



MASTERARBEIT | MASTER'S THESIS

Titel | Title

Investigating the impact of induced heat stress on glycosyl
inositol phospho ceramides in Austrian white wine through
high-resolution mass spectrometry

verfasst von | submitted by

Hoang Anh Nguyenová BSc

angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of
Master of Science (MSc)

Wien | Vienna, 2025

Studienkennzahl lt. Studienblatt | Degree
programme code as it appears on the
student record sheet:

UA 066 862

Studienrichtung lt. Studienblatt | Degree
programme as it appears on the student
record sheet:

Masterstudium Chemie

Betreut von | Supervisor:

Ass.-Prof. Dipl.-Ing. Evelyn Rampler Privatdoz. Bakk.
PhD

Acknowledgements

First and foremost, I would like to express my deepest gratitude to my supervisor, Ass.-Prof. Dipl.-Ing. Evelyn Rampler, for her mentorship over the past several months. Thank you for the guidance, for introducing me to the scientific world beyond lectures and seminars, and for the continuous support during this entire project. I felt truly welcomed in the research group and was inspired by attending my first conferences, meeting experts in the field, and gaining a completely new perspective on what I had studied for years. I am especially thankful to Marlene Pühringer and Amirreza Dowlati. Their patience and expertise in mass spectrometry and lipidomics saved me countless times. My sincere thanks also go to the Köllensperger team for creating such a warm and welcoming lab environment. I appreciated everything, from the conversations during lunch to the practical advice during my laboratory work. I am also grateful to Univ.-Prof. DI Dr. Jürgen Zanghellini for his guidance in data analysis and computational systems biology, from which I learned a great deal.

I would like to thank Raimund Schubert from the faculty's workshop for his kind help with all the glassware, Sonja Hager for her support in protein determination, and Prof. Dr. nat. techn. Verena Ibl for the insightful discussion on lipid behavior in plants. I also deeply appreciate the collaboration with the Federal College and Research Institute for Viticulture and Pomology Klosterneuburg, and I thank the team there for providing and helping with the samples without which this project would not be possible.

Lastly, I want to express my utmost gratitude to my parents and my two little siblings for always believing in me and supporting me unconditionally. A heartfelt thank you to Eva Michalcová for always being there for me and for the constant encouragement. I am also thankful to Ronan Smith and the Charlie P's team for always having my back. And last but not least, to my partner Tomáš Tichý: thank you for taking care of me throughout every step of this journey and for letting me take over your precious computer.

Abstract

Glycosyl inositol phospho ceramides (GIPCs) represent a major class of sphingolipids found in plants and fungi. Although many biological roles have been established, their specific mechanisms and roles under environmental stress remains insufficiently understood. In this study, we investigated the temporal response of GIPC in white wine leaf samples (from Grüner Veltliner and Riesling) collected at the Federal College and Research Institute for Viticulture and Pomology Klosterneuburg, which were subjected to artificial heat stress. After extracting lipids using a solvent system of 2-propanol, n-hexane, and water, and adding lactosyl ceramide for relative quantification, high-resolution reversed-phase LC-MS/MS was conducted in CID positive, CID negative, and EAD positive modes. GIPCs were identified using Lipid Data Analyzer (LDA), which incorporates a novel implementation of the EAD mode specifically tailored for GIPC analysis. In total, 100 GIPC species were identified and categorized into two confidence levels: 22 species were partially resolved with at least one MS2 spectral identification and consistent MS1 and retention time patterns, while 78 A-series lipids and 5 higher-class D- and E-series were putatively identified based on ECN retention time trends.

PCA of the most confidently identified lipids showed clustering based on harvesting time, and hierarchical clustering also revealed structural coregulation patterns based on the hydroxylation and unsaturation states. Two-way ANOVA further highlighted differences between wine types, with Riesling exhibiting more dynamic lipid changes over time. A dynamic approach for data analysis was implemented as well to capture time-dependent lipid behavior using the NetworkX Python package. Pearson correlation-based networks were constructed for each time point and condition. Key GIPCs with hydroxylated very-long-chain fatty acids (h-VLFCAs) consistently proved as central hubs, with their biological relevance assessed through eigenvector centrality. The dynamic trends corresponded with temperature differences between control and heat-stressed samples, showing denser, more connected networks that indicate increased membrane-level coordination in response to heat stress. Combining this dynamic perspective with static analysis supports the systemic role of h-VLFCAs in membrane stabilization under heat stress.

Our LC-MS/MS approach proved to be a successful method for the detection and annotation of GIPCs, demonstrating its suitability for GIPC-focused lipidomics. It provides a solid foundation for future studies involving enhanced EAD-based fragmentation, integrated network analyses, and multi-omics strategies to further explore the role of GIPCs as potential biomarkers in crop biotechnology.

Zusammenfassung

Glycosyl-Inositolphospho-Ceramide (GIPCs) stellen eine wichtige Klasse von Sphingolipiden in Pflanzen und Pilzen dar. Obwohl viele biologische Funktionen bereits bekannt sind, bleiben ihre spezifischen Mechanismen und Rollen unter wachsenden Temperaturen weiterhin unzureichend verstanden. In dieser Studie wurde das Zeitverhalten von GIPCs in Blattproben österreichischer Weißweinsorten (Grüner Veltliner und Riesling) untersucht, die am HBLA und Bundesamt für Wein- und Obstbau Klosterneuburg einer künstlichen Hitzebelastung ausgesetzt wurden. Nach der Lipidextraktion mit einem Lösungsmittelsystem aus 2-Propanol, n-Hexan und Wasser zusammen mit Lactosylceramid als dem internen Standard, wurde eine hochauflösende LC-MS/MS-Analyse in den Modi CID positiv, CID negativ und EAD positiv durchgeführt. Die Identifizierung der GIPCs erfolgte mit dem Softwaretool Lipid Data Analyzer (LDA), unter Implementierung einer neuen EAD-Methode optimiert speziell für GIPCs. Insgesamt wurden 100 GIPC-Spezies identifiziert und in zwei Stufen kategorisiert: 22 Spezies wurden teilweise aufgelöst, basierend auf mindestens einer MS2-Fragmentidentifizierung sowie konsistenten MS1- und Retentionszeitmuster, während 78 Lipide der A-Serie sowie 5 höherklassige Serien (D- und E-Serien) basierend auf ECN-trends putativ identifiziert wurden.

Die PCA der zuverlässigsten Lipide zeigte eine zeitabhängige Clusterbildung, und die hierarchische Clusteranalyse offenbarte eine strukturelle Koregulation basierend auf Hydroxylierungs- und Doppelbindungsgrad. Die Zwei-Wege-ANOVA zeigte darüber hinaus Unterschiede zwischen den Weinsorten, wobei Riesling deutlichere Veränderungen über die Zeit aufwies. Zusätzlich wurde ein dynamischer Ansatz implementiert, um zeitabhängige Veränderungen im Lipidverhalten mittels des Python-Pakets NetworkX zu erfassen. Für jeden Zeitpunkt und jede Bedingung wurden Pearson-korrelationsbasierte Netzwerke konstruiert. Dabei zeigten sich GIPCs mit hydroxilierten langkettigen Fettsäuren (h-VLCFAs) konsistent als zentrale Knoten, deren biologische Relevanz anhand der Eigenvektor-Zentralität bestimmt wurde. Die dynamischen Trends stimmten mit den Temperaturunterschieden zwischen Kontroll- und Hitzestressbedingungen überein: dichtere und stärker vernetzte Netzwerke deuteten auf eine verstärkte Membranorganisation als Reaktion auf Hitzestress hin. Die Kombination aus dynamischer und statischer Analyse unterstützt die systemische Rolle von h-VLCFAs bei der Membranstabilisierung unter Hitzestress.

Unser LC-MS/MS-Ansatz erwies sich als erfolgreiche Methode zur Detektion und Annotation von GIPCs und bestätigt sich als passend für GIPC-zentrierte Lipidomik. Er bildet eine solide Grundlage für zukünftige Studien mit erweiterter EAD-Fragmentierung, integrierten Netzwerkanalysen, und multi-omics Strategien, um die Rolle von GIPCs als potenzielle Biomarker in der Pflanzenbiotechnologie weiter zu untersuchen.

Contents

Abstract	iv
1 Introduction	1
1.1 Glycosyl Inositol Phosphoceramides	1
1.1.1 Structural Diversity	2
1.1.2 Biological Role	4
1.1.3 Thermal Stress	4
1.2 Technical Approach	5
1.2.1 Extraction Methods	5
1.2.2 Multi-mode Mass Spectrometry	5
1.2.3 Annotation Problem	6
1.3 Objective of the Study	6
2 Material and Methods	7
2.1 Material	7
2.2 Growth Conditions and Heat Stress Application	7
2.3 Sample Preparation	8
2.4 Extraction	9
2.4.1 Final Extraction Method	9
2.5 LC-MS/MS Workflow	10
2.5.1 High-Performance Liquid Chromatography	10
2.5.2 High-Resolution Mass Spectrometry	11
2.6 GIPC Annotation & Identification	13
2.6.1 Lipid Data Analyzer	13
2.6.2 Skyline	15
2.6.3 Other Software Tools	15
2.7 Data Preprocessing	15
2.7.1 Normalization Methods	15
2.7.1.1 Dry Weight Determination	15
2.7.1.2 Protein Determination	16
2.8 Data Quality Assessment	17
2.8.1 Calculated Resolution	17
2.8.2 Resolution Comparison Between EAD and CID	18
2.8.3 Technical Replicates Comparison	19
2.8.4 Extraction Replicates Comparison	19
2.8.5 Lactosyl Ceramide Variability	21
2.8.6 Suggestions for Optimal Strategy	22

2.9	Static Data Analysis	23
2.10	Dynamic Data Analysis	23
2.11	User Interface and Workflow Automation	23
3	Results	25
3.1	Introduction to GIPC Identification	25
3.2	GIPC Identification - CID Mode	26
3.2.1	CID Positive Mode	26
3.2.2	CID Negative Mode	26
3.3	GIPC Identification - EAD Mode	27
3.4	Pooled Samples & Matching	27
3.5	Unknown Fragments - Fragmentor	34
3.6	ECN Number	35
3.6.1	GIPC Series Class A	36
3.6.2	Higher-order GIPC Series Identification	39
	Summary of Identified GIPCs	43
3.7	Preprocessing Effects	44
3.8	Static Data Analysis	47
3.8.1	Principal Component Analysis	47
3.8.2	Hierarchical Clustering	48
3.8.3	Two-way ANOVA	50
	Control X Wine	50
	Time X Condition	50
	Time X Wine	50
3.8.4	CV Analysis	50
3.9	Dynamic Data Analysis	54
3.9.1	Network Analysis	55
4	Discussion	59
4.1	Biological Interpretation	59
4.1.1	Connections between the Static and Dynamic Analysis	60
4.1.2	What does the literature say?	60
4.2	Normalization Debate	61
4.3	Limitations and Future Directions	61
5	Conclusion	63
	Bibliography	64
A	Tools and Software Used	69
A.1	Word Processing Software	69
A.2	Bibliography Management	69
A.3	Data Analysis	69
A.4	Use of AI Tools	70
B	Normalization Table	71

C	GIPC ID Lists GV Sampling 1	73
D	GIPC ID Lists R Sampling 1	89
E	GIPC ID Lists GV Sampling 2	99
F	GIPC ID Lists R Sampling 2	112
G	GIPC ID Lists GV Sampling 4	125
H	GIPC ID Lists R Sampling 4	136
I	GIPC ID Lists GV Sampling 5	148
J	GIPC ID Lists R Sampling 5	159

List of Figures

1.1	GIPC Lipid Structure	2
1.2	GIPC Classification	3
2.1	Temperature Recording: Control vs Loggers	8
2.2	Final Extraction Method	9
2.3	LC-MS/MS Workflow	10
2.4	Dry Weight Ratios	16
2.5	BSA Calibration Row	17
2.6	Mass Resolution for MS1 and MS2: Mode Comparison	18
2.7	LacCer Detection Without Standard	21
2.8	LacCer Replicates: Peak Areas	21
2.9	LacCer Replicates: Proportion Comparison	22
2.10	User Interface	24
3.1	GIPC Fragmentation Description	25
3.2	MS2 spectra of GIPC A in all acquisition modes	28
3.3	Fragmentor Visualization	34
3.4	ECN Model	35
3.5	ECN Model: A Series	36
3.6	ECN Model: C_HEX_NH_D Series	39
3.7	ECN Model: D_2HEX_PEN_NAc Series	40
3.8	ECN Model: D_2HEX_PEN and D_HEX_2PEN Series	41
3.9	ECN Model: E_2HEX_2PEN_NH and E_3HEX_PEN_NAC Series	42
3.10	Areas of GIPC Species (with highest confidence identification)	45
3.11	PC1 vs PC2 Plots	47
3.12	PC2 vs PC3 & PC1 vs PC2 Plots	48
3.13	Hierarchical Clustering	49
3.14	Two-Way ANOVA: Condition $\times$ Wine Type	51
3.15	Two-Way ANOVA: Time $\times$ Condition	52
3.16	Two-Way ANOVA: Time $\times$ Wine Type	53
3.17	CV Distributions	54
3.18	Network Densities	55
3.19	Network Graphs	56
3.20	Network Graphs – Riesling	57
3.21	Network Graphs – Grüner Veltliner	58

List of Tables

2.1	Chemical List	7
2.2	LC gradient	11
2.3	MS Parameters	12
2.4	Mass Resolution: m/z 1262	17
2.5	Mass Resolution: m/z 956	18
2.6	Technical Replicate Summary	19
2.7	Comparison of RSD values across normalization methods	20
3.1	GIPC ID List: Partially Resolved Lipids	29
3.2	GIPC ID List: Putatively Identified Lipids	37
3.4	Time-course Expression Patterns	46
3.5	PCA: Explained Variance	47
B.1	Dry Weight and Protein Content	71
C.1	GIPC ID List: S1_GV_c replicates	74
C.2	GIPC ID List: S1_GV_s replicates	83
D.1	GIPC ID List: S1_R_c replicates	90
D.2	GIPC ID List: S1_R_s replicates	95
E.1	GIPC ID List: S2_GV_c replicates	100
E.2	GIPC ID List: S2_GV_s replicates	106
F.1	GIPC ID List: S2_R_c replicates	113
F.2	GIPC ID List: S2_R_s replicates	118
G.1	GIPC ID List: S4_GV_c replicates	126
G.2	GIPC ID List: S4_GV_s replicates	131
H.1	GIPC ID List: S4_R_c replicates	137
H.2	GIPC ID List: S4_R_s replicates	142
I.1	GIPC ID List: S5_GV_c replicates	149
I.2	GIPC ID List: S5_GV_s replicates	154
J.1	GIPC ID List: S5_R_c replicates	160
J.2	GIPC ID List: S5_R_s replicates	165

List of Abbreviations

ACN	Acetonitrile
AF	Ammonium Formate
BHT	Butylated Hydroxytoluene
BSA	Bovine Serum Albumin
CAD	Collision Activated Dissociation
CE	Collision Energy
CHEBI	Chemical Entities of Biological Interest
CID	Collision Induced Dissociation
CV	Coefficient of Variation
d	Dihydroxylated
DHA	Dihydroxyacetophenone
DW	Dry Weight
EAD	Electron Activated Dissociation
ECN	Equivalent Carbon Number
ELR	EarlyLeave Removal
ESI	Electrospray Ionization
FA	Formic Acid
FA	Fatty Acid
FWMH	Full Width at Half Maximum
GINT	Glucosamine Inositol Phosphorylceramide Transferase
GIPC	Glycosyl Inositol Phosphoceramides
GMT	Glycosylinositol Phosphorylceramide Mannosyl Transferase
GV	Grüner Veltliner
h	Hydroxylated
HPLC	High Performance Liquid Chromatography
HRMS/MS	High-Resolution Tandem Mass Spectrometry
IDA	Information Dependent Acquisition
IPCS	Inositol Phosphate Ceramide Synthase
IPA	Isopropanol
IPUT	Inositol Phosphorylceramide Ulucuronosyltransferase
LacCer	Lactosyl Ceramide
LC	Liquid Chromatography
LC-MS	Liquid Chromatography - Mass Spectrometry
LCB	Long Chain Base
LDA	Lipid Data Analyzer
LOH	Lag1 Homolog
MALDI	Matrix Assisted Laser Desorption/Ionization

MRM	M ultiple R eaction M onitoring
MS	M ass S pectrometry
n	Nonhydroxylated
NLP	N ecrosis and L ethal P rotein (ethylene-inducing peptide)
PCA	P rincipal C omponent A nalysis
PC	P rincipal C omponent
q	Q uadruplehydroxylated
R	R iesling
RP	R eversed P hase
RSD	R elative S tandard D eviation
RT	R etention T ime
STD	S tandard D eviation
t	T rihydroxylated
THF	T etrahydrofuran
VLCFA	V ery L ong C hain F atty A cid

Chapter 1

Introduction

Wine lipidomics in general started with a focus on targeting different fatty acids in grapes and wines before the 1980s, using primarily gas chromatography techniques. This focus was later expanded to include lipids that support wine yeast growth and serve as aroma precursors, particularly during the 1980s to 2000s¹. More recently, liquid chromatography coupled with MS-based analytical methods has become the standard for detecting a broader range of lipids, with comprehensive datasets like MS-DIAL, LipidMatch, and LIPID MAPS gaining popularity within the research community. One of the first extensive studies diving into the grape lipidome of *Sauvignon blanc* using shotgun lipidomics appeared only recently². Today, lipidomics applications in wine research extend to grape development, vine stress management, and predicting wine origin and authenticity.

1.1 Glycosyl Inositol Phosphoceramides

Glycosyl Inositol Phosphoceramides (GIPCs) are sphingolipids, a class of membrane lipids, and have been reported to constitute up to 40% of total lipids in plant plasma membranes³. They were first discovered in the 1950s by Carter et al.⁴, from plant seeds such as soybean, corn, flaxseed, and peanut. Initially called "phytoglycolipids" due to their phytosphingosine-type long-chain base, GIPCs remain incompletely characterized even after more than 60 years because of their complex structure. To this day, no commercialized standards are available and automated annotation systems are still under development⁵.

The three main components GIPCs are:

- Ceramide Moiety: consists of a long-chain base (LCB) connected to a fatty acid via an amide bond (typically trihydroxylated), along with a very long-chain fatty acid (VLCFA)
- Core Structure: an inositol phosphate group attached to the ceramide, with glucuronic acid always being the first attached sugar
- Polar Head Group: contains variable sugar units (*more in 'Structural Diversity'*)

The synthesis of GIPCs begins in the endoplasmic reticulum, where ceramide is synthesised. Serine and palmitoyl-CoA are condensed by serine palmitoyltransferase to form 3-ketosphinganine, which is reduced to sphinganine by 3-keto sphinganine reductase. This can be hydroxylated to form various long-chain bases (LCBs). These are converted to ceramide by ceramide synthases (Lag1 Homolog - LOH enzymes)⁶. Once the ceramide is processed (phosphorylated and glucosylated), the addition of the inositol phosphate head group from phosphatidylinositol is catalyzed

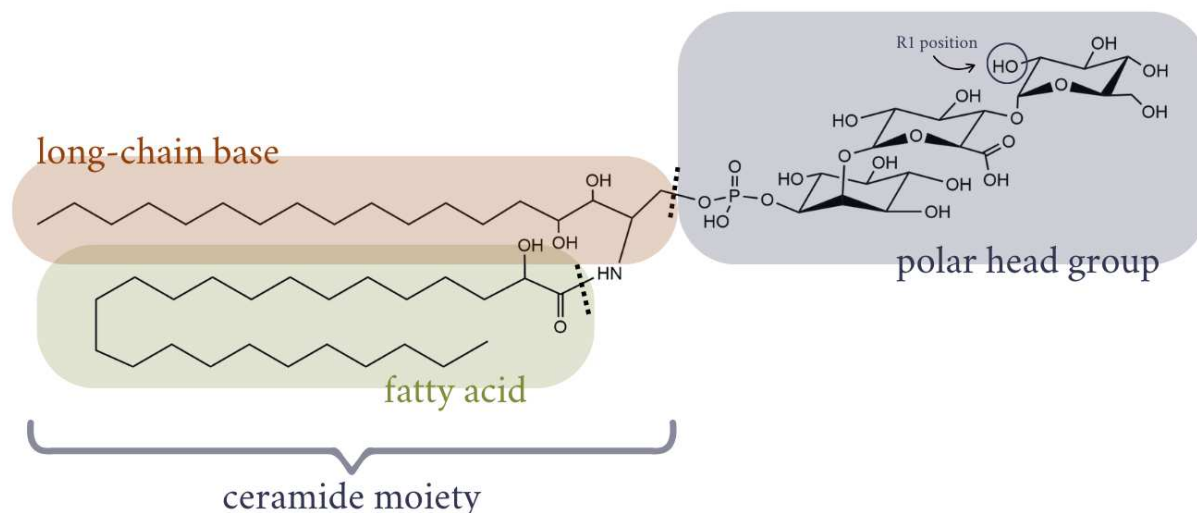


FIGURE 1.1: Structure of a GIPC lipid: Example of A-OH-18:0;O3/24:0, highlighting the long-chain base, fatty acid, and polar head group.

by inositol phosphate ceramide synthases (IPCSs), and this happens in the Golgi apparatus. This step requires specific transporters to transfer nucleotide sugars from cytosol into the Golgi lumen⁷. Final steps in GIPC formation include adding a glucuronic acid residue to the core structure via an $\alpha(1\rightarrow4)$ linkage along with further modifications involving N-acetylglucosamine, mannose, galactose, and other sugars⁶.

1.1.1 Structural Diversity

As mentioned above, GIPCs are known for their structural diversity due to both their complex core structure and the different saccharide components in the polar head group. The saccharides responsible for various modifications include arabinose, galactose, mannose, and fucose⁸. For instance, 19 saccharides were identified in tobacco leaf GIPCs by Kaul et al.⁹, and 20 saccharides in *Candida albicans* by Trinel et al.¹⁰. A Tansley review of different plant sphingolipids by Pata et al.¹¹ explores the vast variability in GIPC structures, classifying species into certain classes depending on their varying polar head groups. This classification depends on the number of saccharide units attached to the polar head, ranging from series 0, which includes only one saccharide, to series A with two saccharides, up to series F, which contains seven saccharides. As the series increases, distinguishing between classes and subclasses becomes more difficult, since the number of branching combinations of polar heads increases¹².

Focusing on plant-specific variations, there are differences documented in the distribution of GIPCs between monocot and dicot plants. Buré et al.¹² reported that series B dominates in monocots, whereas in dicots, series A seems to be more dominant. It is also important to note that differences exist between cell cultures and tissues in both monocots and dicots. Plant cell cultures tend to contain more highly glycosylated GIPCs. The diversity between cell types may reflect different membrane organization strategies; however, further research is necessary to understand the significance of these variations.

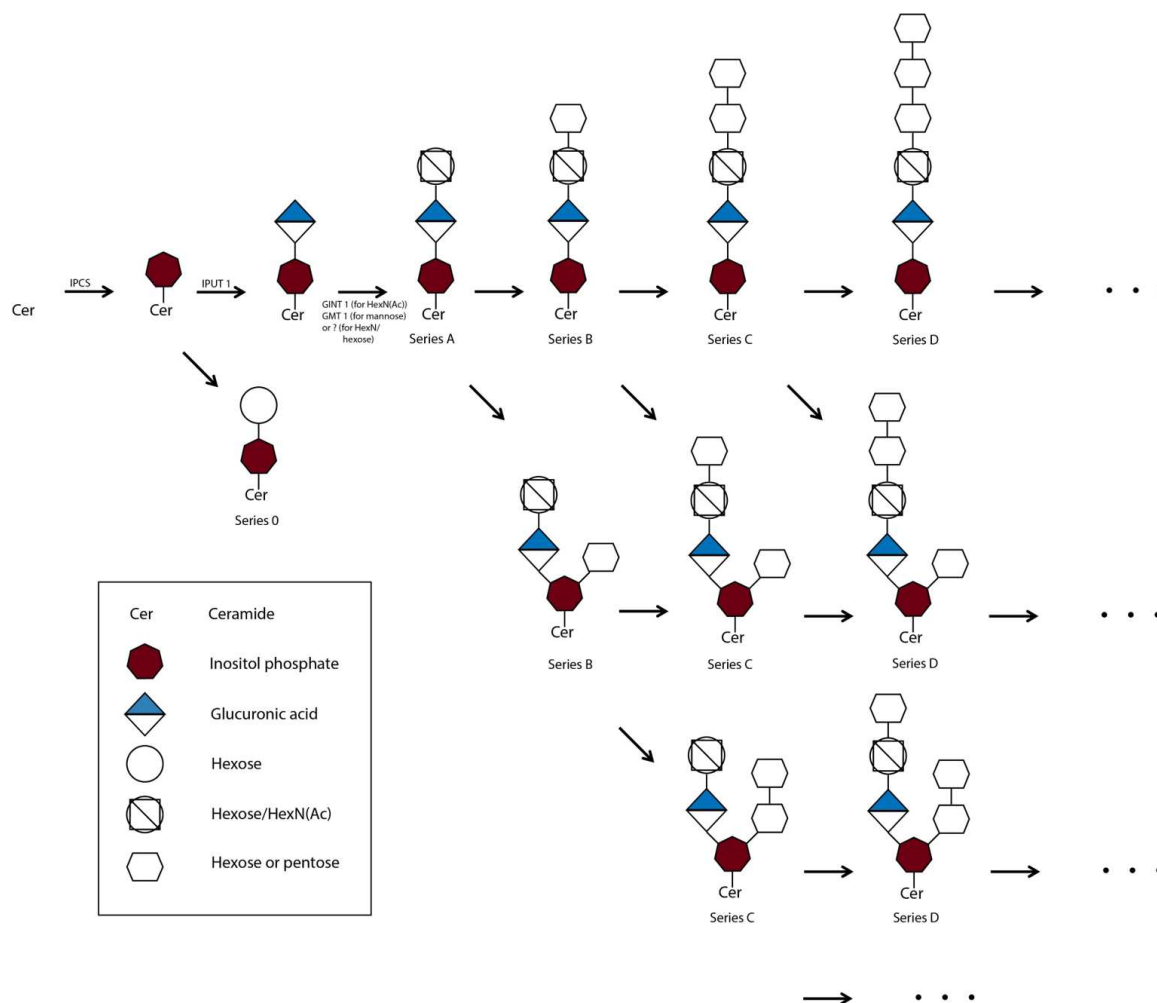


FIGURE 1.2: Different glycan structures according to GIPC series A to F with optional glycan branching possibilities. IPCS = inositol phosphorylceramide synthase; IPUT 1 = inositol phosphorylceramide glucuronosyltransferase 1; GMT 1 = glycosylinositol phosphorylceramide mannosyl transferase 1; GINT 1 = glucoseamine inositol phosphorylceramide transferase 1. Figure created by Pühringer et al.¹³

To understand the structure of each GIPC species in following chapters, let us break down the nomenclature of the lipid GIPC **A-OH-18:1;O3/24:0;O**. The "A" refers to the class described above. "OH" indicates different functional headgroups found in plant species; other key functional groups include NAc (N-acetyl) or NH (amine)⁴. "18:1;O3" represent the long-chain base component, with "O3" indicating the hydroxylation state of the long-chain base, "18" the number of carbon atoms, and "1" the number of double bonds. The nomenclature follows the standardized IUPAC-IUB guidelines¹⁴, which were later updated by Liebisch et al.¹⁵. The "24:0;O" part represents the fatty acid component, analogous to the LCB nomenclature. An alternative, combined notation for lipids identified at lower structural levels, where individual chain fragments are not determined, includes only the total number of carbon atoms, double bonds, and overall hydroxylation state for the entire species.

GIPC Nomenclature Cheat-Sheet**A-OH-18:1;O3/24:0;O**

- **A** – class
- **OH** – functional headgroup (others: NAc, NH)
- **18:1;O3** – long-chain base: tri-OH, 18 C, 1 double bond
- **24:0;O** – fatty acid: mono-OH, 24 C, 0 double bonds

1.1.2 Biological Role

Although some gaps remain in the study of GIPCs, a comprehensive list of their biological roles has already been established. Beginning with their role in membrane organization, these lipids are known to contribute to membrane integrity by providing a significantly higher negative charge on the membrane surface (ζ -potential of -26mV)¹⁶, and by forming membrane microdomains with sterols - an essential factor in the plasma membrane organization¹⁷. Morital et al.¹⁸ also stated that GIPCs serve as anchors for GPI proteins in the outer leaflet of the plasma membrane, important for cell-to-cell recognition and the signal transduction cascade. A function analogous to animal gangliosides. Furthermore, the diversity of polar head groups in GIPCs has been shown to play a role as receptors for pathogen toxins, such as necrosis and ethylene-inducing peptides 1 (NLPs), as reported by Lenarčič et al.¹⁹.

