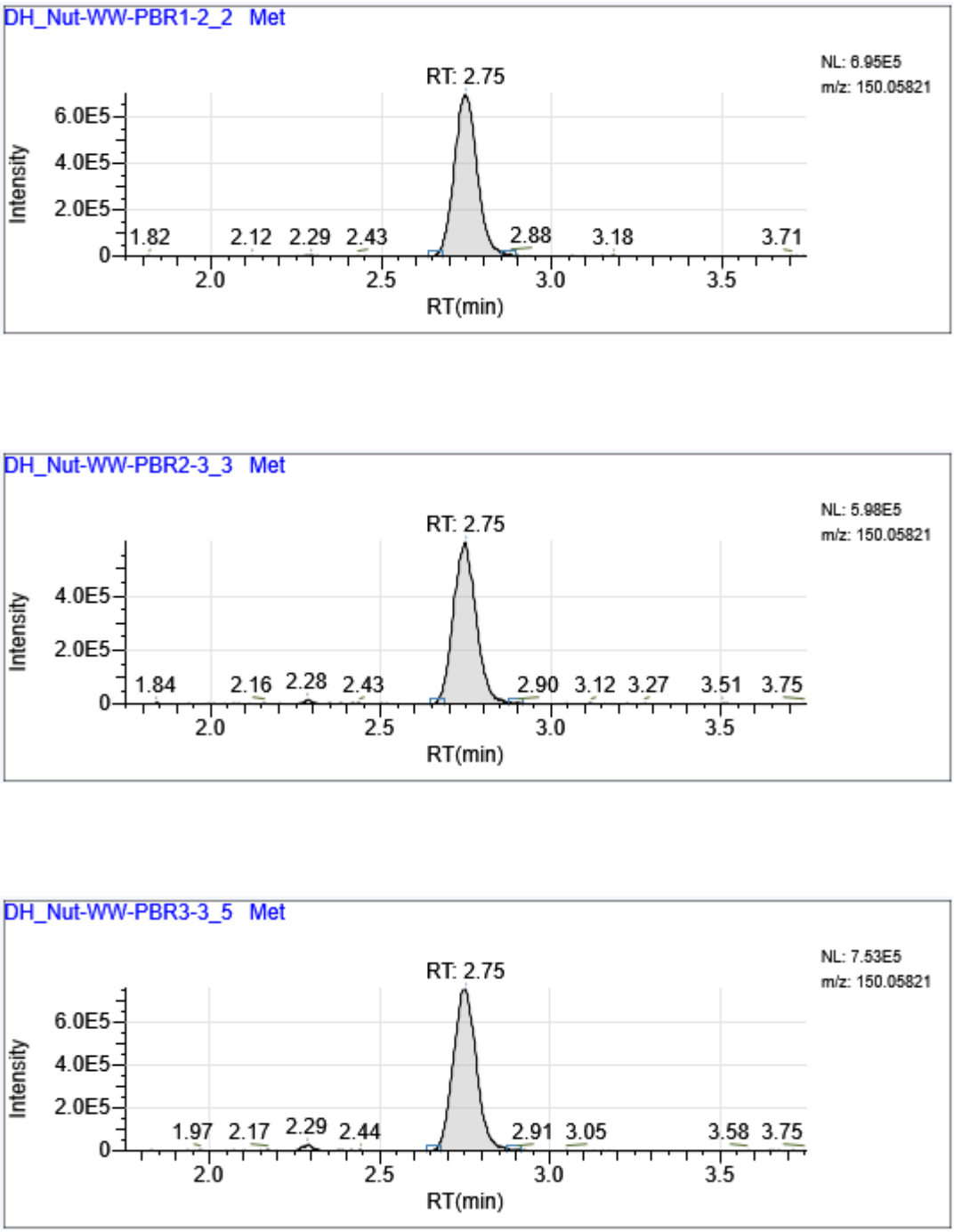


Figure B.16. LC-MS chromatograms of MET of FWW PBRs 1-2-3.

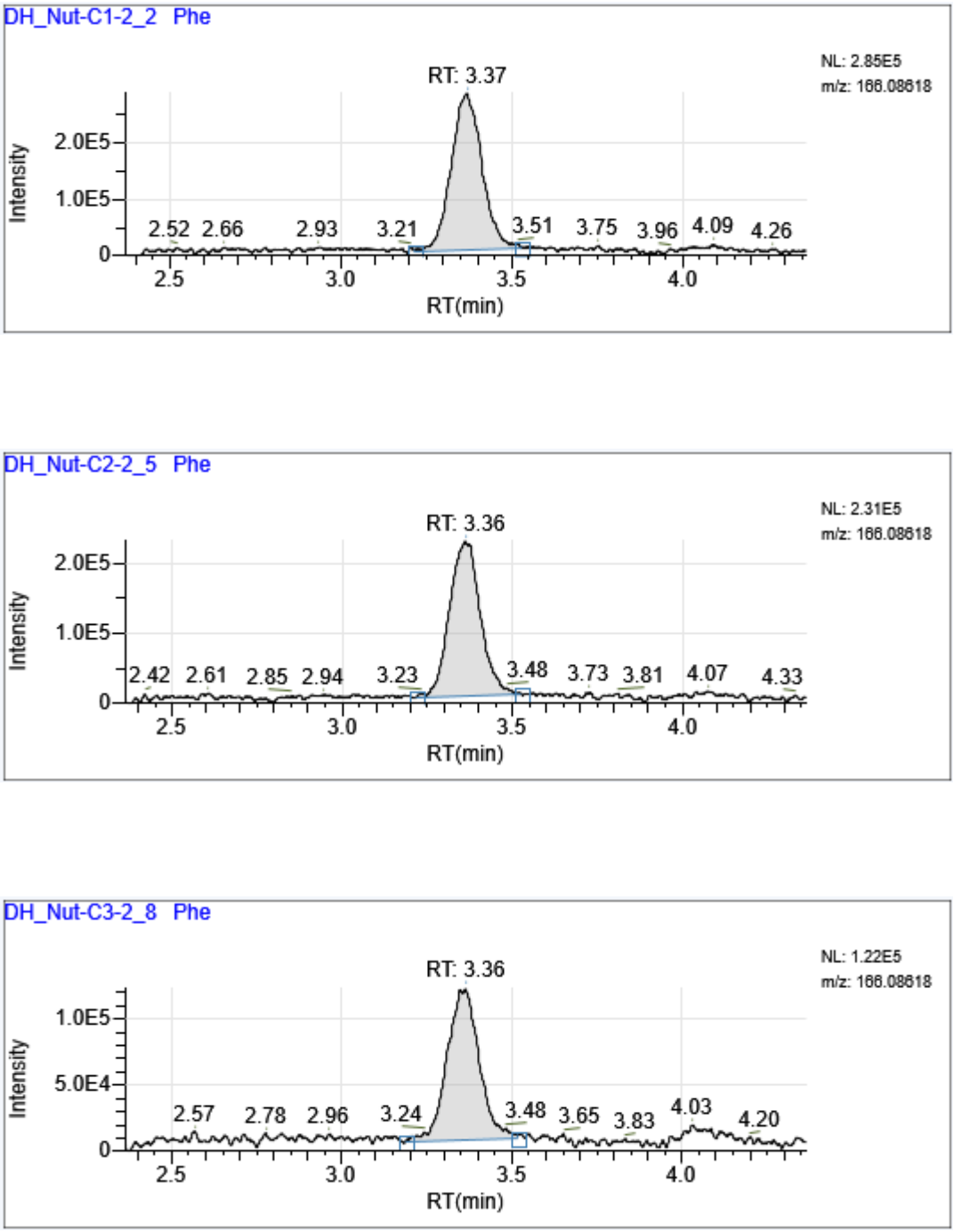


Figure B.17. LC-MS chromatograms of PHE of Control PBRs 1-2-3.

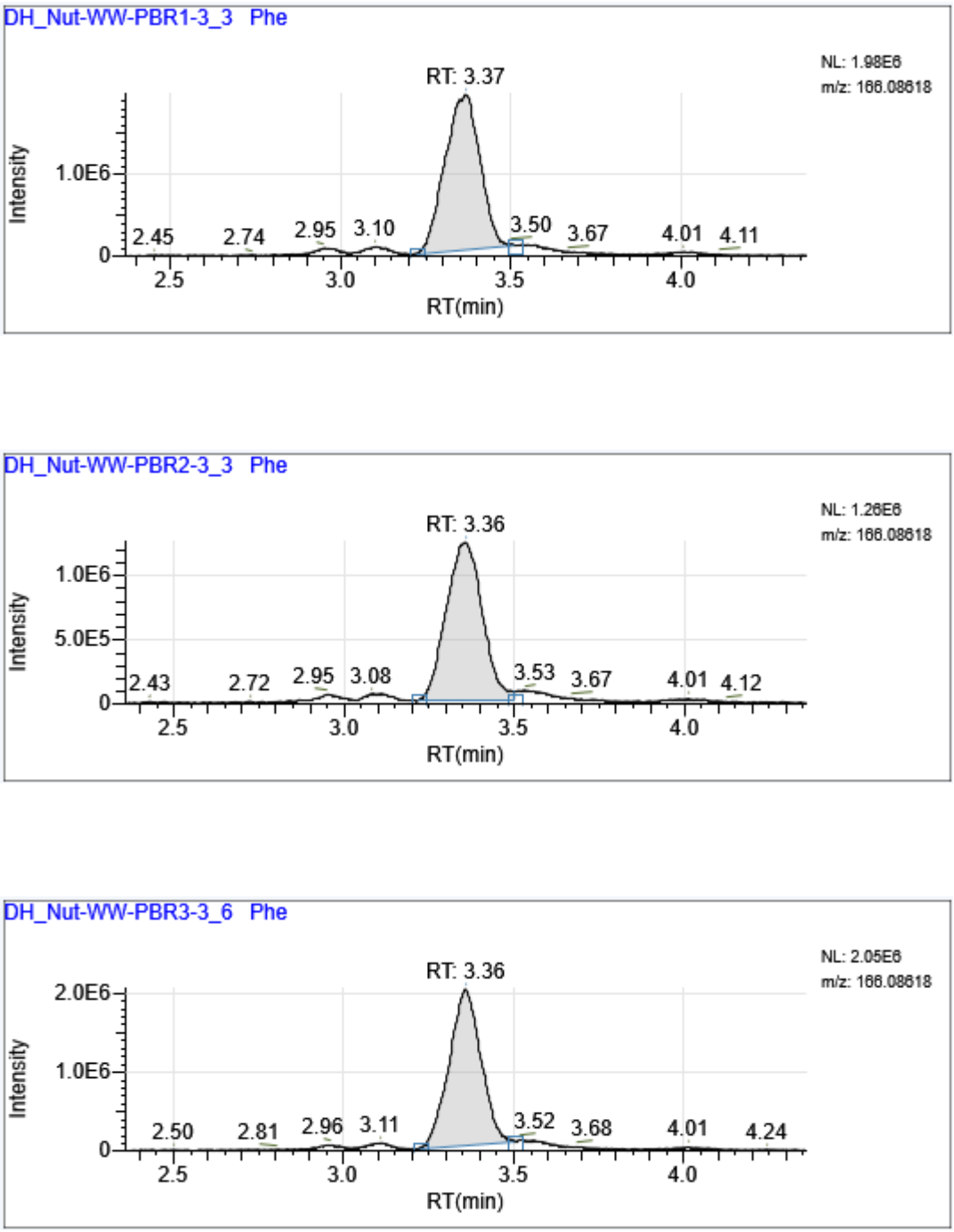


Figure B.18. LC-MS chromatograms of PHE of FWW PBRs 1-2-3.

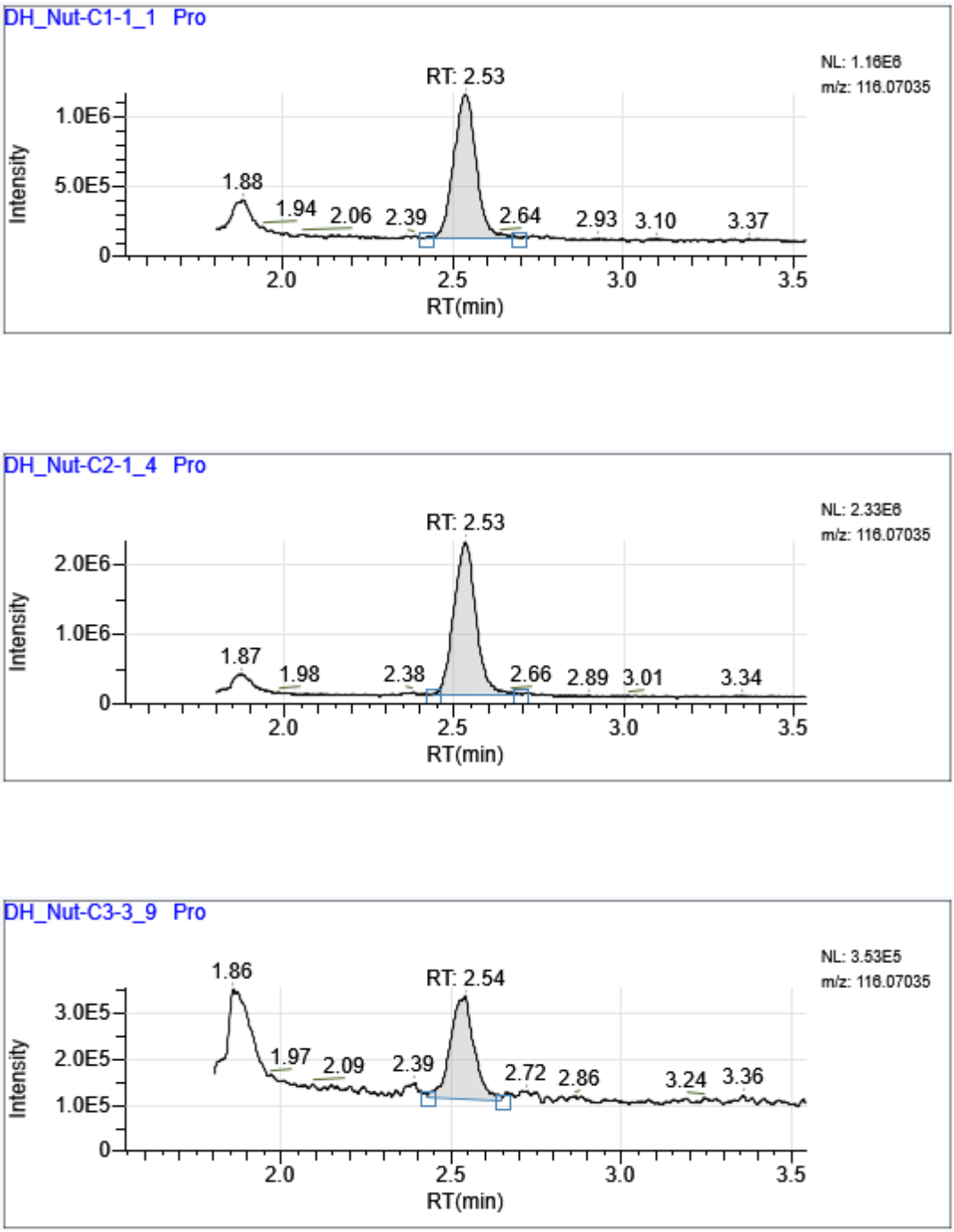


Figure B.19. LC-MS chromatograms of PRO of Control PBRs 1-2-3.

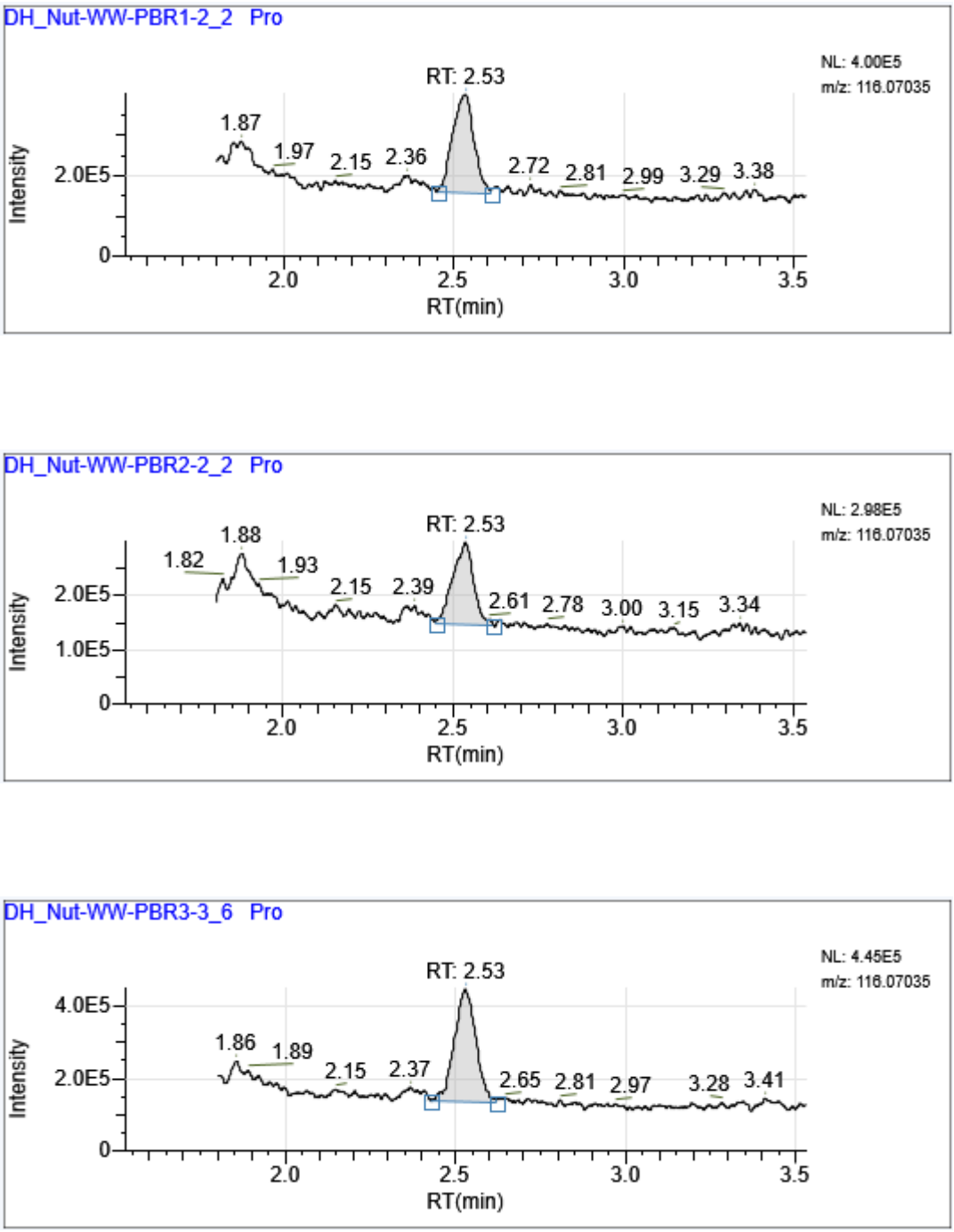


Figure B.20. LC-MS chromatograms of PRO of FWW PBRs 1-2-3

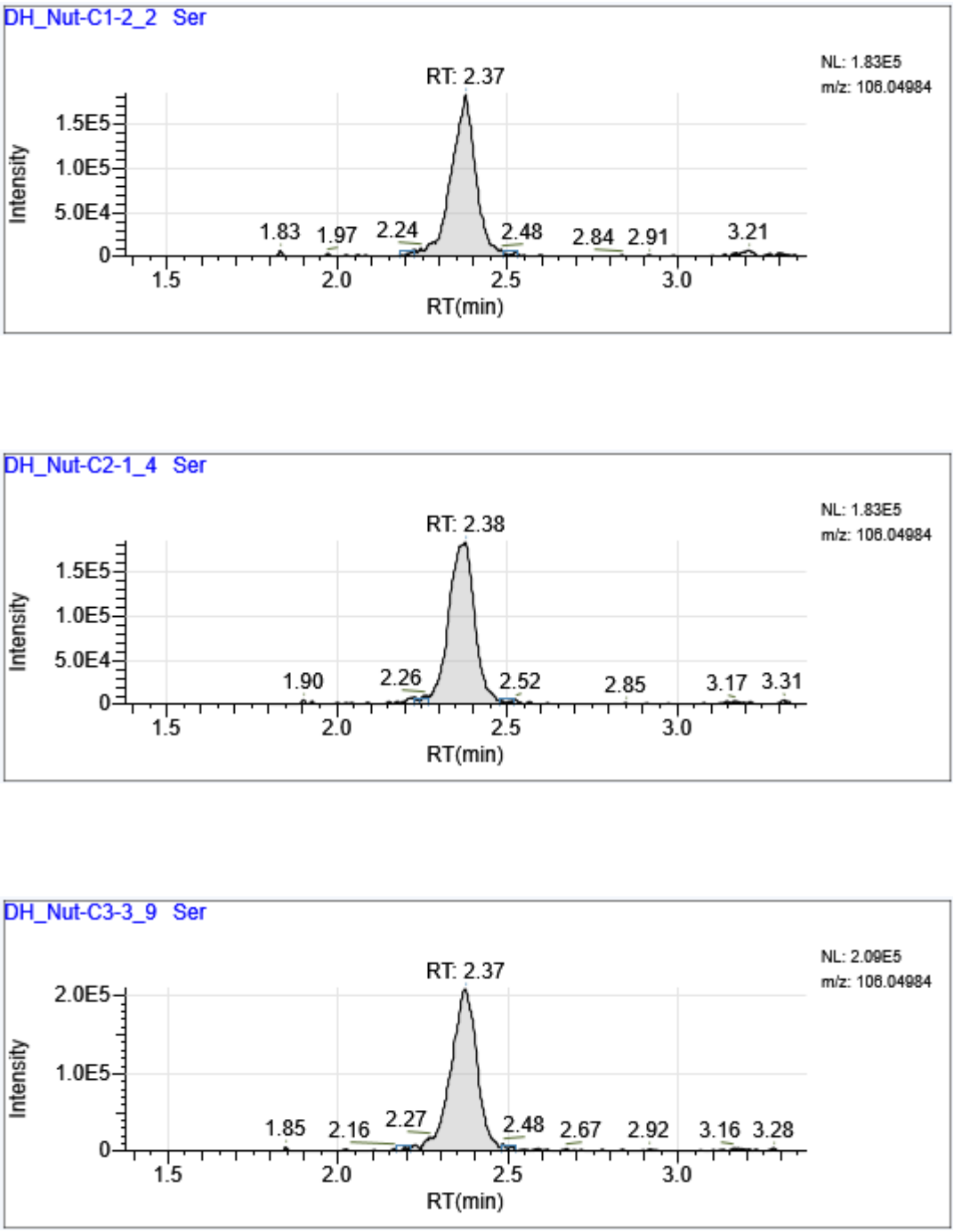


Figure B.21. LC-MS chromatograms of SER of Control PBRs 1-2-3.