1.1.3 Thermal Stress

One crucial role - and a key motivation for this study - was the role of GIPCs in environmental adaptation. In plants, the membrane viscosity changes in response to temperature changes, a phenomenon known as homeoviscosity. This can occur, for example, by transforming some sterols into specific plasma membrane components, such as β -sitosterol and stigmasterol, as part of the adaptation to temperature fluctuations²⁰. As mentioned above, GIPCs are known to interact with these sterols, and their potential functions in the fluidity of the membrane have already been discussed. Cassim et al.³ proposed that GIPCs support the homeoviscosity and help regulate plasma membrane fluidity during thermal adaptation. So far, a significant accumulation of GIPCs has been observed during drought stress in plants. The suggested mechanism is that overexpression of GIPCs increases the number of sugar head groups, which helps prevent protein precipitation upon desiccation²¹. However, the exact role of GIPCs remains unclear, and their inclusion in glycerolipid-based models for stress responses is still needed. GIPCs have rarely been part of comprehensive lipidome studies, mainly because of the specific analytical steps required for their extraction and chromatographic analysis²².

1.2 Technical Approach

1.2.1 Extraction Methods

Over the years, many extraction methods were established. Starting with the method from Bligh and Dyer²³, sphingolipids were extracted with a two-phase liquid extraction, first with a 2:1:0.35 mixture of MeOH/chloroform/water and afterwards only with chloroform. Supernatants were combined, 0.88% KCl in water was added, and the two created phases were dried for analysis. Nichols et al.²⁴ approached the separation with an extraction using 2-propanol following a mixture of 1:1 chloroform:propanol. In case of Hanson et al.²⁵, the whole extraction was based only on the so-called solvent E, which was a 15:15:5:1:0.018 mixture of ethanol / water / diethylether / pyridine / ammonia. And last but not least, Toledo et al.²⁶ used similar extraction steps as Hanson et al.²⁵, but with a mixture of IPA/hexane/water (55:20:25 v/v/v) instead of solvent E.

Markham et al.²⁷ tested all the previously described methods and looked into the ratio of the soluble and insoluble parts. The last method by Toledo et al.²⁶ proved the lowest percentage of the insoluble part (2%), while the method by Nichols et al.²⁴ had the highest percentage (60%). This statement, combined with the fact that Toledo's method appears to have the least amount of hazardous chemicals compared to the others, led Panzeböck et al.⁵, with Thür et al.²⁸ and Pühringer et al.¹³, to optimize this extraction method further based on the outcomes documented by Markham et al.²⁷

1.2.2 Multi-mode Mass Spectrometry

The structural complexity of GIPCs requires advanced analytical workflows for confident identification. As highlighted by Rampler et al.²⁹, lipid annotation can be classified into different structural levels. From levels G to A, confidence in lipid annotation increases: level G includes only accurate mass information, while subsequent levels include the known compound class, known compound sum formula, known structure and double bond positions, and, up to level A, enantiomer-specific identification. Due to their glycosylation patterns and their long-chain bases, GIPCs often require multiple complementary ionization modes and fragmentation strategies to reach higher annotation levels. This is why a multi-mode mass spectrometry (MS) approach is needed for comprehensive structural coverage in GIPC profiling.

Cacas et al.⁸ reported two mass spectrometry methods. Chromatographic separation was performed using a reversed-phase C18 with a THF/methanol/water mobile phase containing ammonium acetate, which allowed separation of ceramide structures. MALDI-MS analysis (using a TOF-TOF mass spectrometer) in negative mode with a 2,6-dihydroxy-acetophenone (DHA) matrix was a useful tool for initial screening. ESI-MS/MS (with a QTRAP 5500 mass spectrometer) provided more detailed structural information, especially when both positive and negative modes were used along with multiple reaction monitoring (MRM) for targeted analysis. With this method, multiply charged species could also be detected, not just the pseudo-molecular species [M-H]⁻ as in MALDI-MS. In ESI-MS/MS, ionization occurred mainly at the phosphate group and the carboxyl group of hexuronic acid. Fragmentation patterns differed slightly between modes, with MALDI-MS/MS uniquely showing C3PO3 ions (from the carbohydrate moiety), while ESI-MS/MS revealed more ions from the ceramide portion. Isomeric structures were also distinguishable using ESI-MS/MS.

Since Tarazona et al.²², screened multiple lipid classes rather than GIPCs exclusively, SRM transitions in positive ion mode were used, targeting the loss of phosphoinositol-containing head groups. In contrast, an in-house method developed by Panzeböck et al.⁵ used an LC gradient based solely on isopropanol, avoiding the highly flammable THF, which showed even better peak separation. For high-resolution tandem MS, Q Exactive HF instrument with a HESI ion source was used in both positive and negative modes. This method allowed to annotate 84 GIPC species across various plants, identification of the previously unknown 18:2;O3 long chain base, and enabled relative quantification. The benefits of this in-house method included a better extraction efficiency, safer handling, and faster processing time. Pühringer et al.¹³ later enhanced the method for higher series GIPCs, considered more possibilities of branching in the sugar head groups, and achieved detection of double-bond positions. The final MS method used in this study is described in Chapter 2.

1.2.3 Annotation Problem

GIPCs are poorly represented in major databases. LIPID MAPS has only one single entry for the GIPC 18:1;O3/24:0;O (GlcN-alpha1-4-GlcUA-alpha1-2-PI-Cer (18:1;O3/24:0 (2OH))) from 2021, while ChEBI lists either a phosphoethanolamine glycosyl inositol phosphoceramide zwitterion (ChEBI: 143881, year 2003) or five GIPC-anchor amidated amino acid residues from 2020. These limitations complicate the automation of GIPCs. Common manual approaches include accurate mass measurements, characteristic fragmentation patterns, and matching retention times in positive and/or negative modes, as well as compliance with the equivalent carbon number (ECN)²⁹. With additional decision rule sets based on fragment rules and intensity rules, automated annotation tools are now emerging for GIPC, one such tool being the software Lipid Data Analyzer (LDA). The annotation strategy in this project relies heavily on the work of Panzeböck et al.⁵, Pühringer et al.¹³, and Hartler et al.³⁰ and is further discussed in Chapter 2.

1.3 Objective of the Study

The objective of this study was to examine the behavior of glycosyl inositol phosphoceramides (GIPCs) in response to abiotic heat stress in white wine leaf samples. Two wine types, Grüner Veltliner (GV) and Riesling (R), were analyzed at four different time points under both control and heat-stress conditions. Using LC-MS/MS, lipids were analyzed across three acquisition modes: collision-induced dissociation (CID) in positive and negative ion modes, and electron-activated dissociation (EAD) in positive mode. The goal was to characterize lipids up to the structural level C, achieving sufficient identification based on accurate masses, characteristic fragmentation patterns, and retention times following the ECN model. After identification, relative quantification of GIPC was performed, and the data were used for multivariate statistical analyses such as principal component analysis, ANOVA, hierarchical clustering, and network analysis. This approach aimed to track potential shifts in GIPC composition in response to heat stress, providing a deeper understanding of membrane lipid remodeling under abiotic stress conditions.

Chapter 2

Material and Methods

2.1 Material

The plant material used for this project was wine leaves (*Vitis vinifera*) collected between July and September 2022 from the Federal College and Research Institute for Viticulture and Pomology, more specifically the types Grüner Veltliner and Riesling. For each wine type there were four different plant samplings collected, and four replicates were analyzed for control and artificial heat stress application. The fifth replicates for both control and stress conditions for each of the four samplings were used for dry weight determination. In total, 64 samples were analyzed, with 32 replicates for each wine type. The naming of the samples, for example *S1_GV_c_3*, would stand for: [number of the sampling]_[wine type (GV - Grüner Veltliner, R - Riesling)]_[condition (control/stress)]_[number of the replicate]. Regarding the chemicals used for analysis, all chemicals were LC-MS grade and are listed in Table 2.1.

TABLE 2.1: List of chemicals used in this study.

Chemical	Abbreviation/Formula	Provider
Formic acid	FA	VWR
n-hexane	-	(Vienna, Austria)
Methanol	MeOH	Honeywell
Acetonitrile	ACN	(Offenbach, Germany)
2-propanol	IPA	
Water	H ₂ O	
Potassium hydroxide	KOH	Carl Roth (Karlsruhe, Germany)
Butylated hydroxytoluene	BHT	Sigma-Aldrich
Ammonium formate	AF	(Vienna, Austria)
C16 Lactosyl(β) Ceramide (18:1;O1/16:0)	LacCer	Avanti Polar Lipids, Inc. (Alabaster, Alabama, USA)

2.2 Growth Conditions and Heat Stress Application

To monitor the abiotic stress response of the plants, an artificial heat stress was applied by putting on an aluminium foil over the samples. There were three EasyLog loggers used as temperature sensors to measure the temperatures inside the foil. The maximum, minimum, and average temperatures for each month from June 2022 to September 2022 were obtained from the

TIMEANDDATE³¹ database. The differences between these temperatures, as well as the recorded logger temperatures, were plotted against each other, as shown in Figure 2.1. A clear trend was observed, with the largest differences in maximum temperatures in July, followed by a gradual decrease.

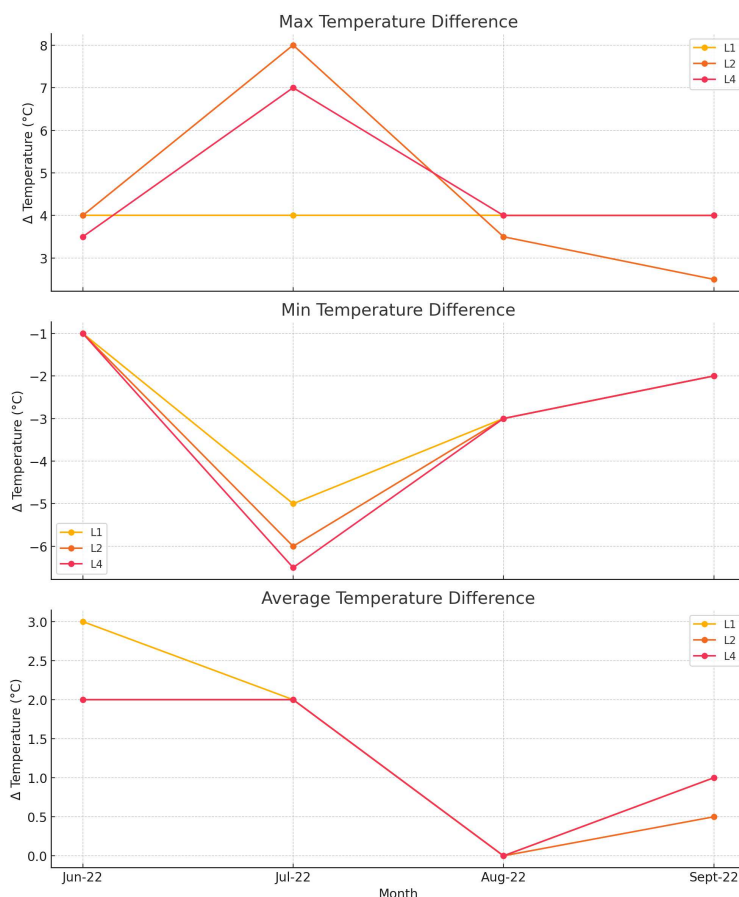


FIGURE 2.1: Temperature differences between the loggers (inside the foil) and the outside environment from June to September 2022: maximum (top), minimum (middle), and average (bottom) values.

2.3 Sample Preparation

The sample preparation was carried out by Marlene Pühringer and Nina Thür (University of Vienna, Austria), where the wine samples were first frozen and homogenized using a ball mill at a frequency of 30 s^{-1} . To do this, the metal part had to be cooled with liquid nitrogen first. Subsequently, the plant material was stored in a freezer at -80°C and kept there until further analysis. Although it has been years since the samples were taken out to continue the project, according to Shin et al.³², who conducted some stability tests on glycosphingolipids, the long-term stability showed results within acceptance criteria. This was confirmed by Sidhu et al.³³ for even longer periods at both -20°C and -80°C .

2.4 Extraction

2.4.1 Final Extraction Method

Approximately 100mg of homogenized wine leaf material was weighed into glass vials using a CPA225D balance (Sartorius, Vienna, Austria). After adding the C16 Lactosyl(β) Ceramide standard diluted in IPA and water, the mixture was vortexed. Subsequently, a 0.01% BHT solution in IPA was added, as an antioxidant, commonly used to stabilize lipids and prevent oxidative degradation³⁴. After a second vortexing step, an IPA/n-hexane mixture was added. According to Shahidi et al.³⁵, n-hexane has proved to be an efficient solvent for selectively extracting non-polar lipids. The contents of the glass vial were shaken, then centrifuged at 4°C for phase separation (Hermle Centrifuge Z-446K). The low temperature helps reduce the enzymatic activity and prevent lipid hydrolysis. The supernatant was removed, and a second mixture containing 0.01% BHT/IPA/n-hexane/water was added. Shaking and centrifugation steps were repeated. Afterwards, the supernatants were combined and dried in a Genevac EZ-2 Series Personal Evaporator (SP Scientific, Ipswich, UK), with a maximal temperature of 30°C using the 'LOW BP' program. After overnight drying, 0.1M KOH in MeOH was added to hydrolyze esterified lipids - i.e., to convert triglycerides and other esterified lipids into free fatty acids and glycerol³⁶. This was again shaken and after a pH adjustment to approximately 6-7, the solvent was dried overnight. On the third day of extraction, an IPA:water mixture (65:35) was added, and ClariSteps filters (Sartorius, Vienna, Austria) were used to filter 1 ml (for preexperiment) or 0.5 ml of the sample into HPLC vials. In the final extraction protocol, inserts were placed in the HPLC glass vials, and 200 μ L was transferred. The detailed extraction steps with the exact volumes are shown in Figure 2.2.



FIGURE 2.2: Workflow of the final two-step liquid-liquid extraction method used for GIPC sample preparation.

2.5 LC-MS/MS Workflow

The overall workflow after extraction involves obtaining MS1 and MS2 spectra using high-performance liquid chromatography coupled with the ZenoTOF 7600. Each sample was measured in CID positive mode. Sample pools for each wine type (GV/Riesling) and each condition (control/stress) were then prepared by combining the extracts and analyzed in CID negative mode and EAD positive mode. Lipids were annotated using the LDA software, and matching across all three modes was used to identify the GIPC species present in the samples. Peak integration was performed using Skyline, and further analysis steps were carried out using Python scripts. These will be elaborated in later chapters.

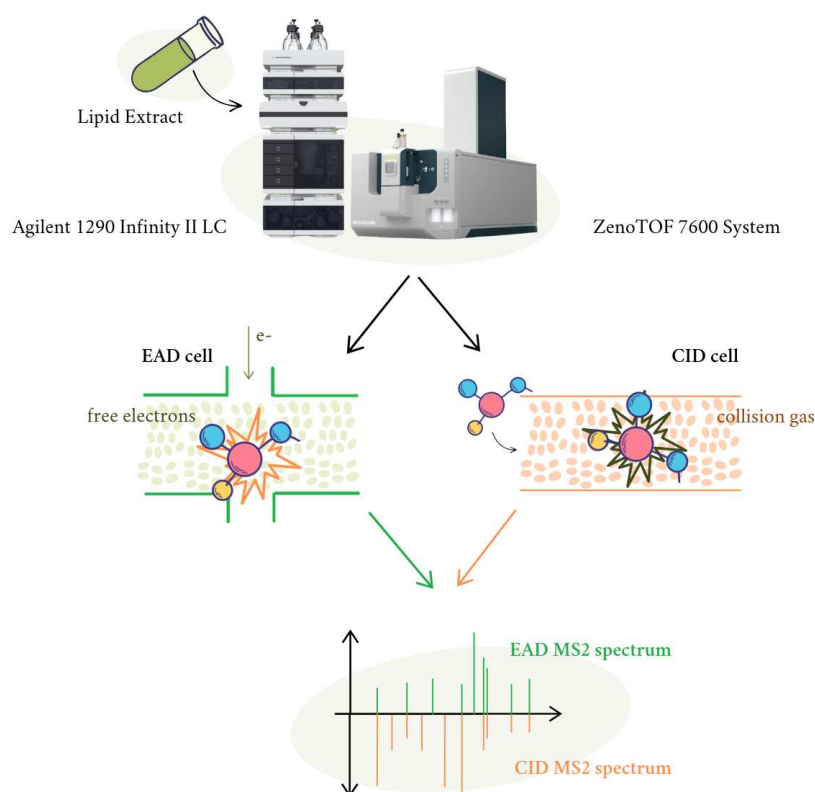


FIGURE 2.3: RP HRMSn method for qualitative and relative quantitative analysis of GIPCs. Figure inspired by Katharina Hohenwallner, Evelyn Rampler, and Che et al.³⁷; stock instrument photos from Agilent³⁸ and Sciex³⁹.

2.5.1 High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) was performed on an HPLC system (Agilent 1260 Infinity II system with a C18 reversed-phase (RP) Acquity UPLC HSS T3 column (PREMIER, 2.1 × 150mm, 1.8 μ m, Waters, Vienna, Austria). Chromatographic elution was performed at a column temperature of 40°C, and a binary pump (G7210A) was employed with a flow rate of 0.25 mL/min throughout each run. The mobile phases consisted of:

- Solvent A: 60% ACN + 40% H₂O + 0.1%FA and 10mM ammonium formate (AF)
- Solvent B: 90% IPA + 10% ACN + 0.1% FA and 10mM AF

A gradient elution was applied as described in Table 2.2. The total run time was 30 minutes. Samples were maintained at 10°C in the multisamples, and 5 μ L was injected per run.

TABLE 2.2: LC gradient program used for C18 reversed-phase separation.

Time (min)	A (%)	B (%)	Flow (mL/min)	Pressure (bar)
2.0	70	30	0.25	1300
3.0	45	55	0.25	1300
13.0	33	67	0.25	1300
18.0	0	100	0.25	1300
26.0	0	100	0.25	1300
26.1	70	30	0.25	1300
30.0	70	30	0.25	1300

2.5.2 High-Resolution Mass Spectrometry

Liquid chromatography was coupled with a ZenoTOF 7600 system (SCIEX, Concord, Canada) equipped with a TurbolonSpray ion source. Data acquisition was performed using SCIEX OS software version 3.3.1.43 and ClearCore2.Service 3.3.1.43. All measurements were carried out in information-dependent acquisition (IDA) mode with a total run time of 30 minutes per injection. Ions were detected within the 2-30-minute window for each polarity and fragmentation mode. As previously mentioned, analyses were performed in both positive and negative ionization modes.

Ion source parameters were kept constant for all modes: curtain gas at 35, collision gas (CAD) at 7, ion source gas 1 at 50 psi, ion source gas 2 at 60 psi, and source temperature at 450°C. A spray voltage of +5500 V was used in both positive modes, and -4000 V in negative mode. Declustering potentials were set to ± 120 V, and the TOF-MS1 scan range was m/z 500-2000 with an accumulation time of 100 ms. For each survey scan, up to five precursors exceeding 100 counts/s were selected for MS/MS acquisition. Previously selected candidate ions were excluded for 3 seconds after two occurrences.

All MS scans in positive EAD, positive CID, and negative CID modes were collected within the m/z range of 50-2000. For positive CID, collision energy (CE) was 50 ± 10 V. For negative CID, it was -50 ± 10 V. In EAD experiments, the electron kinetic energy was set to 15 eV, with a reaction time of 30 ms and an electron beam current of 5500 nA. In all MS2 methods, the accumulation time was 65 ms for EAD and 100 ms for CID. Zeno pulsing was enabled in all MS2 scans with a threshold of 40,000 cps to improve sensitivity. Q1 resolution was set to unit resolution throughout. A reduced inclusion list targeting specific precursor m/z values was used for CID negative and EAD positive analyses.

TABLE 2.3: Mass Spectrometry Parameters used for EAD and CID experiments with the ZenoTOF™ 7600 System

Parameter	EAD Positive	CID Positive	CID Negative
General			
Set run time (min)	30	30	30
Workflow	Small molecules	Small molecules	Small molecules
Intact protein mode	False	False	False
Ion Source			
Source name	TurboIonSpray	TurboIonSpray	TurboIonSpray
Curtain gas	35	35	35
CAD gas	7	7	7
Ion source gas 1 (psi)	50	50	50
Ion source gas 2 (psi)	60	60	60
Temperature (°C)	450	450	450
Spray voltage (V)	+5500	+5500	−4000
MS1 Survey Scan			
Polarity	Positive	Positive	Negative
TOF m/z range	500–2000	500–2000	500–2000
Accumulation time (s)	0.1	0.1	0.1
Declustering potential (V)	+120	+120	−120
IDA Criteria			
Max candidate ions	5	5	5
Intensity threshold (cps)	100	100	100
Exclude after (occurrences)	2	2	2
Exclusion duration (s)	3	3	3
Inclusion list used	No	Yes	Yes
MS2 Scan			
Fragmentation mode	EAD	CID	CID
m/z range	50–2000	50–2000	50–2000
Accumulation time (s)	0.065	0.1	0.1
Collision energy (V)	12	50 ± 10	−50 ± 10
Zeno pulsing	Enabled	Enabled	Enabled
Zeno threshold (cps)	40000	40000	40000
Q1 resolution	Unit	Unit	Unit
EAD-Specific Settings			
Electron kinetic energy (eV)	15	–	–
Electron beam current (nA)	5500	–	–
Reaction time (ms)	30	–	–
Software Version			
Acquisition software	SCIEX OS 3.3.1.43, ClearCore2.Service 3.3.1.43		

2.6 GIPC Annotation & Identification

2.6.1 Lipid Data Analyzer

Lipid Data Analyzer by Hartler et al.⁴⁰ is one of several available tools for identifying (and optionally quantifying) lipids from LC-MS data. In 2020, Hartler et al.³⁰ expanded the software to include sphingolipids and demonstrated that LDA can identify even isobaric and isomeric lipid species. Several extensions were added, such as a decision rule set that provides users with flexibility in searching for other lipid classes, intensity rules, and an adaptable range of hydroxylation stages. The selling point of LDA is its aim to prevent overannotation by only assigning structural details backed by spectral evidence.

In this project, LDA is used to annotate the fragments in the MS2 spectrum to generate an ID list of all found GIPCs. These GIPCs need to be detected across all three modes (negative/positive CID and positive EAD) to confirm the species, at least at the molecular species level.

LISTING 2.1: GIPC fragmentation file for +H detection of lipids with the OH functional group in series A. All fragmentation rules written and improved by Lisa Panzeböck and Marlene Pühringer (University of Vienna, Vienna, Austria).

```
[GENERAL]
AmountOfChains=2
AmountOfLCBs=1
ChainLibrary=fattyAcidChains.xlsx
LCBLibrary=dLCB.xlsx
CAtomsFromName=D*(\d+):\d+
DoubleBondsFromName=D*\d+:(\d+)
BasePeakCutoff=0.05%
ChainCutoff=10%
SpectrumCoverage=10%
FaHydroxylationRange=0-1
LcbHydroxylationRange=2-3
RetentionTimePostprocessing=true
SingleChainIdentification=true

[HEAD]
!FRAGMENTS
Name=Precursor      Formula=$PRECURSOR      Charge=1 MSLevel=2  mandatory=false
Name=C3P03_B1_437   Formula=C12H22O15P      Charge=1 MSLevel=2  mandatory=false
Name=Z0              Formula=$PRECURSOR-C18H31O20P Charge=1 MSLevel=2  mandatory=false
Name=ZOmH2O          Formula=$PRECURSOR-C18H33O21P Charge=1 MSLevel=2  mandatory=false
Name=B2_hydroxy      Formula=C12H19O11       Charge=1 MSLevel=2  mandatory=false
Name=B1_hydroxy      Formula=C6H11O5         Charge=1 MSLevel=2  mandatory=false
Name=C2_hydroxy      Formula=C12H21O12       Charge=1 MSLevel=2  mandatory=false
Name=IP              Formula=C6H14O9P        Charge=1 MSLevel=2  mandatory=false

[CHAINS]
!FRAGMENTS
Name=W              Formula=$LCB-HO          Charge=1 MSLevel=2  mandatory=false  oh=1,2,3
Name=W-H2O          Formula=$LCB-H3O2        Charge=1 MSLevel=2  mandatory=false  oh=2,3
Name=W-2H2O         Formula=$LCB-H5O3        Charge=1 MSLevel=2  mandatory=false  oh=3
```

Name=W_MS3	Formula=\$LCB-HO	Charge=1	MSLevel=3	mandatory=false	oh=1,2,3
Name=W-H2O_MS3	Formula=\$LCB-H3O2	Charge=1	MSLevel=3	mandatory=false	oh=2,3
Name=W-2H2O_MS3	Formula=\$LCB-H5O3	Charge=1	MSLevel=3	mandatory=false	oh=3
Name=V	Formula=\$CHAIN+H2N	Charge=1	MSLevel=2	mandatory=false	
Name=V_2	Formula=\$CHAIN+HN	Charge=1	MSLevel=2	mandatory=false	
Name=V_MS3	Formula=\$CHAIN+H2N	Charge=1	MSLevel=3	mandatory=false	
Name=V_MS3_2	Formula=\$CHAIN+HN	Charge=1	MSLevel=3	mandatory=false	

LISTING 2.2: GIPC fragmentation file for LDA -H detection of lipids with the OH (series A). All fragmentation rules written and improved by Lisa Panzeböck and Marlene Pühringer (University of Vienna, Vienna, Austria).

```
[GENERAL]
AmountOfChains=2
AmountOfLCBs=1
ChainLibrary=fattyAcidChains.xlsx
LCBLibrary=dLCB.xlsx
CAtomsFromName=D*(\d+):\d+
DoubleBondsFromName=D*\d+:(\d+)
BasePeakCutoff=0.05%
ChainCutoff=10%
SpectrumCoverage=10%
FaHydroxylationRange=0-1
LcbHydroxylationRange=2-3
RetentionTimePostprocessing=true
SingleChainIdentification=true

[HEAD]
!FRAGMENTS
Name=Precursor      Formula=$PRECURSOR      Charge=1 MSLevel=2  mandatory=false
Name=C3P03_B1_437   Formula=C12H22015P      Charge=1 MSLevel=2  mandatory=false
Name=Z0              Formula=$PRECURSOR-C18H31020P Charge=1 MSLevel=2  mandatory=false
Name=Z0mH2O          Formula=$PRECURSOR-C18H33021P Charge=1 MSLevel=2  mandatory=false
Name=B2_hydroxy      Formula=C12H19011       Charge=1 MSLevel=2  mandatory=false
Name=B1_hydroxy      Formula=C6H1105         Charge=1 MSLevel=2  mandatory=false
Name=C2_hydroxy      Formula=C12H21012       Charge=1 MSLevel=2  mandatory=false
Name=IP              Formula=C6H1409P        Charge=1 MSLevel=2  mandatory=false

[CHAINS]
!FRAGMENTS
Name=W              Formula=$LCB-HO          Charge=1 MSLevel=2  mandatory=false  oh=1,2,3
Name=W-H2O          Formula=$LCB-H3O2        Charge=1 MSLevel=2  mandatory=false  oh=2,3
Name=W-2H2O         Formula=$LCB-H5O3        Charge=1 MSLevel=2  mandatory=false  oh=3
Name=W_MS3          Formula=$LCB-HO          Charge=1 MSLevel=3  mandatory=false  oh=1,2,3
Name=W-H2O_MS3      Formula=$LCB-H3O2        Charge=1 MSLevel=3  mandatory=false  oh=2,3
Name=W-2H2O_MS3     Formula=$LCB-H5O3        Charge=1 MSLevel=3  mandatory=false  oh=3
Name=V              Formula=$CHAIN+H2N       Charge=1 MSLevel=2  mandatory=false
Name=V_2            Formula=$CHAIN+HN        Charge=1 MSLevel=2  mandatory=false
Name=V_MS3          Formula=$CHAIN+H2N       Charge=1 MSLevel=3  mandatory=false
Name=V_MS3_2        Formula=$CHAIN+HN        Charge=1 MSLevel=3  mandatory=false
```

2.6.2 Skyline

Skyline, a tool initially developed for targeted mass spectrometry (MS) proteomics workflows, is used in this project to integrate detected GIPCs. The software gained popularity as an open-source, vendor-independent tool that facilitates the transfer of methods between different labs and instrument platforms for data processing⁴¹. In this case, after identifying GIPCs through their MS2 fragments, a transition list was edited, and the chromatograms of the identified GIPCs were manually reviewed. The corresponding areas were then exported for further data analysis.