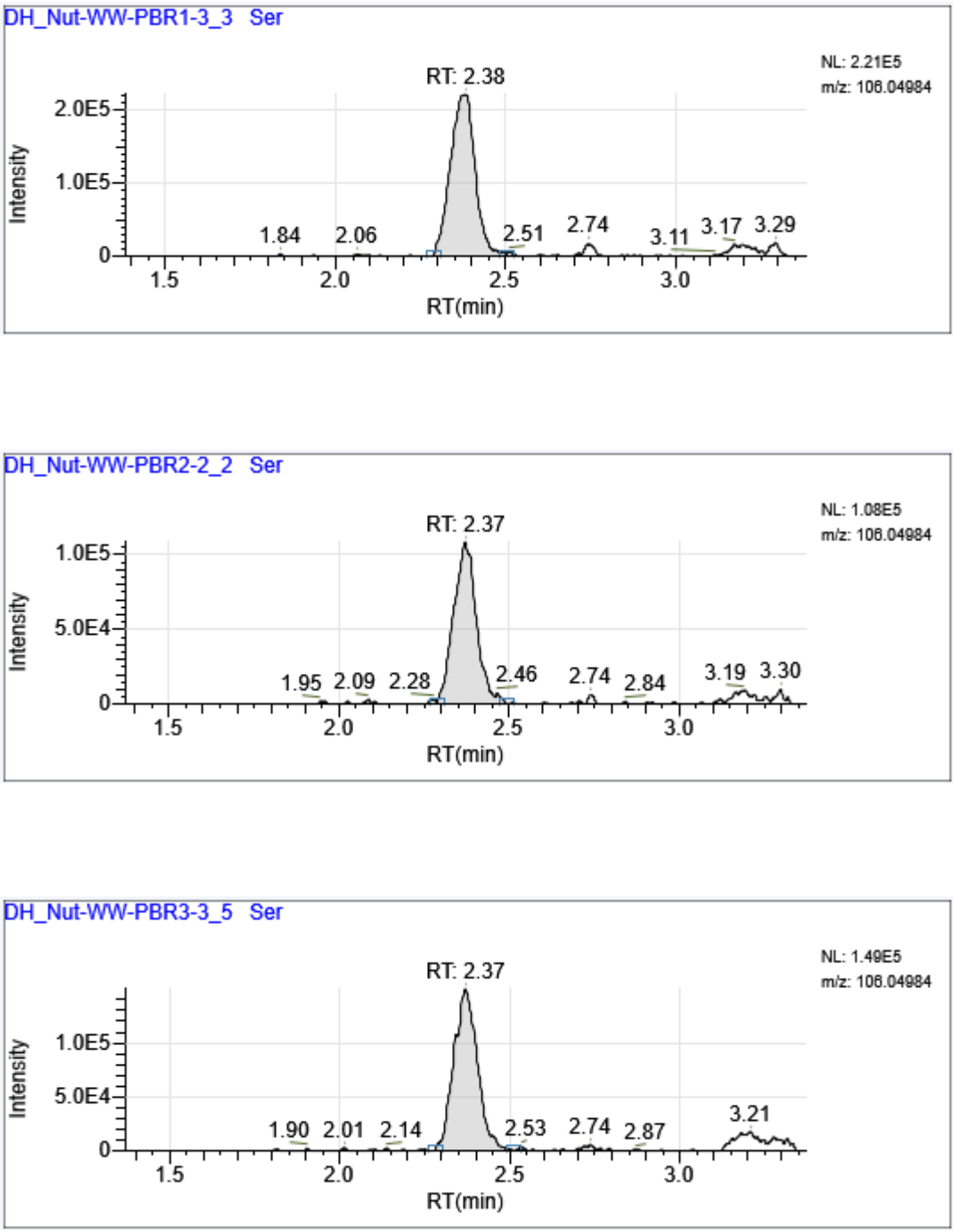
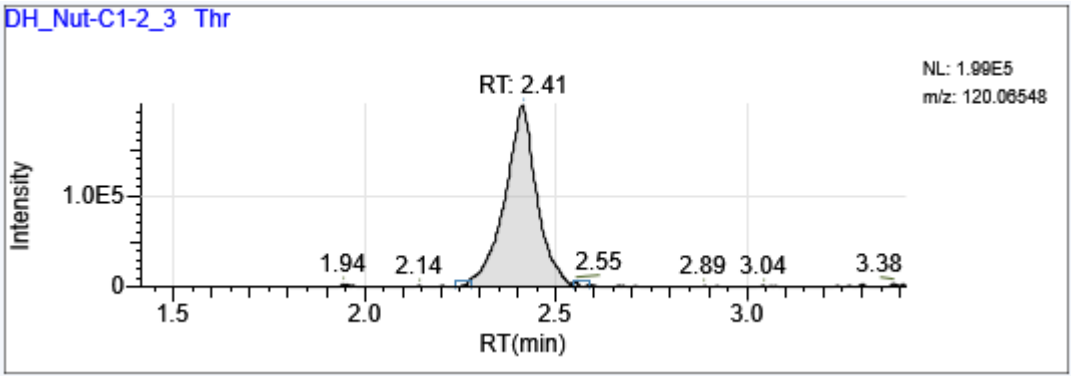
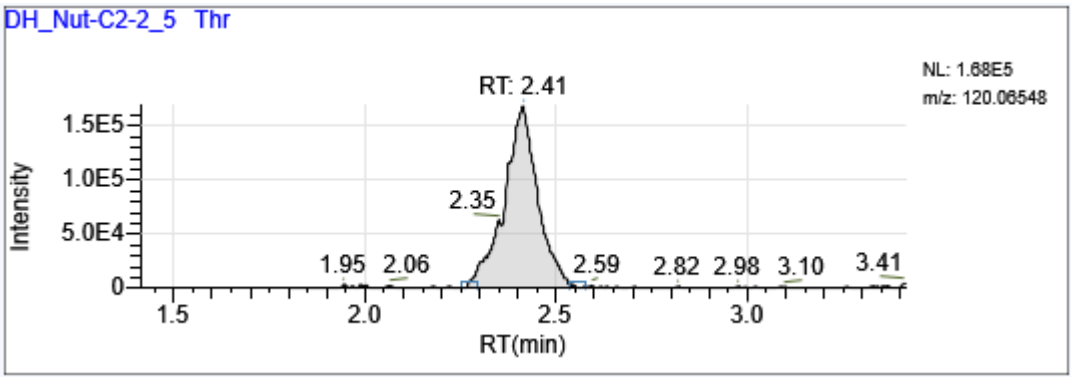


Figure B.22. LC-MS chromatograms of SER of FWW PBRs 1-2-3.





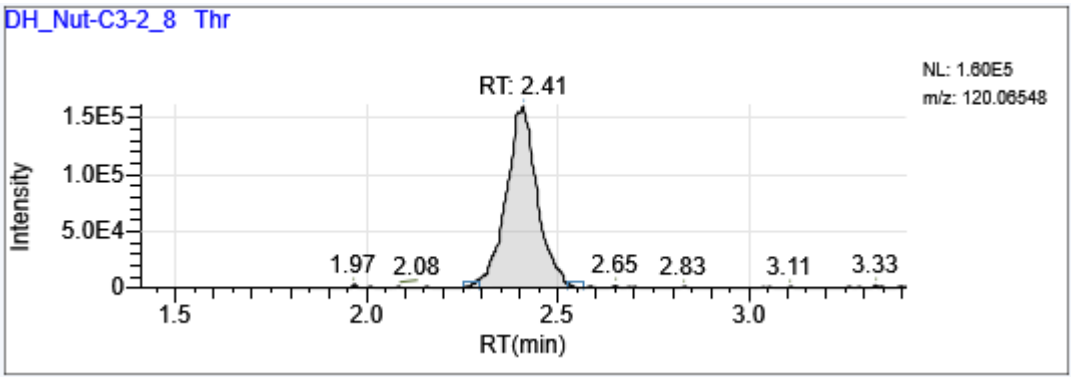


Figure B.23. LC-MS chromatograms of THR of Control PBRs 1-2-3.

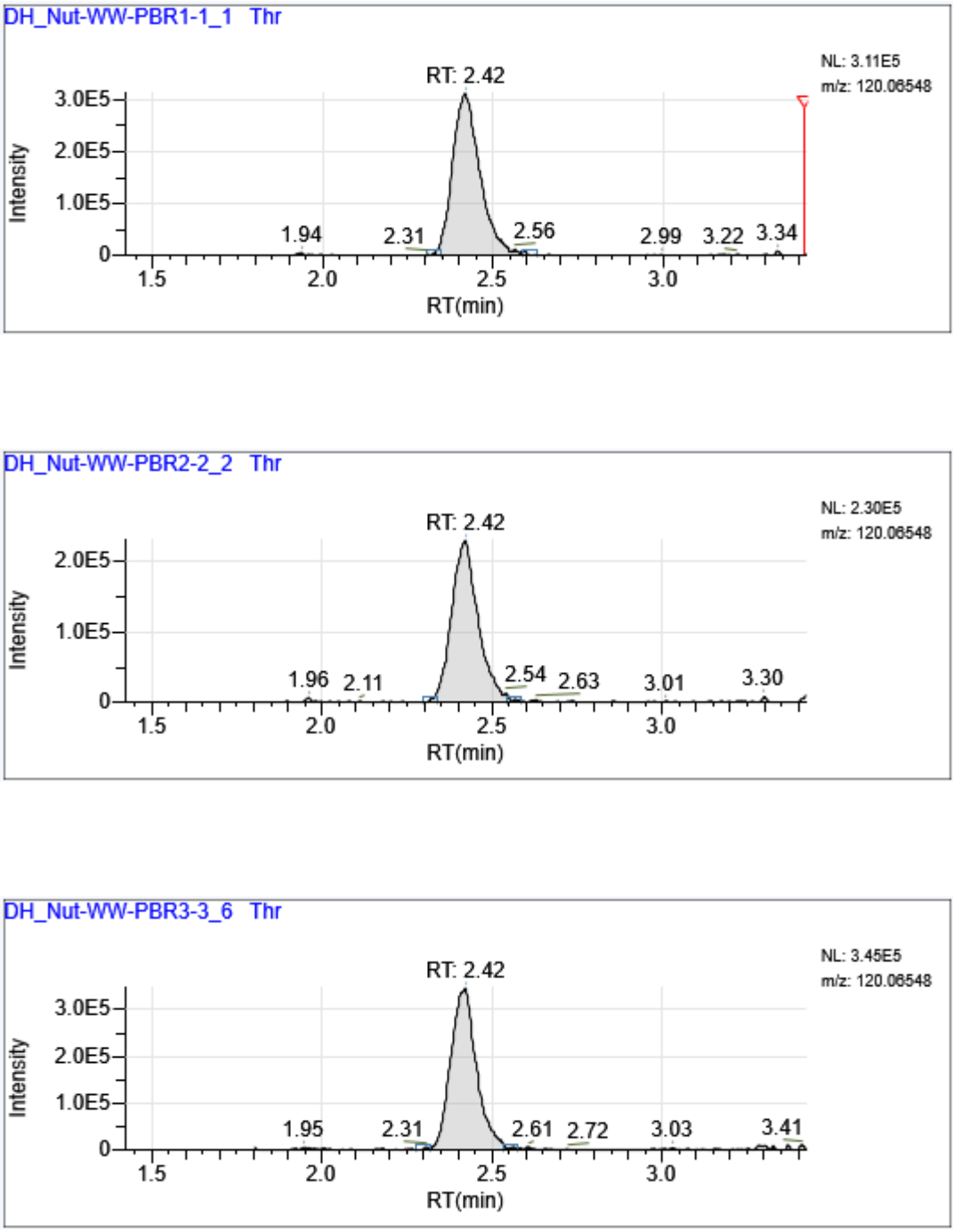


Figure B.24. LC-MS chromatograms of THR of FWW PBRs 1-2-3.

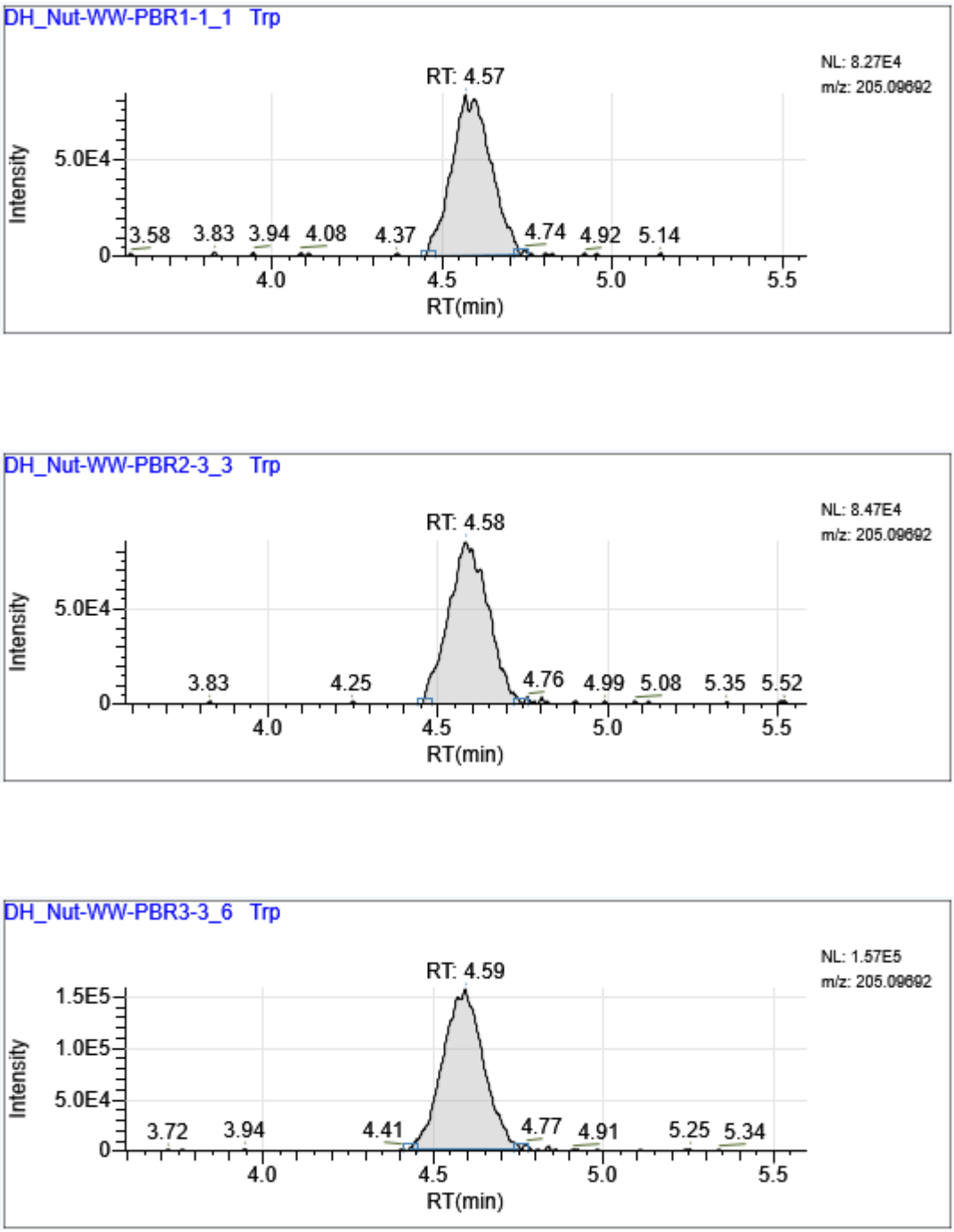


Figure B.25. LC-MS chromatograms of TRP of FWW PBRs 1-2-3

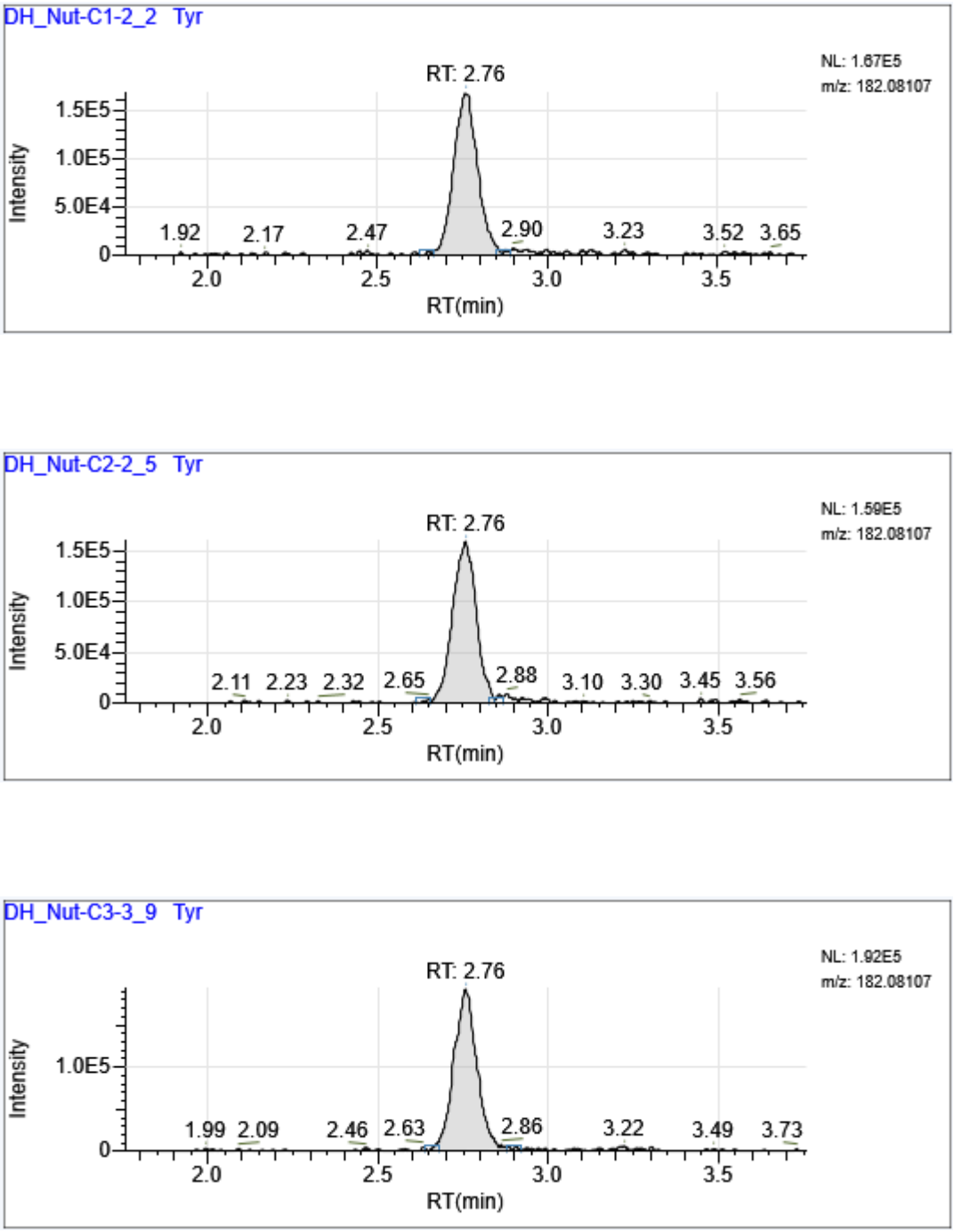


Figure B.26. LC-MS chromatograms of TYR of Control PBRs 1-2-3

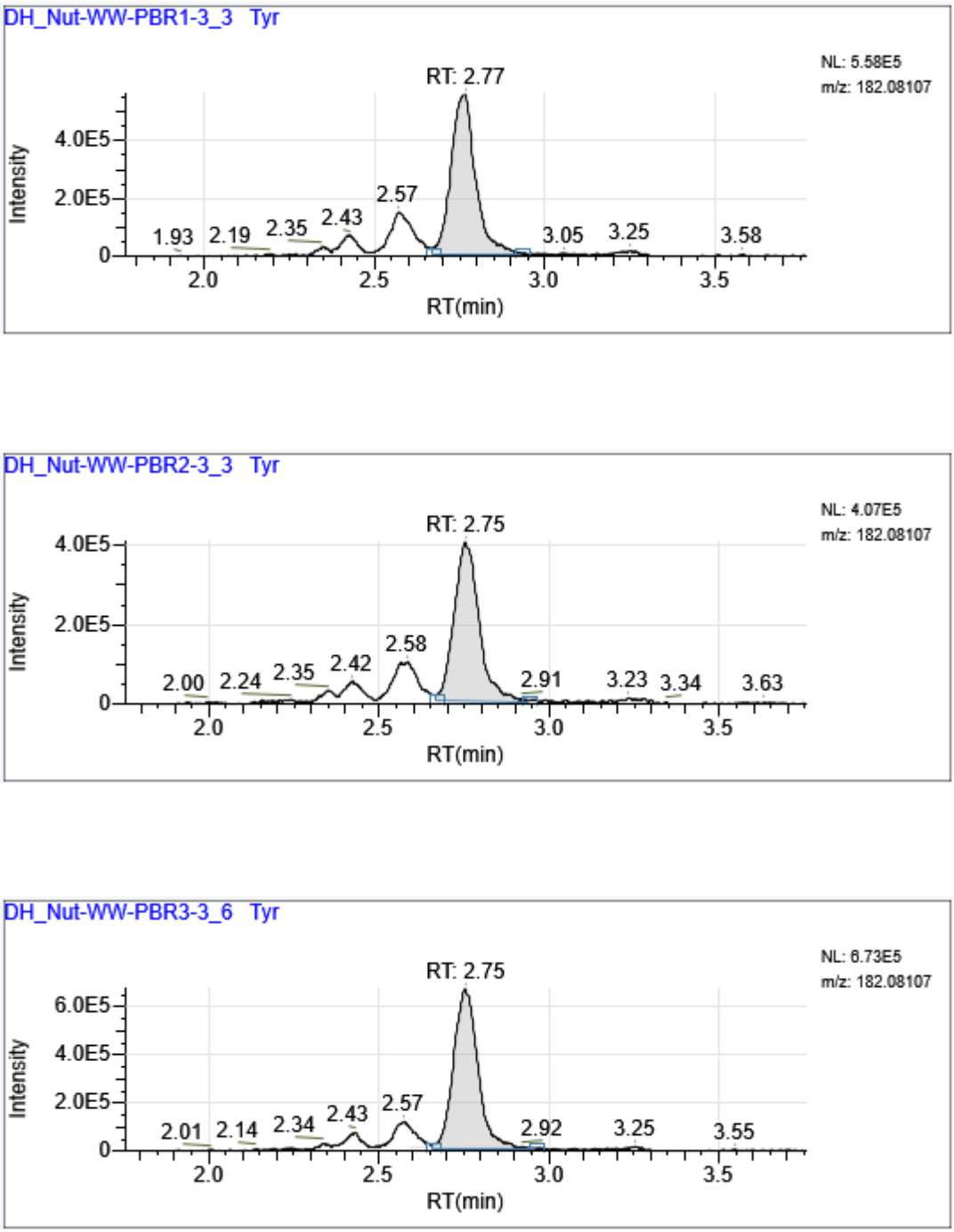


Figure B.27. LC-MS chromatograms of TYR of FWW PBRs 1-2-3.

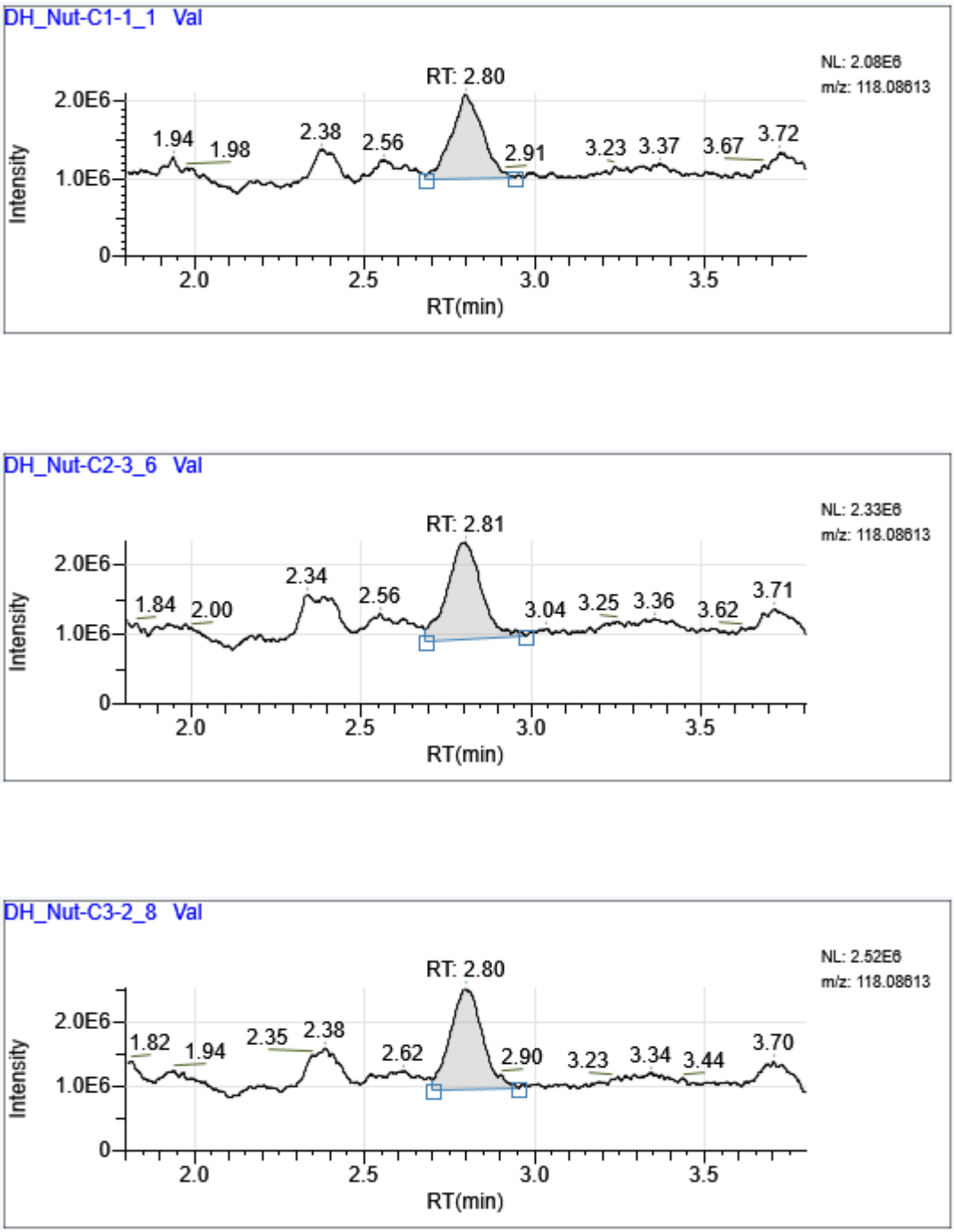


Figure B.28. LC-MS chromatograms of VAL of Control PBRs 1-2-3.

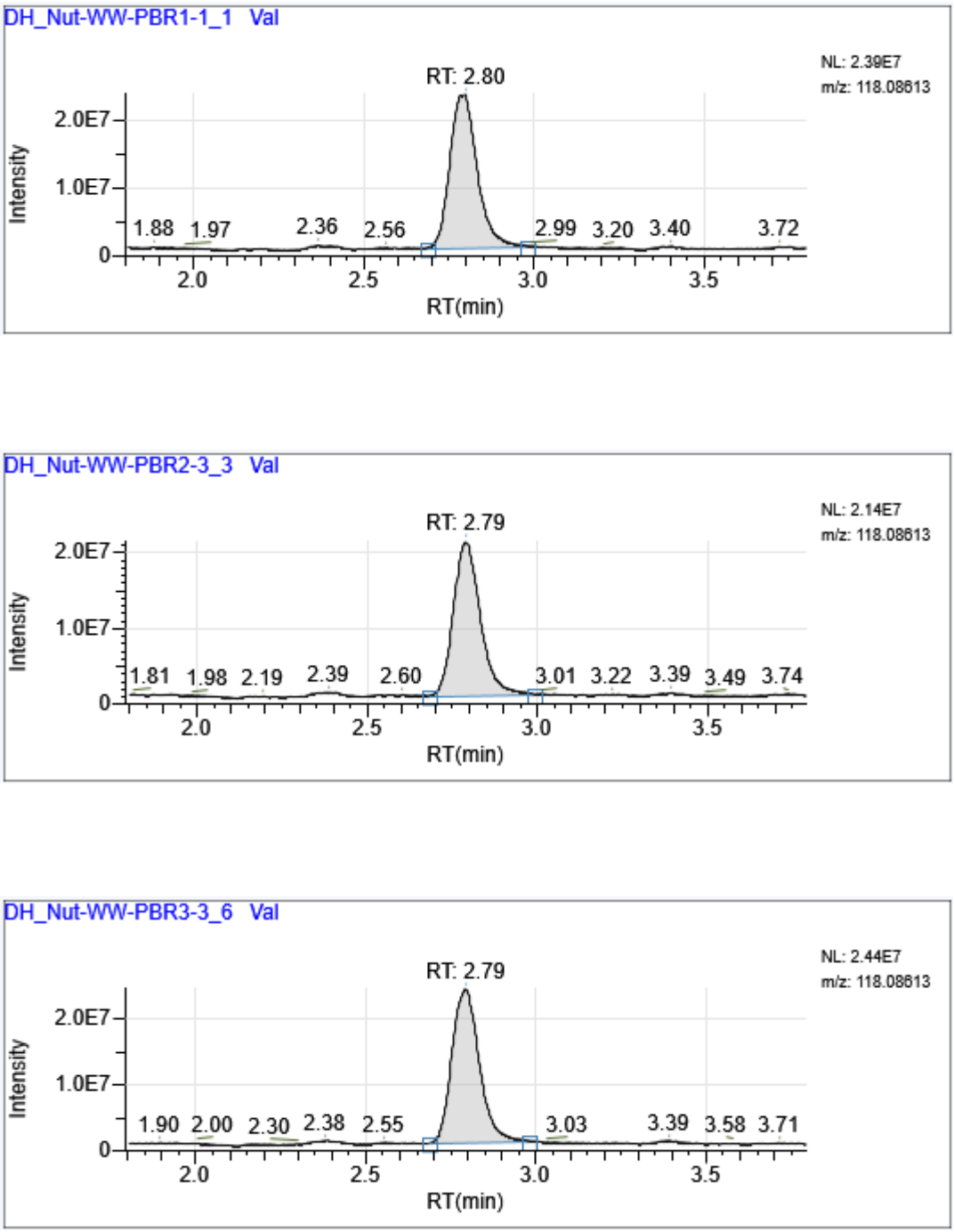


Figure B.29. LC-MS chromatograms of VAL of FWW PBRs 1-2-3.

APPENDIX C

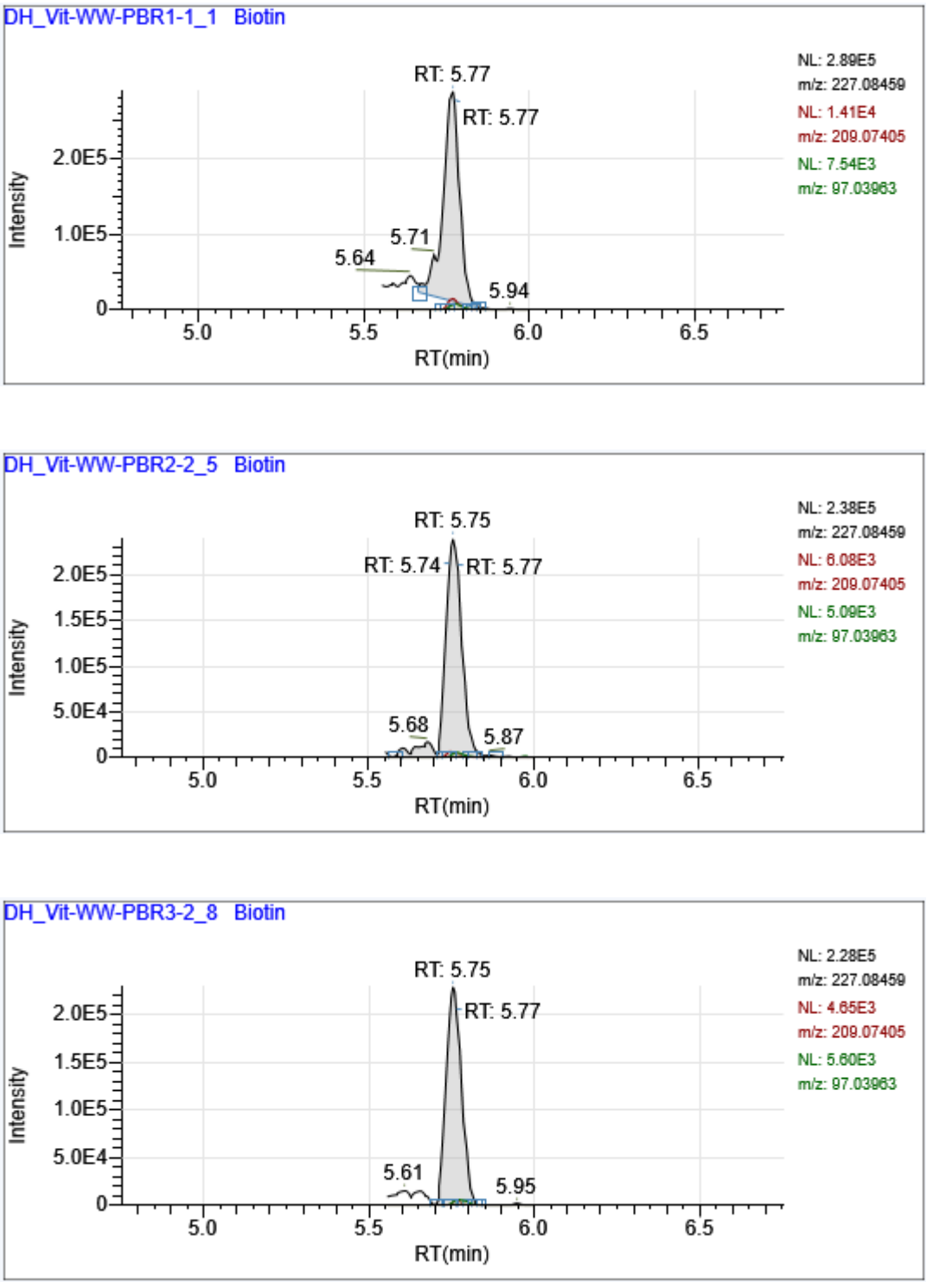


Figure C.1. LC-MS chromatograms of Biotin of FWW PBRs 1-2-3.

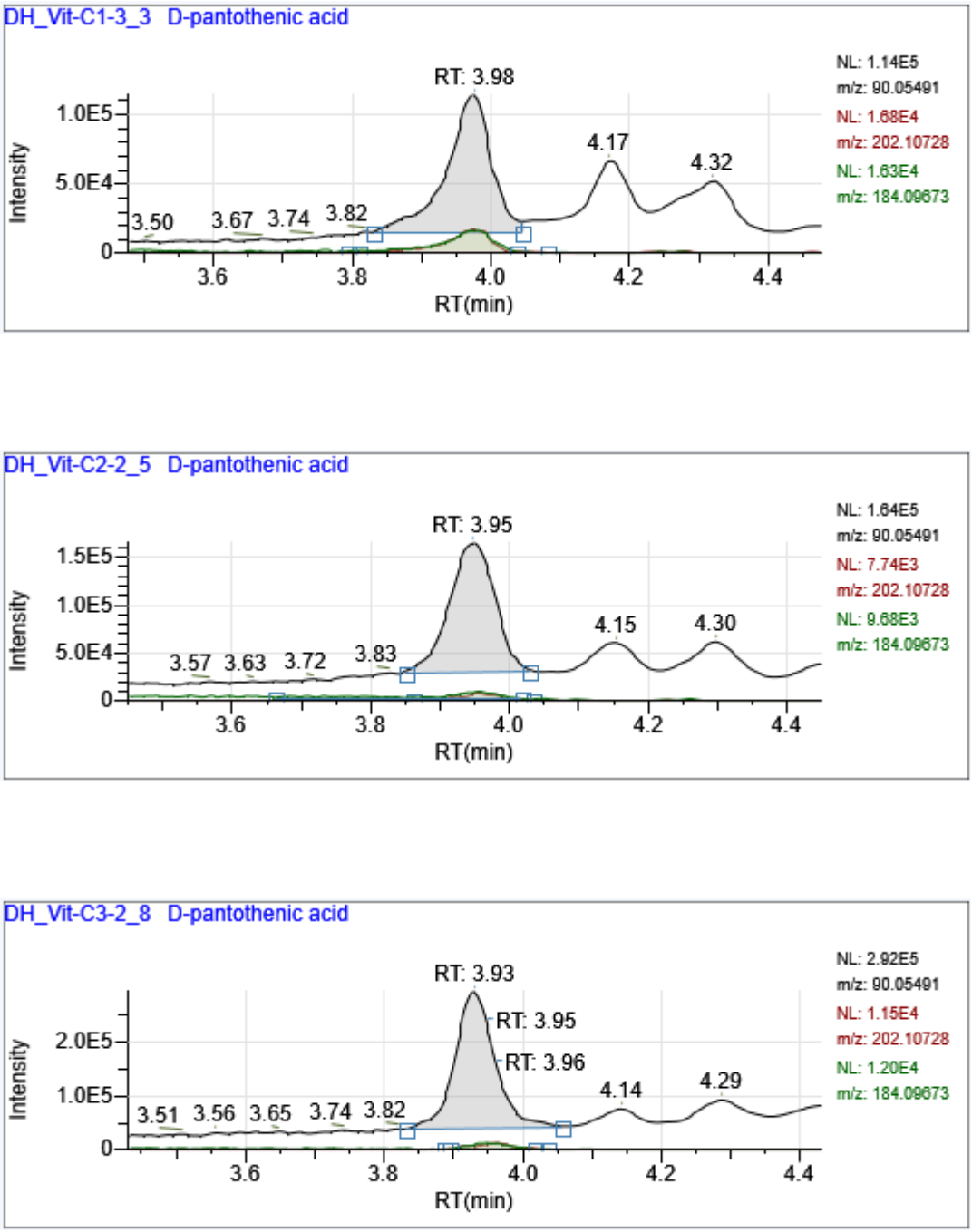


Figure C.2. LC-MS chromatograms of Pantothenic acid of Control PBRs 1-2-3.

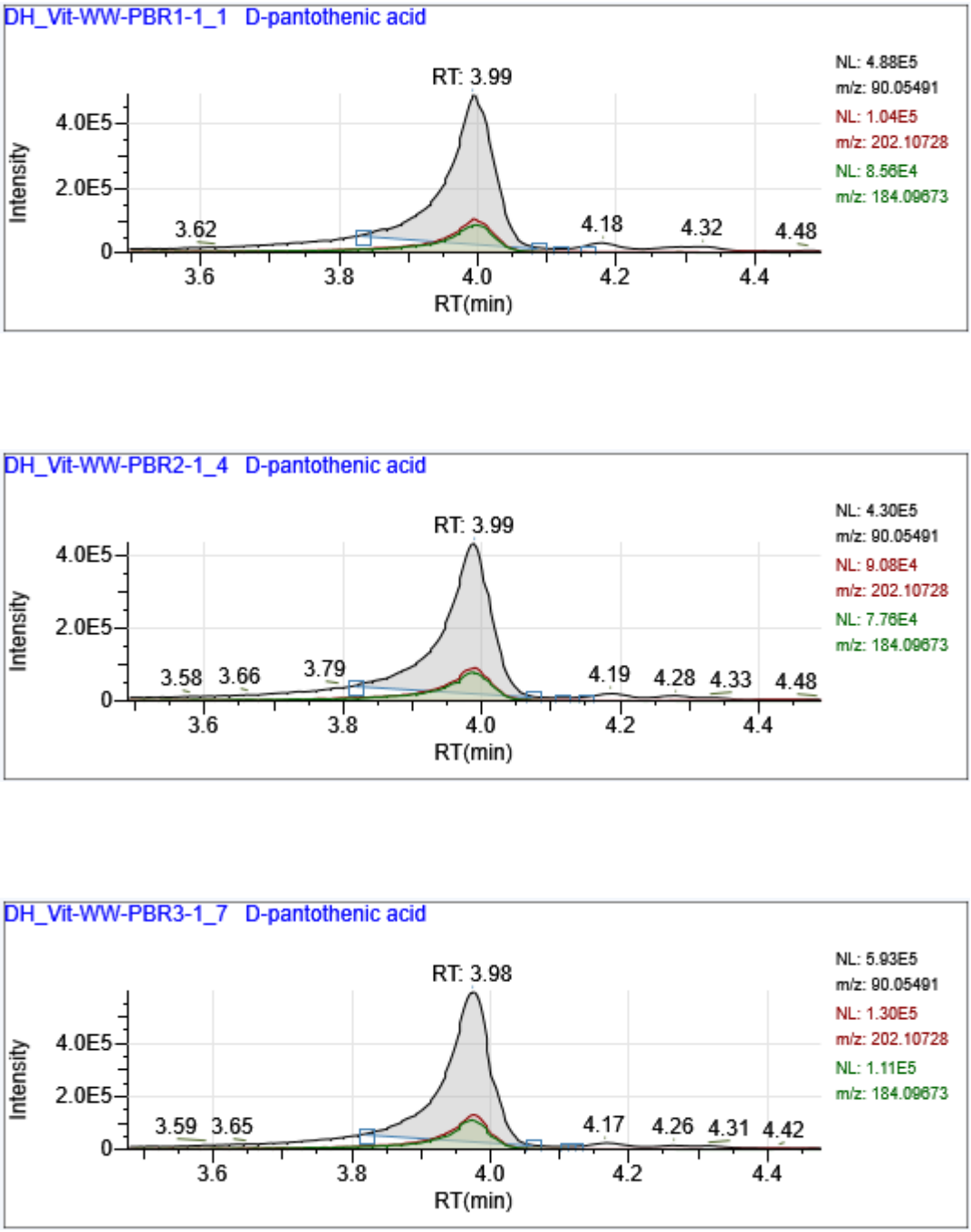


Figure C.3. LC-MS chromatograms of Pantothenic acid of FWW PBRs 1-2-3.

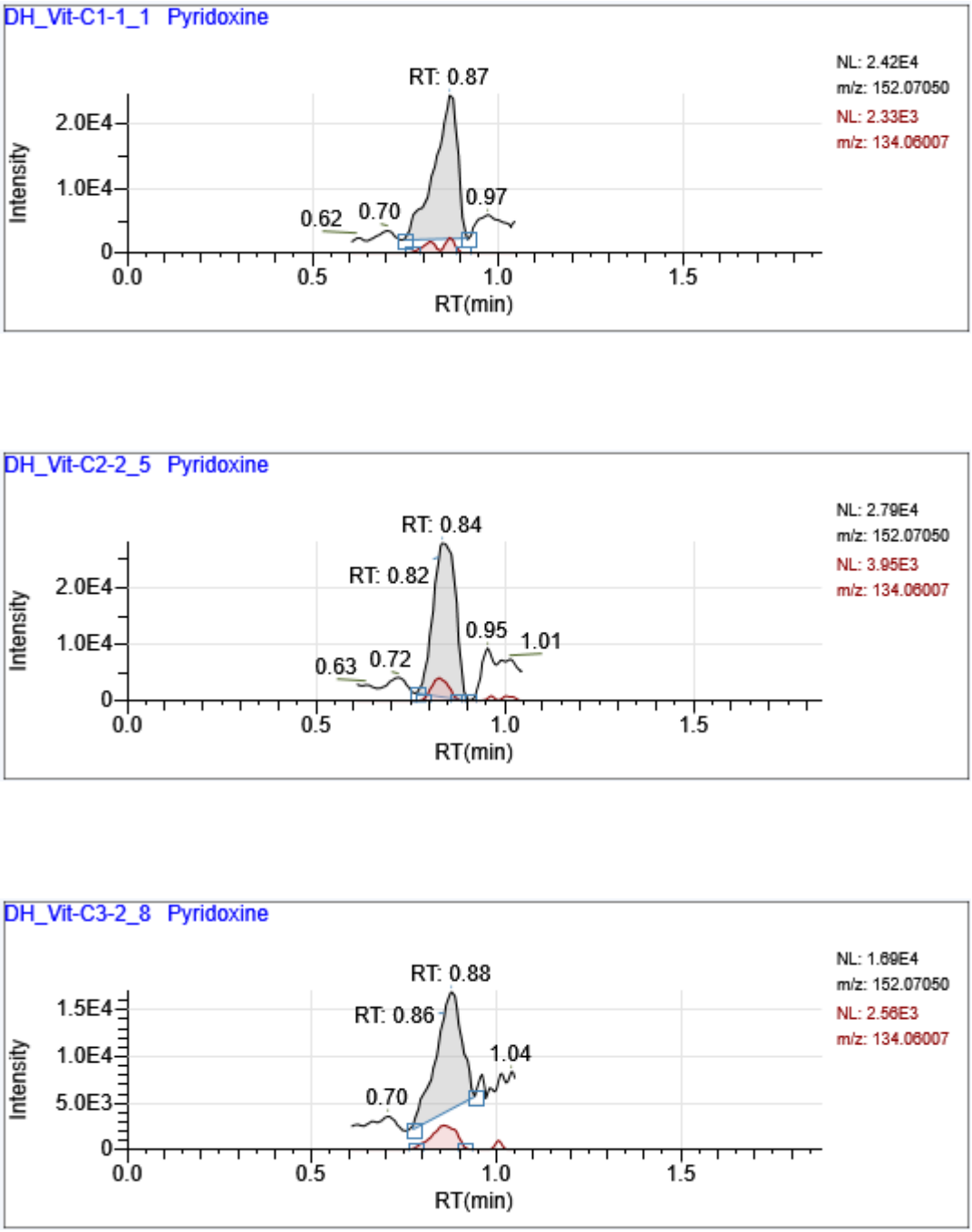


Figure C.4. LC-MS chromatograms of Pyridoxine of Control PBRs 1-2-3.

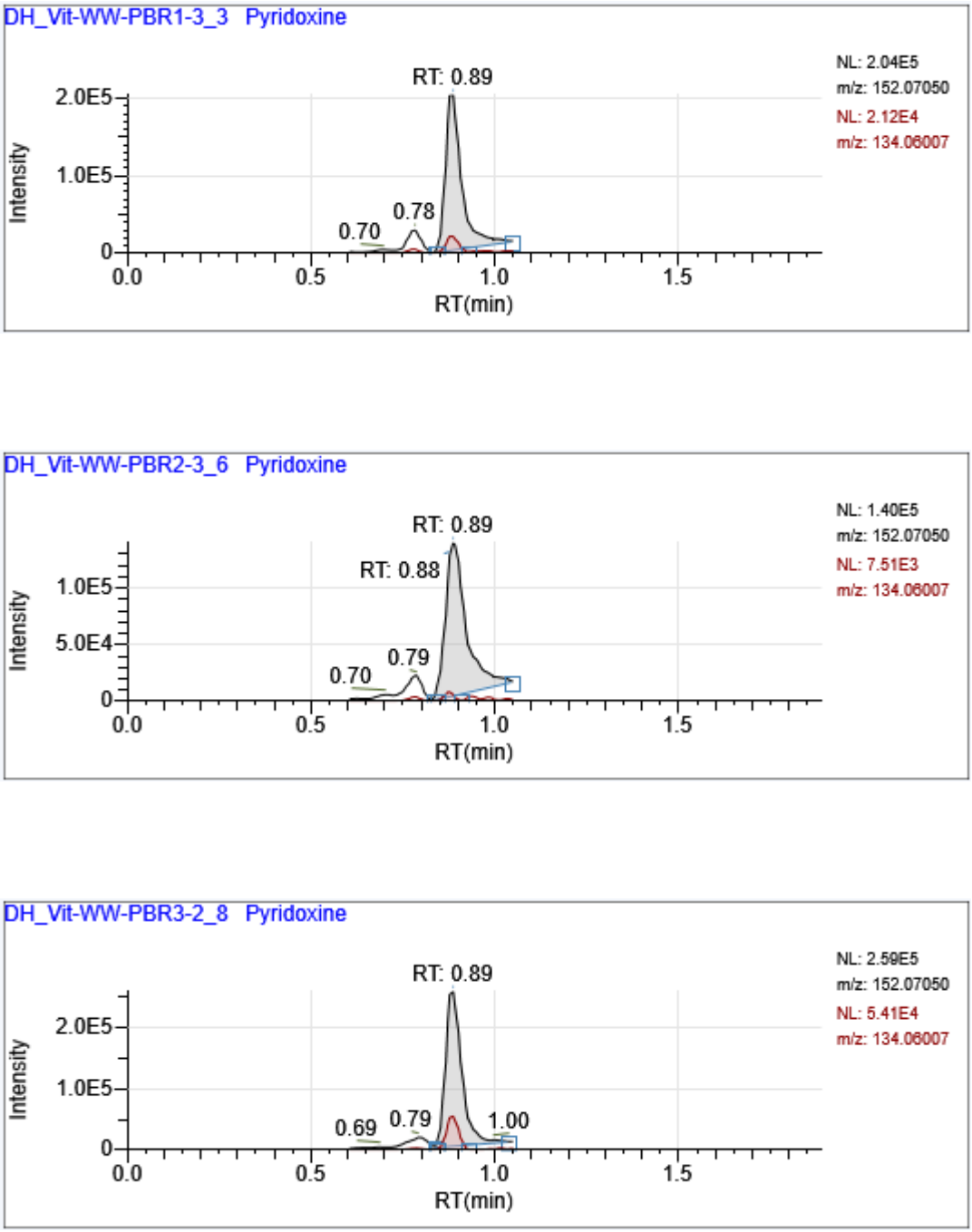


Figure C.5. LC-MS chromatograms of Pyridoxine of FWW PBRs 1-2-3.