2.6.3 Other Software Tools

Fragmentor, an extension of the Sciex Software Program, was used to search for fragments not detected by LDA. This approach was inspired by the work of Panzeböck et al.⁵ and Pühringer et al.¹³, who identified additional fragments using Orbitrap-methods. Important considerations include the age of the material and the technical limitations of the ZenoTOF instrument. The goal was to identify fragments that could reveal additional structural features of the lipid species, such as the positions of double bonds. To enable the use of Fragmentor, the chemical structures were drawn in ChemDraw (Version 19.1).

2.7 Data Preprocessing

First and foremost, after parsing the sample identifiers to identify time points, wine type, and condition, the average background signal from blank samples was calculated and subtracted from each molecule's area. Negative values were set to zero, and the areas were normalized (see normalization description below). A log transformation was then applied to the area values. This transformation reduces heteroscedasticity and enhances the robustness of downstream statistical analyses such as ANOVA and network construction. Missing values were imputed using the formula: $(\text{min_value} - 1)$.

2.7.1 Normalization Methods

The multi-factor normalization approach included three factors: the amount of internal standard (LacCer), the dry weight, and the protein content. Scaling was incorporated to preserve the magnitude of values throughout the process. This was achieved by calculating the median values for each normalization factor and applying sequential normalization using scaling factors. This method ensures that the normalized values do not become too small, maintaining a reasonable value range.

Dry Weight Determination

One critical factor is the varying water content across samples. Every fifth replicate from each sampling point, wine type, and condition was selected. Approximately 100 mg of frozen, homogenized leaf samples were weighed and dried in an oven at 100°C for approximately 20 hours, after which the dry weight was determined.

As seen in Figure 2.4, the dry weight ratios under stress conditions were mostly lower than those under control conditions, indicating a lower water content in stressed samples. The exceptions were the S2_GV and S5_R samples.

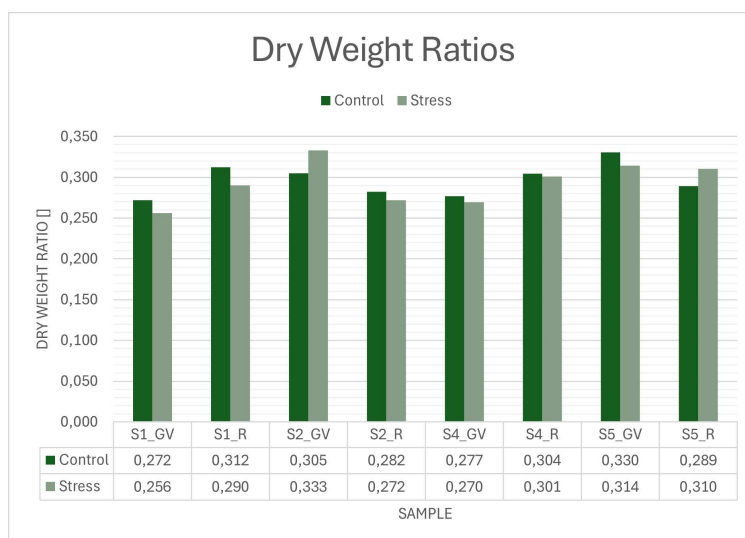


FIGURE 2.4: Comparison of dry weight ratios between control and heat-stressed leaf samples across different time points and wine types. Lower ratios in stressed samples indicate reduced water content due to heat exposure.

Protein Determination

Another normalization to consider is the protein content in the samples. Pellets containing proteins and other cell wall materials such as cellulose, hemicellulose, and pigments (chlorophylls, etc.) were stored at -80°C before the sample preparation. A 0.2 M NaOH solution was prepared, and the dried protein pellets were resuspended in 1 mL of this solution. The mixture was then shaken overnight at 40°C . Before shaking, the supernatants were green, likely due to chlorophyll, but overnight they turned slightly brownish, indicating possible chlorophyll degradation and/or polyphenol oxidation. On the next day, 1:10 and 1:100 dilutions were prepared, along with the BSA standards for the calibration row and the working reagent (Micro BCA Protein Assay Kit, Thermo Scientific). All solutions were transferred into 96 well plates (Greiner CELLSTAR 96 well plates), shaken, incubated at 37°C for 1.5 hours, and sent to the Food Chemistry and Toxicology Department of the University of Vienna to be measured using a BioTek SynergyH1 microplate reader (supervised by Sonja Hager) and Gen5 software. It should be noted that pigment interference or Cu^{2+} reduction by plant phenolics may still be present. However, as this step is used solely for normalization, the resulting absorbance differences were still considered valid for use. The calibration curve (Figure 2.5) showed a better R^2 when fitted with the exponential function compared to the linear one. This exponential model was therefore used to calculate the protein content of the samples.

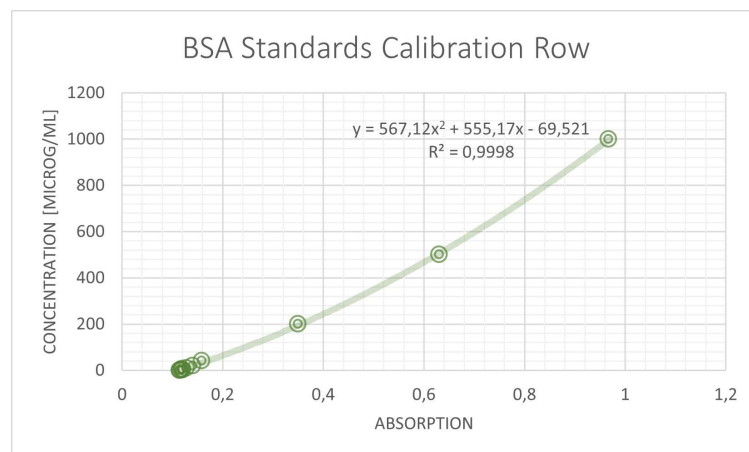


FIGURE 2.5: BSA calibration curve used for protein quantification. The data were fitted with an exponential model, and was subsequently used to determine protein concentrations in the wine leaf samples.

2.8 Data Quality Assessment

2.8.1 Calculated Resolution

The mass resolution R is characterized as:

$$R = \frac{m}{\Delta m} \quad (2.1)$$

where m is the m/z value of the peak and Δm the full width at half maximum (FWHM).

The replicates of the sample *S1_GV_c_4* were selected. Their .wiff files were converted to .mzML format using ProteoWizard's msconvert tool, and a Python script was written to calculate the FWHM-based resolution using pyteomics, mzml, and scipy. The script computes the average resolution across the entire spectrum for both MS1 and MS2, as well as the resolution at m/z 1262 (± 0.5 Da tolerance), since this is the precursor ion for the most abundant GIPC species in our samples: A-OH-18:1;O3/24:0;O. The results are shown in Table 2.4. ZenoTOF instruments are known to achieve a resolution of over 42,000 at m/z 956, which was also tested to assess instrument performance (see Table 2.5). Although the resolution for MS1 is higher at m/z 956, the MS2 spectra unfortunately showed significantly lower resolution.

TABLE 2.4: Mass Resolution for MS1 and MS2: Average and m/z 1262

Sample	Avg MS1	Avg MS2	1262_MS1	1262_MS2
S1_GV_c_4_rep1	22671.8	31654.9	23576.2	27010.1
S1_GV_c_4_rep2	21620.3	41446.2	22074.2	35535.9
S1_GV_c_4_rep3	21529.9	37532.3	22342.1	32347.5
S1_GV_c_4_rep4	20914.3	34181.0	21328.6	35754.4
Average	21684.1	36203.6	22330.3	32662.0
StdDev	631.7	3675.3	809.5	3530.9

TABLE 2.5: Mass Resolution for MS1 and MS2: m/z 956

Sample	956_MS1	956_MS2
S1_GV_c_4_rep1	32917.2	18131.3
S1_GV_c_4_rep2	30288.8	16856.3
S1_GV_c_4_rep3	30422.9	19940.2
S1_GV_c_4_rep4	29639.2	15196.5

Both the average and the resolution at m/z 1262 showed lower values for the MS1 spectra, typically ranging from 20,000 to 24,000. In contrast, the MS2 spectra exhibited higher resolution, with the second replicate reaching an average of 41,446.18. However, it is worth noting that the standard deviation was significantly smaller than that in MS2 (around one-fifth), indicating more consistent resolution in MS1 despite its lower values.

2.8.2 Resolution Comparison Between EAD and CID

Since the application of EAD is less common than CID positive, we decided to expand the resolution analysis across a wider m/z range to investigate the differences between CID and EAD. Resolution values were extracted every 200 Da from m/z 500 to 2,000 and plotted using .mzml files from the pooled control sample *GIPC_Control_Pool*.

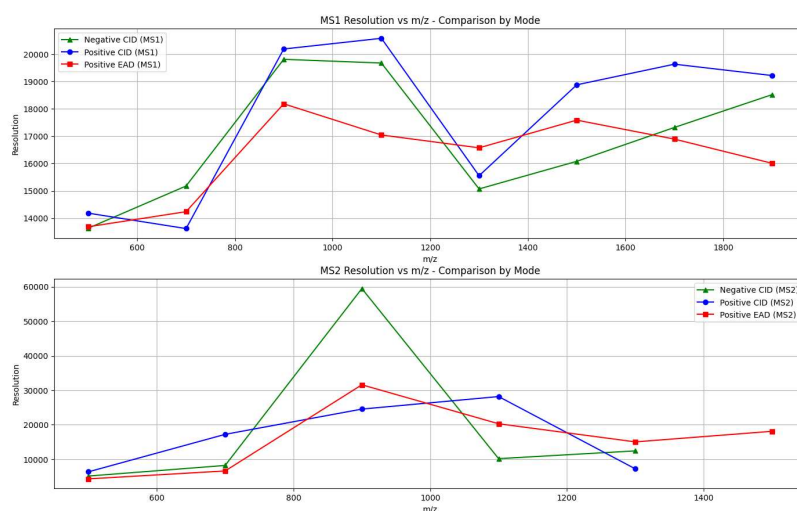


FIGURE 2.6: Comparison of MS1 and MS2 mass resolution across acquisition modes (CID negative, CID positive, and EAD positive) in 200 Da intervals from m/z 500 to 2,000.

As shown in Figure 2.6, MS1 resolution reached values above 20,000 across all modes. Positive CID mode showed the highest MS1 resolution in the m/z ranges 900 - 1,100 and 1,500 - 2,000. Negative CID followed a similar trend but with generally lower values. EAD in positive mode, although having lower resolution in certain regions, proved to be most consistent after m/z 900. All three acquisition modes displayed low resolution below m/z 700 and a drop at around m/z 1,300.

For MS2 spectra, negative CID peaked at a resolution of nearly 60,000 around m/z 900, then dropped to 10,000 at m/z 1,100. Both positive CID and EAD reached their highest resolution values near 30,000, after which the resolution decreased. The high peak in case of negative CID (MS2), almost reaching 60,000, raised questions about whether it was an outlier; however, this resolution peak was observed in other samples such as *GV_Stress_Pool*.

2.8.3 Technical Replicates Comparison

To assess measurements consistency across technical replicates, one pooled sample (*GV_Control_Pool_pos_CID*) in CID mode was measured three times. All detected molecular areas in Skyline were normalized by the internal LacCer standard. The mean, standard deviation, relative abundance (percentage), and relative standard deviation (RSD) were calculated. Lower RSD values indicate better reproducibility between the technical replicates.

TABLE 2.6: Summary of technical replicates including mean values, standard deviation (Std), percentage, and relative standard deviation (RSD).

Molecule	Mean	Std	Percentage	RSD
A-OH-18:0;O3/22:0;O	0.4167	0.0153	4.2	3.66
A-OH-18:0;O3/23:0;O	0.3623	0.0142	3.6	3.91
A-OH-18:0;O3/24:0;O	0.9167	0.0256	9.2	2.8
A-OH-18:0;O3/25:0;O	0.1987	0.0132	2.0	6.64
A-OH-18:1;O3/22:0	0.0988	0.0026	1.0	2.59
A-OH-18:1;O3/23:0	0.1623	0.0098	1.6	6.05
A-OH-18:1;O3/24:0	0.2771	0.0067	2.8	2.41
A-OH-18:1;O3/25:0	0.1219	0.0077	1.2	6.36
A-OH-18:1;O3/20:0;O	0.0933	0.0035	0.9	3.77
A-OH-18:1;O3/21:0;O	0.0655	0.0033	0.7	5.11
A-OH-18:1;O3/22:0;O	1.6444	0.054	16.5	3.28
A-OH-18:1;O3/23:0;O	1.3848	0.0336	13.9	2.43
A-OH-18:1;O3/24:0;O	3.4881	0.0943	35.0	2.7
A-OH-18:1;O3/25:0;O	0.7561	0.0185	7.6	2.44

The **average RSD** across all molecules in the three replicates was **3.87%**, which is still considered a good level of reproducibility.

2.8.4 Extraction Replicates Comparison

Additionally, three extraction replicates of the sample *S1_GV_c_4* were randomly taken during the experimental phase. Their RSD values, with and without different normalization strategies, are compared in Table 2.7. To enhance readability, lipid species are listed in descending order of their signal intensities.

The data show that without any normalization steps, we obtain the lowest RSD values, with the average increasing from 27% (no normalization) to 45% after full normalization. In both the unnormalized and dry weight-normalized conditions, a trend is observed: lower-abundance species produce higher RSD values, indicating greater variability in measurement precision.

TABLE 2.7: RSD values of A-OH lipids across different normalization strategies: no normalization, only dry weight, only LacCer internal standard, and full normalization.

Molecule	No normalization			Only dry weight			Only LacCer			With normalization		
	Mean	Std	RSD [%]	Mean	Std	RSD [%]	Mean	Std	RSD [%]	Mean	Std	RSD [%]
A-OH-18:1;O3/24:0;O	3267128.0	378304.1	12	116601.6	10100.0	9	3.94	1.50	38	3.94	1.50	38
A-OH-18:1;O3/22:0;O	852890.3	190372.9	22	30344.2	5807.7	19	1.05	0.48	46	1.05	0.48	46
A-OH-18:1;O3/23:0;O	572256.0	68346.4	12	20505.1	2830.1	14	0.75	0.28	38	0.75	0.28	38
A-OH-18:0;O3/24:0;O	625090.3	73386.9	12	22300.4	1838.5	8	0.68	0.24	35	0.68	0.24	35
A-OH-18:1;O3/25:0;O	451688.7	107094.6	24	16064.0	3302.6	21	0.56	0.26	47	0.56	0.26	47
A-OH-18:0;O3/22:0;O	178659.7	42166.8	24	6354.2	1299.8	20	0.22	0.10	47	0.22	0.10	47
A-OH-18:0;O3/23:0;O	125412.0	13095.3	10	4482.7	425.1	9	0.15	0.06	37	0.15	0.06	37
A-OH-18:0;O3/25:0;O	108567.3	27799.8	26	3858.5	864.0	22	0.14	0.08	56	0.14	0.08	56
A-OH-18:1;O3/24:0	114993.3	40673.4	35	4080.0	1343.4	33	0.13	0.06	48	0.13	0.06	48
A-OH-18:1;O3/23:0	32848.0	11622.9	35	1164.5	379.7	33	0.04	0.02	55	0.04	0.02	55
A-OH-18:1;O3/25:0	35480.7	14654.1	41	1257.2	491.9	39	0.05	0.03	61	0.05	0.03	61
A-OH-18:1;O3/22:0	8111.3	5300.6	65	294.5	203.8	69	0.01	0.00	39	0.01	0.00	39
Average RSD			27			25			45			45

2.8.5 Lactosyl Ceramide Variability

During the extraction of the replicates of sample *S1_GV_c_1*, the LacCer standard was intentionally skipped to observe whether a significant amount of LacCer is naturally present. As shown in Figure 2.7, a detectable amount of LacCer appears at a retention time of approximately 15.2–15.3 minutes, corresponding to about one-twentieth (5%) of the signal observed when the LacCer standard is added. Since these values will be used for data normalization rather than absolute quantification, the analysis will proceed as planned.

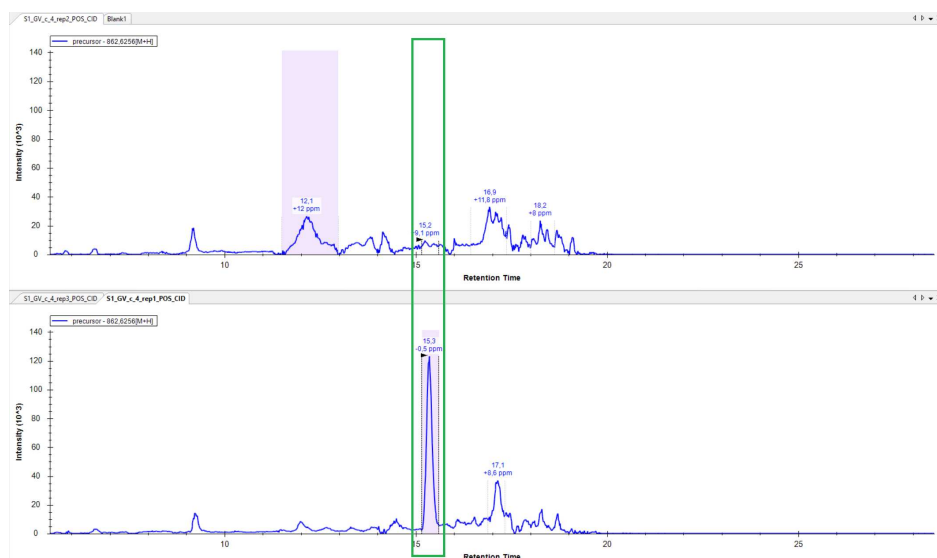


FIGURE 2.7: Chromatographic detection of LacCer in sample *S1_GV_c_1* without (top) and with (bottom) the addition of the LacCer standard.

However, from Table 2.7, it is clear that the RSD values increase significantly after normalization to the internal standard. This observation raised concerns about the consistency of the internal standard, prompting us to examine the variability of LacCer further to evaluate whether this variability might contribute to the data's variation.

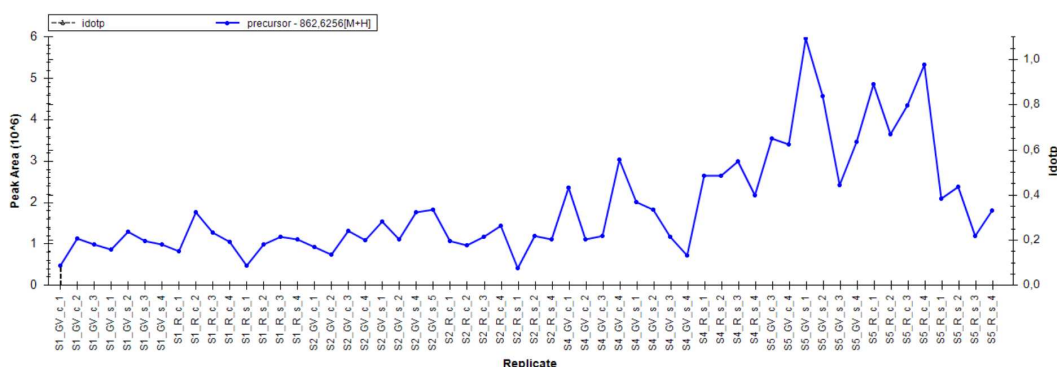


FIGURE 2.8: Peak areas of Lactosyl Ceramide samples measured in CID positive mode. Screenshot of the Skyline software.

Figure 2.9 shows the differences of LacCer between two extraction replicates *S1_GV_c_2* and *S1_GV_c_3*. The internal standard displays a noticeably different intensity ratio compared to the

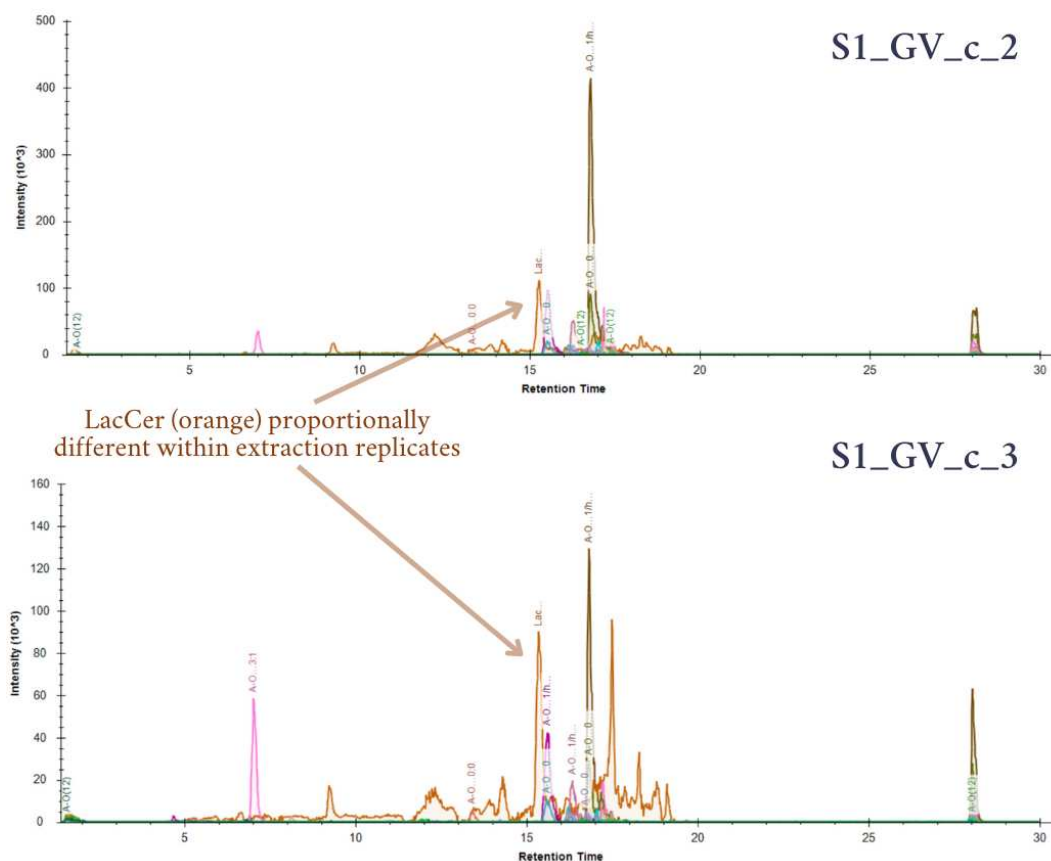


FIGURE 2.9: Spectral comparison between extraction replicates *S1_GV_c_2* and *S1_GV_c_3*, highlighting the variability of the Lactosyl Ceramide internal standard relative to other detected lipids..

rest of the lipid profile. Additionally, all peak areas across individual samples measured in positive CID mode are plotted in Figure 2.8. There, a trend appears, with variability increasing even more, especially in samples measured later in the sequence, which could suggest reduced accuracy in later measurements. Neither the low concentration of the internal standard nor carry-over seem to be the issue, since the intensities were sufficient, and LacCer was not detected in technical blanks.

2.8.6 Suggestions for Optimal Strategy

Based on the assessment of different normalization steps and the evaluation of data quality, here is a short summary:

- After testing multiple normalization strategies, normalizing to dry weight emerged as the most robust one. It consistently resulted in lower RSD values across lipid species.
- The use of LacCer as an internal standard introduced high variability, which likely contributes to high RSD values. This suggests that LacCer may not be a suitable internal standard under the current experimental conditions. Future workflows might consider either finding a more stable standard or normalizing only to the dry weight.

- Normalization to protein was also considered as an alternative and is discussed later in Section 3 (under *Static Data Analysis*). The summary of all findings is to be found in Section 4.
- Additionally, a trend of increasing variability was observed in samples measured later in the LC-MS run sequence, indicating reduced accuracy and compromising data quality.

2.9 Static Data Analysis

A JSON file containing the normalized values was loaded, and the data was scaled using Standard Scaler before performing PCA. Visualizations of PC1 vs. PC2 (as well as PC1 vs. PC3 and PC2 vs. PC3) were generated. Samples were consistently color-coded by wine type and condition.

The next step was hierarchical clustering of both samples and lipid species. Ward's method with Euclidean distance was applied, and a heatmap visualization with dendrograms was created.

Lastly, a two-way ANOVA analysis was performed to analyze each molecule individually, including significant effects and interactions (Time $\times$ Condition, Time $\times$ Wine, and Condition $\times$ Wine). The Benjamini-Hochberg FDR multiple testing correction was applied, and interaction plots for significant molecules were constructed. These steps form the statistical groundwork, which is followed by the temporal and network-based workflow. Principal component analysis (PCA) and data preprocessing were performed using the `scikit-learn` library. For statistical analyses, the `statsmodels` package was utilized for the ANOVA step, visualizations were created with `seaborn` and `matplotlib`, and `pandas` was used for general data manipulation and organization.

2.10 Dynamic Data Analysis

For the dynamic data analysis, lipid-lipid correlation networks were constructed based on grouped data by time, wine type, and condition. Pearson correlation coefficients were calculated for all lipid pairs, with significance filtering applied ($|r| \geq 0.7$, $p < 0.05$). The coefficient of variation (CV) was also calculated from individual sample values.

Significant correlations were then used to create graphs, where lipids served as nodes and edges represented the correlations. Eigenvector centrality was calculated for each node, and a color gradient was applied to indicate the centralities for each lipid. This network-based approach was inspired by a recent study by Silva et al.⁴², where lipidomics correlations were also modeled using the `NetworkX` package. This method allows for the identification of subnetwork structures and lipid co-regulation patterns, which would not be visible using univariate statistics.

2.11 User Interface and Workflow Automation

A User Interface was built using a simple web-based architecture with a Python backend and a web frontend. The backend was developed with `FastAPI`, a Python web framework known for its high performance and automatic API documentation⁴³. The main application structure includes static files (HTML, CSS, and JavaScript), a data directory for raw data and results, and a processing pipeline with separate endpoints for different types of analysis.

The data upload system consists of a simple file upload interface for Skyline exports and normalization files (e.g., dry weights, protein content). The analysis pipeline is divided into the normalization stage, where the user can choose whether to skip the protein normalization or not, the static analysis (PCA, hierarchical clustering, ANOVA), and the dynamic analysis (network analysis). The results viewing system has dedicated HTML pages for each analysis type (`static.html` and `dynamic.html`). There is an additional pipeline overview available at `pipeline.html`, which helps users understand the steps of each analysis.

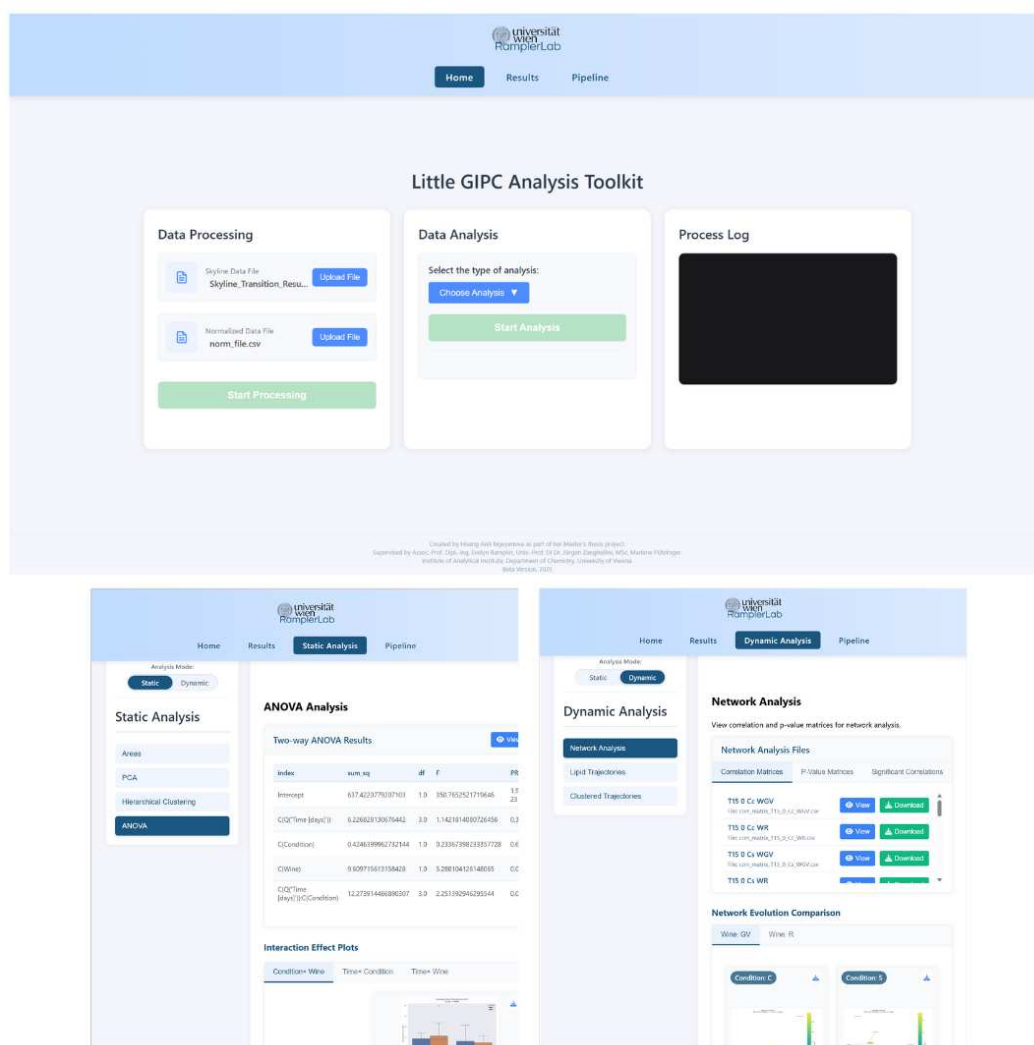


FIGURE 2.10: Screenshot of the web-based user interface developed for automated GIPC data analysis (GIPC Analysis Toolkit).

The application automatically opens a browser window and runs on localhost port 5000. Threading is used to open the browser after server startup. The main purpose of this side project was to create a user-friendly web interface tailored specifically for this project, particularly in terms of handling identifiers. Despite its specificity, the tool offers a comprehensive overview of the entire analysis workflow with visually appealing plot outputs, including GIFs and condition comparisons. The interface is also easily editable for future use.

Chapter 3

Results

3.1 Introduction to GIPC Identification

Matching MS2 spectra across all acquisition modes provides the most robust characterization of the GIPC species. In this study, however, we will identify the species on multiple identification levels. These are categorized into two groups: partially resolved lipids and putatively identified lipids. The highest confidently partially resolved lipids will correspond most closely to identification Level C from the framework described by Rampler et al.²⁹. In Figure 3.1, the annotated fragments identified by LDA are shown and will be discussed later in this chapter.

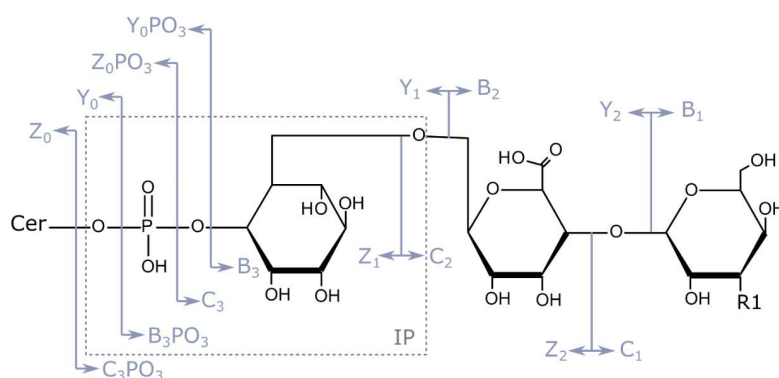


FIGURE 3.1: Structure and fragmentation description of GIPC series A (Hex(R1)-HexA-IPC) with R1 being either OH, NH_2 or NAc created by Panzeböck et al.⁴⁴.

Partially Resolved Lipids

- *a) Highest Confidence Identification*

GIPC species with matching MS2 spectra across all acquisition modes in at least some pooled samples. These lipids form the most reliable subset of the data.

- *b) Limited MS2-based Confirmation*

Species detected with only one MS2 spectrum, showing consistent retention times and ECN behavior across acquisition modes. Fragmentation information may be incomplete; however, identification is still supported by chromatographic consistency.

Putatively Identified Lipids

Lipids included in this category could not be structurally resolved beyond general class-level assignment. In particular, the fine structure—such as glycan branching or ceramide hydroxylation—could not be determined.

- *a) Individual species from the GIPC A-series* These lipids show consistent ECN behavior and retention time alignment in at least two modes, supporting their classification within the A-series.
- *b) Putative identification of higher-class GIPC series*
Entire GIPC series may be tentatively annotated if at least three lipids are detected within that series and their retention times follow a linear ECN-consistent trend.

3.2 GIPC Identification - CID Mode

3.2.1 CID Positive Mode

All samples were separately measured using the CID method in positive mode, and LDA was employed to determine the observed MS2 spectra. The CID positive mode was chosen despite EAD yielding more fragments because, although fewer fragments were observed, the carbon chains were more frequently identified, providing a more detailed description of the species.

The most common fragments observed in single samples were: IP (the main fragment confirming the identity of GIPCs), as well as Z0, Z0mH₂O, W, W-H₂O, B2_hydroxy, C3PO3_B1_459, and occasionally B1_hydroxy and B1_amine. GIPC class A was the only class in which [+Na] adducts were found. Notably, B2_hydroxy and C3PO3_B1_459 were only detectable in the [+Na] spectra. In pooled samples, where concentrations were higher, the number of detected fragments typically increased (see ID lists in Appendices).

A notable difference between [+H] and [+Na] adducts was observed for fragment Z0, which is most abundant in [+H], but appeared at much lower intensity in [+Na]. Regarding the precursor ion, in the [+H] mode, almost no precursor signal remained, whereas in the [+Na] mode, the intensity was generally higher. Another important feature was the chain identification being detected only in the [+H] mode. In contrast, in [+Na] mode, identification was limited to the molecular species level.

In higher GIPC classes (C, E, and D), patterns were observed; however, known fragments were rarely identified. These unidentified fragments will be further analyzed using Fragmentor to evaluate whether they could represent potential overannotation of the GIPC species. The results of this analysis are further described in the subsection *Unknown Fragments*.

3.2.2 CID Negative Mode

The main limitation of CID in negative mode is the significantly lower signal intensity compared to positive CID. This can be attributed to differences in ionization efficiency. GIPCs often contain amine or a hydroxyl groups, which are known to protonate easily, facilitating the formation of [M+H]⁺ and [M+Na]⁺ adducts. In contrast, the negative mode proved to be less efficient, as GIPCs are typically neutral or zwitterionic, making deprotonization more difficult. Additionally,

positive ions - particularly those from glycan moieties - are generally more stable than negative product ions, where glycosidic bonds may not break cleanly, leading to unstable intermediate ions.⁴⁵ However, the negative mode is known to complement the positive mode by providing some finer details about the structure of the species⁴⁶.

Due to very low abundancies in negative CID mode, fragments were only detected in pooled samples. Fragments observed across nearly all class A GIPCs were C3PO3_hydroxy (m/z 597) and Y1 (m/z 922). Additional fragments such as Z0PO3 (m/z 686) and IPmH2O (m/z 241) were also detected. Unfortunately, [M-2H] ions were not detected.

3.3 GIPC Identification - EAD Mode

Based on the species identified in CID positive mode, the mass inclusion lists were refined to improve fragment detection in EAD mode. Preliminary experiments already confirmed that EAD yields a higher number of fragments. By reducing the inclusion, we aimed to enhance fragment coverage.

The fact that EAD outperforms CID is not a recent statement. The key difference lies in the mechanism difference. CID is often limited to labile bonds, while EAD introduces electrons that can break bonds along the backbone (C-C; C-N) without the need for energy redistribution. This is called non-ergodic fragmentation, which results in b-, c-, x-, and z-type ions, in contrast to CID, which generates only y- and b-ions, resulting in a narrower ion map⁴⁷.

For the first time in this project, fragments from class A GIPCs with functional groups other than OH were found (specifically NAc and NH). In subclass A_NAc, the lipid A-NAc-18:1;O2/25:2 was identified with following fragments: IP (m/z 261), W-H2O(18:1;O2) (m/z 264), W(18:1;O2) (m/z 282) and C3PO3_B1 (m/z 437). In subclass A_NH, multiple lipids were observed with fragments including: IP, V (m/z 410), C3PO3_B1, Z0mH2O, and Z0.

As expected, OH species were detected most frequently. Fragmentation profiles were largely similar to those found in CID positive mode, though more fragments were observed and several new ones appeared. New identified [M+H] fragments were: B1_hydroxy (m/z 163) and W-2H2O(18:1;O3) (m/z 262). In contrast to positive CID [M+Na] mode, B1_hydroxy appeared in [M+Na], as well as C2_hydroxy (m/z 379). For a more detailed overview, see the ID lists in the Appendices.

3.4 Pooled Samples & Matching

Pools were collected separately for each wine type and condition. However, dividing the samples by these subgroups did not provide sufficient intensities for detecting MS2 spectra in the CID negative mode. Consequently, comparative analysis was limited to broader pools: *GIPC_Control_Pool* and *GIPC_Stress_Pool*.

Notably, the control pool yielded more MS2 spectra in the CID negative mode than the stress pool. Moreover, only species containing a double bond were detected under these conditions. Additionally, retention times slightly deviated from the other modes with shifts of up to 0.4 minutes in the case of A-OH-18:1;O3/24:0 and A-OH-18:1;O3/24:0;O (*GIPC_Stress_Pool*). Despite these limitations, the CID negative mode still provided helpful additional evidence for identification.

A total of 12 GIPCs were classified with the highest confidence and used for the data analysis described later in this chapter. A full overview of the pooled samples and matched acquisition modes is provided in Table 3.2, with lipids identified with the highest confidence in bold and those with limited MS2-based confirmation in *italics*.

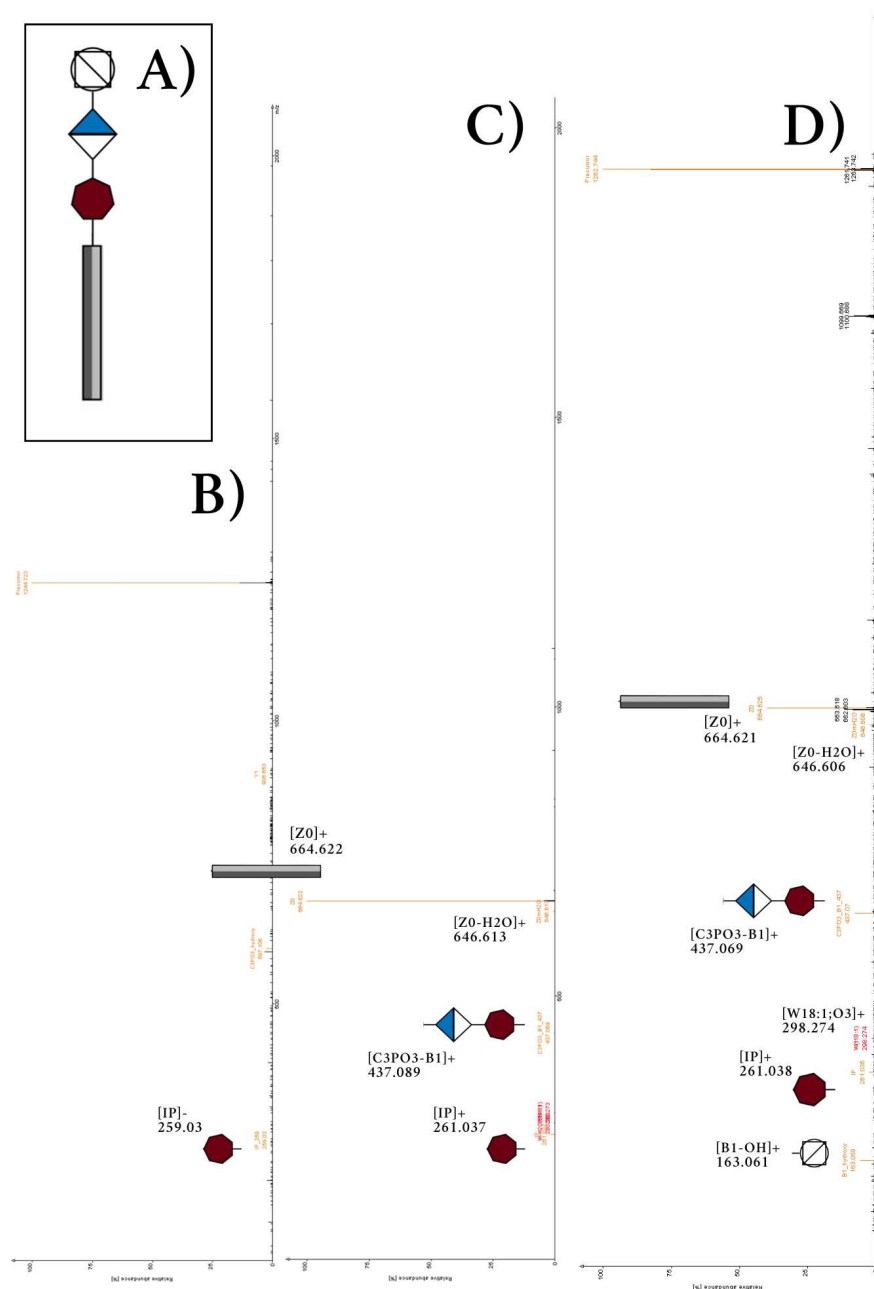


FIGURE 3.2: a) Schematic structure of GIPC A (Hex(R1)-HexA-IPC), illustrated by Pühringer et al.¹³ (see description in Figure 1.2); b) MS2 spectrum of A-OH-18:1;O3/24:0 acquired in CID negative mode; c) MS2 spectrum of the same species in CID positive mode; d) MS2 spectrum acquired in EAD positive mode.

TABLE 3.1: GIPC ID List: Pooled control and stress samples with matching retention times across all three acquisition modes for **Partially Resolved lipids**. Lipids with the highest confidence identification are shown in **bold**, while lipids with limited MS2-based confirmations are in *gray italics*.

Composition	CID positive			EAD positive			CID negative		
	m/z	Rt (min)	Fragments MS2	m/z	Rt (min)	Fragments MS2	m/z	Rt (min)	Fragments MS2
GIPC_Control_Pool Sample									
<i>A-NH-18:0;O2/24:1</i>				<i>1251.739</i>	<i>13.93</i>	<i>IP:261.034, V_2(24:1):381.363, C3PO3_B1_437:437.068, Z0mH2O:636.593</i>			
<i>A-NH-18:0;O2/26:1</i>				<i>1279.766</i>	<i>16.25</i>	<i>IP:261.035, V(26:1):410.395, C3PO3_B1_437:437.068, Z0mH2O:664.622</i>			
<i>A-NH-18:0;O2/27:1</i>				<i>1279.782</i>	<i>16.61</i>	<i>IP:261.036, V_2(27:1):423.065, C3PO3_B1_437:437.065, Z0mH2O:678.642, Z0:696.657</i>			
<i>A-OH-38:1;O4</i>				<i>1136.572</i>	<i>17.19</i>	<i>V_2(19:1):311.274</i>			
<i>A-OH-40:1;O3</i>				<i>1218.706</i>	<i>14.93</i>	<i>B1_hydroxy:163.06, IP:261.038, Z0:620.595</i>			
A-OH-18:0;O3/22:0;O	1236.7161	15.51	IP:261.035, W-H₂O(18:0;O3):282.278, Z0mH₂O:620.591, Z0:638.604	1236.7194	15.46	B1_hydroxy:163.058, IP:261.036, W-H₂O(18:0;O3):282.286, W(18:0;O3):300.304, C3PO3_B1_437:437.068, Z0mH₂O:620.62, Z0:638.589			
A-OH-18:1;O3/22:0	1218.7104	14.69	IP:261.031, W-H₂O(18:1;O3):280.259, W(18:1;O3):298.272, C3PO3_B1_437:437.086, Z0:620.595	1218.7078	14.65	W(18:1;O3):298.275, C3PO3_B1_437:437.065, Z0:620.591			
A-OH-18:1;O3/22:0;O	1234.7059	14.14	IP:261.037, W-H₂O(18:1;O3):280.261, W(18:1;O3):298.276, C3PO3_B1_437:437.07, Z0mH₂O:618.585, Z0:636.591	1234.7058	14.26	B1_hydroxy:163.061, IP:261.035, W-H₂O(18:1;O3):280.259, W(18:1;O3):298.279, C3PO3_B1_437:437.073, Z0mH₂O:618.577	1232.6887	14.61	C3PO3_hydroxy:597.103, Z0PO3:714.532, Y1:894.613
A-OH-18:0;O3/23:0;O	1250.7369	16	IP:261.037, W-H₂O(18:0;O3):282.28, C3PO3_B1_437:437.067, Z0mH₂O:634.61, Z0:652.62	1250.7437	16.01	B1_hydroxy:163.063, IP:261.041, W(18:0;O3):300.327, C3PO3_B1_437:437.074, Z0mH₂O:634.61, Z0:652.617			

Composition	m/z	Rt (min)	Fragments MS2	m/z	Rt (min)	Fragments MS2	m/z	Rt (min)	Fragments MS2
A-OH-18:1;O3/23:0	1232.7221	15.71	IP:261.033, W-2H ₂ O(18:1;O3):262.253, W-H ₂ O(18:1;O3):280.264, W(18:1;O3):298.277, Z0mH ₂ O:616.636, Z0:634.614	1232.729	15.66	B1_hydroxy:163.058, IP:261.041, W(18:1;O3):298.272, C3PO3_B1_437:437.065, Z0:634.613	1232.7383	15.75	C3PO3_hydroxy:597.103
A-OH-18:1;O3/23:0;O	1248.7211	15.13	IP:261.035, W-H ₂ O(18:1;O3):280.259, W(18:1;O3):298.276, C3PO3_B1_437:437.07, Z0mH ₂ O:632.596, Z0:650.608	1248.7209	15.5	B1_hydroxy:163.057, IP:261.033, W(18:1;O3):298.287, C3PO3_B1_437:437.076, Z0mH ₂ O:632.595, Z0:650.603	1246.7031	15.69	C3PO3_hydroxy:597.103, Y1:908.623
A-OH-18:0;O3/24:0;O	1264.7484	16.59	IP:261.035, W-H ₂ O(18:0;O3):282.28, W(18:0;O3):300.29, Z0mH ₂ O:648.626, Z0:666.64	1264.75	16.67	B1_hydroxy:163.061, IP:261.035, C3PO3_B1_437:437.073, Z0mH ₂ O:648.618, Z0:666.643			
A-OH-18:1;O3/24:0	1246.7382	16.36	IP:261.256, W-H ₂ O(18:1;O3):280.278, W(18:1;O3):298.266, Z0mH ₂ O:630.645, Z0:648.626	1246.7397	16.47	B1_hydroxy:163.06, IP:261.036, W-H ₂ O(18:1;O3):262.251, W-H ₂ O(18:1;O3):280.258, W(18:1;O3):298.278, C3PO3_B1_437:437.071, Z0mH ₂ O:630.607, Z0:648.628	1244.7234	16.73	IP_259:259.03, C3PO3_hydroxy:597.106, Y1:906.653
A-OH-18:1;O3/24:0;O	1262.7322	16.66	IP:261.163, W-H ₂ O(18:1;O3):280.261, W(18:1;O3):298.273, C3PO3_B1_437:437.073, Z0mH ₂ O:646.628, Z0:664.626	1262.7343	16.33	B1_hydroxy:163.061, IP:261.038, W-H ₂ O(18:1;O3):280.266, W(18:1;O3):298.271, C3PO3_B1_437:437.069, Z0mH ₂ O:646.609, Z0:664.621	1260.7201	16.45	C3PO3_hydroxy:597.107, Y1:922.629
A-OH-18:0;O3/25:0;O	1278.7637	16.98	B1_hydroxy:163.061, IP:261.038, W-H ₂ O(18:0;O3):282.29, W(18:0;O3):300.285, Z0mH ₂ O:662.64, Z0:680.654	1278.7599	17.03	B1_hydroxy:163.06, IP:261.038, C3PO3_B1_437:437.067, Z0mH ₂ O:662.624, Z0:680.653			
A-OH-18:1;O3/25:0	1260.7567	16.86	IP:261.037, W-H ₂ O(18:1;O3):280.261, W(18:1;O3):298.274, Z0:662.639						

Composition	m/z	Rt (min)	Fragments MS2	m/z	Rt (min)	Fragments MS2	m/z	Rt (min)	Fragments MS2
A-OH-18:1;O3/25:0;O	1276.7438	16.98	IP:261.038, W-H ₂ O(18:1;O3):280.264, W(18:1;O3):298.264, B2_hydroxy:339.299, C3PO3_B1_437:437.073, Z0mH ₂ O:660.647, Z0:678.642	1276.7496	16.71	B1_hydroxy:163.06, IP:261.04, W(18:1;O3):298.272, C3PO3_B1_437:437.064, Z0mH ₂ O:660.64, Z0:678.637	1274.7318	16.96	C3PO3_hydroxy:597.114, Y1:936.643
GIPC_Stress_Pool Sample									
A-NAc-39:0;O4				1263.729	17	IP:261,033, C3PO3_B1_437:437.067			
A-NH-18:1;O3/23:1				1251.72	13.93	IP:261,033, W-2H ₂ O(18:1;O3):262.246, W-H ₂ O(18:1;O3):280.264, W(18:1;O3):298.277, Z0mH ₂ O:636.588			
A-NH-18:1;O3/25:1 A-OH-39:1;O4	1279.7523	16.39	Z0mH ₂ O:664.62, Z0:682.626	1220.685	13.1	B1_hydroxy:163.056, IP:261.290, C3PO3_B1_437:437.061, Z0:622.57			
A-OH-18:0;O3/22:0;O	1236.7203	15.14	IP:261.035, W-H ₂ O(18:0;O3):282.282, C3PO3_B1_437:437.085, Z0mH ₂ O:620.582, Z0:638.606	1236.7161	15.07	B1_hydroxy:163.057, IP:261.037, W(18:0;O3):300.286, C3PO3_B1_437:437.081, Z0mH ₂ O:620.596, Z0:638.594			
A-OH-18:1;O3/22:0;O	1234.7024	14.65	IP:261.035, W-2H ₂ O(18:1;O3):262.248, W-H ₂ O(18:1;O3):280.264, W(18:1;O3):298.271, C3PO3_B1_437:437.073, Z0mH ₂ O:618.572, Z0:636.592	1234.7019	14.22	B1_hydroxy:163.059, IP:261.038, W(18:1;O3):298.269, C3PO3_B1_437:437.072, Z0mH ₂ O:618.569, Z0:636.594	1232.6814	14.64	C3PO3_hydroxy:597.098, Z0PO3:714.542, Y1:894.597
A-OH-18:0;O3/23:0;O	1250.7367	16.03	IP:261.035, W-H ₂ O(18:0;O3):282.278, C3PO3_B1_437:437.049, Z0mH ₂ O:634.613, Z0:652.62	1250.7347	16.05	B1_hydroxy:163.059, IP:261.038, W(18:0;O3):300.281, C3PO3_B1_437:437.070, Z0mH ₂ O:634.601, Z0:652.623			

Composition	m/z	Rt (min)	Fragments MS2	m/z	Rt (min)	Fragments MS2	m/z	Rt (min)	Fragments MS2
A-OH-18:1;O3/23:0	1232.7244	15.65	IP:261.035, W-H ₂ O(18:1;O3):280.256, W(18:1;O3):298.271, Z0mH ₂ O:616.595, Z0:634.613	1232.7188	15.71	B1_hydroxy:163.052, IP:261.029, C3PO3_B1_437:437.067, Z0:634.62	1246.696	15.75	C3PO3_hydroxy:597.098, Z0PO3:728.552, Y1:908.617
A-OH-18:1;O3/23:0;O	1248.7192	15.94	IP:261.035, W-2H ₂ O(18:1;O3):262.255, W(18:1;O3):298.276, C3PO3_B1_437:437.073, Z0mH ₂ O:632.597, Z0:650.606	1248.7125	15.90	B1_hydroxy:163.060, IP:261.031, C3PO3_B1_437:437.052, Z0mH ₂ O:632.62, Z0:650.609			
A-OH-18:0;O3/24:0	1248.7573	16.87	IP:261.037, W(18:0;O3):300.287, Z0:650.639	1246.7375	16.39	B1_hydroxy:163.055, IP:261.037, W-H ₂ O(18:1;O3):280.266, C3PO3_B1_437:437.075, Z0:648.632			
A-OH-18:0;O3/24:0;O	1264.7513	16.54	IP:261.037, W-H ₂ O(18:0;O3):282.282, Z0mH ₂ O:648.626, Z0:666.636	1264.7481	16.63	B1_hydroxy:163.063, IP:261.037, W-H ₂ O(18:1;O3):282.759, C3PO3_B1_437:437.060, Z0mH ₂ O:648.621, Z0:666.638			
A-OH-18:1;O3/24:0	1246.7379	16.38	IP:261.039, W-H ₂ O(18:1;O3):280.261, W(18:1;O3):298.273, Z0mH ₂ O:630.622, Z0:648.626	1246.7375	16.39	B1_hydroxy:163.055, IP:261.037, W-H ₂ O(18:1;O3):280.266, C3PO3_B1_437:437.075, Z0:648.632	1244.7206	16.79	C3PO3_hydroxy:597.097, Y1:906.645
A-OH-18:1;O3/24:0;O	1262.7372	16.83	IP:261.039, W-H ₂ O(18:1;O3):280.243, W(18:1;O3):298.271, C3PO3_B1_437:437.072, Z0mH ₂ O:646.617, Z0:664.622	1262.7287	16.97	B1_hydroxy:163.059, IP:261.036, W(18:1;O3):298.272, C3PO3_B1_437:437.067, Z0mH ₂ O:646.584, Z0:664.611	1260.7136	16.50	C3PO3_hydroxy:597.109, Y1:922.644
A-OH-18:0;O3/25:0;O	1278.7620	17.01	B1_hydroxy:163.051, IP:261.037, W-H ₂ O(18:0;O3):282.273, W(18:0;O3):300.285, C3PO3_B1_437:437.061, Z0mH ₂ O:662.639, Z0:680.653	1278.7689	17.02	B1_hydroxy:163.058, IP:261.031, C3PO3_B1_437:437.058, Z0mH ₂ O:662.654, Z0:680.641			

Composition	m/z	Rt (min)	Fragments MS2	m/z	Rt (min)	Fragments MS2	m/z	Rt (min)	Fragments MS2
A-OH-18:1;O3/25:0	1260.7518	16.84	IP:261.033, W(18:1;O3):298.271, C3PO3_B1_437:437.070, Z0:662.643	1260.7536	16.94	B1_hydroxy:163.059, IP:261.038, W-H ₂ O(18:1;O3):280.264, C3PO3_B1_437:437.070, Z0:662.635			
A-OH-18:1;O3/25:0;O	1276.7496	16.65	IP:261.037, W-H ₂ O(18:1;O3):280.261, W(18:1;O3):298.271, C3PO3_B1_437:437.060, Z0mH ₂ O:660.631, Z0:678.641	1276.7486	16.72	B1_hydroxy:163.059, IP:261.037, W(18:1;O3):298.279, C3PO3_B1_437:437.066, Z0mH ₂ O:660.625, Z0:678.639			
A-OH-18:1;O3/26:0;O	1290.7562	17.03	B1_hydroxy:163.147, IP:261.041, W-H ₂ O(18:1;O3):280.26, W(18:1):298.27, C3PO3_B1_437:437.069, Z0mH ₂ O:674.647, Z0:692.653						
A-OH-44:3;O4	1286.7294	16.6	IP:261.04, Z0:648.619						

3.5 Unknown Fragments - Fragmentor

In case of class A GIPCs, a consistently observed but unassigned fragment was detected at m/z around 621 with relatively high abundance - up to 75% of nearby fragment intensities ([+Na] mode). Other recurrent fragments included signals at m/z 665, 693-694 and 778-779 in the [+H] mode, as well as 667 in the [+Na] mode. Fragmentor was used and successfully identified the m/z 621 fragment as a modified C3PO3 fragment with an additional carbon bond (see Figure 3.3); the software did not recognize other fragments.

In GIPC classes C to E, certain spectral patterns were observed in the MS2 spectra, but no fragments could be definitely identified. This has prompted a focus on the identification at the MS1 level, to have at least a tentative identification of higher series in the wine leaf samples.