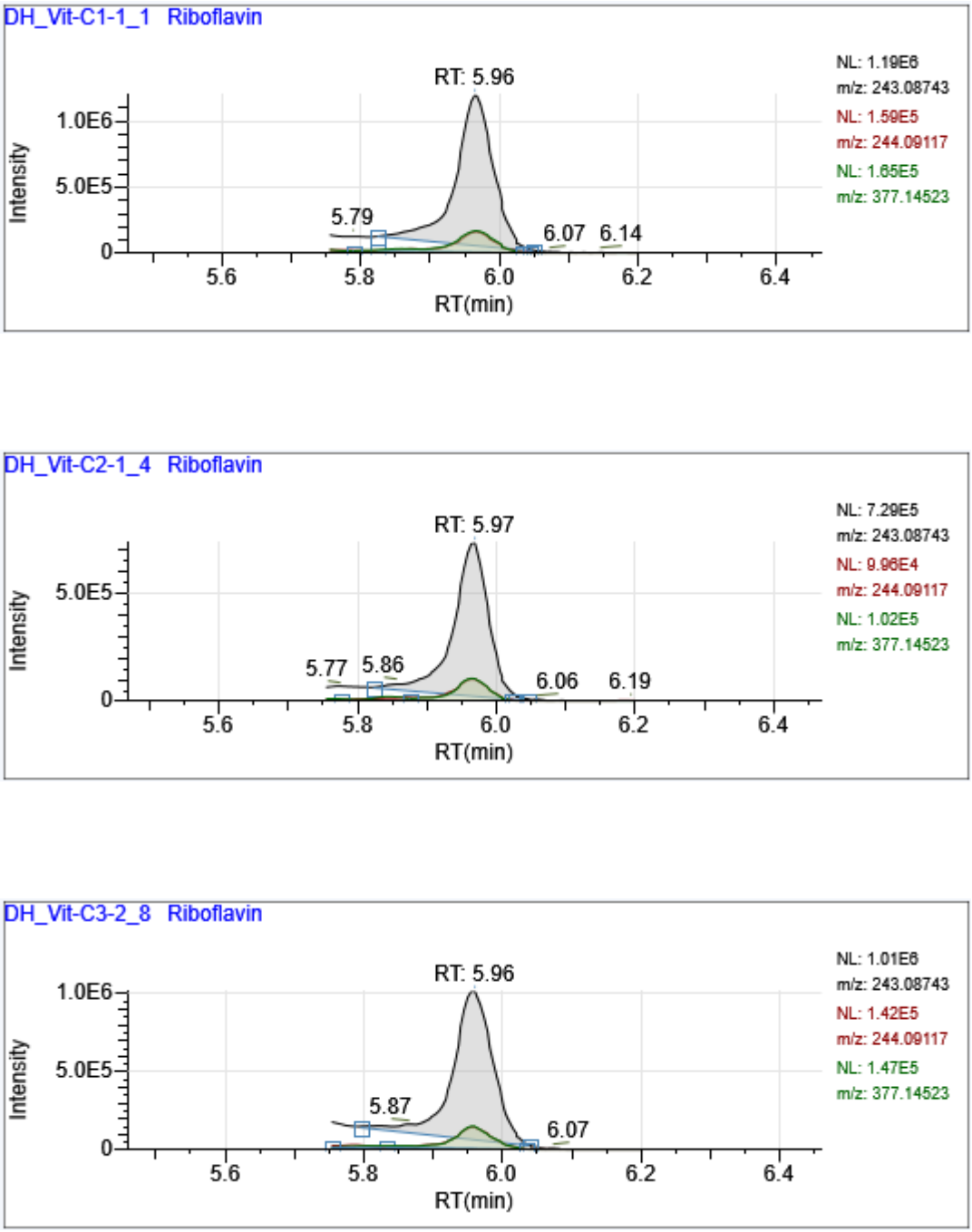


Figure C.6. LC-MS chromatograms of Riboflavin of Control PBRs 1-2-3.

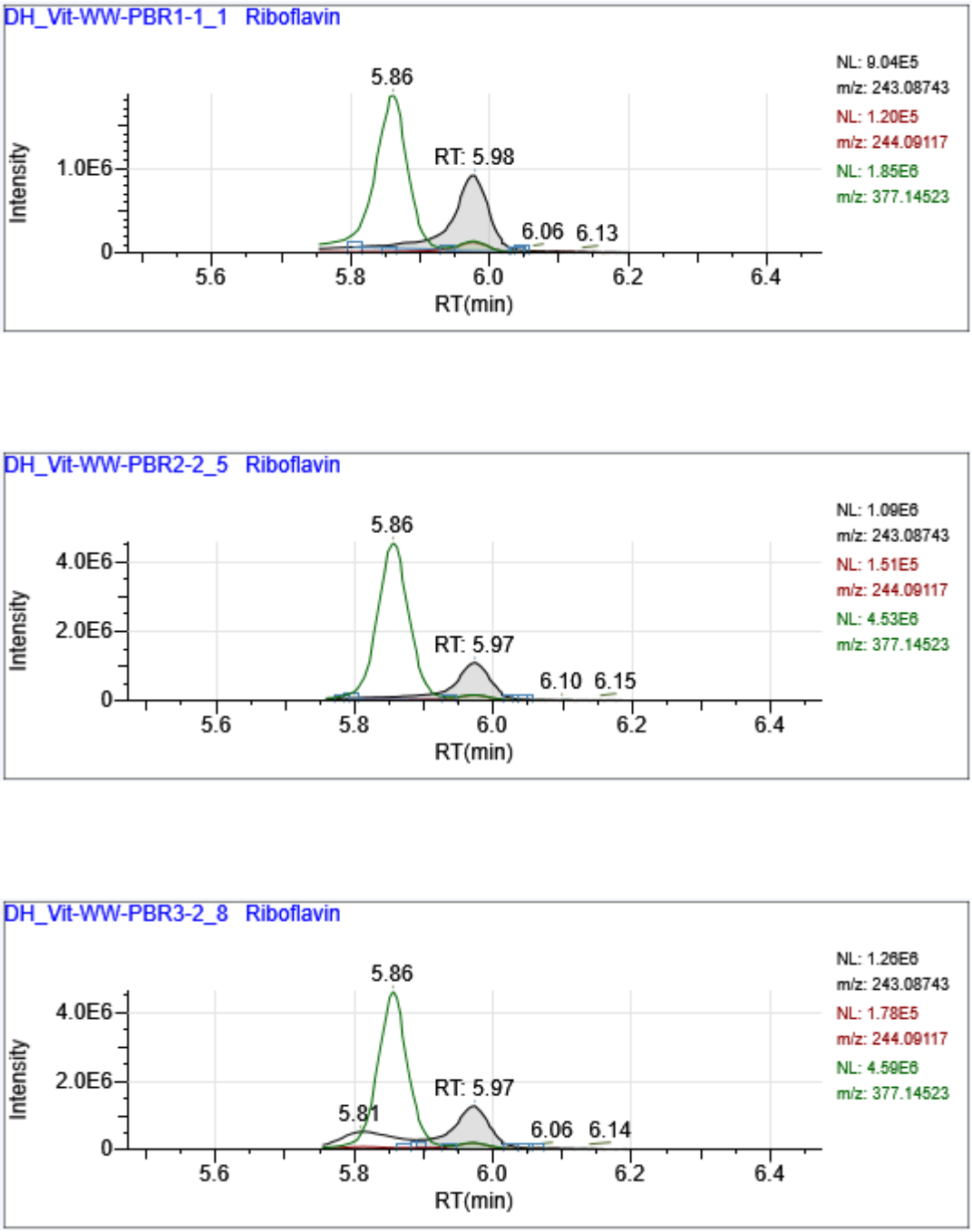


Figure C.7. LC-MS chromatograms of Riboflavin of FWW PBRs 1-2-3.

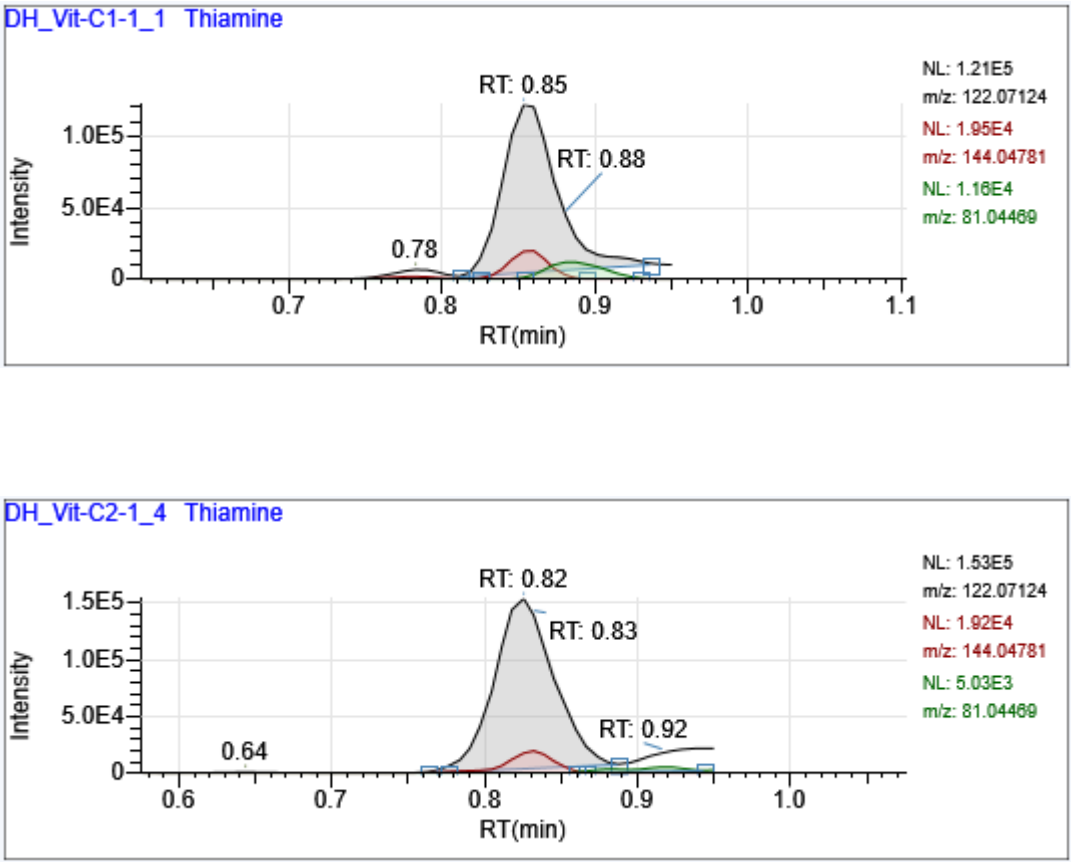


Figure C.8. LC-MS chromatograms of Thiamine of Control PBRs 1-2.

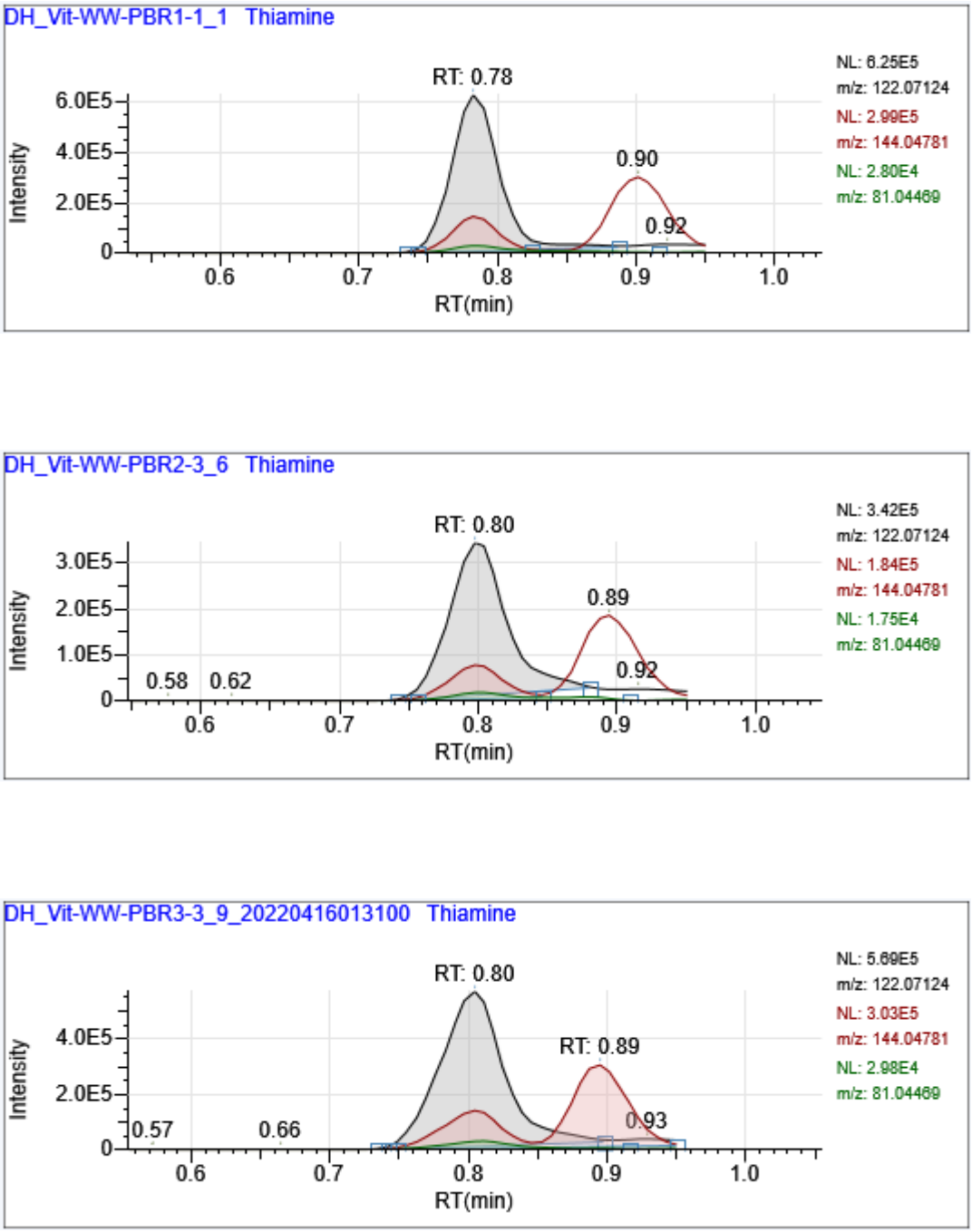


Figure C.9. LC-MS chromatograms of Thiamine of FWW PBRs 1-2-3.

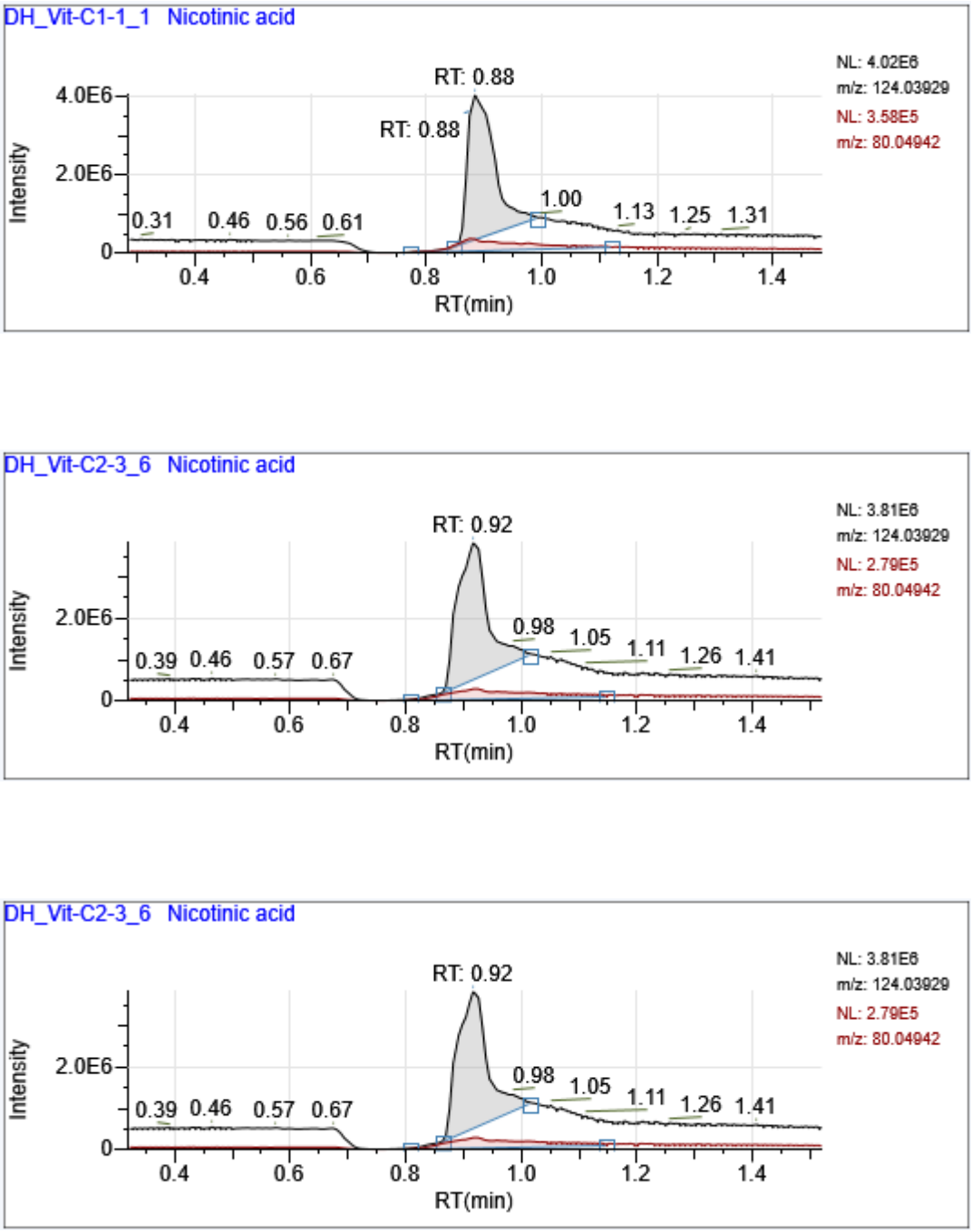


Figure C.10. LC-MS chromatograms of Nicotinic acid of Control PBRs 1-2-3.

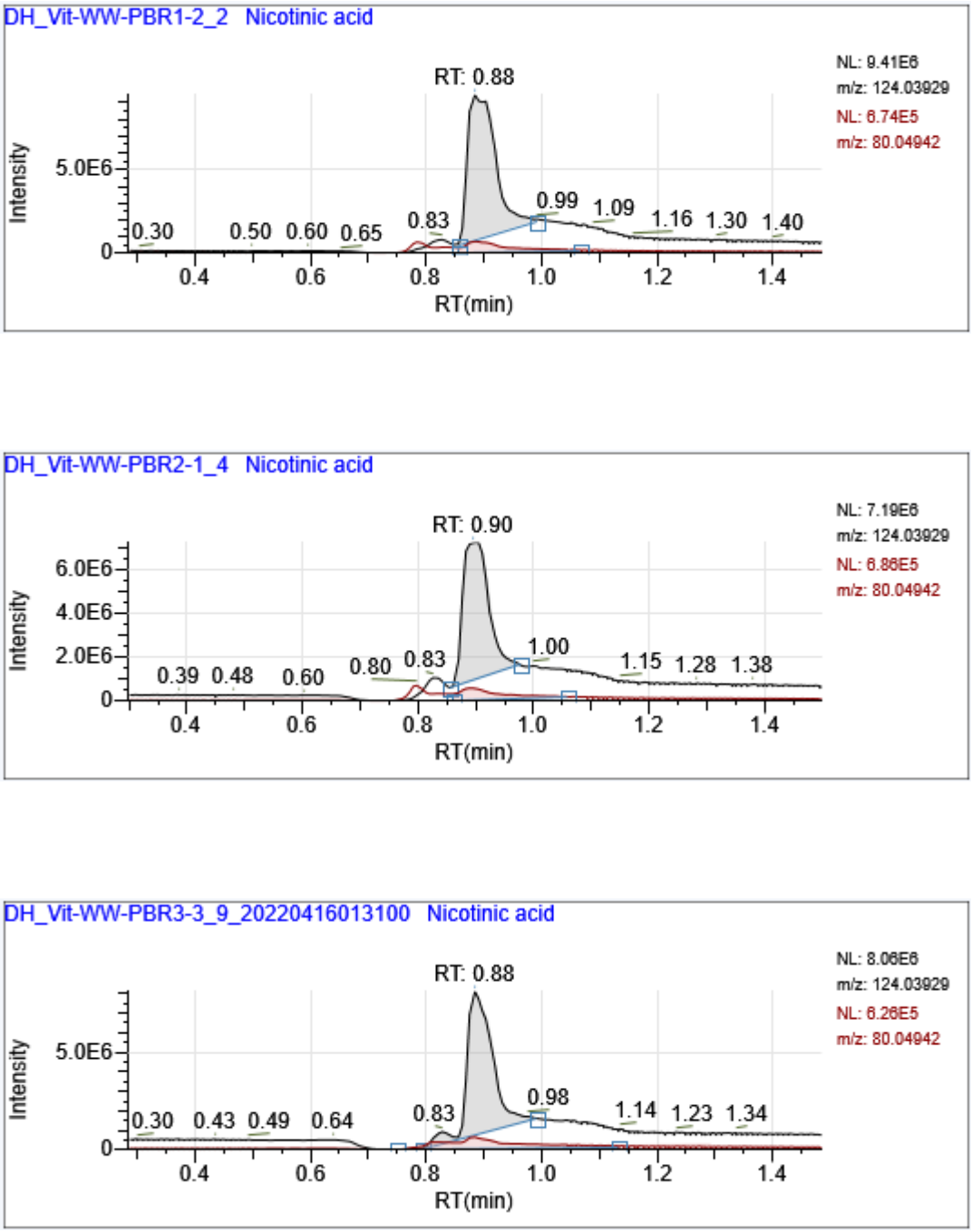


Figure C.11. LC-MS chromatograms of Nicotinic acid of FWW PBRs 1-2-3.

APPENDIX D

Table D.1. LC-MS data for amino acids analysis.

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Blank (UPW)	ALA	2,58	2,67	0.10	90,0549	90,05483	-.75000 (ppm)
Blank (UPW)	ALA	2,58	2,55	-0.02	90,0549	90,05493	.35135 (ppm)
Control PBR 1.1	ALA	2,58	2,63	0.05	90,0549	90,05508	1.96102 (ppm)
Control PBR 1.2	ALA	2,58	2,63	0.05	90,0549	90,05511	2.38461 (ppm)
Control PBR 1.3	ALA	2,58	2,62	0.05	90,0549	90,05508	1.96102 (ppm)
Control PBR 2.1	ALA	2,58	2,62	0.05	90,0549	90,05512	2.46933 (ppm)
Control PBR 2.2	ALA	2,58	2,63	0.06	90,0549	90,05508	1.96102 (ppm)
Control PBR 2.3	ALA	2,58	2,62	0.05	90,0549	90,05507	1.87630 (ppm)
Control PBR 3.1	ALA	2,58	2,62	0.04	90,0549	90,05507	1.87630 (ppm)
Control PBR 3.2	ALA	2,58	2,62	0.05	90,0549	90,05508	2.04574 (ppm)
Control PBR 3.3	ALA	2,58	2,63	0.05	90,0549	90,05508	1.96102 (ppm)
Reagent Blank 1	ALA	2,58	2,63	0.05	90,0549	90,05496	.69023 (ppm)
Reagent Blank 2	ALA	2,58	2,49	-0.09	90,0549	90,0552	3.31653 (ppm)
Reagent Blank 3	ALA	2,58	2,81	0.23	90,0549	90,05498	.85966 (ppm)
FWW PBR 1.1	ALA	2,58	2,62	0.05	90,0549	90,05492	.18191 (ppm)
FWW PBR 1.2	ALA	2,58	2,62	0.05	90,0549	90,05495	.60551 (ppm)
FWW PBR 1.3	ALA	2,58	2,63	0.05	90,0549	90,05505	1.62214 (ppm)
FWW PBR 2.1	ALA	2,58	2,62	0.05	90,0549	90,05508	1.96102 (ppm)
FWW PBR 2.2	ALA	2,58	2,63	0.05	90,0549	90,05507	1.87630 (ppm)
FWW PBR 2.3	ALA	2,58	2,62	0.04	90,0549	90,05506	1.79158 (ppm)
FWW PBR 3.1	ALA	2,58	2,63	0.06	90,0549	90,05507	1.87630 (ppm)
FWW PBR 3.2	ALA	2,58	2,62	0.05	90,0549	90,0551	2.21517 (ppm)
FWW PBR 3.3	ALA	2,58	2,62	0.05	90,0549	90,0551	2.21517 (ppm)
Blank (UPW)	ARG	5,78	5,58	-0.20	175,11885	175,11899	.78825 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Blank (UPW)	ARG	5,78	5,59	-0.19	175,11885	175,11888	.17831 (ppm)
Control PBR 1.1	ARG	5,78	5,91	0.13	175,11885	175,119	.87538 (ppm)
Control PBR 1.2	ARG	5,78	5,91	0.13	175,11885	175,11923	2.18239 (ppm)
Control PBR 1.3	ARG	5,78	5,89	0.11	175,11885	175,11929	2.53093 (ppm)
Control PBR 2.1	ARG	5,78	5,89	0.11	175,11885	175,11919	1.92099 (ppm)
Control PBR 2.2	ARG	5,78	5,9	0.12	175,11885	175,11922	2.09526 (ppm)
Control PBR 2.3	ARG	5,78	5,88	0.10	175,11885	175,11928	2.44379 (ppm)
Control PBR 3.1	ARG	5,78	5,89	0.11	175,11885	175,11922	2.09526 (ppm)
Control PBR 3.2	ARG	5,78	5,9	0.12	175,11885	175,11919	1.92099 (ppm)
Control PBR 3.3	ARG	5,78	5,91	0.13	175,11885	175,11923	2.18239 (ppm)
Reagent Blank 1	ARG	5,78	5,99	0.21	175,11885	175,11893	.43971 (ppm)
Reagent Blank 2	ARG	5,78	5,91	0.13	175,11885	175,11911	1.48532 (ppm)
Reagent Blank 3	ARG	5,78	5,94	0.16	175,11885	175,11908	1.31105 (ppm)
FWW PBR 1.1	ARG	5,78	5,92	0.14	175,11885	175,11896	.61398 (ppm)
FWW PBR 1.2	ARG	5,78	5,9	0.12	175,11885	175,1192	2.00812 (ppm)
FWW PBR 1.3	ARG	5,78	5,9	0.12	175,11885	175,11919	1.92099 (ppm)
FWW PBR 2.1	ARG	5,78	5,9	0.12	175,11885	175,11926	2.35666 (ppm)
FWW PBR 2.2	ARG	5,78	5,91	0.13	175,11885	175,11923	2.18239 (ppm)
FWW PBR 2.3	ARG	5,78	5,89	0.11	175,11885	175,1192	2.00812 (ppm)
FWW PBR 3.1	ARG	5,78	5,9	0.12	175,11885	175,11925	2.26952 (ppm)
FWW PBR 3.2	ARG	5,78	5,89	0.11	175,11885	175,11931	2.61806 (ppm)
FWW PBR 3.3	ARG	5,78	5,9	0.12	175,11885	175,11919	1.92099 (ppm)
Blank (UPW)	ASP	2,2	N/F	N/F	134,04466	N/F	N/F
Blank (UPW)	ASP	2,2	2,02	-0.18	134,04466	134,0443	-2.71354 (ppm)
Control PBR 1.1	ASP	2,2	2,19	-0.01	134,04466	134,04501	2.63664 (ppm)
Control PBR 1.2	ASP	2,2	2,2	0.00	134,04466	134,04494	2.06747 (ppm)
Control PBR 1.3	ASP	2,2	2,2	0.00	134,04466	134,04503	2.75048 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Control PBR 2.1	ASP	2,2	2,2	0.00	134,04466	134,04494	2.06747 (ppm)
Control PBR 2.2	ASP	2,2	2,2	0.00	134,04466	134,04494	2.06747 (ppm)
Control PBR 2.3	ASP	2,2	2,2	0.00	134,04466	134,04497	2.29514 (ppm)
Control PBR 3.1	ASP	2,2	2,2	0.00	134,04466	134,04492	1.95364 (ppm)
Control PBR 3.2	ASP	2,2	2,2	0.00	134,04466	134,04494	2.06747 (ppm)
Control PBR 3.3	ASP	2,2	2,2	0.00	134,04466	134,04492	1.95364 (ppm)
Reagent Blank 1	ASP	2,2	2,22	0.02	134,04466	134,04465	-.09537 (ppm)
Reagent Blank 2	ASP	2,2	2,15	-0.05	134,04466	134,0439	-5.67321 (ppm)
Reagent Blank 3	ASP	2,2	2,12	-0.08	134,04466	N/A	N/A
FWW PBR 1.1	ASP	2,2	2,2	0.00	134,04466	134,04474	.58764 (ppm)
FWW PBR 1.2	ASP	2,2	2,21	0.01	134,04466	134,04457	-.66453 (ppm)
FWW PBR 1.3	ASP	2,2	2,2	0.00	134,04466	134,04494	2.06747 (ppm)
FWW PBR 2.1	ASP	2,2	2,19	-0.01	134,04466	134,04492	1.95364 (ppm)
FWW PBR 2.2	ASP	2,2	2,2	0.00	134,04466	134,04486	1.49831 (ppm)
FWW PBR 2.3	ASP	2,2	2,2	0.00	134,04466	134,04486	1.49831 (ppm)
FWW PBR 3.1	ASP	2,2	2,2	0.00	134,04466	134,04494	2.06747 (ppm)
FWW PBR 3.2	ASP	2,2	2,2	0.00	134,04466	134,04495	2.18131 (ppm)
FWW PBR 3.3	ASP	2,2	2,2	0.00	134,04466	134,04497	2.29514 (ppm)
Blank (UPW)	CYS	2,25	2,16	-0.09	122,02701	122,02772	5.79864 (ppm)
Blank (UPW)	CYS	2,25	2,33	0.08	122,02701	122,02778	6.29881 (ppm)
Control PBR 1.1	CYS	2,25	2,46	0.21	122,02701	N/A	N/A
Control PBR 1.2	CYS	2,25	2,42	0.17	122,02701	122,02805	8.48709 (ppm)
Control PBR 1.3	CYS	2,25	2,05	-0.20	122,02701	122,02764	5.17342 (ppm)
Control PBR 2.1	CYS	2,25	2,11	-0.14	122,02701	122,02784	6.79899 (ppm)
Control PBR 2.2	CYS	2,25	2,17	-0.08	122,02701	122,02783	6.73647 (ppm)
Control PBR 2.3	CYS	2,25	2,38	0.13	122,02701	N/A	N/A
Control PBR 3.1	CYS	2,25	2,26	0.01	122,02701	N/A	N/A