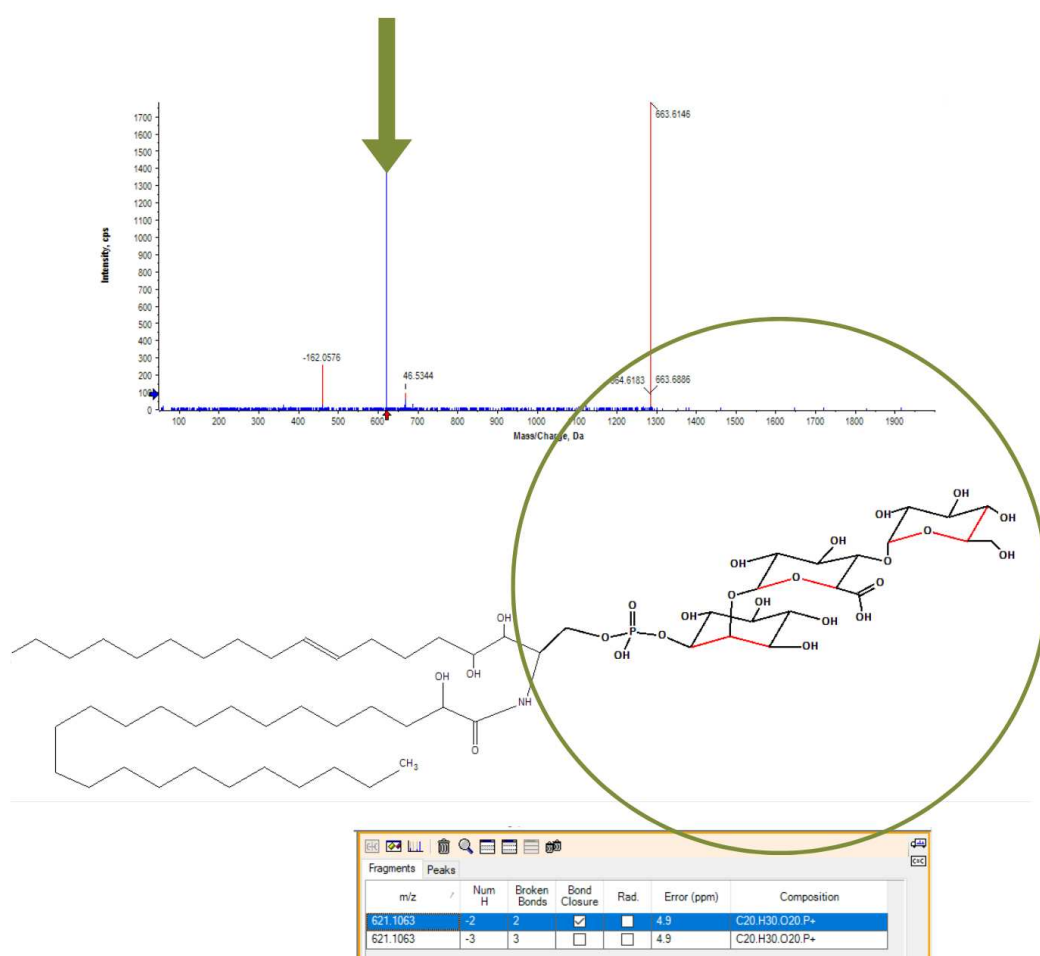


FIGURE 3.3: Fragmentor screenshot displaying the signal at m/z 621 in sample S1_GV_c_1, corresponding to the species A-OH-18:1;O3/24:0;O.

3.6 ECN Number

Numerous publications have demonstrated that employing chromatographic separation enables the prediction of retention times (RT) of compounds based on their physicochemical properties. The Effective Carbon Number (ECN) is an effect during a reversed phase (RP) separation: the species with a smaller number of carbons in the acyl chain should elute earlier, as well as with the increasing degree of unsaturation⁴⁸. This can be very practical in multiple cases, such as:

- A quick estimation and comparison between the GIPC species depending on their differing chain lengths as well as the degrees of unsaturation.
- Since these two characteristics are now clustered into one value, it is also an easier way to group the GIPCs and observe any possible patterns.
- Possible automatization (or at least a partial one) of the identification process of GIPCs, if the dataset is too big.

For this, the chain composition needs to be identified, and the ECN formula is applied:

$$ECN = (\text{Total number of Carbons}) - 2 * (\text{Total number of Double Bonds}) \quad (3.1)$$

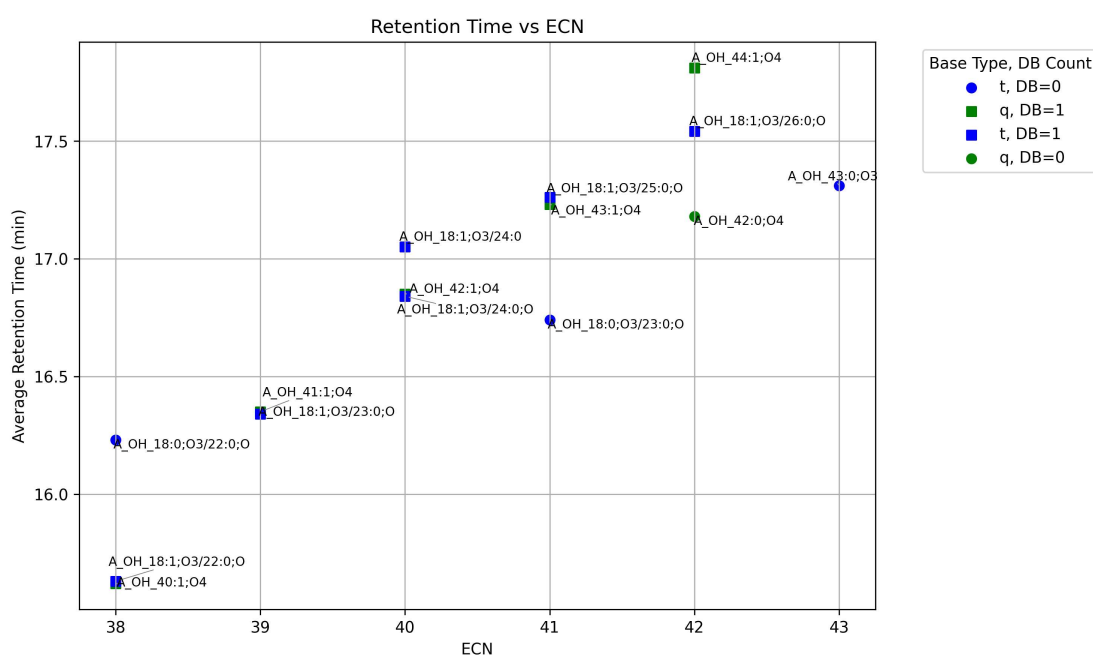


FIGURE 3.4: ECN Numbers of the GIPC-A-OH species detected in the sample *S1_GV_c_1* plotted against the retention times.

Figure 3.4 confirms the theory of the ECN model, showing us the moderately linear increase with the increase in carbon number in species with no or a single double bond. The species with the same carbon number but with a higher degree of unsaturation show earlier elution times, leading to the species A-OH-18:1;O3/22:0;O and A-OH-40:1;O4 having the shortest average retention times, and A-OH-44:1;O4 having the highest one.

3.6.1 GIPC Series Class A

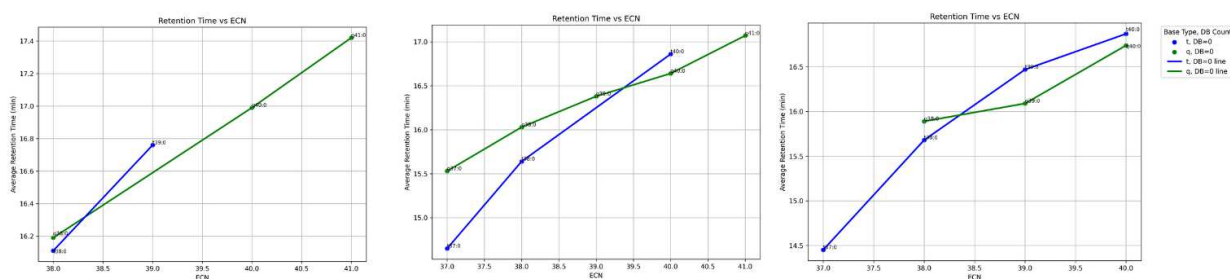
For all functional groups - NAc, NH, and OH - a linear retention time behavior was observed across all three acquisition modes, with species grouped by hydroxylation state.

A_NAc class did not exhibit any dihydroxylated species in any mode in comparison to A_NH and A_OH, where all known hydroxylation variants were consistently detected, as illustrated in Figure 3.5. Classes A_NH and A_OH also exhibit a higher number of species across all modes.

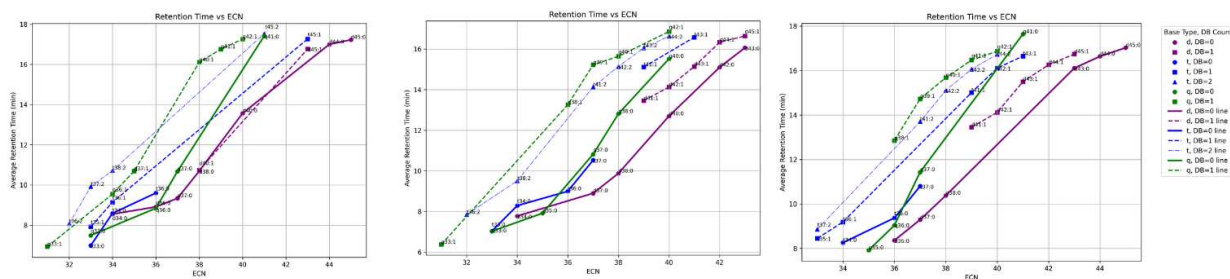
Furthermore, multiple saturation states were identified within each hydroxylation category, with ECN values reaching up to 45.

The lipids that are considered identified on putative level exhibited matching retention times in at least two modes and aligned with the linear ECN model across all three modes, as described earlier in this chapter. The complete list of species is provided in Table 3.2.

Class A - Functional Group NAc



Class A - Functional Group NH



Class A - Functional Group OH

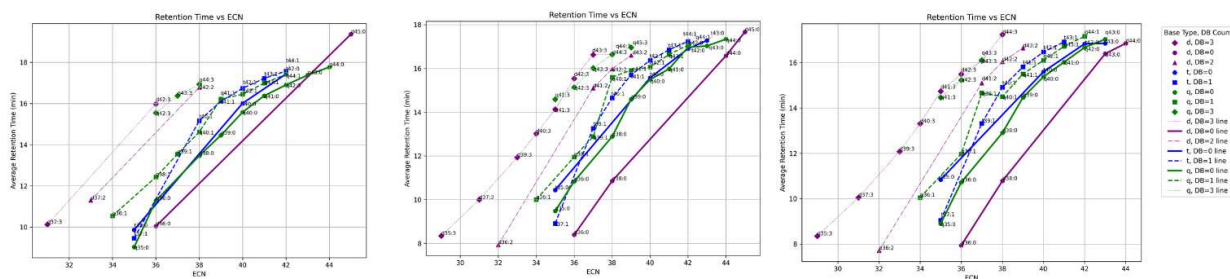


FIGURE 3.5: ECN model for GIPC Series A lipids containing functional groups NAc, NH, and OH. Shown are all detected MS1 spectra across three acquisition modes: negative mode (left), positive CID (center), and positive EAD (right).

TABLE 3.2: Identification of **putatively identified** A-series lipids under different ionization modes with matching retention times.

Lipid A_NAc	CID neg	CID pos RT [min]	EAD pos	Lipid A_NH	CID neg	CID pos RT [min]	EAD pos	Lipid A_OH	CID neg	CID pos RT [min]	EAD pos
37:0;O3		14.65	14.45	34:0;O2	8.55	7.78		36:0;O2		8.39	7.94
38:0;O3	16.11	15.64	15.68	36:0;O3	8.91		8.36	38:0;O2		10.85	10.78
39:0;O3	16.76		16.47	37:0;O2	9.34	8.9	9.29	44:0;O2		16.58	16.85
40:0;O3		16.86	16.87	38:0;O2	10.69	9.88	10.38	36:2;O2		7.93	7.71
37:0;O4		15.53	14.45	43:0;O2		16.06	16.11	41:2;O2		15.1	15.1
38:0;O4	16.19	16.03	15.89	44:0;O2	17.00		16.64	42:2;O2	16.78	15.98	16.04
39:0;O4		16.38	16.09	45:0;O2	17.23		17.03	43:2;O2		16.60	16.64
40:0;O4	16.99	16.64	16.74	41:1;O2		13.46	13.45	35:3;O2		8.34	8.35
41:0;O4	17.42	17.07		42:1;O2		14.13	14.12	37:3;O2	10.12	10.00	10.05
				43:1;O2		15.14	15.50	39:3;O2		11.93	12.08
				44:1;O2		16.33	16.26	40:3;O2		13.02	13.31
				45:1;O2	16.77	16.64	16.74	41:3;O2		14.13	14.74
				33:0;O3	6.99	7.04		42:3;O2	15.98	15.54	15.49
				34:0;O3	8.59	8.29	8.26	43:3;O2		16.63	16.09
				36:0;O3	9.60	9.00	9.37	44:3;O2		16.64	17.24
				37:0;O3		10.53	10.80	35:0;O3	9.87	10.44	10.84
				35:1;O3	7.92		8.45	40:0;O3	16.01	15.57	15.60
				36:1;O3	9.14		9.19	42:0;O3	17.43	16.90	16.83
				41:1;O3		15.11	15.01	43:0;O3		17.28	16.85
				42:1;O3			16.11	37:1;O3	9.46	8.91	9.04
				43:1;O3		16.58	16.65	39:1;O3		13.26	13.32
				45:1;O3	17.27			40:1;O3	15.16	14.65	14.91
				36:2;O3	8.07	7.86		41:1;O3	16.11	15.70	15.81
				37:2;O3	9.92		8.86	42:1;O3	16.74	16.37	16.47
				38:2;O3	10.72	9.51		43:1;O3	17.22	16.85	16.91

Continued on next page

Continued from previous page

Lipid A_NAc	CID neg	CID pos RT [min]	EAD pos	Lipid A_NH	CID neg	CID pos RT [min]	EAD pos	Lipid A_OH	CID neg	CID pos RT [min]	EAD pos
				41:2;O3		14.13	13.70	44:1;O3	17.58	17.24	
				42:2;O3		15.14	15.11	35:0;O4	9.03	9.48	8.90
				43:2;O3		16.58	16.06	36:0;O4	11.28	10.83	10.72
				44:2;O3		16.63	16.73	38:0;O4	13.46	12.88	12.90
				33:0;O4	7.49	7.03		39:0;O4	14.46	14.59	14.46
				35:0;O4		7.92	7.92	40:0;O4	15.58	15.49	15.38
				36:0;O4	8.84		9.04	41:0;O4	16.38	15.98	16.02
				37:0;O4	10.68	10.82	11.43	42:0;O4	16.90	16.97	16.67
				38:0;O4		12.81		43:0;O4	17.38	17.03	17.03
								44:0;O4	17.77	17.34	17.03
				41:0;O4	17.42		17.64	36:1;O4	10.54	10.00	10.05
				33:1;O4	6.93	6.38		38:1;O4	12.43	11.95	11.97
				36:1;O4	9.55			39:1;O4	13.54	12.88	14.66
				37:1;O4	10.69			40:1;O4	14.62	15.60	14.50
				38:1;O4		13.26	12.85	41:1;O4	16.22	15.92	15.50
				39:1;O4		15.22	14.73	42:1;O4	16.45	16.06	16.10
				40:1;O4	16.11	15.64	15.68	43:1;O4	16.99	16.64	16.72
				41:1;O4	16.76		16.47	44:1;O4	17.42	17.07	17.16
				42:1;O4	17.26	16.86	16.87	41:3;O4		14.59	14.46
								42:3;O4	15.55	15.14	15.23
								43:3;O4	16.38	16.02	16.11
								44:3;O4	16.94	16.64	

3.6.2 Higher-order GIPC Series Identification

The idea is now also to apply the ECN model to the higher-order GIPC classes (C, D, E) as additional identification evidence. Given the limited structural information available—primarily precursor m/z values and retention times—this step can support the tentative identification of GIPCs within higher structural series, which would also align with the existing literature.

In the ECN plots listed below, lipid species are connected based on the number of double bonds they contain. A thick red line connects lipids with zero double bonds, while a blue line connects those with a single double bond.

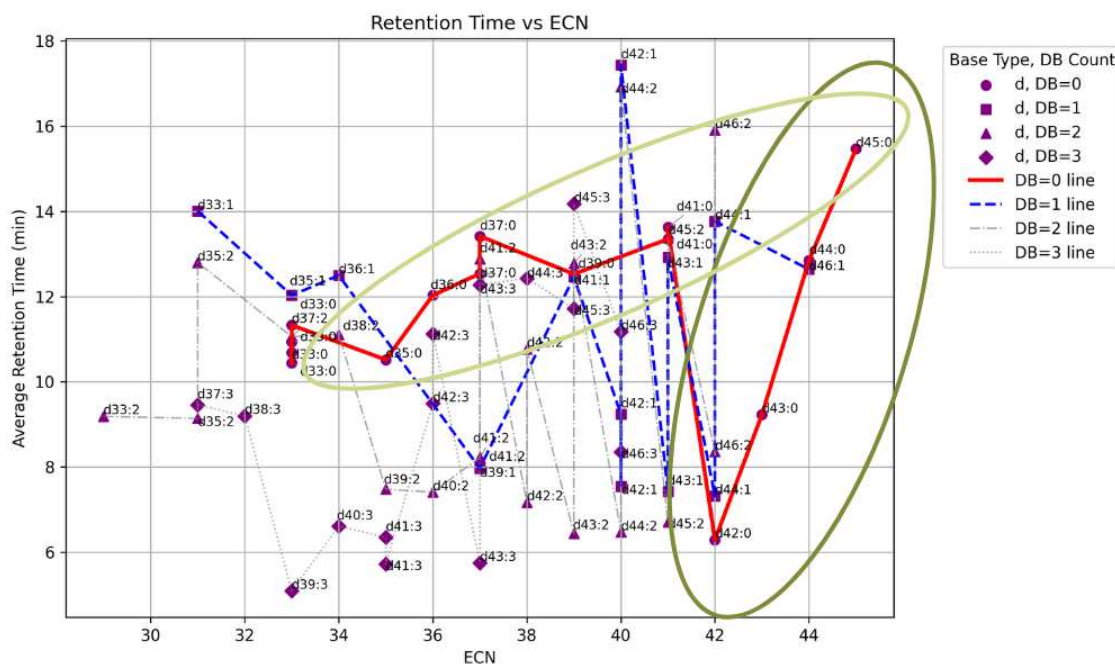


FIGURE 3.6: ECN model for C_HEX_NH series lipids (dihydroxylated). The two highlighted circles indicate linear retention time trends.

Let us examine the GIPC species where some unknown MS2 fragments were detected to see whether there could be further evidence. Among the species from the C Series, only the series C_HEX_NH exhibited a consistent retention time pattern, specifically the dihydroxylated ones across the 42:0, 43:0, 44:0 and 45:0 species. However, since it is already documented that the majority of GIPCs in plants are tri- or tetrahydroxylated, we concluded, based on the observed retention time pattern of the class C GIPCs, that any GIPC series lacking these lipids with suitable retention times will be excluded from further considerations (as observed here).

In the D series four promising subseries were identified based on the presence of MS2 fragments. Looking at the dihydroxylated lipids from the D_2HEX_PEN_NAC Series, the lipids from 37:0 to 46:0 exhibit a roughly linear increase in retention time, although 41:0 deviates slightly. Lipids with a single double bond - 45:1, 43:1 and 41:1 - are as expected under the red line, having lower retention times than the ones without any double bond. However, one would expect a linear trend between them as well, which, unfortunately, is not the case. Trihydroxylated lipids in this series follow an alignment with the ECN model as well, observed for 40:0 to 46:0 (DB=1), 41:1 to 44:1 (DB=1) and 40:2 to 46:2 (DB=2).

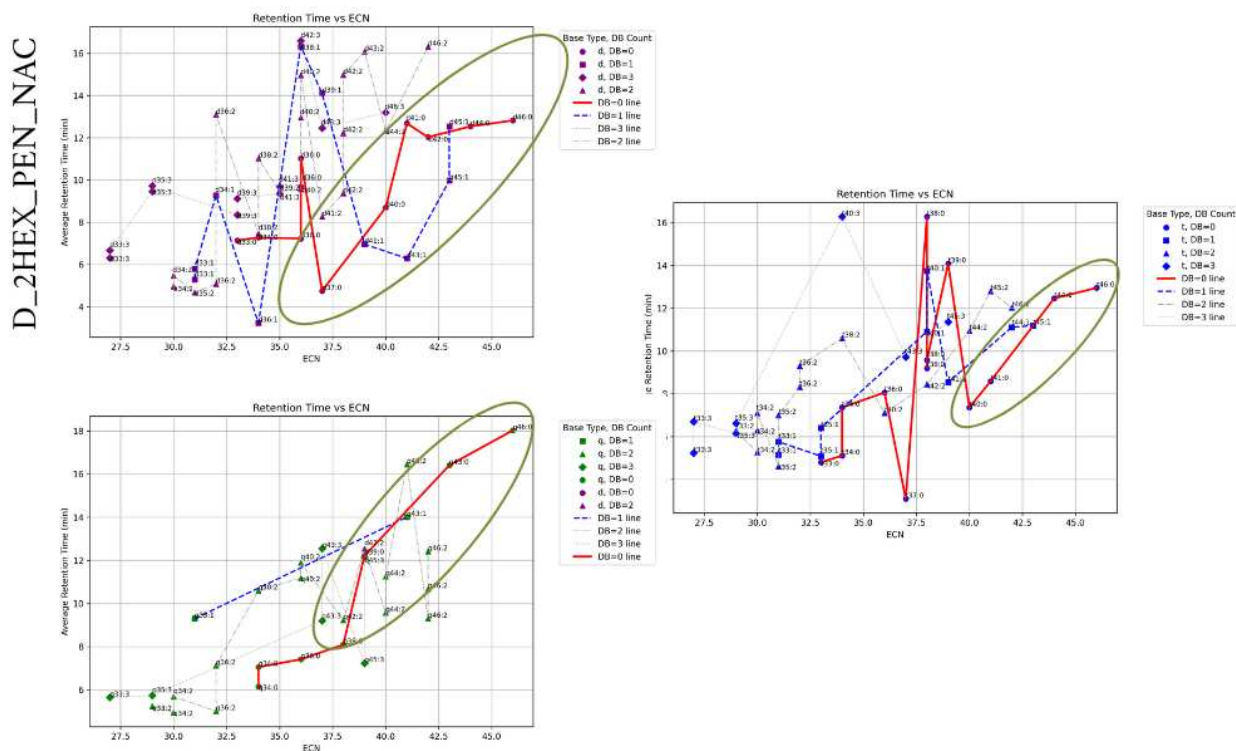


FIGURE 3.7: ECN model for D_2HEX_PEN_NAc series lipids. The different symbols represent distinct hydroxylation states: d (top) for dihydroxylated, t (right) for trihydroxylated, and q (bottom) for tetrahydroxylated species.

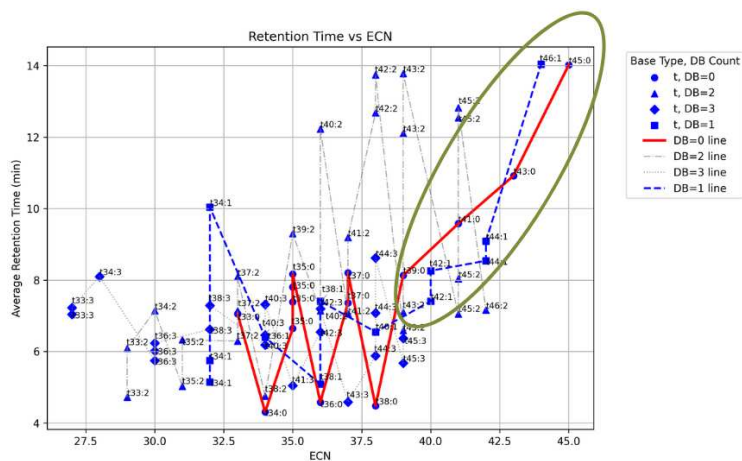
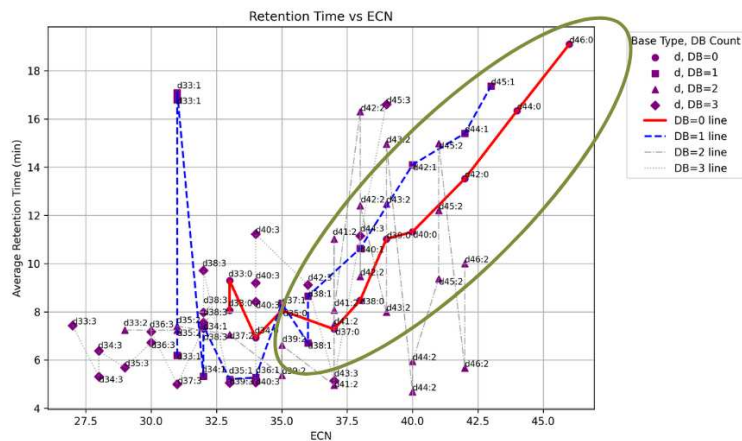
For the D_2HEX_PEN_NH series, both di- and trihydroxylated lipids were examined. The dihydroxylated lipids, ranging from 40:0 to 46:0, exhibit a linear retention time trend. Lipids with one double bond also appear to be linear from 40:1, 41:1 to 44:1, 45:1. While trihydroxylated lipids also follow the linear increase, the retention time increase with ECN number seems to be steeper compared to the dihydroxylated ones.

The last final D series group considered was D_HEX_2PEN_NH. The dihydroxylated species show a promising increase from 38:0 up to 46:0. Similarly, the tetrahydroxylated species show a trend from 38:0 to 44:0.

The lipids from the E series showed the least convincing results, with the least amount of lipids showing the expected behavior. However, E_2HEX_2PEN_NH and E_3HEX_PEN_NAC did show a somewhat linear increase among both di- and trihydroxylated lipids without double bonds.

From a literature perspective, Cacas et al.⁸ reported D_HEX_2PEN_OH in *Sauvignon blanc* and *Merlot* grape berries, though this series differs functionally from those discussed in our analysis. Within the E Series, only E_3PEN_HEX_NAC and E_3PEN_HEX_OH were reported in the study. In his study, E_PEN_3HEX_NAC, which was discussed in our results, was found only in *Nicotiana tabacum* and *Nicotiana benthamiana*, and E_2HEX_2PEN_NH only in *Castanea sativa* and *Spinacia oleracea*. Unfortunately, for D_HEX_2PEN_OH and E_HEX_3PEN_OH, we did not obtain sufficient MS1 spectra to create ECN plots. All retention times were approximately 1 minute, which was deemed unreliable for interpretation.