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Control PBR 3.2	CYS	2,25	2,08	-0.17	122,02701	122,02829	10.48780 (ppm)
Control PBR 3.3	CYS	2,25	2,15	-0.10	122,02701	N/A	N/A
Reagent Blank 1	CYS	2,25	2,12	-0.13	122,02701	122,02771	5.73611 (ppm)
Reagent Blank 2	CYS	2,25	2,37	0.12	122,02701	N/A	N/A
Reagent Blank 3	CYS	2,25	2,28	0.03	122,02701	122,02753	4.29810 (ppm)
FWW PBR 1.1	CYS	2,25	2,15	-0.10	122,02701	122,02783	6.73647 (ppm)
FWW PBR 1.2	CYS	2,25	2,48	0.23	122,02701	N/A	N/A
FWW PBR 1.3	CYS	2,25	2,1	-0.15	122,02701	122,02823	9.98762 (ppm)
FWW PBR 2.1	CYS	2,25	2,1	-0.15	122,02701	N/A	N/A
FWW PBR 2.2	CYS	2,25	2,12	-0.13	122,02701	122,02811	9.04979 (ppm)
FWW PBR 2.3	CYS	2,25	2,14	-0.11	122,02701	122,028	8.11196 (ppm)
FWW PBR 3.1	CYS	2,25	2,47	0.22	122,02701	122,02802	8.23700 (ppm)
FWW PBR 3.2	CYS	2,25	2,15	-0.10	122,02701	N/A	N/A
FWW PBR 3.3	CYS	2,25	2,47	0.22	122,02701	122,02798	7.92439 (ppm)
Blank (UPW)	GLU	2,34	N/F	N/F	148,06038	N/F	N/F
Blank (UPW)	GLU	2,34	2,34	0.00	148,06038	148,06021	-1.14020 (ppm)
Control PBR 1.1	GLU	2,34	2,38	0.04	148,06038	148,06053	1.02402 (ppm)
Control PBR 1.2	GLU	2,34	2,38	0.04	148,06038	148,06035	-.21268 (ppm)
Control PBR 1.3	GLU	2,34	2,38	0.04	148,06038	148,06049	.71484 (ppm)
Control PBR 2.1	GLU	2,34	2,38	0.04	148,06038	148,06038	-.00656 (ppm)
Control PBR 2.2	GLU	2,34	2,37	0.03	148,06038	148,06036	-.10962 (ppm)
Control PBR 2.3	GLU	2,34	2,37	0.03	148,06038	148,06039	.09650 (ppm)
Control PBR 3.1	GLU	2,34	2,37	0.03	148,06038	148,06046	.50873 (ppm)
Control PBR 3.2	GLU	2,34	2,38	0.04	148,06038	148,06046	.50873 (ppm)
Control PBR 3.3	GLU	2,34	2,38	0.04	148,06038	148,06044	.40567 (ppm)
Reagent Blank 1	GLU	2,34	2,36	0.02	148,06038	148,0602	-1.24326 (ppm)
Reagent Blank 2	GLU	2,34	2,38	0.04	148,06038	148,06046	.50873 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Reagent Blank 3	GLU	2,34	2,39	0.05	148,06038	148,0605	.81790 (ppm)
FWW PBR 1.1	GLU	2,34	2,37	0.03	148,06038	148,06018	-1.34632 (ppm)
FWW PBR 1.2	GLU	2,34	2,38	0.04	148,06038	148,06027	-.72797 (ppm)
FWW PBR 1.3	GLU	2,34	2,38	0.04	148,06038	148,06047	.61178 (ppm)
FWW PBR 2.1	GLU	2,34	2,38	0.04	148,06038	148,06047	.61178 (ppm)
FWW PBR 2.2	GLU	2,34	2,38	0.04	148,06038	148,06053	1.02402 (ppm)
FWW PBR 2.3	GLU	2,34	2,37	0.03	148,06038	148,06046	.50873 (ppm)
FWW PBR 3.1	GLU	2,34	2,38	0.04	148,06038	148,06042	.30261 (ppm)
FWW PBR 3.2	GLU	2,34	2,38	0.04	148,06038	148,06059	1.43625 (ppm)
FWW PBR 3.3	GLU	2,34	2,38	0.04	148,06038	148,06047	.61178 (ppm)
Blank (UPW)	GLY	2,53	2,75	0.22	76,03927	N/A	N/A
Blank (UPW)	GLY	2,53	2,53	0.00	76,03927	N/A	N/A
Control PBR 1.1	GLY	2,53	2,6	0.07	76,03927	N/A	N/A
Control PBR 1.2	GLY	2,53	2,58	0.05	76,03927	76,03941	1.78622 (ppm)
Control PBR 1.3	GLY	2,53	2,66	0.13	76,03927	76,03945	2.38823 (ppm)
Control PBR 2.1	GLY	2,53	2,56	0.03	76,03927	76,03949	2.88990 (ppm)
Control PBR 2.2	GLY	2,53	2,56	0.03	76,03927	76,03936	1.18421 (ppm)
Control PBR 2.3	GLY	2,53	2,57	0.04	76,03927	76,03944	2.28789 (ppm)
Control PBR 3.1	GLY	2,53	2,68	0.15	76,03927	76,03941	1.88655 (ppm)
Control PBR 3.2	GLY	2,53	2,65	0.12	76,03927	76,0394	1.68588 (ppm)
Control PBR 3.3	GLY	2,53	2,53	0.00	76,03927	76,03941	1.78622 (ppm)
Reagent Blank 1	GLY	2,53	2,55	0.02	76,03927	76,03938	1.48521 (ppm)
Reagent Blank 2	GLY	2,53	2,6	0.07	76,03927	76,03933	.78287 (ppm)
Reagent Blank 3	GLY	2,53	2,59	0.06	76,03927	76,03932	.68253 (ppm)
FWW PBR 1.1	GLY	2,53	2,57	0.04	76,03927	76,03926	-.12015 (ppm)
FWW PBR 1.2	GLY	2,53	2,55	0.02	76,03927	76,03941	1.78622 (ppm)
FWW PBR 1.3	GLY	2,53	2,56	0.03	76,03927	76,03938	1.48521 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
FWW PBR 2.1	GLY	2,53	2,62	0.09	76,03927	76,03941	1.88655 (ppm)
FWW PBR 2.2	GLY	2,53	2,55	0.02	76,03927	76,03941	1.88655 (ppm)
FWW PBR 2.3	GLY	2,53	2,53	0.00	76,03927	76,03927	-.01981 (ppm)
FWW PBR 3.1	GLY	2,53	2,56	0.03	76,03927	76,03943	2.08722 (ppm)
FWW PBR 3.2	GLY	2,53	2,56	0.03	76,03927	76,03951	3.09057 (ppm)
FWW PBR 3.3	GLY	2,53	2,55	0.02	76,03927	76,03945	2.38823 (ppm)
Blank (UPW)	HIS	4,75	4,73	-0.02	156,07664	N/A	N/A
Blank (UPW)	HIS	4,75	4,94	0.19	156,07664	156,07635	-1.82615 (ppm)
Control PBR 1.1	HIS	4,75	4,79	0.04	156,07664	156,07645	-1.23956 (ppm)
Control PBR 1.2	HIS	4,75	4,79	0.04	156,07664	156,07675	.71573 (ppm)
Control PBR 1.3	HIS	4,75	4,78	0.03	156,07664	156,07686	1.40008 (ppm)
Control PBR 2.1	HIS	4,75	4,77	0.02	156,07664	156,07686	1.40008 (ppm)
Control PBR 2.2	HIS	4,75	4,77	0.02	156,07664	156,07689	1.59561 (ppm)
Control PBR 2.3	HIS	4,75	4,77	0.02	156,07664	156,07675	.71573 (ppm)
Control PBR 3.1	HIS	4,75	4,79	0.04	156,07664	156,07651	-.84850 (ppm)
Control PBR 3.2	HIS	4,75	4,78	0.03	156,07664	156,07675	.71573 (ppm)
Control PBR 3.3	HIS	4,75	4,79	0.04	156,07664	156,07668	.22691 (ppm)
Reagent Blank 1	HIS	4,75	N/F	N/F	156,07664	N/F	N/F
Reagent Blank 2	HIS	4,75	N/F	N/F	156,07664	N/F	N/F
Reagent Blank 3	HIS	4,75	N/F	N/F	156,07664	N/F	N/F
FWW PBR 1.1	HIS	4,75	4,79	0.04	156,07664	156,07658	-.35968 (ppm)
FWW PBR 1.2	HIS	4,75	4,78	0.03	156,07664	156,0769	1.69338 (ppm)
FWW PBR 1.3	HIS	4,75	4,8	0.05	156,07664	156,0769	1.69338 (ppm)
FWW PBR 2.1	HIS	4,75	4,77	0.02	156,07664	156,07689	1.59561 (ppm)
FWW PBR 2.2	HIS	4,75	4,78	0.03	156,07664	156,07695	1.98667 (ppm)
FWW PBR 2.3	HIS	4,75	4,79	0.04	156,07664	156,07684	1.30232 (ppm)
FWW PBR 3.1	HIS	4,75	4,78	0.03	156,07664	156,07695	1.98667 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
FWW PBR 3.2	HIS	4,75	4,79	0.04	156,07664	156,07706	2.67103 (ppm)
FWW PBR 3.3	HIS	4,75	4,78	0.03	156,07664	156,07692	1.79114 (ppm)
Blank (UPW)	ILE	3,23	3,39	0.16	132,10178	132,1019	.89471 (ppm)
Blank (UPW)	ILE	3,23	3,24	0.01	132,10178	132,10194	1.24124 (ppm)
Control PBR 1.1	ILE	3,23	3,24	0.01	132,10178	132,1021	2.39632 (ppm)
Control PBR 1.2	ILE	3,23	3,25	0.02	132,10178	132,10208	2.28081 (ppm)
Control PBR 1.3	ILE	3,23	3,25	0.02	132,10178	132,10217	2.97386 (ppm)
Control PBR 2.1	ILE	3,23	3,24	0.01	132,10178	132,1022	3.20487 (ppm)
Control PBR 2.2	ILE	3,23	3,24	0.01	132,10178	132,10222	3.32038 (ppm)
Control PBR 2.3	ILE	3,23	3,23	0.00	132,10178	132,10216	2.85835 (ppm)
Control PBR 3.1	ILE	3,23	3,22	-0.01	132,10178	132,1021	2.39632 (ppm)
Control PBR 3.2	ILE	3,23	3,23	0.00	132,10178	132,1021	2.39632 (ppm)
Control PBR 3.3	ILE	3,23	3,24	0.01	132,10178	132,10216	2.85835 (ppm)
Reagent Blank 1	ILE	3,23	3,25	0.02	132,10178	132,10196	1.35675 (ppm)
Reagent Blank 2	ILE	3,23	3,25	0.02	132,10178	132,10199	1.58776 (ppm)
Reagent Blank 3	ILE	3,23	3,27	0.04	132,10178	132,10204	1.93428 (ppm)
FWW PBR 1.1	ILE	3,23	3,25	0.02	132,10178	132,10196	1.35675 (ppm)
FWW PBR 1.2	ILE	3,23	3,25	0.02	132,10178	132,10214	2.74284 (ppm)
FWW PBR 1.3	ILE	3,23	3,25	0.02	132,10178	132,10204	1.93428 (ppm)
FWW PBR 2.1	ILE	3,23	3,12	-0.11	132,10178	132,10208	2.28081 (ppm)
FWW PBR 2.2	ILE	3,23	3,24	0.01	132,10178	132,10205	2.04979 (ppm)
FWW PBR 2.3	ILE	3,23	3,24	0.01	132,10178	132,1021	2.39632 (ppm)
FWW PBR 3.1	ILE	3,23	3,25	0.02	132,10178	132,10211	2.51182 (ppm)
FWW PBR 3.2	ILE	3,23	3,24	0.01	132,10178	132,10214	2.74284 (ppm)
FWW PBR 3.3	ILE	3,23	3,25	0.02	132,10178	132,10208	2.28081 (ppm)
Blank (UPW)	LEU	3,25	3,39	0.14	132,10178	132,1019	.89471 (ppm)
Blank (UPW)	LEU	3,25	3,24	-0.01	132,10178	132,10194	1.24124 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Control PBR 1.1	LEU	3,25	3,24	-0.01	132,10178	132,1021	2.39632 (ppm)
Control PBR 1.2	LEU	3,25	3,25	0.00	132,10178	132,10208	2.28081 (ppm)
Control PBR 1.3	LEU	3,25	3,25	0.00	132,10178	132,10217	2.97386 (ppm)
Control PBR 2.1	LEU	3,25	3,24	-0.01	132,10178	132,1022	3.20487 (ppm)
Control PBR 2.2	LEU	3,25	3,24	-0.01	132,10178	132,10222	3.32038 (ppm)
Control PBR 2.3	LEU	3,25	3,23	-0.02	132,10178	132,10216	2.85835 (ppm)
Control PBR 3.1	LEU	3,25	3,22	-0.03	132,10178	132,1021	2.39632 (ppm)
Control PBR 3.2	LEU	3,25	3,23	-0.02	132,10178	132,1021	2.39632 (ppm)
Control PBR 3.3	LEU	3,25	3,24	-0.01	132,10178	132,10216	2.85835 (ppm)
Reagent Blank 1	LEU	3,25	3,25	0.00	132,10178	132,10196	1.35675 (ppm)
Reagent Blank 2	LEU	3,25	3,25	0.00	132,10178	132,10199	1.58776 (ppm)
Reagent Blank 3	LEU	3,25	3,27	0.02	132,10178	132,10204	1.93428 (ppm)
FWW PBR 1.1	LEU	3,25	3,25	0.00	132,10178	132,10196	1.35675 (ppm)
FWW PBR 1.2	LEU	3,25	3,25	0.00	132,10178	132,10214	2.74284 (ppm)
FWW PBR 1.3	LEU	3,25	3,25	0.00	132,10178	132,10204	1.93428 (ppm)
FWW PBR 2.1	LEU	3,25	3,24	-0.01	132,10178	132,10213	2.62733 (ppm)
FWW PBR 2.2	LEU	3,25	3,24	-0.01	132,10178	132,10205	2.04979 (ppm)
FWW PBR 2.3	LEU	3,25	3,24	-0.01	132,10178	132,1021	2.39632 (ppm)
FWW PBR 3.1	LEU	3,25	3,25	0.00	132,10178	132,10211	2.51182 (ppm)
FWW PBR 3.2	LEU	3,25	3,24	-0.01	132,10178	132,10214	2.74284 (ppm)
FWW PBR 3.3	LEU	3,25	3,25	0.00	132,10178	132,10208	2.28081 (ppm)
Blank (UPW)	LYS	5,59	N/F	N/F	147,11276	N/F	N/F
Blank (UPW)	LYS	5,59	N/F	N/F	147,11276	N/F	N/F
Control PBR 1.1	LYS	5,59	5,7	0.11	147,11276	N/A	N/A
Control PBR 1.2	LYS	5,59	5,7	0.11	147,11276	147,11292	1.05388 (ppm)
Control PBR 1.3	LYS	5,59	5,68	0.09	147,11276	147,11299	1.57249 (ppm)
Control PBR 2.1	LYS	5,59	5,69	0.10	147,11276	147,11295	1.26132 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Control PBR 2.2	LYS	5,59	5,69	0.10	147,11276	147,11293	1.15760 (ppm)
Control PBR 2.3	LYS	5,59	5,69	0.10	147,11276	147,11295	1.26132 (ppm)
Control PBR 3.1	LYS	5,59	5,67	0.08	147,11276	147,11284	.53527 (ppm)
Control PBR 3.2	LYS	5,59	5,7	0.11	147,11276	147,11272	-.29450 (ppm)
Control PBR 3.3	LYS	5,59	5,7	0.11	147,11276	147,11284	.53527 (ppm)
Reagent Blank 1	LYS	5,59	N/F	N/F	147,11276	N/F	N/F
Reagent Blank 2	LYS	5,59	N/F	N/F	147,11276	N/F	N/F
Reagent Blank 3	LYS	5,59	N/F	N/F	147,11276	N/F	N/F
FWW PBR 1.1	LYS	5,59	5,72	0.13	147,11276	147,11264	-.81311 (ppm)
FWW PBR 1.2	LYS	5,59	5,69	0.10	147,11276	147,11284	.53527 (ppm)
FWW PBR 1.3	LYS	5,59	5,7	0.11	147,11276	147,11295	1.26132 (ppm)
FWW PBR 2.1	LYS	5,59	5,7	0.11	147,11276	147,11296	1.36504 (ppm)
FWW PBR 2.2	LYS	5,59	5,68	0.09	147,11276	147,11287	.74271 (ppm)
FWW PBR 2.3	LYS	5,59	5,7	0.11	147,11276	147,11288	.84644 (ppm)
FWW PBR 3.1	LYS	5,59	5,71	0.12	147,11276	147,11301	1.67621 (ppm)
FWW PBR 3.2	LYS	5,59	5,68	0.09	147,11276	147,11301	1.67621 (ppm)
FWW PBR 3.3	LYS	5,59	5,69	0.10	147,11276	147,11293	1.15760 (ppm)
Blank (UPW)	MET	2,75	N/F	N/F	150,05821	N/F	N/F
Blank (UPW)	MET	2,75	2,78	0.03	150,05821	N/A	N/A
Control PBR 1.1	MET	2,75	2,75	0.00	150,05821	150,05858	2.45566 (ppm)
Control PBR 1.2	MET	2,75	2,75	0.00	150,05821	150,05858	2.45566 (ppm)
Control PBR 1.3	MET	2,75	2,74	-0.01	150,05821	150,05853	2.15060 (ppm)
Control PBR 2.1	MET	2,75	2,75	0.00	150,05821	150,05864	2.86240 (ppm)
Control PBR 2.2	MET	2,75	2,75	0.00	150,05821	150,05861	2.65903 (ppm)
Control PBR 2.3	MET	2,75	2,76	0.01	150,05821	150,05861	2.65903 (ppm)
Control PBR 3.1	MET	2,75	2,74	-0.01	150,05821	150,05852	2.04891 (ppm)
Control PBR 3.2	MET	2,75	2,75	0.00	150,05821	150,05864	2.86240 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Control PBR 3.3	MET	2,75	2,76	0.01	150,05821	150,05847	1.74385 (ppm)
Reagent Blank 1	MET	2,75	2,68	-0.07	150,05821	150,0571	-7.40787 (ppm)
Reagent Blank 2	MET	2,75	N/F	N/F	150,05821	N/F	N/F
Reagent Blank 3	MET	2,75	N/F	N/F	150,05821	N/F	N/F
FWW PBR 1.1	MET	2,75	2,75	0.00	150,05821	150,05829	.52362 (ppm)
FWW PBR 1.2	MET	2,75	2,75	0.00	150,05821	150,05833	.82868 (ppm)
FWW PBR 1.3	MET	2,75	2,75	0.00	150,05821	150,05852	2.04891 (ppm)
FWW PBR 2.1	MET	2,75	2,75	0.00	150,05821	150,05865	2.96408 (ppm)
FWW PBR 2.2	MET	2,75	2,74	-0.01	150,05821	150,05858	2.45566 (ppm)
FWW PBR 2.3	MET	2,75	2,75	0.00	150,05821	150,05847	1.74385 (ppm)
FWW PBR 3.1	MET	2,75	2,75	0.00	150,05821	150,05852	2.04891 (ppm)
FWW PBR 3.2	MET	2,75	2,75	0.00	150,05821	150,05856	2.35397 (ppm)
FWW PBR 3.3	MET	2,75	2,74	-0.01	150,05821	150,05859	2.55734 (ppm)
Blank (UPW)	PHE	3,36	3,56	0.20	166,08618	166,08624	.37737 (ppm)
Blank (UPW)	PHE	3,36	3,58	0.22	166,08618	166,08633	.92861 (ppm)
Control PBR 1.1	PHE	3,36	3,37	0.01	166,08618	166,08649	1.84733 (ppm)
Control PBR 1.2	PHE	3,36	3,37	0.01	166,08618	166,08653	2.12295 (ppm)
Control PBR 1.3	PHE	3,36	3,36	0.00	166,08618	166,08658	2.39857 (ppm)
Control PBR 2.1	PHE	3,36	3,36	0.00	166,08618	166,08664	2.76606 (ppm)
Control PBR 2.2	PHE	3,36	3,36	0.00	166,08618	166,08653	2.12295 (ppm)
Control PBR 2.3	PHE	3,36	3,36	0.00	166,08618	166,0863	.74486 (ppm)
Control PBR 3.1	PHE	3,36	3,36	0.00	166,08618	166,0867	3.13355 (ppm)
Control PBR 3.2	PHE	3,36	3,36	0.00	166,08618	166,08658	2.39857 (ppm)
Control PBR 3.3	PHE	3,36	3,36	0.00	166,08618	166,08646	1.66359 (ppm)
Reagent Blank 1	PHE	3,36	3,4	0.04	166,08618	166,08641	1.38797 (ppm)
Reagent Blank 2	PHE	3,36	3,36	0.00	166,08618	166,08675	3.40917 (ppm)
Reagent Blank 3	PHE	3,36	3,3	-0.06	166,08618	166,08667	2.94981 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
FWW PBR 1.1	PHE	3,36	3,34	-0.02	166,08618	166,0842	-11.93358 (ppm)
FWW PBR 1.2	PHE	3,36	3,35	-0.01	166,08618	166,0856	-3.48129 (ppm)
FWW PBR 1.3	PHE	3,36	3,37	0.01	166,08618	166,08615	-.17387 (ppm)
FWW PBR 2.1	PHE	3,36	3,33	-0.03	166,08618	166,08394	-13.49541 (ppm)
FWW PBR 2.2	PHE	3,36	3,35	-0.01	166,08618	166,08565	-3.20567 (ppm)
FWW PBR 2.3	PHE	3,36	3,36	0.00	166,08618	166,08594	-1.46009 (ppm)
FWW PBR 3.1	PHE	3,36	3,35	-0.01	166,08618	166,08609	-.54136 (ppm)
FWW PBR 3.2	PHE	3,36	3,37	0.01	166,08618	166,08638	1.20422 (ppm)
FWW PBR 3.3	PHE	3,36	3,36	0.00	166,08618	166,08629	.65299 (ppm)
Blank (UPW)	PRO	2,55	2,57	0.02	116,07035	116,07043	.72861 (ppm)
Blank (UPW)	PRO	2,55	2,48	-0.07	116,07035	116,0704	.46569 (ppm)
Control PBR 1.1	PRO	2,55	2,53	-0.02	116,07035	116,07077	3.62077 (ppm)
Control PBR 1.2	PRO	2,55	2,54	-0.01	116,07035	116,07079	3.75223 (ppm)
Control PBR 1.3	PRO	2,55	2,53	-0.02	116,07035	116,07076	3.55504 (ppm)
Control PBR 2.1	PRO	2,55	2,53	-0.02	116,07035	116,07079	3.75223 (ppm)
Control PBR 2.2	PRO	2,55	2,53	-0.02	116,07035	116,07073	3.29211 (ppm)
Control PBR 2.3	PRO	2,55	2,53	-0.02	116,07035	116,07075	3.42357 (ppm)
Control PBR 3.1	PRO	2,55	2,53	-0.02	116,07035	116,07083	4.14661 (ppm)
Control PBR 3.2	PRO	2,55	2,53	-0.02	116,07035	116,07088	4.60673 (ppm)
Control PBR 3.3	PRO	2,55	2,54	-0.01	116,07035	116,07083	4.14661 (ppm)
Reagent Blank 1	PRO	2,55	N/F	N/F	116,07035	N/F	N/F
Reagent Blank 2	PRO	2,55	N/F	N/F	116,07035	N/F	N/F
Reagent Blank 3	PRO	2,55	N/F	N/F	116,07035	N/F	N/F
FWW PBR 1.1	PRO	2,55	2,52	-0.03	116,07035	116,07058	1.97750 (ppm)
FWW PBR 1.2	PRO	2,55	2,53	-0.02	116,07035	116,07065	2.56907 (ppm)
FWW PBR 1.3	PRO	2,55	2,53	-0.02	116,07035	116,0708	3.88369 (ppm)
FWW PBR 2.1	PRO	2,55	2,53	-0.02	116,07035	116,07067	2.76627 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
FWW PBR 2.2	PRO	2,55	2,53	-0.02	116,07035	116,07085	4.27807 (ppm)
FWW PBR 2.3	PRO	2,55	2,53	-0.02	116,07035	116,07078	3.68650 (ppm)
FWW PBR 3.1	PRO	2,55	2,54	-0.01	116,07035	116,07079	3.81796 (ppm)
FWW PBR 3.2	PRO	2,55	2,54	-0.01	116,07035	116,07083	4.14661 (ppm)
FWW PBR 3.3	PRO	2,55	2,53	-0.02	116,07035	116,07079	3.75223 (ppm)
Blank (UPW)	SER	2,33	2,4	0.07	106,04984	106,0498	-.40492 (ppm)
Blank (UPW)	SER	2,33	2,44	0.11	106,04984	N/A	N/A
Control PBR 1.1	SER	2,33	2,36	0.03	106,04984	106,05002	1.68138 (ppm)
Control PBR 1.2	SER	2,33	2,37	0.04	106,04984	106,05005	1.96915 (ppm)
Control PBR 1.3	SER	2,33	2,38	0.05	106,04984	106,05004	1.89721 (ppm)
Control PBR 2.1	SER	2,33	2,38	0.05	106,04984	106,05005	1.96915 (ppm)
Control PBR 2.2	SER	2,33	2,38	0.05	106,04984	106,04997	1.24973 (ppm)
Control PBR 2.3	SER	2,33	2,36	0.03	106,04984	106,04995	1.03391 (ppm)
Control PBR 3.1	SER	2,33	2,37	0.04	106,04984	106,05005	1.96915 (ppm)
Control PBR 3.2	SER	2,33	2,37	0.04	106,04984	106,05	1.46556 (ppm)
Control PBR 3.3	SER	2,33	2,37	0.04	106,04984	106,05003	1.82527 (ppm)
Reagent Blank 1	SER	2,33	2,37	0.04	106,04984	106,04996	1.10585 (ppm)
Reagent Blank 2	SER	2,33	2,39	0.06	106,04984	106,04948	-3.42647 (ppm)
Reagent Blank 3	SER	2,33	2,37	0.04	106,04984	106,04991	.67420 (ppm)
FWW PBR 1.1	SER	2,33	2,37	0.04	106,04984	106,0498	-.40492 (ppm)
FWW PBR 1.2	SER	2,33	2,38	0.05	106,04984	106,04984	.02673 (ppm)
FWW PBR 1.3	SER	2,33	2,38	0.05	106,04984	106,04999	1.39362 (ppm)
FWW PBR 2.1	SER	2,33	2,35	0.02	106,04984	106,04965	-1.77181 (ppm)
FWW PBR 2.2	SER	2,33	2,37	0.04	106,04984	106,05008	2.25692 (ppm)
FWW PBR 2.3	SER	2,33	2,36	0.03	106,04984	106,05007	2.18498 (ppm)
FWW PBR 3.1	SER	2,33	2,38	0.05	106,04984	106,04984	-.04521 (ppm)
FWW PBR 3.2	SER	2,33	2,37	0.04	106,04984	106,04998	1.32168 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
FWW PBR 3.3	SER	2,33	2,37	0.04	106,04984	106,05	1.53750 (ppm)
Blank (UPW)	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Blank (UPW)	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Control PBR 1.1	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Control PBR 1.2	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Control PBR 1.3	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Control PBR 2.1	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Control PBR 2.2	TAU	1,85	1,82	-0.03	126,0219	126,02187	-.21049 (ppm)
Control PBR 2.3	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Control PBR 3.1	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Control PBR 3.2	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Control PBR 3.3	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Reagent Blank 1	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Reagent Blank 2	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Reagent Blank 3	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
FWW PBR 1.1	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
FWW PBR 1.2	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
FWW PBR 1.3	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
FWW PBR 2.1	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
FWW PBR 2.2	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
FWW PBR 2.3	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
FWW PBR 3.1	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
FWW PBR 3.2	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
FWW PBR 3.3	TAU	1,85	N/F	N/F	126,0219	N/F	N/F
Blank (UPW)	THR	2,37	2,37	0.00	120,06548	120,06548	-.03778 (ppm)
Blank (UPW)	THR	2,37	2,13	-0.24	120,06548	120,06539	-.73676 (ppm)
Control PBR 1.1	THR	2,37	2,41	0.04	120,06548	120,06571	1.93207 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Control PBR 1.2	THR	2,37	2,42	0.05	120,06548	120,06569	1.74144 (ppm)
Control PBR 1.3	THR	2,37	2,41	0.04	120,06548	120,06571	1.93207 (ppm)
Control PBR 2.1	THR	2,37	2,41	0.04	120,06548	120,06565	1.42372 (ppm)
Control PBR 2.2	THR	2,37	2,41	0.04	120,06548	120,06563	1.23309 (ppm)
Control PBR 2.3	THR	2,37	2,41	0.04	120,06548	120,06564	1.29664 (ppm)
Control PBR 3.1	THR	2,37	2,41	0.04	120,06548	120,06567	1.61435 (ppm)
Control PBR 3.2	THR	2,37	2,41	0.04	120,06548	120,0657	1.80498 (ppm)
Control PBR 3.3	THR	2,37	2,41	0.04	120,06548	120,06562	1.16955 (ppm)
Reagent Blank 1	THR	2,37	2,43	0.06	120,06548	N/A	N/A
Reagent Blank 2	THR	2,37	2,15	-0.22	120,06548	120,06557	.78829 (ppm)
Reagent Blank 3	THR	2,37	2,43	0.06	120,06548	120,06523	-2.07118 (ppm)
FWW PBR 1.1	THR	2,37	2,42	0.05	120,06548	120,06548	.02576 (ppm)
FWW PBR 1.2	THR	2,37	2,42	0.05	120,06548	120,06552	.34348 (ppm)
FWW PBR 1.3	THR	2,37	2,42	0.05	120,06548	120,06564	1.29664 (ppm)
FWW PBR 2.1	THR	2,37	2,44	0.07	120,06548	120,06553	.40702 (ppm)
FWW PBR 2.2	THR	2,37	2,42	0.05	120,06548	120,06569	1.74144 (ppm)
FWW PBR 2.3	THR	2,37	2,42	0.05	120,06548	120,06558	.85183 (ppm)
FWW PBR 3.1	THR	2,37	2,42	0.05	120,06548	120,06572	1.99562 (ppm)
FWW PBR 3.2	THR	2,37	2,42	0.05	120,06548	120,06568	1.67790 (ppm)
FWW PBR 3.3	THR	2,37	2,42	0.05	120,06548	120,06566	1.48727 (ppm)
Blank (UPW)	TRP	4,56	4,74	0.18	205,09692	N/A	N/A
Blank (UPW)	TRP	4,56	4,36	-0.20	205,09692	205,09669	-1.09730 (ppm)
Control PBR 1.1	TRP	4,56	4,61	0.05	205,09692	205,09746	2.62259 (ppm)
Control PBR 1.2	TRP	4,56	4,6	0.04	205,09692	205,09743	2.47380 (ppm)
Control PBR 1.3	TRP	4,56	4,6	0.04	205,09692	205,09735	2.10181 (ppm)
Control PBR 2.1	TRP	4,56	4,57	0.01	205,09692	205,09637	-2.65966 (ppm)
Control PBR 2.2	TRP	4,56	4,59	0.03	205,09692	205,09743	2.47380 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Control PBR 2.3	TRP	4,56	4,58	0.02	205,09692	205,09743	2.47380 (ppm)
Control PBR 3.1	TRP	4,56	4,59	0.03	205,09692	205,09746	2.62259 (ppm)
Control PBR 3.2	TRP	4,56	4,59	0.03	205,09692	205,09708	.76264 (ppm)
Control PBR 3.3	TRP	4,56	4,55	-0.01	205,09692	205,09744	2.54820 (ppm)
Reagent Blank 1	TRP	4,56	N/F	N/F	205,09692	N/F	N/F
Reagent Blank 2	TRP	4,56	4,62	0.06	205,09692	205,09494	-9.65307 (ppm)
Reagent Blank 3	TRP	4,56	4,58	0.02	205,09692	205,09605	-4.22202 (ppm)
FWW PBR 1.1	TRP	4,56	4,57	0.01	205,09692	205,09703	.53945 (ppm)
FWW PBR 1.2	TRP	4,56	4,6	0.04	205,09692	205,09703	.53945 (ppm)
FWW PBR 1.3	TRP	4,56	4,59	0.03	205,09692	205,09729	1.80422 (ppm)
FWW PBR 2.1	TRP	4,56	4,59	0.03	205,09692	205,09697	.24186 (ppm)
FWW PBR 2.2	TRP	4,56	4,58	0.02	205,09692	205,09737	2.17621 (ppm)
FWW PBR 2.3	TRP	4,56	4,58	0.02	205,09692	205,09711	.91144 (ppm)
FWW PBR 3.1	TRP	4,56	4,59	0.03	205,09692	205,09734	2.02741 (ppm)
FWW PBR 3.2	TRP	4,56	4,59	0.03	205,09692	205,09737	2.17621 (ppm)
FWW PBR 3.3	TRP	4,56	4,59	0.03	205,09692	205,09727	1.72982 (ppm)
Blank (UPW)	TYR	2,75	2,77	0.02	182,08107	182,08115	.41872 (ppm)
Blank (UPW)	TYR	2,75	2,76	0.01	182,08107	182,07996	-6.11785 (ppm)
Control PBR 1.1	TYR	2,75	2,75	0.00	182,08107	182,08156	2.68137 (ppm)
Control PBR 1.2	TYR	2,75	2,76	0.01	182,08107	182,0815	2.34617 (ppm)
Control PBR 1.3	TYR	2,75	2,75	0.00	182,08107	182,08144	2.01096 (ppm)
Control PBR 2.1	TYR	2,75	2,76	0.01	182,08107	182,08148	2.26236 (ppm)
Control PBR 2.2	TYR	2,75	2,76	0.01	182,08107	182,08139	1.75955 (ppm)
Control PBR 2.3	TYR	2,75	2,75	0.00	182,08107	182,08138	1.67575 (ppm)
Control PBR 3.1	TYR	2,75	2,76	0.01	182,08107	182,08151	2.42997 (ppm)
Control PBR 3.2	TYR	2,75	2,75	0.00	182,08107	182,08145	2.09476 (ppm)
Control PBR 3.3	TYR	2,75	2,76	0.01	182,08107	182,08141	1.84335 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Reagent Blank 1	TYR	2,75	2,75	0.00	182,08107	182,08139	1.75955 (ppm)
Reagent Blank 2	TYR	2,75	2,91	0.16	182,08107	N/A	N/A
Reagent Blank 3	TYR	2,75	2,75	0.00	182,08107	182,08121	.75392 (ppm)
FWW PBR 1.1	TYR	2,75	2,76	0.01	182,08107	182,08112	.25111 (ppm)
FWW PBR 1.2	TYR	2,75	2,75	0.00	182,08107	182,08116	.50252 (ppm)
FWW PBR 1.3	TYR	2,75	2,77	0.02	182,08107	182,08144	2.01096 (ppm)
FWW PBR 2.1	TYR	2,75	2,75	0.00	182,08107	182,0815	2.34617 (ppm)
FWW PBR 2.2	TYR	2,75	2,76	0.01	182,08107	182,08144	2.01096 (ppm)
FWW PBR 2.3	TYR	2,75	2,75	0.00	182,08107	182,08141	1.84335 (ppm)
FWW PBR 3.1	TYR	2,75	2,76	0.01	182,08107	182,08139	1.75955 (ppm)
FWW PBR 3.2	TYR	2,75	2,75	0.00	182,08107	182,08147	2.17856 (ppm)
FWW PBR 3.3	TYR	2,75	2,75	0.00	182,08107	182,08147	2.17856 (ppm)
Blank (UPW)	VAL	2,84	2,87	0.03	118,08613	118,08618	.43731 (ppm)
Blank (UPW)	VAL	2,84	2,69	-0.15	118,08613	118,08617	.37270 (ppm)
Control PBR 1.1	VAL	2,84	2,8	-0.04	118,08613	118,0864	2.24636 (ppm)
Control PBR 1.2	VAL	2,84	2,8	-0.04	118,08613	118,08638	2.11714 (ppm)
Control PBR 1.3	VAL	2,84	2,81	-0.03	118,08613	118,08643	2.56940 (ppm)
Control PBR 2.1	VAL	2,84	2,82	-0.02	118,08613	118,0864	2.31097 (ppm)
Control PBR 2.2	VAL	2,84	2,8	-0.04	118,08613	118,08633	1.72949 (ppm)
Control PBR 2.3	VAL	2,84	2,81	-0.03	118,08613	118,08638	2.11714 (ppm)
Control PBR 3.1	VAL	2,84	2,8	-0.04	118,08613	118,08635	1.85871 (ppm)
Control PBR 3.2	VAL	2,84	2,8	-0.04	118,08613	118,08635	1.85871 (ppm)
Control PBR 3.3	VAL	2,84	2,79	-0.05	118,08613	118,08637	2.05253 (ppm)
Reagent Blank 1	VAL	2,84	2,75	-0.09	118,08613	118,08621	.69575 (ppm)
Reagent Blank 2	VAL	2,84	2,78	-0.06	118,08613	118,08617	.30810 (ppm)
Reagent Blank 3	VAL	2,84	2,74	-0.10	118,08613	118,08619	.50192 (ppm)
FWW PBR 1.1	VAL	2,84	2,8	-0.04	118,08613	118,08596	-1.43634 (ppm)

Sample Name	Amino Acid	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
FWW PBR 1.2	VAL	2,84	2,79	-0.05	118,08613	118,08594	-1.63017 (ppm)
FWW PBR 1.3	VAL	2,84	2,79	-0.05	118,08613	118,08622	.76036 (ppm)
FWW PBR 2.1	VAL	2,84	2,79	-0.05	118,08613	118,08617	.30810 (ppm)
FWW PBR 2.2	VAL	2,84	2,79	-0.05	118,08613	118,08618	.43731 (ppm)
FWW PBR 2.3	VAL	2,84	2,79	-0.05	118,08613	118,08618	.43731 (ppm)
FWW PBR 3.1	VAL	2,84	2,79	-0.05	118,08613	118,08615	.17888 (ppm)
FWW PBR 3.2	VAL	2,84	2,79	-0.05	118,08613	118,08618	.43731 (ppm)
FWW PBR 3.3	VAL	2,84	2,79	-0.05	118,08613	118,0862	.56653 (ppm)

Table D.2. MS ion inclusion list for amino acids analysis.

Mass [m/z]	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	(N)CE type	Comment
126,02190	M+	1	Positive	1,60	2,10	25	NCE	N=Tau; A=M+; T=XIC
76,03927	M+	1	Positive	2,28	2,78	25	NCE	N=Gly; A=M+; T=XIC
78,03970	M+	1	Positive	2,25	2,75	25	NCE	N=GlyIS; A=M+; T=XIC
90,05490	M+	1	Positive	2,33	2,83	25	NCE	N=Ala; A=M+; T=XIC
94,08003	M+	1	Positive	2,33	2,83	25	NCE	N=AlaIS; A=M+; T=XIC
106,04984	M+	1	Positive	2,08	2,58	25	NCE	N=Ser; A=M+; T=XIC
116,07035	M+	1	Positive	2,30	2,80	25	NCE	N=Pro; A=M+; T=XIC
118,08613	M+	1	Positive	2,59	3,09	25	NCE	N=Val; A=M+; T=XIC
120,06548	M+	1	Positive	2,12	2,62	25	NCE	N=Thr; A=M+; T=XIC
122,02701	M+	1	Positive	2,00	2,50	25	NCE	N=Cys; A=M+; T=XIC

Mass [m/z]	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	(N)CE type	Comment
126,13644	M+	1	Positive	2,59	3,09	25	NCE	N=ValIS; A=M+; T=XIC
132,10178	M+	1	Positive	2,98	3,48	25	NCE	N=Ile; A=M+; T=XIC
132,10178	M+	1	Positive	3,21	3,29	25	NCE	N=Leu; A=M+; T=XIC
134,04466	M+	1	Positive	1,95	2,45	25	NCE	N=Asp; A=M+; T=XIC
135,12065	M+	1	Positive	2,77	3,77	25	NCE	N=LeuIS; A=M+; T=XIC
137,06361	M+	1	Positive	1,93	2,43	25	NCE	N=AspIS; A=M+; T=XIC
147,11276	M+	1	Positive	5,09	6,09	25	NCE	N=Lys; A=M+; T=XIC
148,06038	M+	1	Positive	2,09	2,59	25	NCE	N=Glu; A=M+; T=XIC
150,05821	M+	1	Positive	2,50	3,00	25	NCE	N=Met; A=M+; T=XIC
151,07938	M+	1	Positive	2,10	2,60	25	NCE	N=GluIS; A=M+; T=XIC
153,07706	M+	1	Positive	2,50	3,00	25	NCE	N=MetIS; A=M+; T=XIC
156,07664	M+	1	Positive	4,50	5,00	25	NCE	N=His; A=M+; T=XIC
166,08618	M+	1	Positive	3,11	3,61	25	NCE	N=Phe; A=M+; T=XIC
172,10626	M+	1	Positive	3,11	3,61	25	NCE	N=PheIS; A=M+; T=XIC
175,11885	M+	1	Positive	5,53	6,03	25	NCE	N=Arg; A=M+; T=XIC
176,10167	M+	1	Positive	2,30	2,80	25	NCE	N=Cit; A=M+; T=XIC
178,11542	M+	1	Positive	2,25	2,75	25	NCE	N=CitIS; A=M+; T=XIC

Mass [m/z]	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	(N)CE type	Comment
180,14630	M+	1	Positive	5,39	5,89	25	NCE	N=ArgIS; A=M+; T=XIC
182,08107	M+	1	Positive	2,50	3,00	25	NCE	N=Tyr; A=M+; T=XIC
188,10112	M+	1	Positive	2,50	3,00	25	NCE	N=TyrIS; A=M+; T=XIC
205,09692	M+	1	Positive	4,31	4,81	25	NCE	N=Trp; A=M+; T=XIC

APPENDIX E

Table E.1. LC-MS data of vitamin analysis.

Sample Name	Vitamin	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Blank (ACN)	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Blank (ACN)	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Blank (ACN)	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Control PBR 1.1	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Control PBR 1.2	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Control PBR 1.3	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Control PBR 2.1	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Control PBR 2.2	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Control PBR 2.3	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Control PBR 3.1	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Control PBR 3.2	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Control PBR 3.3	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Reagent Blank 1	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Reagent Blank 2	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
Reagent Blank 3	Biotin	5,77	N/F	N/F	227,08459	N/F	N/F
FWW PBR 1.1	Biotin	5,77	5,77	-0.01	227,08459	227,0849	1.34389 (ppm)
FWW PBR 1.2	Biotin	5,77	5,76	-0.01	227,08459	227,0849 9	1.74705 (ppm)
FWW PBR 1.3	Biotin	5,77	5,75	-0.02	227,08459	227,0849 5	1.54547 (ppm)
FWW PBR 2.1	Biotin	5,77	5,75	-0.02	227,08459	227,0849 9	1.74705 (ppm)
FWW PBR 2.2	Biotin	5,77	5,75	-0.02	227,08459	227,0849 6	1.61266 (ppm)
FWW PBR 2.3	Biotin	5,77	5,75	-0.03	227,08459	227,0849 6	1.61266 (ppm)
FWW PBR 3.1	Biotin	5,77	5,75	-0.03	227,08459	227,0848 5	1.14230 (ppm)
FWW PBR 3.2	Biotin	5,77	5,75	-0.02	227,08459	227,0849 9	1.74705 (ppm)
FWW PBR 3.3	Biotin	5,77	5,75	-0.03	227,08459	227,0848 5	1.14230 (ppm)

Sample Name	Vitamin	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Blank (ACN)	Pantothenic acid	3,95	N/F	N/F	90,05491	N/F	N/F
Blank (ACN)	Pantothenic acid	3,95	N/F	N/F	90,05491	N/F	N/F
Blank (ACN)	Pantothenic acid	3,95	N/F	N/F	90,05491	N/F	N/F
Control PBR 1.1	Pantothenic acid	3,95	3,96	0.01	90,05491	90,05502	1.18607 (ppm)
Control PBR 1.2	Pantothenic acid	3,95	3,97	0.01	90,05491	90,05501	1.10135 (ppm)
Control PBR 1.3	Pantothenic acid	3,95	3,98	0.02	90,05491	90,05495	.50832 (ppm)
Control PBR 2.1	Pantothenic acid	3,95	3,97	0.01	90,05491	90,05496	.59304 (ppm)
Control PBR 2.2	Pantothenic acid	3,95	3,95	-0.01	90,05491	90,05498	.76247 (ppm)
Control PBR 2.3	Pantothenic acid	3,95	3,93	-0.02	90,05491	90,05495	.42360 (ppm)
Control PBR 3.1	Pantothenic acid	3,95	3,92	-0.03	90,05491	90,05496	.59304 (ppm)
Control PBR 3.2	Pantothenic acid	3,95	3,93	-0.03	90,05491	90,05495	.42360 (ppm)
Control PBR 3.3	Pantothenic acid	3,95	3,92	-0.03	90,05491	90,05495	.50832 (ppm)
Reagent Blank 1	Pantothenic acid	3,95	N/F	N/F	90,05491	N/F	N/F
Reagent Blank 2	Pantothenic acid	3,95	N/F	N/F	90,05491	N/F	N/F
Reagent Blank 3	Pantothenic acid	3,95	N/F	N/F	90,05491	N/F	N/F
FWW PBR 1.1	Pantothenic acid	3,95	3,99	0.04	90,05491	90,05497	.67775 (ppm)
FWW PBR 1.2	Pantothenic acid	3,95	4	0.04	90,05491	90,05496	.59304 (ppm)
FWW PBR 1.3	Pantothenic acid	3,95	3,99	0.04	90,05491	90,05499	.93191 (ppm)
FWW PBR 2.1	Pantothenic acid	3,95	3,99	0.03	90,05491	90,05502	1.18607 (ppm)
FWW PBR 2.2	Pantothenic acid	3,95	3,98	0.03	90,05491	90,05499	.93191 (ppm)
FWW PBR 2.3	Pantothenic acid	3,95	3,98	0.02	90,05491	90,05499	.93191 (ppm)
FWW PBR 3.1	Pantothenic acid	3,95	3,98	0.02	90,05491	90,05495	.50832 (ppm)
FWW PBR 3.2	Pantothenic acid	3,95	3,97	0.01	90,05491	90,05492	.16944 (ppm)
FWW PBR 3.3	Pantothenic acid	3,95	3,97	0.02	90,05491	90,05498	.76247 (ppm)
Blank (ACN)	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Blank (ACN)	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Blank (ACN)	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F

Sample Name	Vitamin	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Control PBR 1.1	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Control PBR 1.2	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Control PBR 1.3	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Control PBR 2.1	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Control PBR 2.2	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Control PBR 2.3	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Control PBR 3.1	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Control PBR 3.2	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Control PBR 3.3	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Reagent Blank 1	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Reagent Blank 2	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Reagent Blank 3	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
FWW PBR 1.1	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
FWW PBR 1.2	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
FWW PBR 1.3	Folic acid	5,16	5,37	0.21	295,09363	295,09036	-11.06558 (ppm)
FWW PBR 2.1	Folic acid	5,16	5,14	-0.02	295,09363	295,09222	-4.75716 (ppm)
FWW PBR 2.2	Folic acid	5,16	5,14	-0.02	295,09363	295,09305	-1.96492 (ppm)
FWW PBR 2.3	Folic acid	5,16	5,15	0.00	295,09363	295,09363	.00000 (ppm)
FWW PBR 3.1	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
FWW PBR 3.2	Folic acid	5,16	5,15	0.00	295,09363	295,09259	-3.51616 (ppm)
FWW PBR 3.3	Folic acid	5,16	N/F	N/F	295,09363	N/F	N/F
Blank (ACN)	Pyridoxine	0,8	0,84	0.04	152,0705	N/A	N/A
Blank (ACN)	Pyridoxine	0,8	0,82	0.01	152,0705	152,07066	1.10374 (ppm)
Blank (ACN)	Pyridoxine	0,8	0,81	0.01	152,0705	152,0705	.00000 (ppm)
Control PBR 1.1	Pyridoxine	0,8	0,87	0.07	152,0705	152,07068	1.20408 (ppm)
Control PBR 1.2	Pyridoxine	0,8	0,87	0.07	152,0705	152,07069	1.30442 (ppm)
Control PBR 1.3	Pyridoxine	0,8	0,86	0.06	152,0705	152,07056	.40136 (ppm)

Sample Name	Vitamin	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Control PBR 2.1	Pyridoxine	0,8	0,82	0.02	152,0705	152,07034	-1.00340 (ppm)
Control PBR 2.2	Pyridoxine	0,8	0,84	0.04	152,0705	152,07072	1.50510 (ppm)
Control PBR 2.3	Pyridoxine	0,8	0,84	0.04	152,0705	152,07076	1.70578 (ppm)
Control PBR 3.1	Pyridoxine	0,8	0,9	0.10	152,0705	152,0705	.00000 (ppm)
Control PBR 3.2	Pyridoxine	0,8	0,88	0.08	152,0705	152,07066	1.10374 (ppm)
Control PBR 3.3	Pyridoxine	0,8	0,87	0.07	152,0705	152,07057	.50170 (ppm)
Reagent Blank 1	Pyridoxine	0,8	N/F	N/F	152,0705	N/F	N/F
Reagent Blank 2	Pyridoxine	0,8	N/F	N/F	152,0705	N/F	N/F
Reagent Blank 3	Pyridoxine	0,8	0,89	0.09	152,0705	N/A	N/A
FWW PBR 1.1	Pyridoxine	0,8	0,89	0.08	152,0705	152,07066	1.10374 (ppm)
FWW PBR 1.2	Pyridoxine	0,8	0,89	0.08	152,0705	152,07051	.10034 (ppm)
FWW PBR 1.3	Pyridoxine	0,8	0,89	0.08	152,0705	152,07072	1.50510 (ppm)
FWW PBR 2.1	Pyridoxine	0,8	0,89	0.09	152,0705	152,0706	.70238 (ppm)
FWW PBR 2.2	Pyridoxine	0,8	0,88	0.08	152,0705	152,07057	.50170 (ppm)
FWW PBR 2.3	Pyridoxine	0,8	0,89	0.08	152,0705	152,07053	.20068 (ppm)
FWW PBR 3.1	Pyridoxine	0,8	0,88	0.08	152,0705	152,07071	1.40476 (ppm)
FWW PBR 3.2	Pyridoxine	0,8	0,89	0.08	152,0705	152,07074	1.60544 (ppm)
FWW PBR 3.3	Pyridoxine	0,8	0,88	0.08	152,0705	152,07074	1.60544 (ppm)
Blank (ACN)	Riboflavin	5,98	N/F	N/F	243,08743	N/F	N/F
Blank (ACN)	Riboflavin	5,98	6,01	0.03	243,08743	243,08788	1.82035 (ppm)
Blank (ACN)	Riboflavin	5,98	N/F	N/F	243,08743	N/F	N/F
Control PBR 1.1	Riboflavin	5,98	5,96	-0.02	243,08743	243,08768	1.00433 (ppm)
Control PBR 1.2	Riboflavin	5,98	5,97	-0.01	243,08743	243,08769	1.06710 (ppm)
Control PBR 1.3	Riboflavin	5,98	5,97	-0.01	243,08743	243,08783	1.63204 (ppm)
Control PBR 2.1	Riboflavin	5,98	5,97	-0.01	243,08743	243,08763	.81602 (ppm)
Control PBR 2.2	Riboflavin	5,98	5,96	-0.02	243,08743	243,08757	.56494 (ppm)
Control PBR 2.3	Riboflavin	5,98	5,95	-0.03	243,08743	243,08771	1.12987 (ppm)

Sample Name	Vitamin	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Control PBR 3.1	Riboflavin	5,98	5,96	-0.02	243,08743	243,08755	.50217 (ppm)
Control PBR 3.2	Riboflavin	5,98	5,96	-0.02	243,08743	243,08762	.75325 (ppm)
Control PBR 3.3	Riboflavin	5,98	5,95	-0.03	243,08743	243,08754	.43940 (ppm)
Reagent Blank 1	Riboflavin	5,98	6	0.02	243,08743	N/A	N/A
Reagent Blank 2	Riboflavin	5,98	N/F	N/F	243,08743	N/F	N/F
Reagent Blank 3	Riboflavin	5,98	N/F	N/F	243,08743	N/F	N/F
FWW PBR 1.1	Riboflavin	5,98	5,98	0.00	243,08743	243,08774	1.25542 (ppm)
FWW PBR 1.2	Riboflavin	5,98	5,98	0.00	243,08743	243,08771	1.12987 (ppm)
FWW PBR 1.3	Riboflavin	5,98	5,97	-0.01	243,08743	243,08783	1.63204 (ppm)
FWW PBR 2.1	Riboflavin	5,98	5,97	-0.01	243,08743	243,08775	1.31819 (ppm)
FWW PBR 2.2	Riboflavin	5,98	5,97	-0.01	243,08743	243,08775	1.31819 (ppm)
FWW PBR 2.3	Riboflavin	5,98	5,97	-0.01	243,08743	243,08772	1.19264 (ppm)
FWW PBR 3.1	Riboflavin	5,98	5,97	-0.01	243,08743	243,08771	1.12987 (ppm)
FWW PBR 3.2	Riboflavin	5,98	5,97	-0.01	243,08743	243,08763	.81602 (ppm)
FWW PBR 3.3	Riboflavin	5,98	5,97	-0.01	243,08743	243,08754	.43940 (ppm)
Blank (ACN)	Thiamine	0,75	N/F	N/F	122,07124	N/F	N/F
Blank (ACN)	Thiamine	0,75	N/F	N/F	122,07124	N/F	N/F
Blank (ACN)	Thiamine	0,75	N/F	N/F	122,07124	N/F	N/F
Control PBR 1.1	Thiamine	0,75	0,85	0.10	122,07124	122,07135	.93749 (ppm)
Control PBR 1.2	Thiamine	0,75	0,85	0.10	122,07124	122,07131	.62500 (ppm)
Control PBR 1.3	Thiamine	0,75	0,82	0.07	122,07124	122,07117	-.50000 (ppm)
Control PBR 2.1	Thiamine	0,75	0,82	0.07	122,07124	122,07124	.00000 (ppm)
Control PBR 2.2	Thiamine	0,75	0,85	0.10	122,07124	122,07124	.00000 (ppm)
Control PBR 2.3	Thiamine	0,75	0,87	0.12	122,07124	122,07144	1.68749 (ppm)
Control PBR 3.1	Thiamine	0,75	0,89	0.14	122,07124	122,07109	-1.18749 (ppm)
Control PBR 3.2	Thiamine	0,75	0,92	0.17	122,07124	122,07123	-.06250 (ppm)
Control PBR 3.3	Thiamine	0,75	0,91	0.16	122,07124	122,07133	.74999 (ppm)

Sample Name	Vitamin	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
Reagent Blank 1	Thiamine	0,75	N/F	N/F	122,07124	N/F	N/F
Reagent Blank 2	Thiamine	0,75	N/F	N/F	122,07124	N/F	N/F
Reagent Blank 3	Thiamine	0,75	N/F	N/F	122,07124	N/F	N/F
FWW PBR 1.1	Thiamine	0,75	0,78	0.03	122,07124	122,0712 2	-.12500 (ppm)
FWW PBR 1.2	Thiamine	0,75	0,78	0.03	122,07124	122,0712 6	.18750 (ppm)
FWW PBR 1.3	Thiamine	0,75	0,78	0.03	122,07124	122,0713 3	.81249 (ppm)
FWW PBR 2.1	Thiamine	0,75	0,79	0.04	122,07124	122,0713	.50000 (ppm)
FWW PBR 2.2	Thiamine	0,75	0,79	0.04	122,07124	122,0713 7	1.06249 (ppm)
FWW PBR 2.3	Thiamine	0,75	0,8	0.05	122,07124	122,0713 1	.62500 (ppm)
FWW PBR 3.1	Thiamine	0,75	0,79	0.04	122,07124	122,0712 7	.25000 (ppm)
FWW PBR 3.2	Thiamine	0,75	0,82	0.07	122,07124	122,0713 3	.74999 (ppm)
FWW PBR 3.3	Thiamine	0,75	0,8	0.05	122,07124	122,0713 6	.99999 (ppm)
Blank (ACN)	Nicotinic acid	1,04	0,8	-0.24	124,03929	124,0393 8	.73809 (ppm)
Blank (ACN)	Nicotinic acid	1,04	0,81	-0.23	124,03929	124,0394 7	1.41468 (ppm)
Blank (ACN)	Nicotinic acid	1,04	0,8	-0.24	124,03929	124,0393 4	.43056 (ppm)
Control PBR 1.1	Nicotinic acid	1,04	0,88	-0.16	124,03929	124,0394 1	.98413 (ppm)
Control PBR 1.2	Nicotinic acid	1,04	0,9	-0.14	124,03929	124,0393 4	.43056 (ppm)
Control PBR 1.3	Nicotinic acid	1,04	0,9	-0.14	124,03929	124,0393 4	.43056 (ppm)
Control PBR 2.1	Nicotinic acid	1,04	0,9	-0.14	124,03929	124,0393 1	.18452 (ppm)
Control PBR 2.2	Nicotinic acid	1,04	0,91	-0.13	124,03929	124,0393 3	.30754 (ppm)
Control PBR 2.3	Nicotinic acid	1,04	0,92	-0.12	124,03929	124,0393 4	.36905 (ppm)
Control PBR 3.1	Nicotinic acid	1,04	0,9	-0.14	124,03929	124,0392 5	-.36905 (ppm)
Control PBR 3.2	Nicotinic acid	1,04	0,9	-0.14	124,03929	124,0393 3	.30754 (ppm)
Control PBR 3.3	Nicotinic acid	1,04	0,89	-0.15	124,03929	124,0392 9	.00000 (ppm)
Reagent Blank 1	Nicotinic acid	1,04	0,88	-0.16	124,03929	124,0393 6	.55357 (ppm)
Reagent Blank 2	Nicotinic acid	1,04	0,88	-0.16	124,03929	124,0393 9	.79960 (ppm)
Reagent Blank 3	Nicotinic acid	1,04	0,88	-0.16	124,03929	124,0394	.86111 (ppm)

Sample Name	Vitamin	RT	Actual RT	RT Delta	m/z (Expected)	m/z (Apex)	m/z (Delta)
FWW PBR 1.1	Nicotinic acid	1,04	0,89	-0.15	124,03929	124,03934	.43056 (ppm)
FWW PBR 1.2	Nicotinic acid	1,04	0,88	-0.16	124,03929	124,03932	.24603 (ppm)
FWW PBR 1.3	Nicotinic acid	1,04	0,9	-0.14	124,03929	124,03938	.73809 (ppm)
FWW PBR 2.1	Nicotinic acid	1,04	0,9	-0.14	124,03929	124,03941	.98413 (ppm)
FWW PBR 2.2	Nicotinic acid	1,04	0,9	-0.14	124,03929	124,03937	.61508 (ppm)
FWW PBR 2.3	Nicotinic acid	1,04	0,88	-0.16	124,03929	124,03932	.24603 (ppm)
FWW PBR 3.1	Nicotinic acid	1,04	0,88	-0.16	124,03929	124,03939	.79960 (ppm)
FWW PBR 3.2	Nicotinic acid	1,04	0,89	-0.15	124,03929	124,03941	.92262 (ppm)
FWW PBR 3.3	Nicotinic acid	1,04	0,88	-0.16	124,03929	124,03939	.79960 (ppm)

Table E.2. MS ion inclusion list for vitamin analysis.

Mass [m/z]	Formula [M]	Formula type	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	(N)CE type	Comment
124,03930	C6H5NO2	Chemical formula	+ H	1	Positive	0,07	1,80	35	NCE	Nicotinic acid
170,08117	C8H11NO3	Chemical formula	+ H	1	Positive	0,60	1,05	35	NCE	Pyridoxine
220,11795	C9H17NO5	Chemical formula	+ H	1	Positive	3,00	4,80	35	NCE	D-pantothenic acid
245,09544	C10H16N2O3S	Chemical formula	+ H	1	Positive	5,55	6,00	35	NCE	Biotin
265,11176			+ H	1	Positive	0,50	0,95	35	NCE	Thiamine
377,14556	C17H20N4O6	Chemical formula	+ H	1	Positive	5,75	6,20	35	NCE	Riboflavin
442,14696	C19H19N7O6	Chemical formula	+ H	1	Positive	4,95	5,40	35	NCE	Folic Acid
673,79121				2	Positive	4,00	4,94	35	NCE	Methylcobalamine
678,29098	C63H88CoN14O14P	Chemical formula	+ H	2	Positive	5,41	5,85	35	NCE	Cyanocobalamine

APPENDIX F

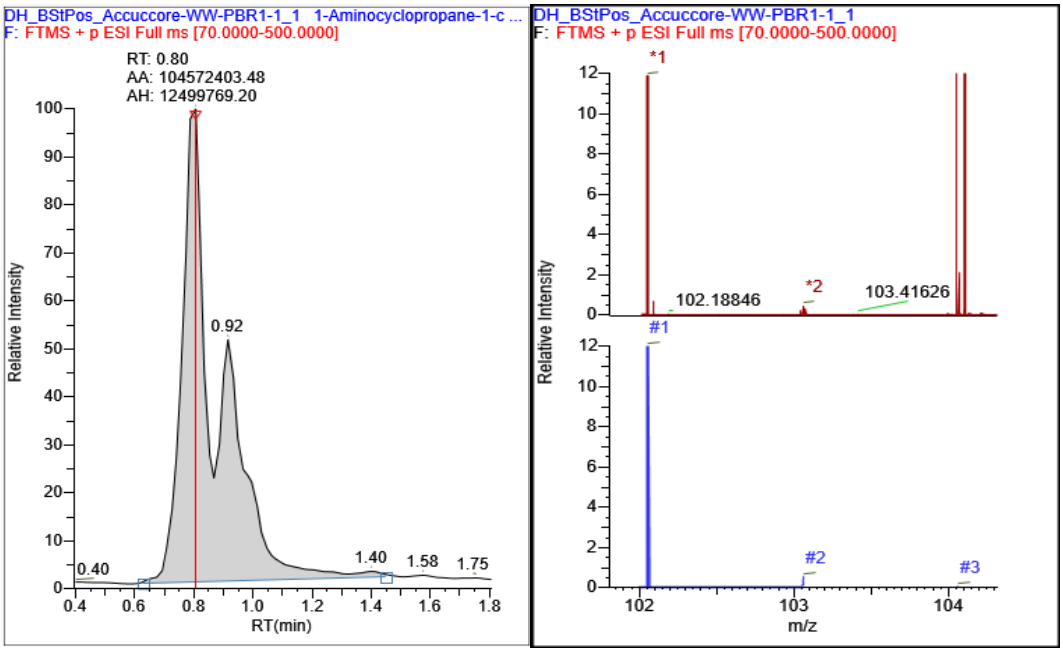


Figure F.1. LC-MS chromatogram and spectrum of 1-aminocyclopropane-1-carboxylic acid of FWW PBR 1.

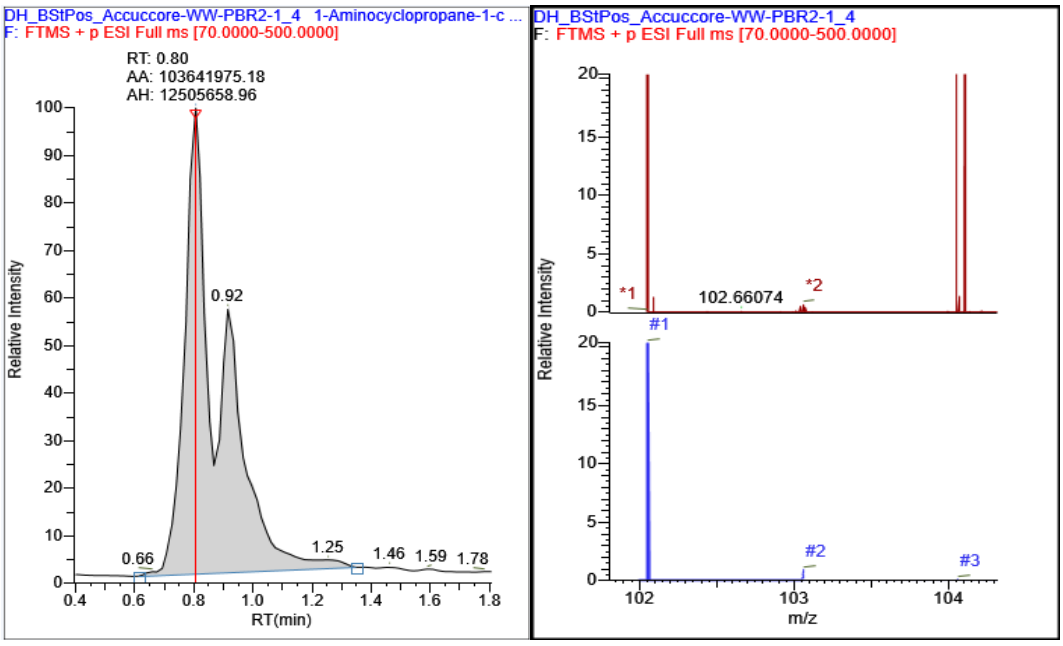


Figure F.2. LC-MS chromatogram and spectrum of 1-aminocyclopropane-1-carboxylic acid of FWW PBR 2.