D_2HEX_PEN_NH



D_HEX_2PEN_NH

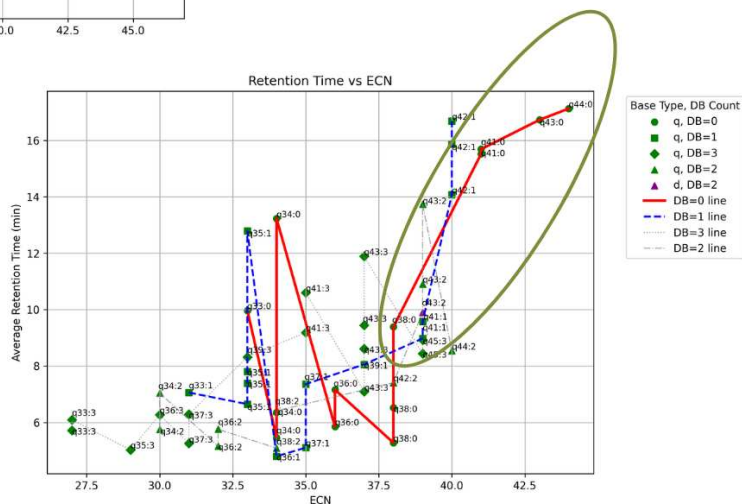
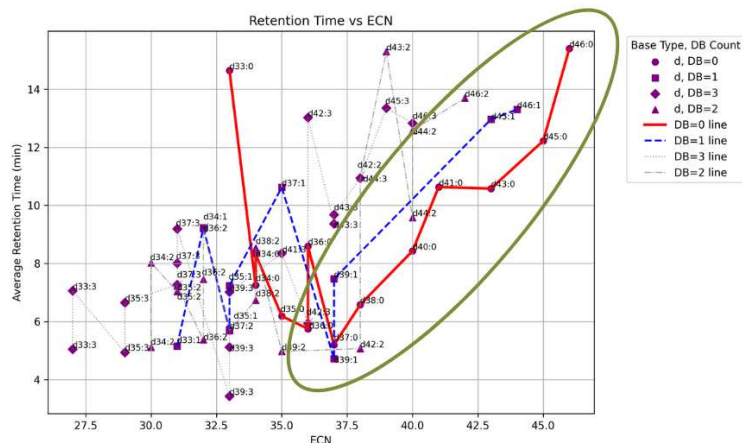
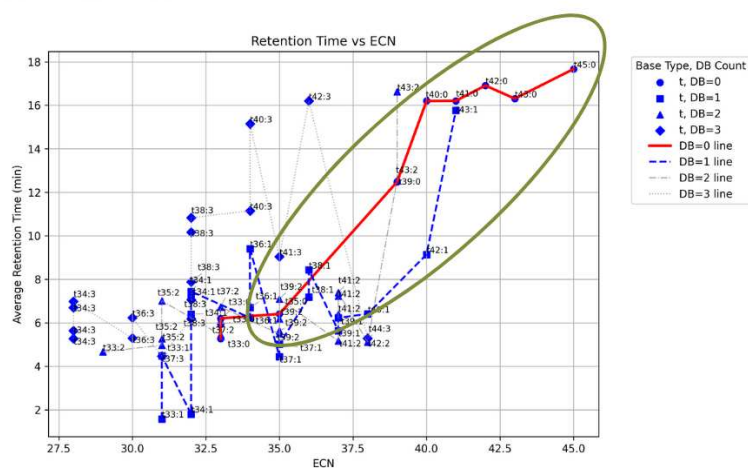
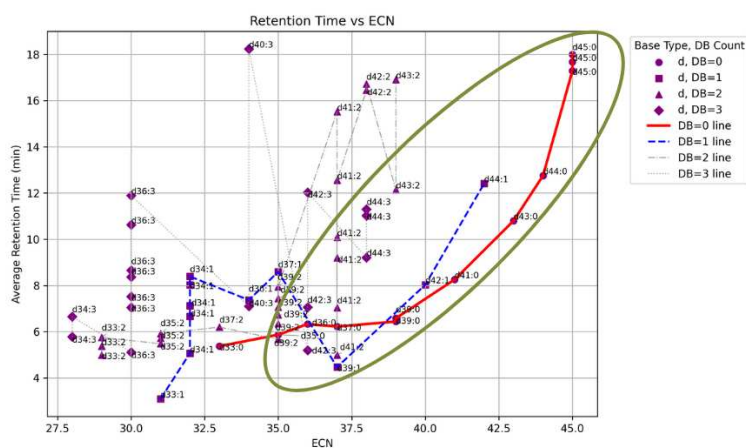


FIGURE 3.8: ECN model for D_2HEX_PEN and D_HEX_2PEN series lipids. Symbols analogous to Figure 3.7.

E_2HEX_2PEN_NH



E_3HEX_PEN_NAC

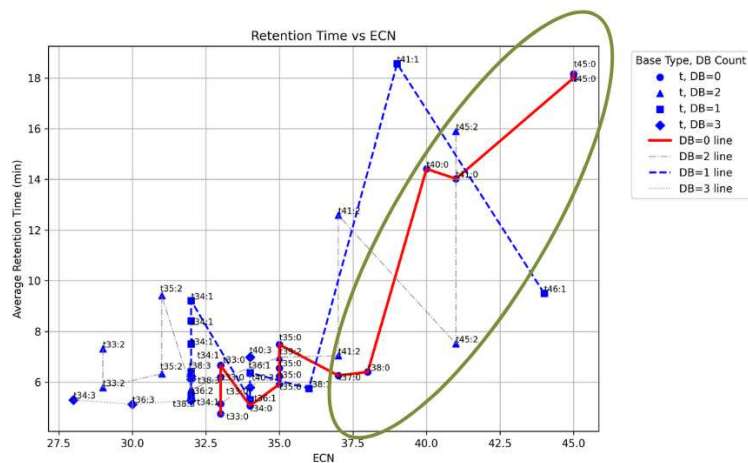
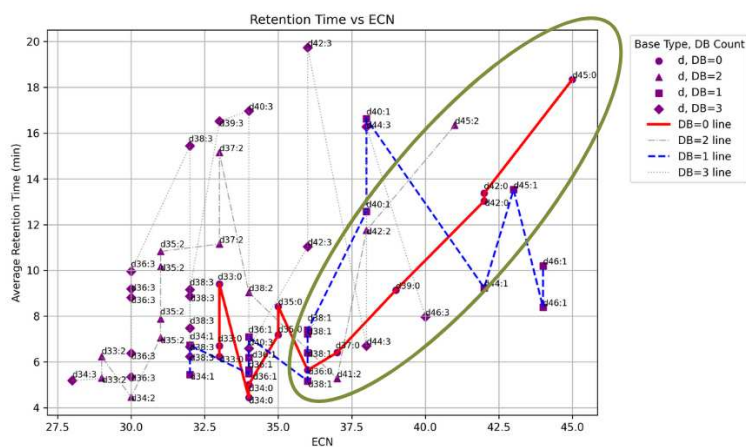


FIGURE 3.9: ECN model for E_2HEX_2PEN_NH and E_3HEX_PEN_NAC series lipids. Symbols are log to Figure 3.7.

Summary of Identified GIPCs

Partially Resolved Lipids – Highest Confidence Identification (n = 12)

A-OH-18:0;O3/22:0;O	A-OH-18:0;O3/23:0;O	A-OH-18:0;O3/24:0;O
A-OH-18:0;O3/25:0;O	A-OH-18:1;O3/22:0	A-OH-18:1;O3/22:0;O
A-OH-18:1;O3/23:0	A-OH-18:1;O3/23:0;O	A-OH-18:1;O3/24:0
A-OH-18:1;O3/24:0;O	A-OH-18:1;O3/25:0	A-OH-18:1;O3/25:0;O

Partially Resolved Lipids – Limited MS2-based Confirmation (n = 10)

A-Nac-39:0;O4	A-NH-18:0;O2/24:1	A-NH-18:0;O2/26:1
A-NH-18:0;O2/27:1	A-NH-18:1;O3/25:1	A-OH-38:1;O4
A-OH-39:1;O4	A-OH-40:1;O3	A-OH-18:1;O3/26:0;O
A-OH-44:3;O4		

Putatively Identified Lipids – GIPC A-Series (n = 78)

A-Nac-37:0;O3	A-Nac-38:0;O3	A-Nac-39:0;O3
A-Nac-40:0;O3	A-Nac-37:0;O4	A-Nac-38:0;O4
A-Nac-40:0;O4	A-Nac-41:0;O4	A-NH-34:0;O2
A-NH-36:0;O2	A-NH-37:0;O2	A-NH-38:0;O2
A-NH-43:0;O2	A-NH-44:0;O2	A-NH-45:0;O2
A-NH-41:1;O2	A-NH-43:1;O2	A-NH-33:0;O3
A-NH-34:0;O3	A-NH-36:0;O3	A-NH-37:0;O3
A-NH-35:1;O3	A-NH-36:1;O3	A-NH-41:1;O3
A-NH-42:1;O3	A-NH-43:1;O3	A-NH-36:2;O3
A-NH-37:2;O3	A-NH-38:2;O3	A-NH-41:2;O3
A-NH-42:2;O3	A-NH-44:2;O3	A-NH-33:0;O4
A-NH-35:0;O4	A-NH-36:0;O4	A-NH-37:0;O4
A-NH-41:0;O4	A-NH-33:1;O4	A-NH-40:1;O4
A-NH-41:1;O4	A-NH-42:1;O4	A-OH-36:0;O2
A-OH-38:0;O2	A-OH-44:0;O2	A-OH-36:2;O2
A-OH-41:2;O2	A-OH-42:2;O2	A-OH-43:2;O2
A-OH-35:3;O2	A-OH-37:3;O2	A-OH-39:3;O2
A-OH-40:3;O2	A-OH-41:3;O2	A-OH-42:3;O2
A-OH-43:3;O2	A-OH-44:3;O2	A-OH-35:0;O3
A-OH-40:0;O3	A-OH-42:0;O3	A-OH-43:0;O3
A-OH-37:1;O3	A-OH-39:1;O3	A-OH-43:1;O3
A-OH-44:1;O3	A-OH-35:0;O4	A-OH-36:0;O4
A-OH-38:0;O4	A-OH-39:0;O4	A-OH-40:0;O4
A-OH-41:0;O4	A-OH-36:1;O4	A-OH-38:1;O4
A-OH-39:1;O4	A-OH-40:1;O4	A-OH-41:1;O4
A-OH-41:3;O4	A-OH-42:3;O4	A-OH-43:3;O4

Putatively Identified Lipids – Higher-Class GIPC Series (n = 5)

D-2HEX-PEN-NAC	D-2HEX-PEN-NH	D-HEX-2PEN-NH
E-2HEX-2PEN-NH	E-3HEX-PEN-NAC	

3.7 Preprocessing Effects

The consequent Figure 3.10 is divided by identified GIPC species with bars in pairs to see the differences between the control and stress conditions. From the raw areas, it can be concluded that A-OH-18:1;O3/24:0;O is by far the most abundant detected GIPC species. Other highly abundant lipids include A-OH-18:1;O3/22:0;O, A-OH-18:1;O3/23:0;O, A-OH-18:1;O3/25:0;O, and A-OH-18:0;O3/24:0;O - all of which contain a hydroxylated fatty acid (FA). Among these hydroxylated FA-containing lipids (hereafter referred to as h-lipids), the one with the lowest abundance proved to be A-OH-18:0/23:0;O, yet it was still more abundant than any of the non-hydroxylated species. The overall least abundant species across all the lipids was A-OH-18:1;O3/22:0. This pattern matches with the GIPC-A-series distribution in spinach reported by Panzeböck et al.⁵

The log-transformed lipid areas provide some insights into the relative abundance changes across different time points for both Grüner Veltliner (GV) and Riesling (R) samples. Table 3.4 shows us whether a lipid at a given time point is overexpressed under stress conditions. What do these tables reveal? First and foremost, lipid abundances change over time. In GV samples, by Day 5, none of the lipids were overexpressed under stressed conditions, even though they were overexpressed by Day 1. By Day 75, the behavior changes substantially once again, suggesting that long-term exposure to heat stress could have altered the lipid expression. On the other hand, examining the R samples reveals a different trend. Day 1 appears to be the one with the most pronounced changes, and the expression of lipids at other time points seems largely unchanged, indicating a prolonged adaptation to stress.

The lipid abundances were evaluated with an additional normalization step to the protein content to understand how much impact this normalization step against the biological mass would have on the interpretation. In lipidomic studies, it is common to normalize to total protein content when using cell extracts or tissue homogenates. However, we cannot automatically assume that the lipid and protein concentrations co-vary⁴⁹. Subsequently, there could be stress-induced proteolysis, which reduces protein without the proportional lipid loss⁵⁰. Moreover, it remains unclear whether the analyzed GIPCs are linked to cytosolic protein changes, as they are primarily associated with membrane dynamics. To address these questions and evaluate the effect of protein normalization, the areas under control and stress conditions were compared both with and without normalization to the protein content. Static analysis was then performed to examine whether the normalization affects the group separations.

In most cases, the normalization step does not significantly change the expression patterns of the lipids. The GIPC abundance here, therefore, does show a good sign of robustness. However, some changes are detectable. At the time points where lipid expression was already variable (specifically, Day 75 in GV and Day 1 in R), the normalization step influenced the output for some lipids.

In the Riesling samples, the only lipids that were overexpressed under stress at all the time points were A-OH-18:0;O3/22:0;O and A-OH-18:0;O3/24:0;O. In the GV samples, excluding Day 5 (where no lipid was overexpressed), additional h-lipids such as A-OH-18:0;O3/23:0;O and A-OH-18:1;O3/22:0 also showed overexpression. Another noticeable observation is the differences in the intensity patterns of the log-transformed areas. The two intermediate time points (Day 5 and Day 15) exhibit higher overall differences in intensities than those at Day 1 and Day 75, suggesting dynamic regulation of lipid levels over the course of the stress response.

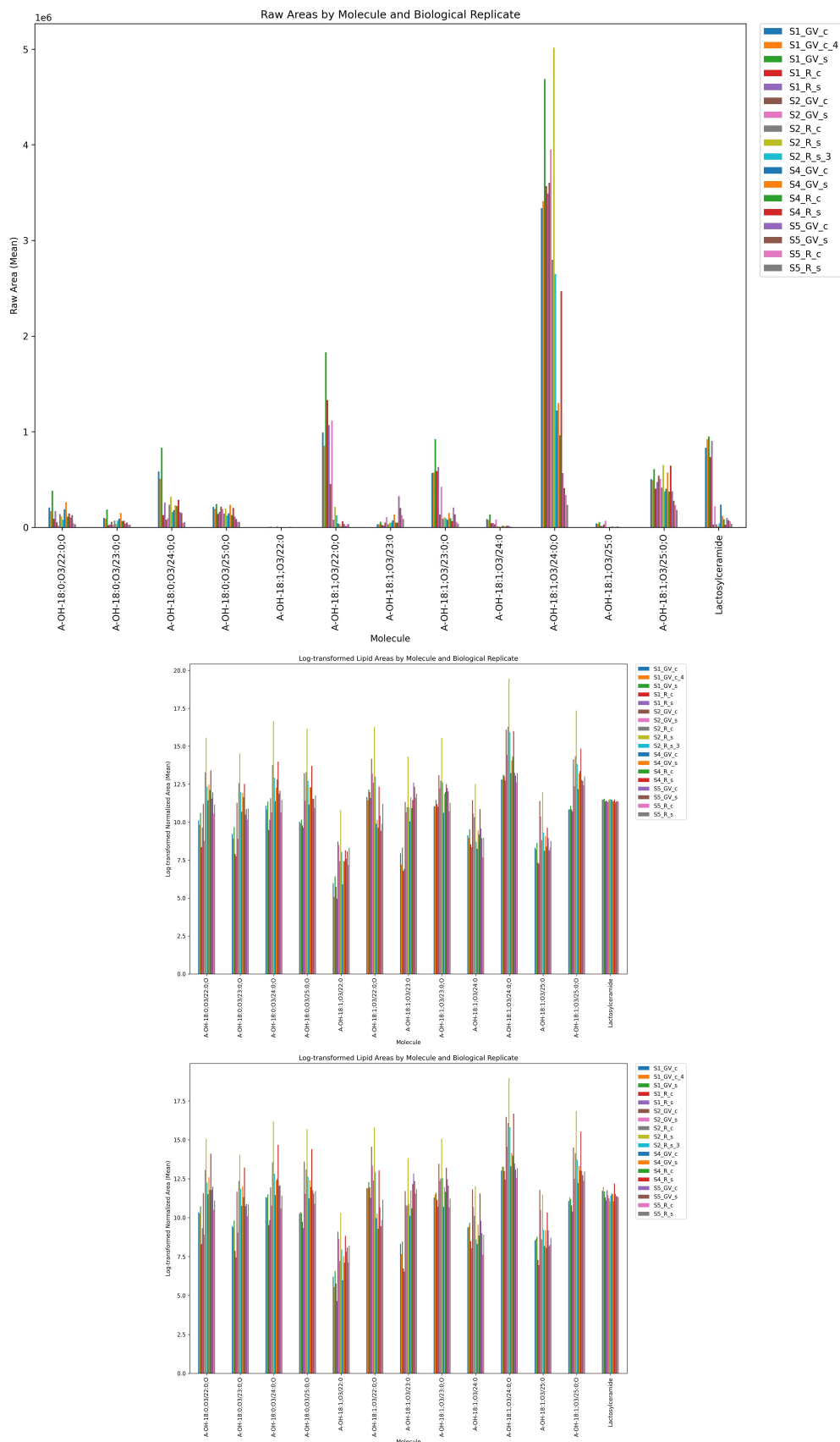


FIGURE 3.10: Raw area values of GIPC with highest confidence identification, averaged for each sampling point and wine type (top); log-transformed areas without protein normalization (middle); and log-transformed areas normalized to protein content (bottom).

TABLE 3.4: Time-course expression patterns of GIPC Species (with highest confidence identification) under stress conditions with and without protein.

Molecule	Day 1		Day 5		Day 15		Day 75	
	No Protein	With Protein	No Protein	With Protein	No Protein	With Protein	No Protein	With Protein
Grüner Veltliner Expression								
A-OH-18:0;O3/22:0;O	Yes	Yes	No	No	Yes	Yes	Yes	Yes
A-OH-18:0;O3/23:0;O	Yes	Yes	No	No	Yes	Yes	Yes	Yes
A-OH-18:0;O3/24:0;O	Yes	Yes	No	No	Yes	Yes	Yes	Yes
A-OH-18:0;O3/25:0;O	Yes	Yes	No	No	Yes	Yes	Yes	No
A-OH-18:1;O3/22:0	Yes	Yes	No	No	Yes	Yes	Yes	Yes
A-OH-18:1;O3/23:0	Yes	Yes	No	No	Yes	Yes	No	No
A-OH-18:1;O3/24:0	Yes	Yes	No	No	Yes	Yes	No	No
A-OH-18:1;O3/25:0	Yes	Yes	No	No	Yes	Yes	No	No
A-OH-18:1;O3/22:0;O	Yes	Yes	No	No	Yes	Yes	No	No
A-OH-18:1;O3/23:0;O	Yes	Yes	No	No	Yes	Yes	No	No
A-OH-18:1;O3/24:0;O	Yes	Yes	No	No	Yes	Yes	No	No
A-OH-18:1;O3/25:0;O	Yes	Yes	No	No	Yes	Yes	Yes	No
Riesling Expression								
A-OH-18:0;O3/22:0;O	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
A-OH-18:0;O3/23:0;O	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
A-OH-18:0;O3/24:0;O	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
A-OH-18:0;O3/25:0;O	No	No	Yes	Yes	Yes	Yes	Yes	Yes
A-OH-18:1;O3/22:0	No	No	Yes	Yes	Yes	Yes	Yes	Yes
A-OH-18:1;O3/23:0	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
A-OH-18:1;O3/24:0	No	No	Yes	Yes	Yes	Yes	Yes	Yes
A-OH-18:1;O3/25:0	No	No	Yes	Yes	Yes	Yes	Yes	Yes
A-OH-18:1;O3/22:0;O	No	No	Yes	Yes	Yes	Yes	Yes	Yes
A-OH-18:1;O3/23:0;O	No	No	Yes	Yes	Yes	Yes	Yes	Yes
A-OH-18:1;O3/24:0;O	No	No	Yes	Yes	Yes	Yes	Yes	Yes
A-OH-18:1;O3/25:0;O	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes

3.8 Static Data Analysis

3.8.1 Principal Component Analysis

After refining the data matrix, normalization, and autoscaling were applied, and then PCA was used. Scores and eigenvalues were calculated using the maximum number of principal components. The explained variance for each principal component was printed to understand better their contribution (Table 3.5). For the normalization method comparison, two different PCA analyses were conducted to see whether some differences might be detected.

TABLE 3.5: Explained Variance by Principal Components in percentages:

Principal Component	Variance
PC1	66.1%
PC2	15.5%
PC3	12.2%
PC4	2.84%
PC5	1.31%
PC6	0.40%
PC7	0.04%
PC8	0.00%
PC9	0.00%
PC10	0.00%
PC11	0.00%
PC12	0.00%

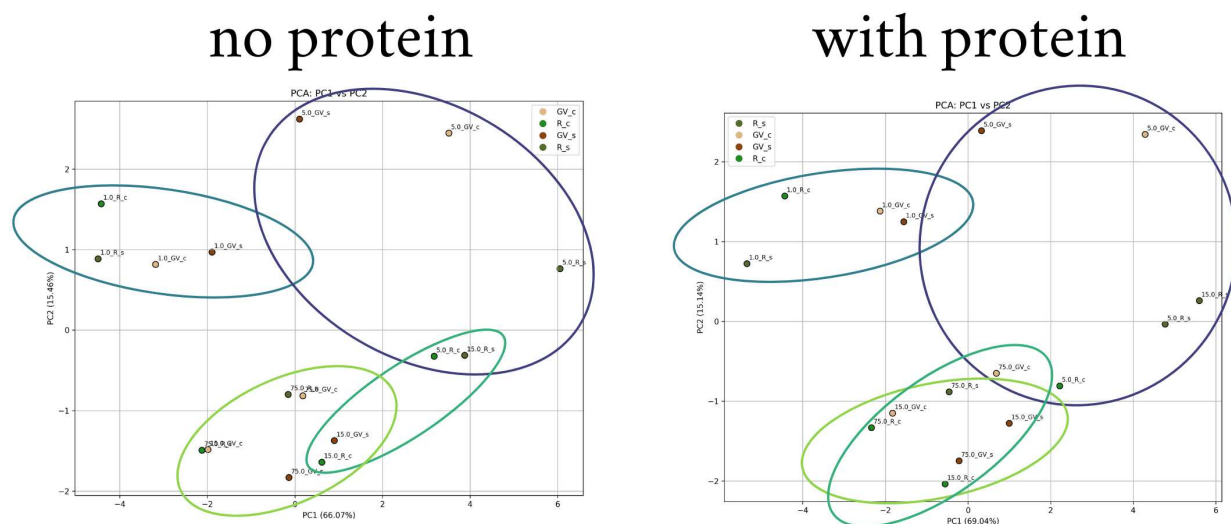


FIGURE 3.11: PC1 vs PC2 plots of GIPC species identified with highest confidence. Left: without protein normalization; Right: with protein normalization. Colored circles indicate clustering by harvesting time point.

In general, across all three PCA plots, it is evident that lipid profiles are clustered according to the time points at which the wine leaves were harvested. Samples from Day 1 appear to be the most distinct, while samples from Day 5 show the highest variability and are most dispersed.

Samples from Days 15 and 75 seem to overlap across all 3 PCA plots (PC1 vs. PC2, PC2 vs. PC3, and PC3 vs. PC1).

When comparing the PCA plots with and without normalization to the protein content, samples within each time point seem to be somewhat differentiated. However, the clusters become less distinct and show increased overlap. This blurring of clusters suggests that protein normalization may introduce noise or even mask some lipidomic shifts that are independent of the total protein content.

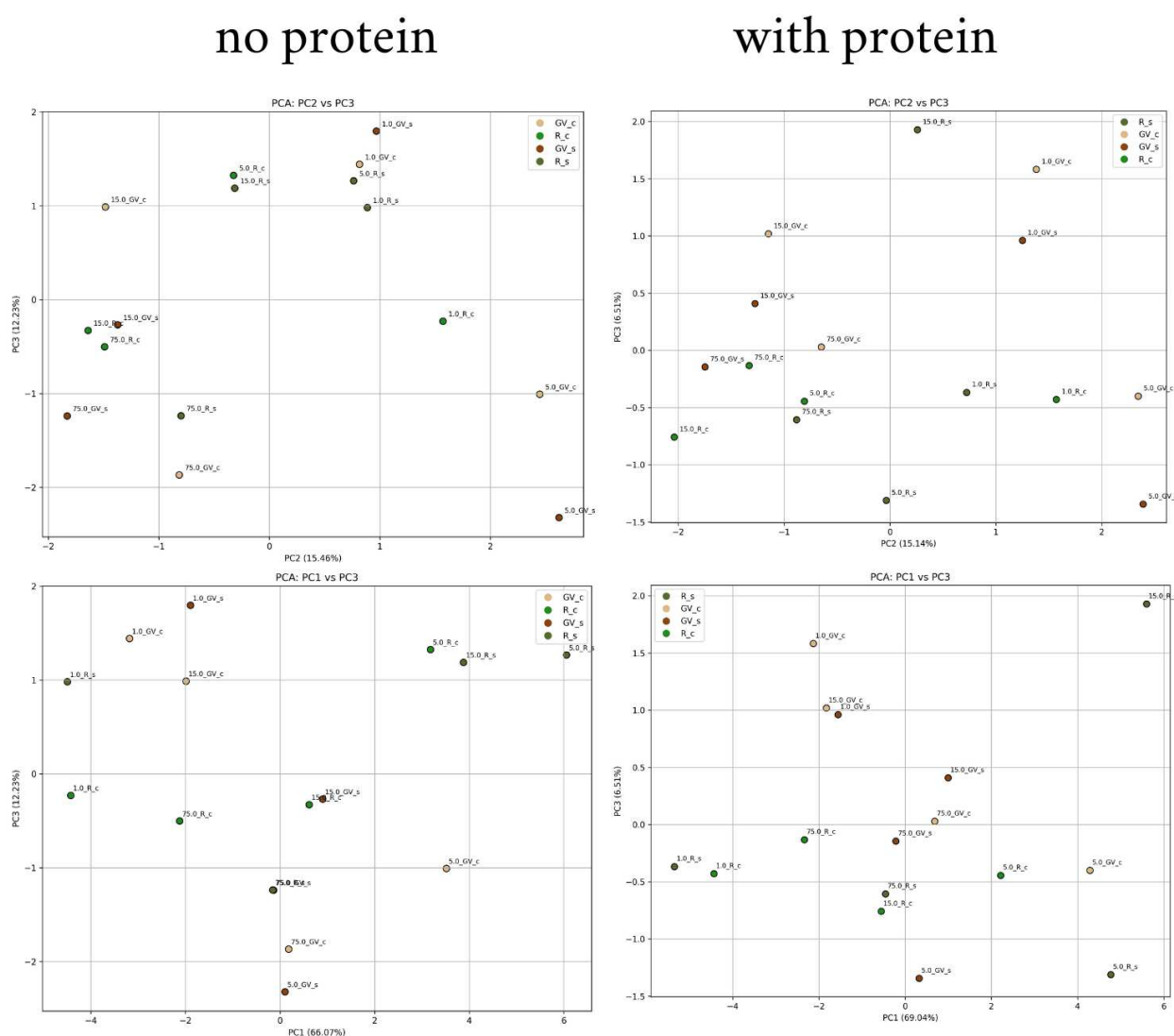


FIGURE 3.12: Principal Component Analysis of GIPC species (highest confidence). Top: PC2 vs PC3 plots; Bottom: PC1 vs PC2 plots. Left: without protein normalization; Right: with protein normalization.

3.8.2 Hierarchical Clustering

The next step in the analysis would be the hierarchical analysis, which generates a heatmap and dendrograms to provide a closer look at the relationships not only among the samples and conditions but also among the lipids. There are two dendrograms visualized: the top one represents the clustering of the species, while the side one shows the clustering of the samples.

Without protein normalization, samples from the same time points tend to cluster together. However, when examining the dendrogram with protein normalization, no trend is observed, as samples from different time points and even different wine types are clustered with each other.

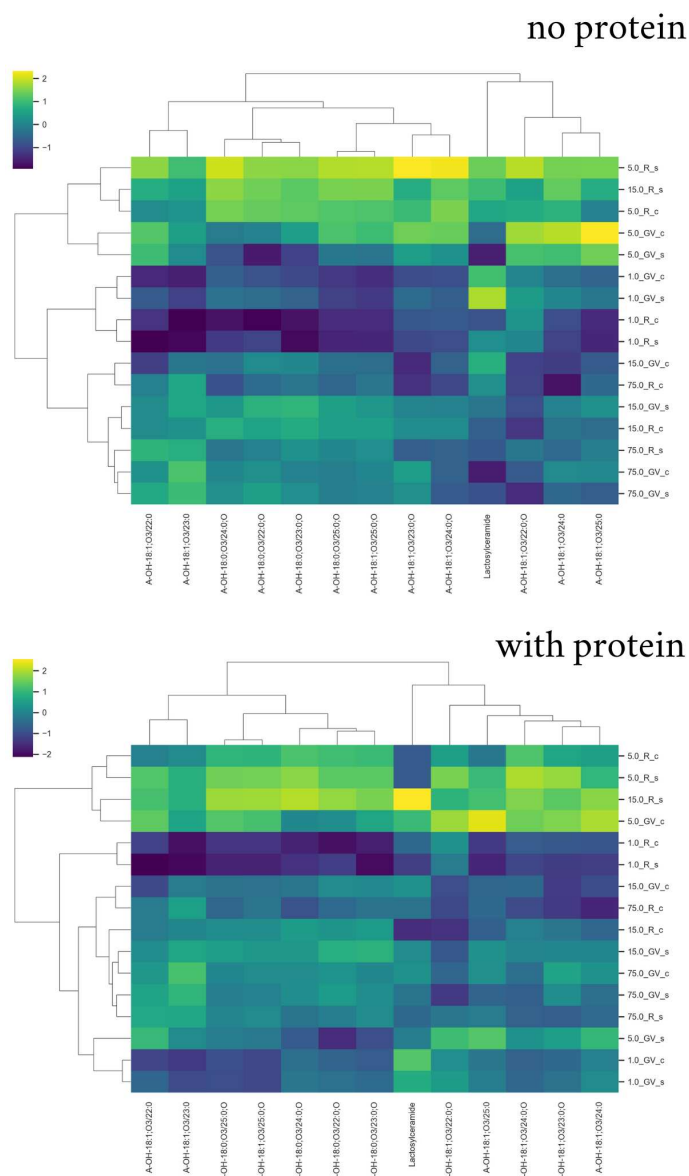


FIGURE 3.13: Hierarchical clustering of GIPC species (highest confidence). Top: without protein normalization; Bottom: with protein normalization. Top dendrograms indicate clustering among lipid species; side dendrograms indicate clustering among samples.