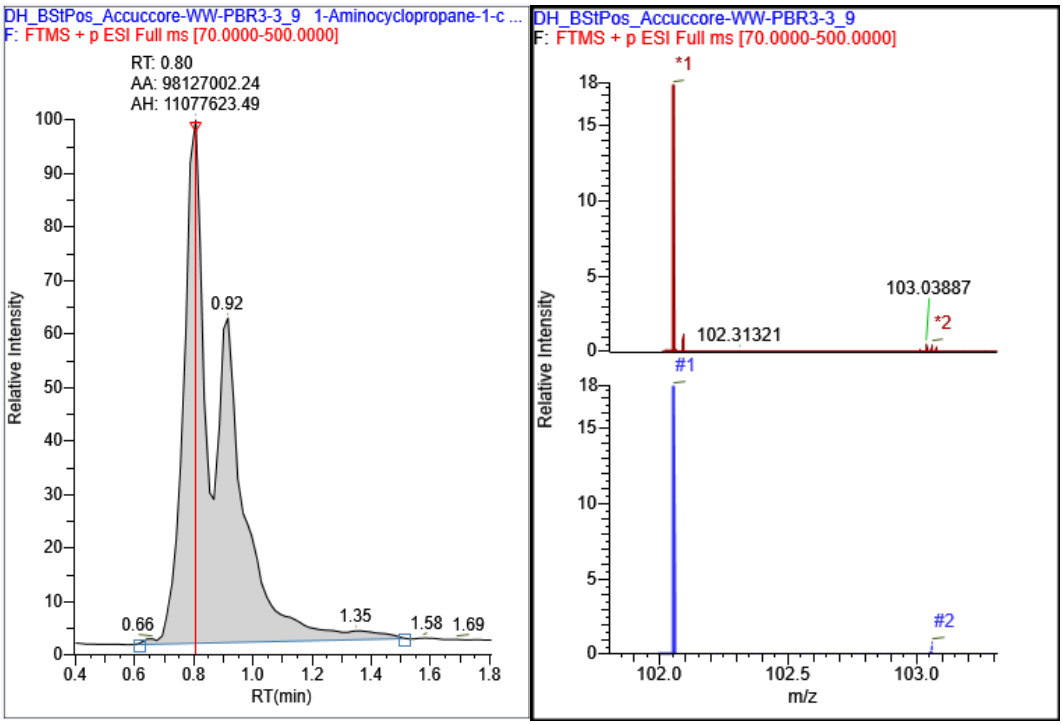


Figure F.3. LC-MS chromatogram and spectrum of 1-aminocyclopropane-1-carboxylic acid of FWW PBR 3.

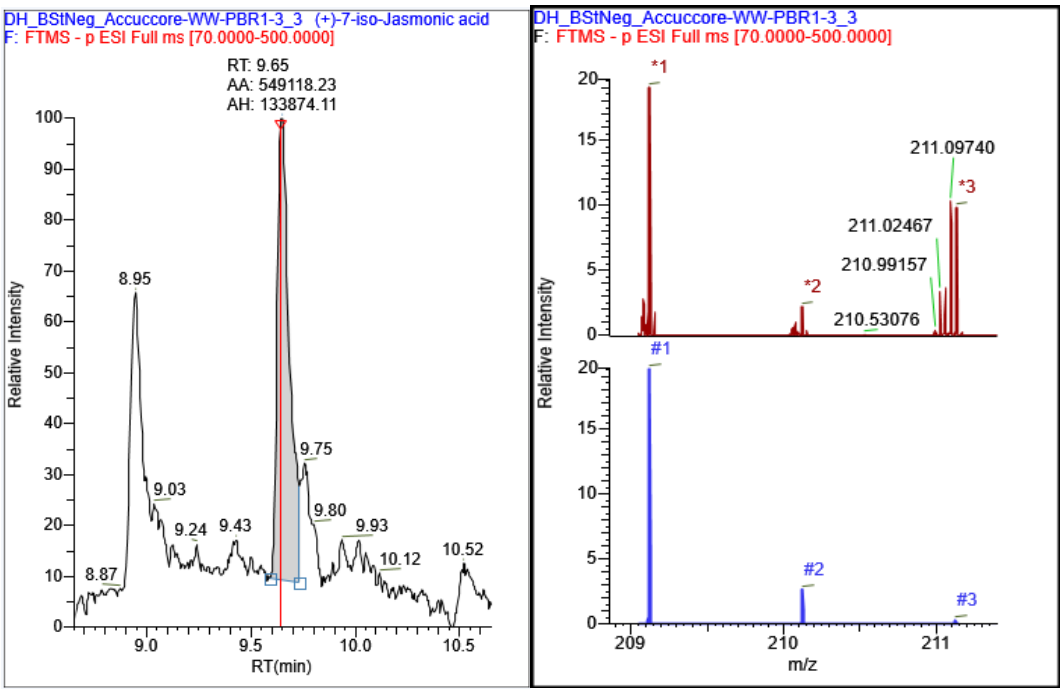


Figure F.4. LC-MS chromatogram and spectrum of (+)-7-isojasmonic acid of FWW PBR 1.

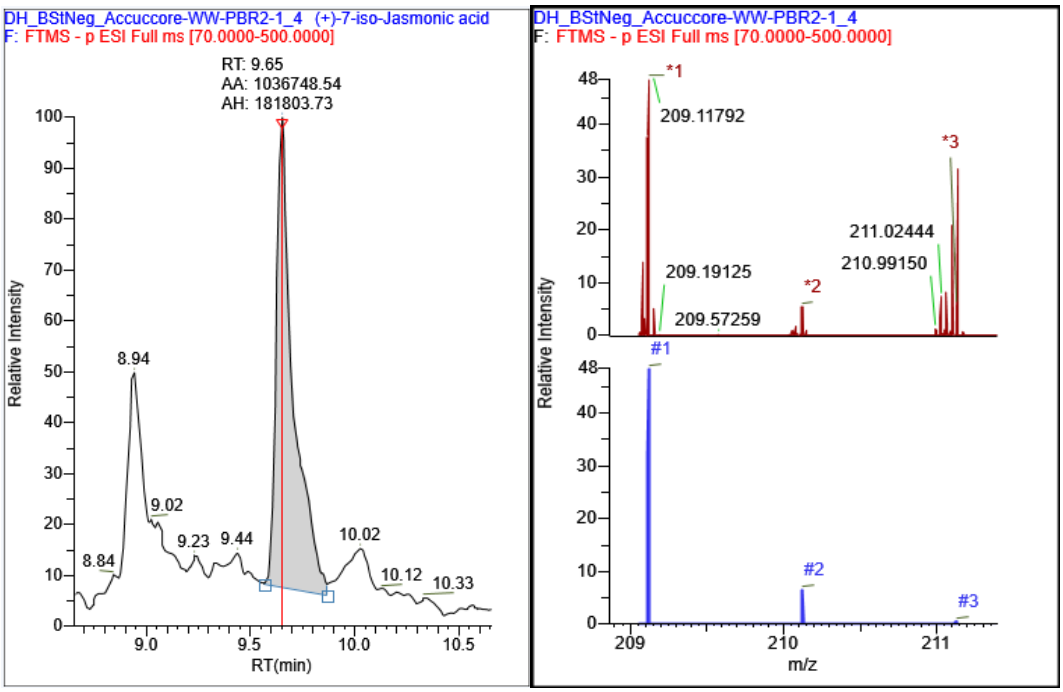


Figure F.5. LC-MS chromatogram and spectrum of (+)-7-isojasmonic acid of FWW PBR 2.

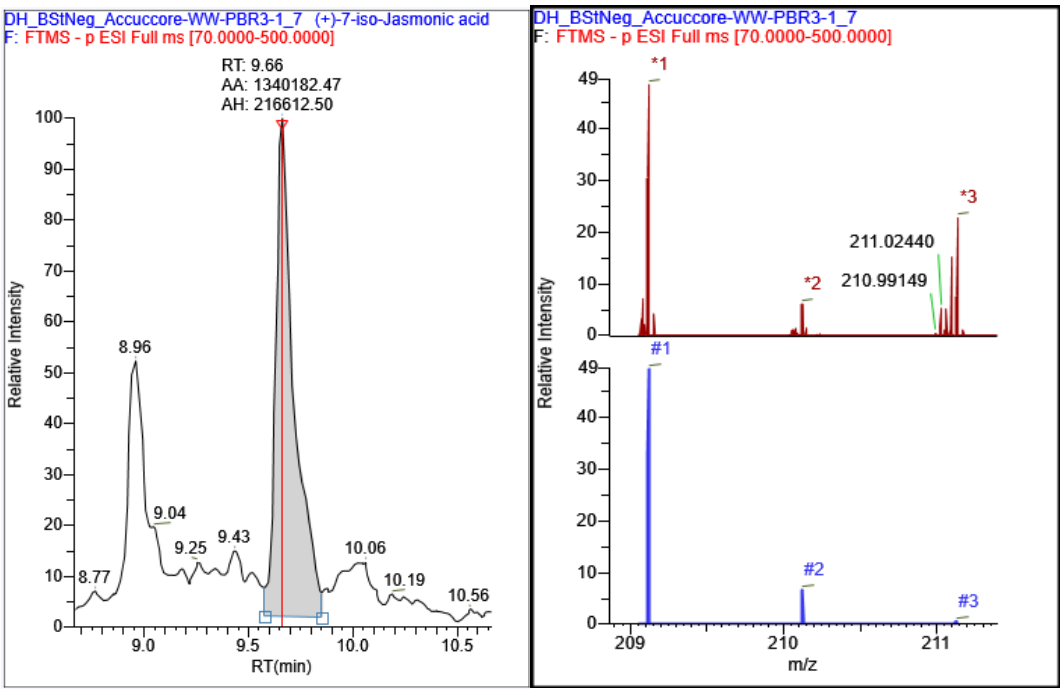


Figure F.6. LC-MS chromatogram and spectrum of (+)-7-isojasmonic acid of FWW PBR 3.

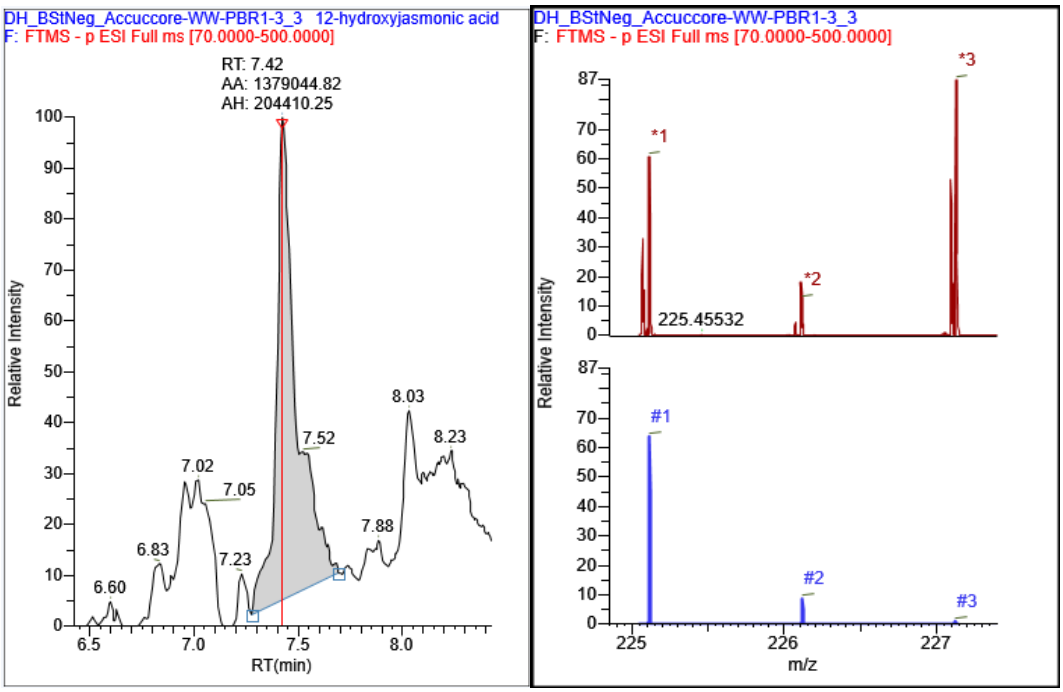


Figure F.7. LC-MS chromatogram and spectrum of 12-hydroxyjasmonic acid of FWW PBR 1.

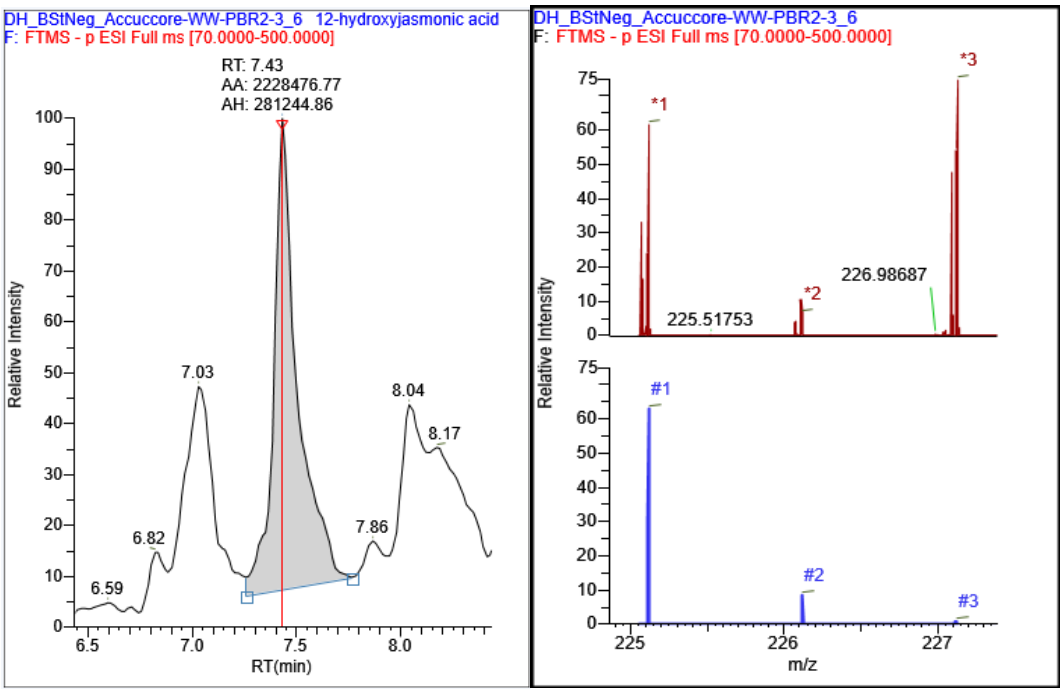


Figure F.8. LC-MS chromatogram and spectrum of 12-hydroxyjasmonic acid of FWW PBR 2.

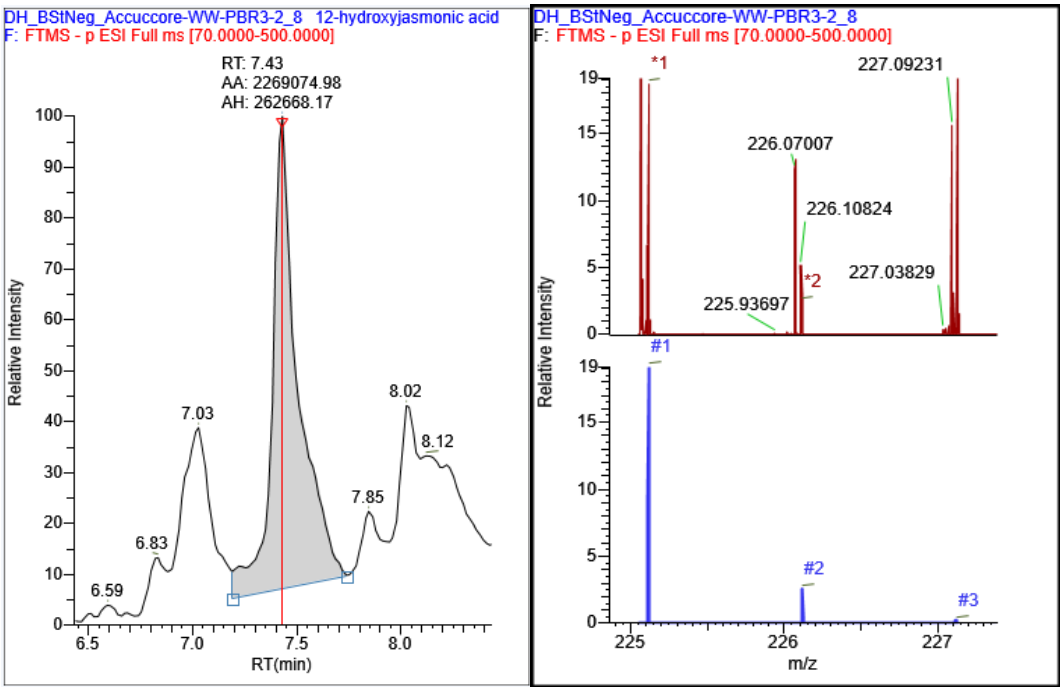


Figure F.9. LC-MS chromatogram and spectrum of 12-hydroxyjasmonic acid of FWW PBR 3.

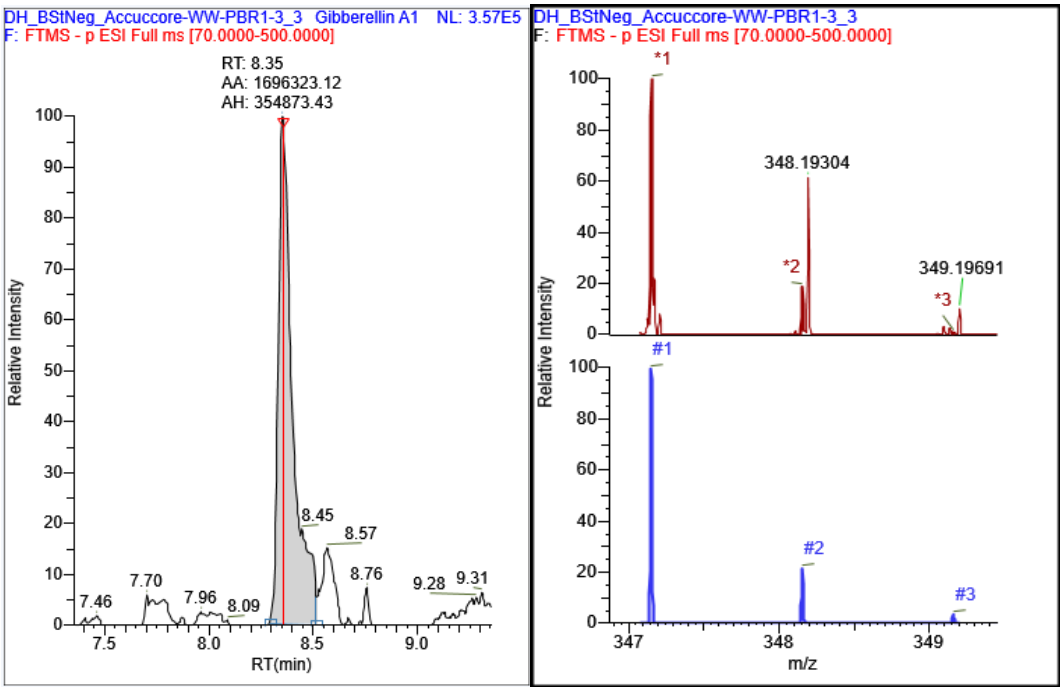


Figure F.10. LC-MS chromatogram and spectrum of Gibberellin A1 of FWW PBR 1.

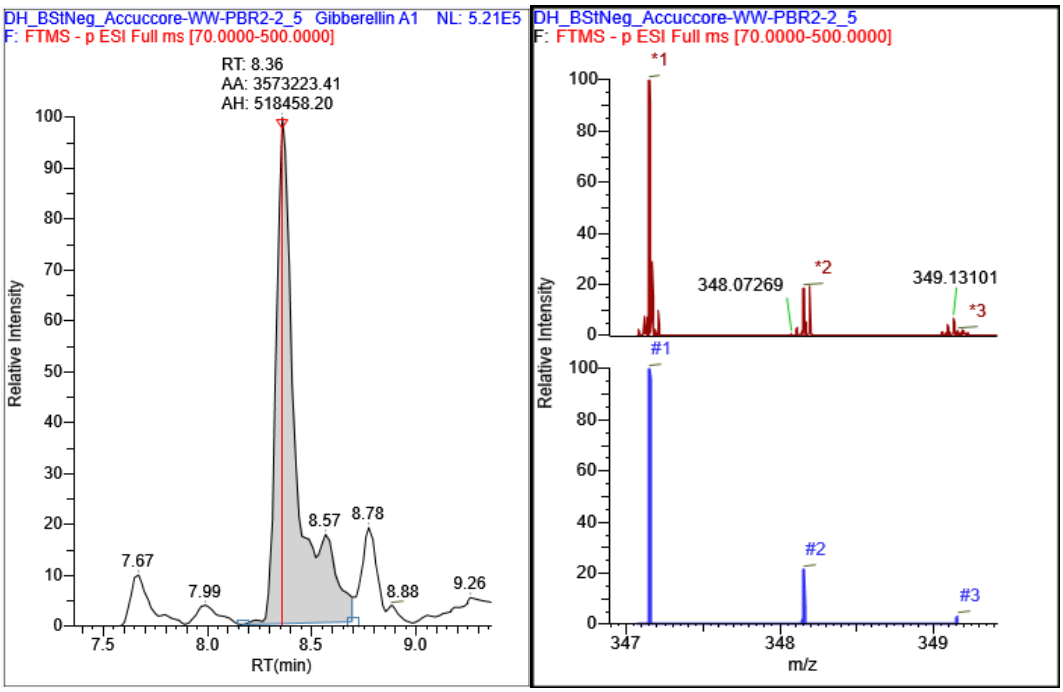


Figure F.11. LC-MS chromatogram and spectrum of Gibberellin A1 of FWW PBR 2.

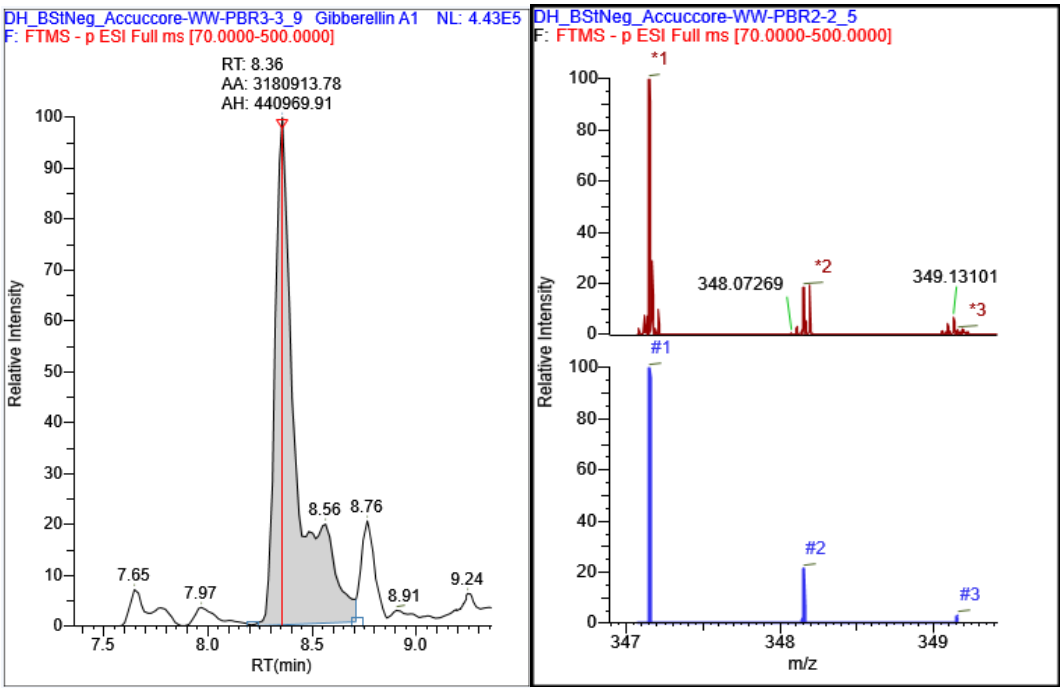


Figure F.12. LC-MS chromatogram and spectrum of Gibberellin A1 of FWW PBR 3.

Table F.1. Qualitative biostimulant analysis results and data.

Sample Name	Name	Class	Empirical Formula	Exact Mass	Polarity	$[M+H]^+/[M-H]^-$ (Theoretical)	$[M+H]^+/[M-H]^-$ (Measured)	Δm/z (ppm)
FWW PBR 1	1-Aminocyclopropane-1-carboxylic acid	Ethylene Precursor	$C_4H_7NO_2$	101,04713	[+]	102,05496	102,05486	-0,98
FWW PBR 2							102,05485	-1,08
FWW PBR 3							102,05486	-0,98
FWW PBR 1	(+)-7-iso-Jasmonic acid	Jasmonates	$C_{12}H_{18}O_4$	210,12559 44	[-]	209,11832	209,11810	-1,05
FWW PBR 2							209,11792	-1,91
FWW PBR 3							209,11801	-1,48
FWW PBR 1	12-hydroxyjasmonic acid		$C_{12}H_{18}O_4$	226,12050 91	[-]	225,11323	225,11319	-0,18
FWW PBR 2							225,11304	-0,84
FWW PBR 3							225,11302	-0,93
FWW PBR 1	Gibberellin A1	Gibberellins	$C_{19}H_{24}O_6$	348,15728 85	[-]	347,15001	347,15044	1,24
FWW PBR 2							347,15030	0,84
FWW PBR3							347,15032	0,89