Regarding lipid clustering, in the unnormalized dataset, lipids are primarily grouped based on their hydroxylation state and the number of double bonds. This indicates that structural properties such as saturation and functional groups play a major role in determining how the lipids co-fluctuate under stress. Exception is the lipid A-OH-18:1;O3/22:0;O which clusters with A-OH-18:1;O3/24:0 and A-OH-18:1;O3/25:0, despite differences in hydroxylation state.

In the protein-normalized dataset, clustering appears to be driven mainly by the number of double bonds, while the hydroxylation state of the lipids has a less pronounced effect. This shift suggests that normalizing to protein content removes the hydroxylation-specific clustering effects.

3.8.3 Two-way ANOVA

Control X Wine

While the log-transformed values have already been looked into in the *Area* section, the two-way ANOVA analysis provides an additional perspective on the overall differences between the control and stressed values, independent of the time points. In GV samples, it is evident that all lipids are consistently overexpressed under stressed conditions. In contrast, this trend is not very clear in the R samples, where the differences are notably less significant. This is particularly true for the species A-OH-18:0;O3/23:0;O and the non-hydroxylated lipids, which have very insignificant differences. However, let us not forget that these are also the species that showed very low overall abundance, which could contribute to the lack of significance.

Time X Condition

What about the relationship between time and condition variables, independent of wine type? Across all lipids, a pattern emerges - a rise in the log-transformed values by Day 5. However, differences occur by Day 15. Here, lipids under stress (red) tend to either increase or remain the same in comparison to Day 5, while control samples show a significant decrease in values, leading to the largest differences between the control and stress conditions in the time series. By Day 75, the log-transformed values appear to converge.

Time X Wine

Looking into the dependencies between time and wine types, Riesling samples display less distinct fluctuations in the log-transformed values, while GV samples tend to jump in their values and show more dynamic changes. The most significant differences between wine types occur at Day 5, followed by Day 15. By Day 75, the values align closely with each other. The overall trend in the log-transformed areas is an initial increase by Day 5, followed by a drop by Day 15. This trend either continues till Day 75 or levels off. In GV samples, this trend is more consistent across lipids. The biggest difference is observed in the lipid A-OH-18:1;O3/23:0, where the values remain the same by Day 5 and 15, followed by an increase by Day 75. Another lipid, A-OH-18:1;O3/20:0;O, also shows a different behavior, exhibiting the most significant drop in abundance at Day 15.

3.8.4 CV Analysis

The distribution of coefficient of variation (CV) values revealed certain patterns across wine types and conditions. In GV, both conditions showed a distinct increase in CV values by Day 5, indicating higher variability among lipid abundances. In R, only the stress condition exhibited a rise at this time point. By Day 15, CV values in GV declined across most species in both conditions, with an increase observed in a few non-hydroxylated lipids under stress. On the other hand, CV values of R samples continued to rise at Day 15 in both conditions. By Day 75, CV values in GV control samples remained relatively stable, while in the stressed condition, most values decreased. In R, however, the majority of lipids showed a continuous rise of CV values, suggesting higher variability in lipid expression. Lipids with consistently high variability across time points included 18:1;O3/22:0 in GV stress and 18:1;O3/25:0 in R (both conditions).

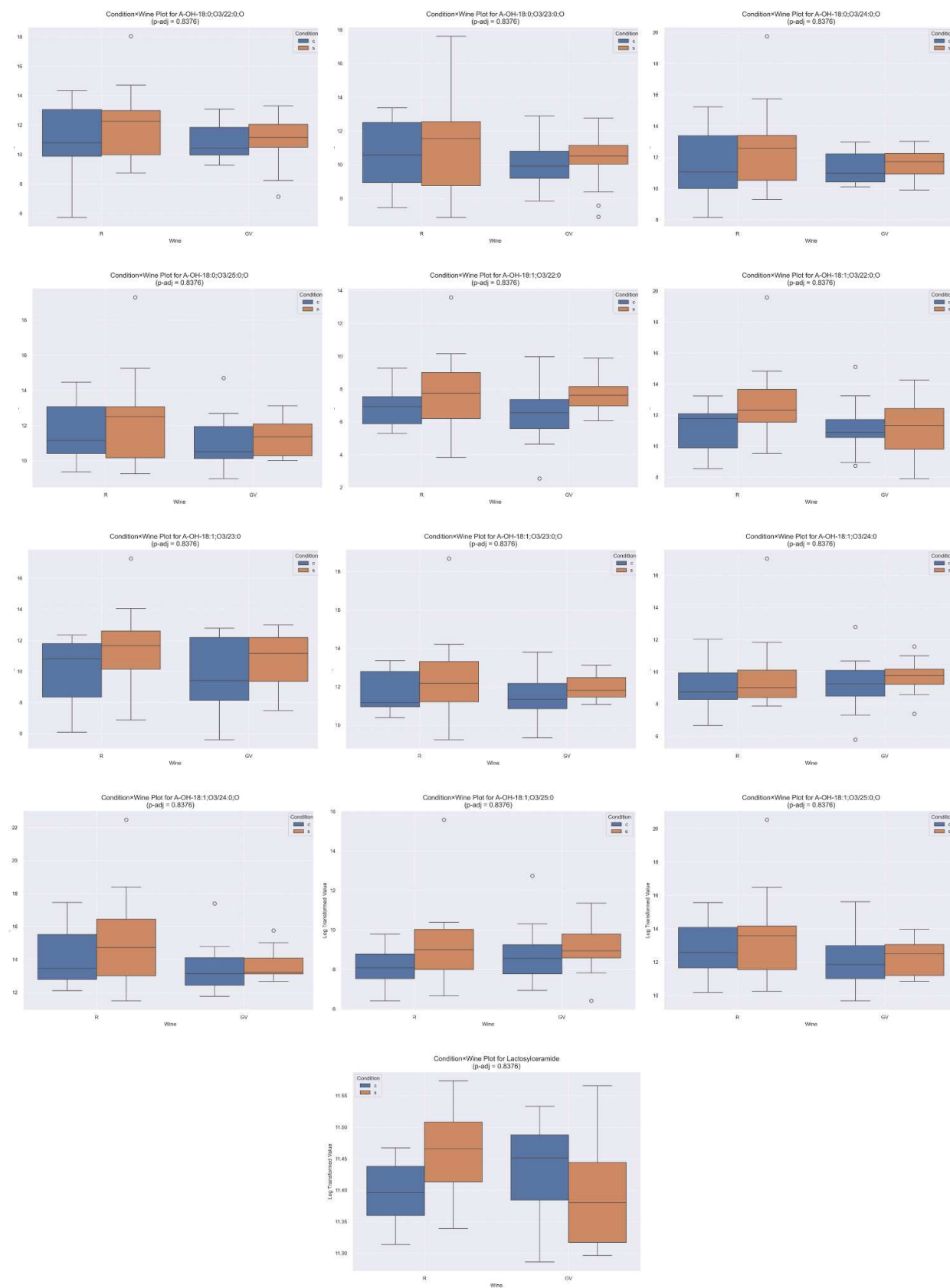


FIGURE 3.14: Interaction plots showing the results of the two-way ANOVA for the Condition × Wine Type interaction.

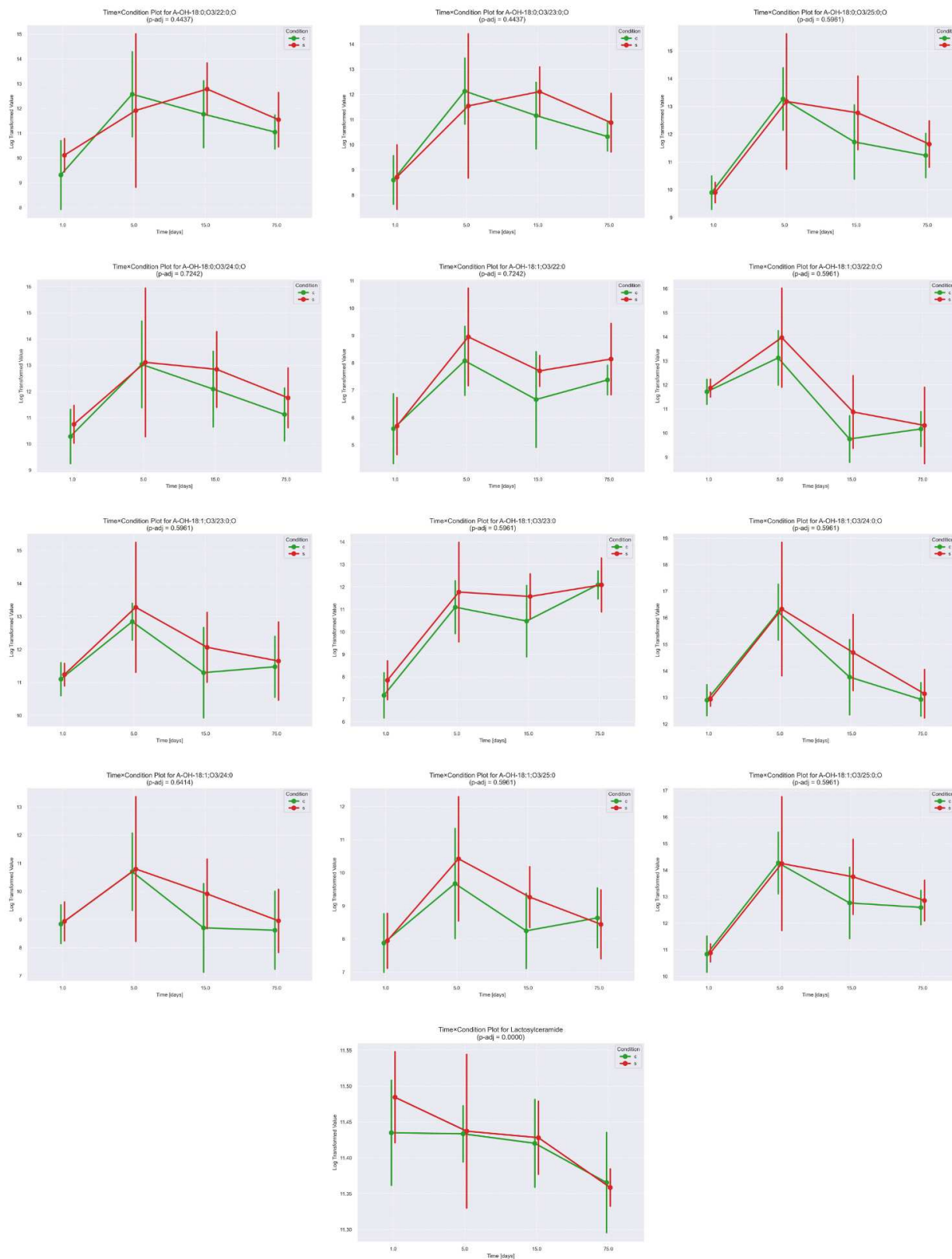


FIGURE 3.15: Interaction plots for the Time $\times$ Condition interaction.

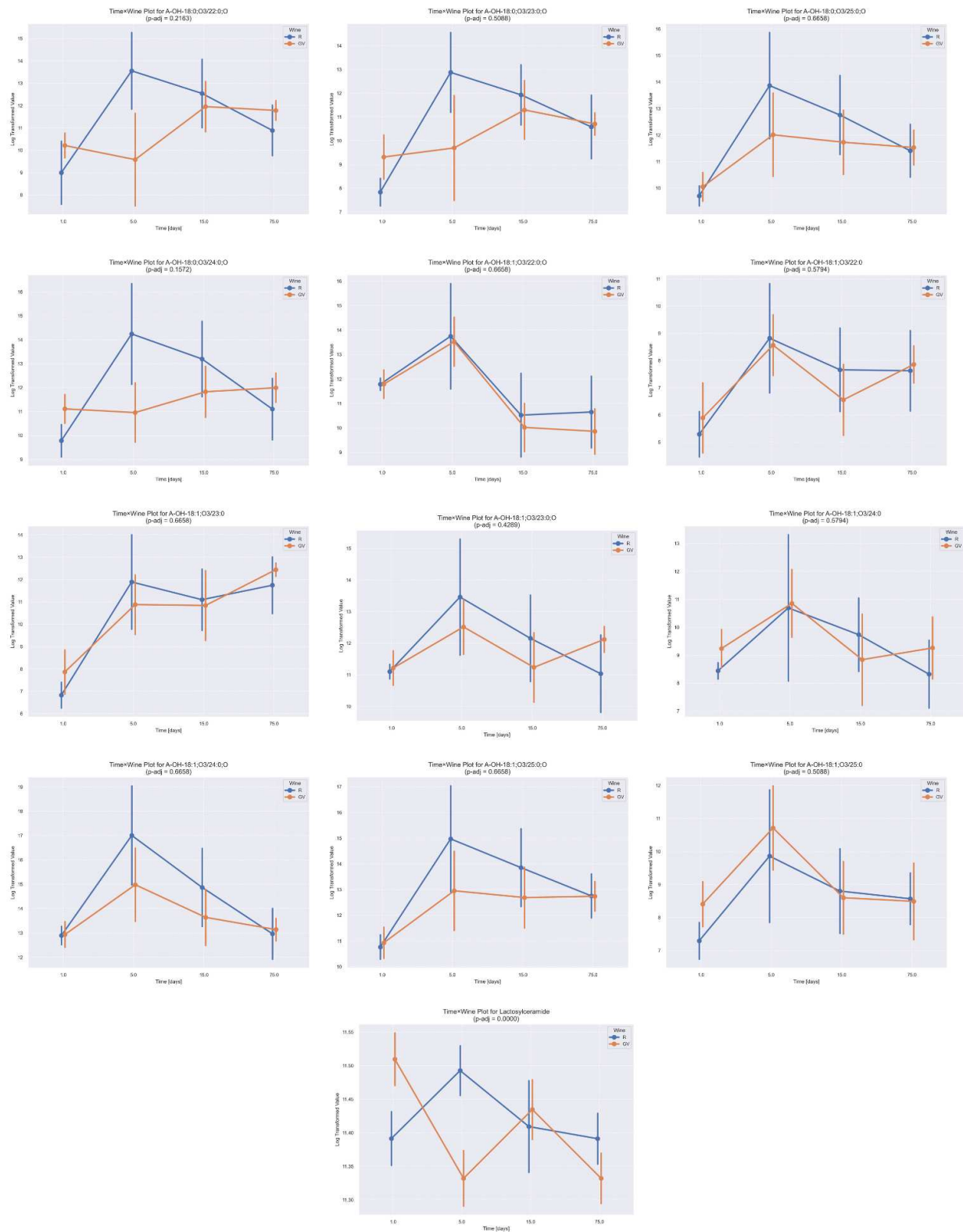
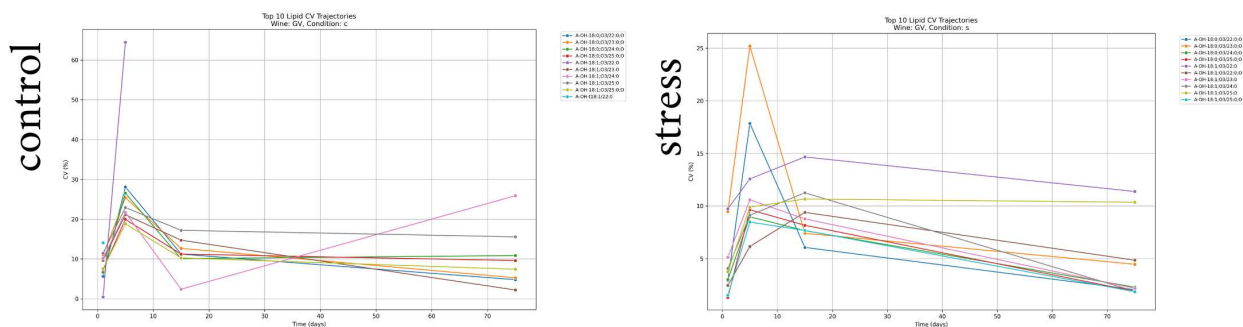


FIGURE 3.16: Interaction plots for the Time × Wine Type interaction.

Grüner Veltliner



Riesling

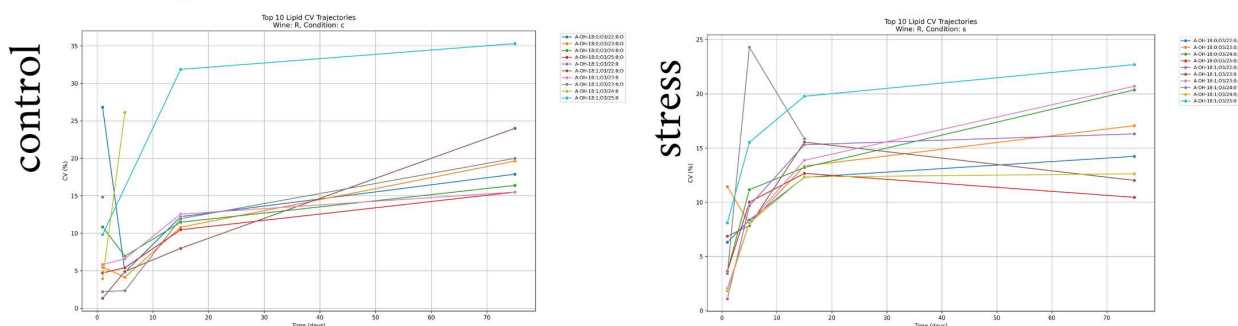


FIGURE 3.17: Coefficient of Variation (CV) distributions for GIPC species across time points. Top: Grüner Veltliner (GV); Bottom: Riesling. Left panels show control conditions, right panels show heat stress conditions.

3.9 Dynamic Data Analysis

How reliable are the results presented in the previous sections? It is well known that PCA and hierarchical clustering are designed for static data - each sample is treated independently. As such, these methods cannot model kinetics, transient responses, or sequences of events, which are often crucial points to consider in biological experiments involving stress or development⁵¹. One would therefore assume that discriminating only by one factor may oversimplify the complex biological regulation occurring in our wine leaf study. To address these limitations, time-aware, longitudinal methods are available - such as EDGE, BETR, hidden Markov models (HMMs), or regression-based techniques - designed to capture temporal dynamics in omics data.

In this study, we employed a hybrid approach, combining classic statistical groundwork with PCA, a two-way ANOVA, and temporal and network-based strategies. While PCA and ANOVA remain the standard workflow for exploratory and factor analysis in lipidomics⁵², the addition of network and dynamic modeling should provide a deeper insight into the systemic behavior of GIPC, considering the wine-specific, stress- as well as the time-dependent conditions. Each analysis step is discussed in Chapter 2.

3.9.1 Network Analysis

To uncover some systemic lipid regulations under heat stress, correlation-based networks were constructed for each wine type, condition, and time point. The number of significant correlations, as well as density, global clustering coefficients, hub structures, and isolated nodes, were looked into.

We first consider the number of significant correlations which reflect coordinated behavior between lipid species. In both wine types under stress, a temporal trend emerged: correlations increased from Day 1, peaked between Day 5 and Day 15, and then declined by Day 75. This may indicate a synchronization of lipid behavior in response to stress, followed by a slight desynchronization over a longer period. Notably, R under stress exhibited the sharpest peak on Day 5, with 50 significant correlations. For control samples, patterns varied depending on the wine type, without exhibiting a uniform trend. For GV, the number of significant correlations declined from 44 on Day 1 to only 6 by Day 75, while in R, correlations increased from 5 on Day 1 to 26 by Day 75.

The network densities indicate how interconnected the lipid system is. Although the overall density levels differed between conditions, being higher in GV control than in GV stress samples, and higher in Riesling stress samples than in their controls, the stressed networks across both wine types followed a uniform temporal trend. Specifically, density increased, peaking at Day 5, and then declined steadily by day 75. The R stress network on Day 5 stood out with the highest overall density, indicating a strong, coordinated lipid response at that point.

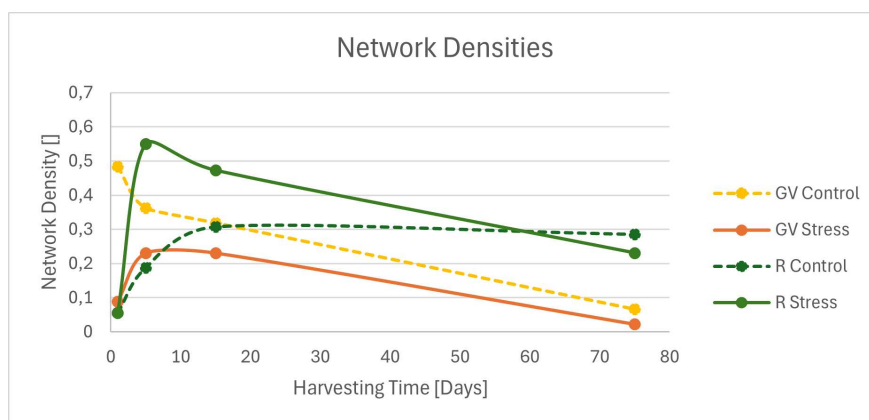


FIGURE 3.18: Network Densities plotted against the harvesting days - dashed lines represent the control networks, while the full ones the stress ones. Orange tones are Grüner Veltliner networks, green tones are Riesling networks.

Global clustering coefficients, indicating the tendency of how lipids tend to form highly connected groups, further support these findings. Coefficients between 0.75 and 1 denote highly coordinated response and shared regulation between the species. In both wine types, stress networks exhibited lower global clustering coefficients compared to their respective control networks. In GV, control networks ranged from 0.7500 to 0.9027, while the stressed networks were lower, at approximately 0.6. There was one exception, where the global clustering coefficient in the stressed network dropped to 0 by Day 75. For R, the control network generated highest clustering coefficients between 0.89 and 1.0, while stressed networks ranged from 0.5 to 0.9.

What about the individual lipids in each network? One important aspect to consider is identifying the hubs of the network, which are the most central molecules driving connectivity. In control networks, the hub lipids were a mixture of hydroxylated and non-hydroxylated fatty acid chains. One non-hydroxylated species, 18:1;O3/23:0, emerged multiple times as a hub, even serving as the main hub in the Day 5 control network of Riesling. By contrast, hubs in stress networks were notably more uniform. Among the top four hub lipids across all stressed networks and time points (a total of 32 lipids), only two had a non-hydroxylated FA chain—this strong enrichment of hydroxylated lipids as hubs indicated a central role in lipid network regulation.

The presence of a 2-hydroxy group on the long-chain base improves hydrogen bonding, allowing for tighter packing within the lipid bilayer. This is crucial for increasing membrane rigidity, especially during heat stress, as it is generally known that membrane fluidity naturally rises under these conditions⁵³. It is proposed that the 2-hydroxy group also serves as a more effective binding site, maybe for lipid-protein interactions, which will stabilize stress-response proteins. By identifying the hubs, we have now obtained some targets that could be traced back to specific ceramide synthase or different FAH isoforms, and possibly validate the hubs using isotope-labelled flux. Looking at the number of double bonds or the length of the chains, no notable trend was detected. Isolated lipids - those without significant correlations - were found across both hydroxylated and non-hydroxylated species. However, some appeared repeatedly as isolates, notably 18:1;O3/22:0, 18:1;O3/24:0, and 18:1;O3/25:0. Among hydroxylated lipids, 18:1;O3/22:0;O was the most isolated one, indicating a minor role in the coordinated response to stress.

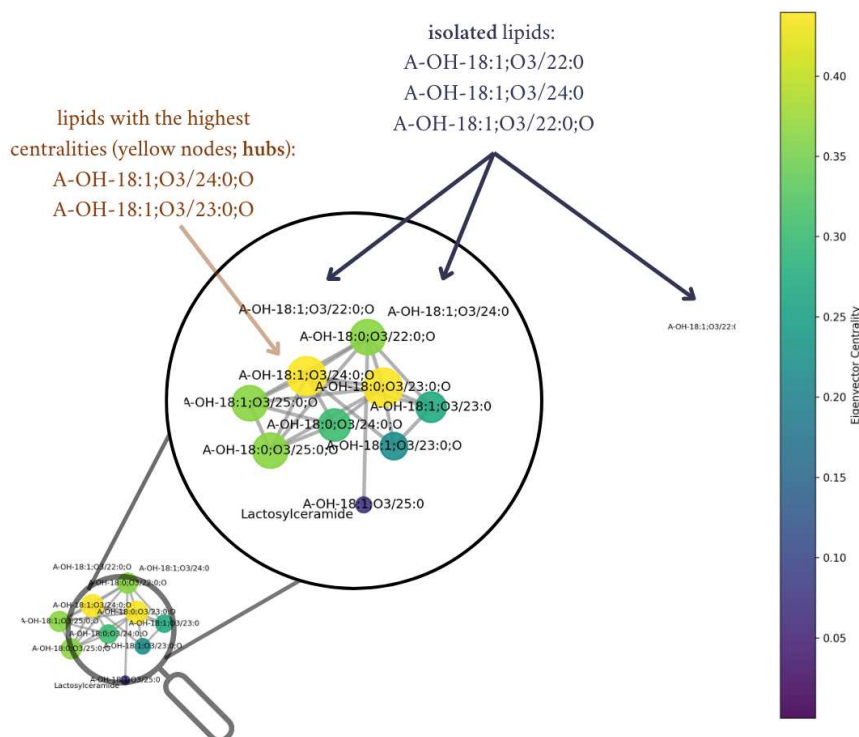


FIGURE 3.19: Network analysis of GIPC species in Riesling samples by Day 75 (stressed condition), highlighting most prominent hubs as well as isolated nodes. Node color gradient indicates eigenvector centrality.

Riesling

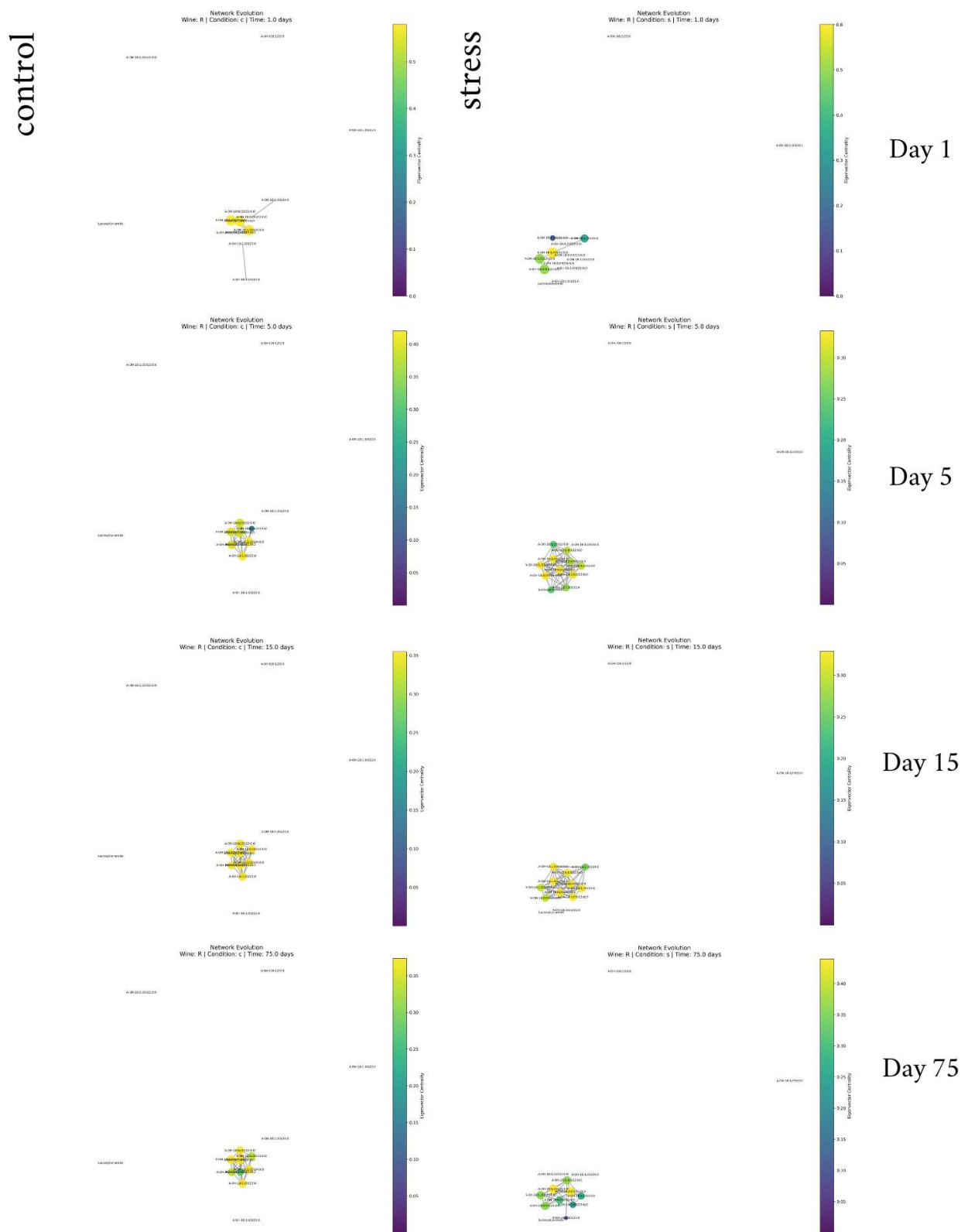


FIGURE 3.20: Network analysis of GIPC species in Riesling samples at each harvest time point. Left: control condition; right: stress condition. Node color gradient indicates eigenvector centrality.

Grüner Veltliner

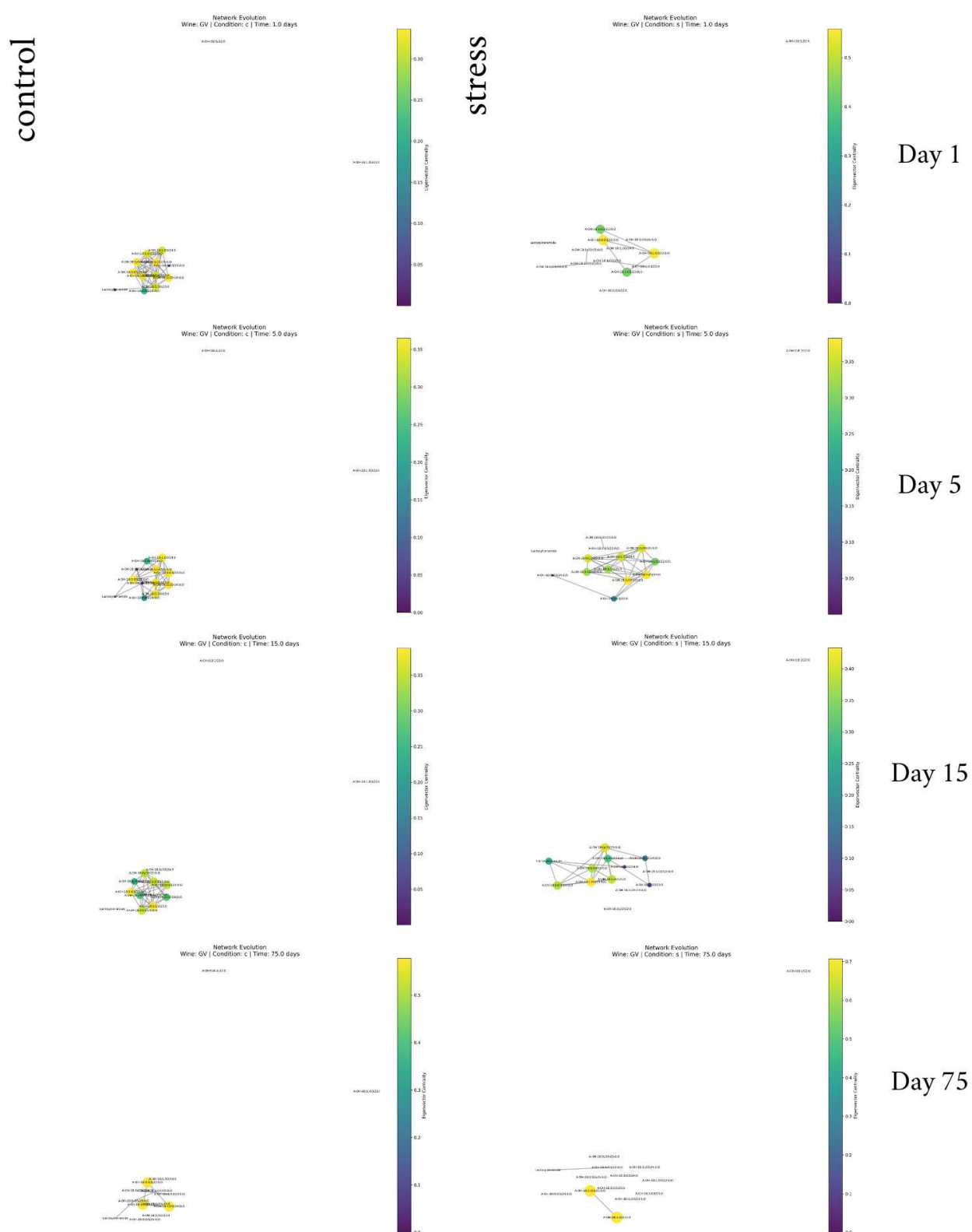


FIGURE 3.21: Network analysis of GIPC species in Grüner Veltliner samples at each harvesting time point. Left: control condition; right: stress condition. Node color gradient indicates eigenvector centrality.

Chapter 4

Discussion

4.1 Biological Interpretation

From the raw data, it is clear that A-OH-18:1;O3/24:0;O dominates the GIPC lipid profile in the wine leave samples across both wine types. GIPCs with a hydroxylated very long fatty acid chain (VLFCA, h-lipids) generally showed higher abundance than their non-hydroxylated counterparts, consistent with findings from spinach reported by Panzeböck et al.⁵

Temporal expression patterns revealed some dynamic shifts: in Grüner Veltliner (GV) samples, stress-induced overexpression was evident at all harvesting time points except for Day 5; in Riesling (R) samples, Day 1 showed some stress-induced changes, and by later time points, nearly all lipids were overexpressed—suggesting a more persistent adaptation under stress. PCA highlighted a clear clustering pattern based on harvesting time points, particularly for Day 1 samples, while Day 5 samples appeared the most dispersed. Hierarchical clustering grouped lipids mainly according to their hydroxylation state and number of double bonds. The two-way ANOVA analysis revealed that GV lipids were broadly overexpressed under stress, while R showed weaker differences, especially in low-abundance non-h-lipids. Stress-related increases peaked by Day 5 and diverged most from controls by Day 15, with lipid levels generally maintained or elevated under stress. Riesling showed more stable patterns over time, whereas GV exhibited stronger shifts, particularly at Days 5 and 15.

Additionally, CV values in GV peaked by Day 5 for both conditions, declined by Day 15, and stabilized or dropped again by Day 75, especially under stress. In Riesling, CV values continued to increase over time. Notably variable lipids were 18:1;O3/22:0 in GV stress and 18:1;O3/25:0 in R, both showing high CVs across multiple time points.

The network analysis revealed that heat stress prompted a more coordinated lipidomic response, with an initial increase in connectivity and denser clustering by Day 5, followed by reduced synchronization by Day 75. Interestingly, while stress networks often exhibited heightened connectivity by Day 5, they consistently exhibited lower global clustering coefficients relative to controls. This suggests that under stress, although lipids interacted more broadly across the network, they formed fewer tightly connected groups, resulting in a more dispersed pattern of coordination. While control networks vary between wine types, one consistent feature was the dominance of h-lipids as hubs in stressed networks, highlighting their role in maintaining membrane stability under thermal stress. These findings support the idea that hydroxylation of fatty acids is functionally prioritized during abiotic stress in a less clustered but more globally responsive stress network.

4.1.1 Connections between the Static and Dynamic Analysis

The integration of static and dynamic analysis provides a clearer understanding of GIPC lipid regulation. Let us revisit the recurring shifts - a prominent peak by Day 5 followed by a sharp decline. This pattern appeared across ANOVA interactions (Time vs Condition, Time vs Wine Type), CV values, and the connectivity and density of stress samples. But what causes this pattern? Is it solely tied to harvesting time, or do other factors contribute?

After reviewing the literature, developmental factors like harvesting time were initially considered possible drivers of the observed lipidomic changes. For instance, Early Leaf Removal (ERL) has been linked to higher phenolic content⁵⁴. Similarly, late-season removal (about 72 days after flowering) increases levels of total and individual amino acids in wine grapes⁵⁵. This could be indirectly responsible for the rise in lipid levels later in the season, indicating physiological changes related to both growth and stress. Additionally, the harvesting times directly influence the amount and makeup of C6 volatiles, which in turn affect membrane lipid composition and the activity of lipid-degrading enzymes⁵⁶. Although these studies focus on berries rather than leaves or even glycosphingolipids, they do pinpoint the significance of timing in understanding lipid data.

The irregular shifts within the time series, however, raised additional questions. Could temperature, rather than development factors, be the underlying cause? Understandably, this variable influences both plant physiology and our data outputs. That is why, in Chapter 2, we also compared outside temperatures in Klosterneuburg with those recorded by loggers beneath the heat-stressed foil. The results showed a consistent trend with our data. This "lurking variable" was ultimately identified—echoing the idea of Judea Peak in "The Book of Why"⁵⁷—indicating that temperature differences have a greater impact on our stressed systems than the development factors.

Continuing with our findings: static results highlighted that h-lipids were more abundant and stable under stress. Although all GIPC A-series lipids are overexpressed (see *Two-way ANOVA*), h-lipids have consistently acted as central hubs in stress conditions. Meanwhile, non-h-species fluctuated in CV analysis and had a higher relative variability. Additionally, their role as hubs was observed only in the control networks. Despite being more flexible and responsive to environmental changes, they appeared to be less structurally significant.

4.1.2 What does the literature say?

To place our findings into a broader biological network, it is essential to connect what is already known about the structural and functional roles of sphingolipids in plants. Our observations—particularly the centrality and stability of hydroxylated lipids (h-lipids) under stress—are supported by several studies in the literature.

Bach et al.⁵⁸ noted that VLCFAs play an essential role in membrane dynamics and structural integrity, e.g., membrane bending and fusion or membrane microdomains. These structural functions may explain the central role of h-lipids in our network analysis. In addition to structural roles, Raffaele et al.⁵⁹ also stated that VLCFAs in plants play a role in the hypersensitive cell death response. While our study focused on abiotic stress rather than biotic stress, the elevated levels and centrality of h-lipids similarly reflect a possible protective or signaling mechanism, helping to maintain cellular integrity during stress adaptation.

Overall, the literature supports our observations that hydroxylated GIPC species with VLFCAs are not only structurally dominant but also functionally prioritized during stress, possibly acting as both membrane stabilizers and potential regulators in stress-responsive pathways.

4.2 Normalization Debate

As part of the data quality assessment (Section 2), multiple normalization strategies were evaluated to identify the most suitable approach for downstream analysis. Normalization to **dry weight** proved to be the most robust, showing lower RSD values than raw data across all lipid species. In contrast, normalization to **total protein content** did not result in any improvements. Overall, this normalization step did not significantly change the expression patterns. However, looking at the different log-transformed areas reveals that protein normalization did influence expression, especially at time points where some changes were already observed (see Day 75 in GV and Day 1 in R). In the PCA analysis, it is also rather noticeable that clusters overlap more after protein normalization, making time-based separation harder to interpret. Comparing the dendrograms from hierarchical clustering, clustering was primarily driven by double-bond numbers. However, the connection between species with the same hydroxylation state was lost. Regarding the ANOVA results, no significant differences were observed.

Similarly, using Lactosyl Ceramide (LacCer) as an **internal standard** was found to introduce significant variability, even among extraction replicates. This variability increased the RSD values, raising questions about the reliability of LacCer as a normalization reference. Future workflows should consider skipping internal standard-based normalization altogether or choosing a more stable internal standard. Overall, normalization to dry weight is recommended as the best approach. Additionally, carefully inspecting the run order for potential time-dependent effects is essential to ensure consistent data quality.

4.3 Limitations and Future Directions

Firstly, a limited number of GIPC species were identified, with only 22 confidently characterized, while the other 78 species and 5 putatively identified higher-class series were annotated. Although no EAD-positive measurements were specifically conducted for GIPCs, our data demonstrate that this mode provides comprehensive fragmentation patterns sufficient for structural elucidation, at least up to Level C. This suggests that EAD-based acquisition could be further optimized with a focus on improving the sensitivity and specificity of the detection, particularly for low-abundance or higher-class GIPCs. In addition, implementing derivatization strategies might be another way on how to improve the detection sensitivity for more challenging lipid species.⁶⁰

A key challenge in GIPC identification is the lack of commercially available standards. Without authentic standards, validation of retention times and fragmentation patterns remains indirect and largely reliant on in silico prediction models. This limitation, in combination with manual spectrum verification, reduces confidence in lower-level identifications which hinders a consistent annotation across studies.

While the Lipid Data Analyzer (LDA) proved to be a valuable tool with integrated fragmentation rules, the annotation process was not fully automated; every fragment documented in this

study had to be manually verified. These limitations likely contribute to the underrepresentation of GIPCs in major databases such as LIPID MAPS.

Another broader limitation is the current lack of network-based lipidomics analyses for glycosphingolipids, and the fact that GIPCs are rarely included in comprehensive lipidome studies. This is mainly due to the specific extraction and chromatographic methods required for their analysis. Our network-based approach could be expanded to include protein-lipid interaction networks or a combination of primary metabolism networks, to potentially reveal how specific GIPCs interact with membrane proteins and metabolites during stress responses. By integrating omics networks, we might find targets for future biotechnological applications and discover a new way to enhance stress tolerance in economically important crops; e.g., by identifying potential biomarkers and by optimizing GIPC profiles in plant cell cultures to increase the production of stress-protective vesicles or other lipid-based products⁶¹. What should also be considered is a more comprehensive experimental design, including multiple tissue types or stress conditions, to uncover spatial and more condition-specific differences in GIPC composition and function across various parts of the plant.

Chapter 5

Conclusion

In this study, an in-house optimized extraction protocol was applied for the analysis of Glycosyl Inositol Phosphoceramides (GIPCs) from wine leaf samples, followed by LC-MS/MS measurements across three acquisition modes: EAD-positive, CID-positive, and CID-negative. The complementary fragmentation patterns allowed for robust structural characterization. Annotation was performed using Lipid Data Analyzer (LDA), confirming the expected dicot-specific distribution of GIPCs. The novelty of this study is the use of EAD-based acquisition for GIPC analysis. Additionally, the fragment ion at m/z 621 was successfully identified in positive mode, confidently assigning the HexA-IPC backbone, especially in the $[Na+]$ mode.

In total, 100 GIPC species were identified across two levels of confidence: 22 species were classified as partially resolved, while 78 lipids from the A-series, along with 5 higher-class series, were putatively identified. Within the partially resolved species, only A-series GIPCs were identified. The majority contained an OH functional group and contained an 18-carbon (C) long-chain base (LCB) that was trihydroxylated and carried either zero or one double bond. The fatty acid chains ranged from 20 to 26C atoms and were either non-hydroxylated or hydroxylated. In contrast, A-series lipids bearing an NH functional group were characterized by an 18C, dihydroxylated LCBs, with all fatty acid chains (ranging from 24C to 27C) being non-hydroxylated and consistently containing a single double bond.

The statistical analysis—including PCA, hierarchical clustering, and two-way ANOVA—revealed time- and condition-dependent shifts in GIPC expression. Dynamic network analysis supported the central role of lipids with a hydroxylated very long fatty acid chain (VLFCFA) under heat stress, showing increased density and centrality in the networks. Notably, changes in expression patterns and network connectivity aligned with recorded temperature differences between ambient and heat-stressed conditions, reflecting a systemic membrane restructuring response, where hydroxylated GIPCs act as stabilizing hubs to maintain membrane integrity under thermal stress.

Understanding GIPC dynamics in wine leaves under abiotic stress gives us additional environmental context. Limitations such as the lack of commercial standards, the need for manual annotation, and the detection of low-abundance species call for methodological improvements. Nonetheless, our method serves as a solid foundation for future studies focusing on enhancing EAD-based fragmentation, exploring derivatization strategies, and expanding network analyses to include protein-lipid interactions and broader stress conditions. These advancements could help characterize the role of GIPCs in plant stress responses and support their application in crop biotechnology.

Bibliography

- (¹) Sherman, E.; Pinu, F. R. Lipidomics in grape and wine research: Past, present and future applications. *Current Opinion in Food Science* **2023**, *52*, 101046.
- (²) Tumanov, S.; Zubenko, Y.; Greven, M.; Greenwood, D. R.; Shmanai, V.; Villas-Boas, S. G. Comprehensive lipidome profiling of Sauvignon blanc grape juice. *Food Chemistry* **2015**, *180*, 249–256.
- (³) Cassim, A. M.; Navon, Y.; Gao, Y.; Decossas, M.; Fouillen, L.; Grélard, A.; Nagano, M.; Lambert, O.; Bahammou, D.; Van Delft, P., et al. Biophysical analysis of the plant-specific GIPC sphingolipids reveals multiple modes of membrane regulation. *Journal of Biological Chemistry* **2021**, 296.
- (⁴) Carter, H. E.; Celmer, W. D.; Galanos, D. S.; Gigg, R.; Lands, W. E.; Law, J. H.; Mueller, K. L.; Nakayama, T.; Tomizawa, H.; Weber, E. Biochemistry of the sphingolipides. X. Phytoglycolipide, a complex phytosphingosine-containing lipide from plant seeds. *Journal of the American Oil Chemists' Society* **1958**, *35*, 335–343.
- (⁵) Panzenboeck, L.; Troppmair, N.; Schlachter, S.; Koellensperger, G.; Hartler, J.; Rampler, E. Chasing the major sphingolipids on earth: automated annotation of plant glycosyl inositol phospho ceramides by glycolipidomics. *Metabolites* **2020**, *10*, 375.
- (⁶) Rennie, E. A.; Ebert, B.; Miles, G. P.; Cahoon, R. E.; Christiansen, K. M.; Stonebloom, S.; Khatab, H.; Twell, D.; Petzold, C. J.; Adams, P. D., et al. Identification of a sphingolipid α -glucuronosyltransferase that is essential for pollen function in Arabidopsis. *The Plant Cell* **2014**, *26*, 3314–3325.
- (⁷) Mortimer, J. C.; Yu, X.; Albrecht, S.; Sicilia, F.; Huichalaf, M.; Ampuero, D.; Michaelson, L. V.; Murphy, A. M.; Matsunaga, T.; Kurz, S., et al. Abnormal glycosphingolipid mannosylation triggers salicylic acid-mediated responses in Arabidopsis. *The Plant Cell* **2013**, *25*, 1881–1894.
- (⁸) Cacas, J.-L.; Bure, C.; Furt, F.; Maalouf, J.-P.; Badoc, A.; Cluzet, S.; Schmitter, J.-M.; Antajan, E.; Mongrand, S. Biochemical survey of the polar head of plant glycosylinositolphosphoceramides unravels broad diversity. *Phytochemistry* **2013**, *96*, 191–200.
- (⁹) Kaul, K.; Lester, R. L. Characterization of inositol-containing phosphosphingolipids from tobacco leaves: isolation and identification of two novel, major lipids: N-acetylglucosamidoglucuronidoinositol phosphorylceramide and glucosamidoglucuronidoinositol phosphorylceramide. *Plant Physiology* **1975**, *55*, 120–129.
- (¹⁰) Trinel, P.-A.; Maes, E.; Zanetta, J.-P.; Delplace, F.; Coddeville, B.; Jouault, T.; Strecker, G.; Poulain, D. Candida albicans phospholipomannan, a new member of the fungal mannose inositol phosphoceramide family. *Journal of Biological Chemistry* **2002**, *277*, 37260–37271.
- (¹¹) Pata, M. O.; Hannun, Y. A.; Ng, C. K.-Y. Plant sphingolipids: decoding the enigma of the Sphinx. *New Phytologist* **2010**, *185*, 611–630.

- (¹²) Buré, C.; Cacas, J.-L.; Wang, F.; Gaudin, K.; Domergue, F.; Mongrand, S.; Schmitter, J.-M. Fast screening of highly glycosylated plant sphingolipids by tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* **2011**, *25*, 3131–3145.
- (¹³) Pühringer, M.; Thür, N.; Schnurer, M.; Lamp, L. M.; Panzenboeck, L.; Hartler, J.; Tanzer, A.; Ibl, V.; Rampler, E. Automated mass spectrometry-based profiling of multi-glycosylated glycosyl inositol phospho ceramides (GIPC) reveals specific series GIPC rearrangements during barley grain development and heat stress response. *The Plant Journal* **2025**, *122*, e70279.
- (¹⁴) IUPAC-IUB The nomenclature of lipids: Recommendations (1976). *Lipids* **1977**, *12*, 455–468.
- (¹⁵) Liebisch, G.; Fahy, E.; Aoki, J.; Dennis, E. A.; Durand, T.; Ejsing, C. S.; Fedorova, M.; Feussner, I.; Griffiths, W. J.; Köfeler, H., et al. Update on LIPID MAPS classification, nomenclature, and shorthand notation for MS-derived lipid structures. *Journal of lipid research* **2020**, *61*, 1539–1555.
- (¹⁶) Jiang, Z.; Zhou, X.; Tao, M.; Yuan, F.; Liu, L.; Wu, F.; Wu, X.; Xiang, Y.; Niu, Y.; Liu, F., et al. Plant cell-surface GIPC sphingolipids sense salt to trigger Ca²⁺ influx. *Nature* **2019**, *572*, 341–346.
- (¹⁷) Grosjean, K.; Mongrand, S.; Beney, L.; Simon-Plas, F.; Gerbeau-Pissot, P. Differential effect of plant lipids on membrane organization: specificities of phytosphingolipids and phytosterols. *Journal of Biological Chemistry* **2015**, *290*, 5810–5825.
- (¹⁸) Morita, N.; Nakazato, H.; Okuyama, H.; Kim, Y.; Thompson Jr, G. A. Evidence for a glycosyl-inositolphospholipid-anchored alkaline phosphatase in the aquatic plant *Spirodela oligorhiza*. *Biochimica et Biophysica Acta (BBA)-General Subjects* **1996**, *1290*, 53–62.
- (¹⁹) Lenarčič, T.; Albert, I.; Böhm, H.; Hodnik, V.; Pirc, K.; Zavec, A. B.; Podobnik, M.; Pahovnik, D.; Žagar, E.; Pruitt, R., et al. Eudicot plant-specific sphingolipids determine host selectivity of microbial NLP cytolysins. *Science* **2017**, *358*, 1431–1434.
- (²⁰) Beck, J. G.; Mathieu, D.; Loudet, C.; Buchoux, S.; Dufourc, E. J. Plant sterols in “rafts”: a better way to regulate membrane thermal shocks. *The FASEB Journal* **2007**, *21*, 1714–1723.
- (²¹) Gasulla, F.; Vom Dorp, K.; Dombrink, I.; Zähringer, U.; Gisch, N.; Dörmann, P.; Bartels, D. The role of lipid metabolism in the acquisition of desiccation tolerance in *C. raterostigma plantagineum*: a comparative approach. *The Plant Journal* **2013**, *75*, 726–741.
- (²²) Tarazona, P.; Feussner, K.; Feussner, I. An enhanced plant lipidomics method based on multiplexed liquid chromatography–mass spectrometry reveals additional insights into cold-and drought-induced membrane remodeling. *The Plant Journal* **2015**, *84*, 621–633.
- (²³) Bligh, E.; Dyer, W. Canadian journal of biochemistry and physiology. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917.
- (²⁴) Nichols, B. Separation of the lipids of photosynthetic tissues: improvements in analysis by thin-layer chromatography. *Biochimica et Biophysica Acta (BBA)-Specialized Section on Lipids and Related Subjects* **1963**, *70*, 417–422.
- (²⁵) Hanson, B. A.; Lester, R. L. The extraction of inositol-containing phospholipids and phosphatidylcholine from *Saccharomyces cerevisiae* and *Neurospora crassa*. *Journal of lipid research* **1980**, *21*, 309–315.

- (²⁶) Toledo, M.; Suzuki, E.; Straus, A.; Takahashi, H. Glycolipids from *Paracoccidioides brasiliensis*. Isolation of a galactofuranose-containing glycolipid reactive with sera of patients with paracoccidioidomycosis. *Journal of Medical and Veterinary Mycology* **1995**, 33, 247–251.
- (²⁷) Markham, J. E.; Li, J.; Cahoon, E. B.; Jaworski, J. G. Separation and identification of major plant sphingolipid classes from leaves. *Journal of Biological Chemistry* **2006**, 281, 22684–22694.
- (²⁸) Thür, N. Glycosyl inositol phospho ceramide analysis using ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry and multistage fragmentation to monitor heat stress in plants, English, Includes illustrations and diagrams. Supervised and evaluated by Evelyn Rampler., Master's thesis, Vienna: University of Vienna, Faculty of Chemistry, 2022, p 74.
- (²⁹) Rampler, E.; Abiead, Y. E.; Schoeny, H.; Ruzs, M.; Hildebrand, F.; Fitz, V.; Koellensperger, G. Recurrent topics in mass spectrometry-based metabolomics and lipidomics—standardization, coverage, and throughput. *Analytical Chemistry* **2020**, 93, 519–545.
- (³⁰) Hartler, J.; Armando, A. M.; Trötzlmüller, M.; Dennis, E. A.; Köfeler, H. C.; Quehenberger, O. Automated annotation of sphingolipids including accurate identification of hydroxylation sites using MS n data. *Analytical chemistry* **2020**, 92, 14054–14062.
- (³¹) Wetter Klosterneuburg, Niederoesterreich, Oesterreich heute und morgen — timeanddate.de, <https://www.timeanddate.de/wetter/oesterreich/klosterneuburg>, [Accessed 06-07-2025].
- (³²) Shin, S.-H.; Park, M.-H.; Byeon, J.-J.; Lee, B. I.; Park, Y.; Ko, A.-r.; Seong, M.-r.; Lee, S.; Kim, M. R.; Seo, J., et al. A liquid chromatography-quadrupole-time-of-flight mass spectrometric assay for the quantification of fabry disease biomarker globotriaosylceramide (GB3) in Fabry model mouse. *Pharmaceutics* **2018**, 10, 69.
- (³³) Sidhu, R.; Mikulka, C. R.; Fujiwara, H.; Sands, M. S.; Schaffer, J. E.; Ory, D. S.; Jiang, X. A HILIC-MS/MS method for simultaneous quantification of the lysosomal disease markers galactosylsphingosine and glucosylsphingosine in mouse serum. *Biomedical Chromatography* **2018**, 32, e4235.
- (³⁴) Cruz, R.; Casal, S.; Mendes, E.; Costa, A.; Santos, C.; Morais, S. Validation of a single-extraction procedure for sequential analysis of vitamin E, cholesterol, fatty acids, and total fat in seafood. *Food Analytical Methods* **2013**, 6, 1196–1204.
- (³⁵) Shahidi, F.; Wanasundara, P. In *Food lipids*; CRC Press: 2002, pp 152–187.
- (³⁶) Ostermann, A. I.; Müller, M.; Willenberg, I.; Schebb, N. H. Determining the fatty acid composition in plasma and tissues as fatty acid methyl esters using gas chromatography—a comparison of different derivatization and extraction procedures. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **2014**, 91, 235–241.
- (³⁷) Che, P.; Davidson, J. T.; Kool, J.; Kohler, I. Electron activated dissociation—a complementary fragmentation technique to collision-induced dissociation for metabolite identification of synthetic cathinone positional isomers. *Analytica Chimica Acta* **2023**, 1283, 341962.
- (³⁸) 1260 Infinity II Manual Preparative LC System — agilent.com, <https://www.agilent.com/en/product/liquid-chromatography/hplc-systems/preparative-hplc-systems/1260-infinity-ii-manual-preparative-lc-system>, [Accessed 05-07-2025].

- (³⁹) ZenoTOF 7600 system — sciex.com, <https://sciex.com/products/mass-spectrometers/qtof-systems/zenotof-7600-system>, [Accessed 04-07-2025].
- (⁴⁰) Hartler, J.; Trötz Müller, M.; Chitraju, C.; Spener, F.; Köfeler, H. C.; Thallinger, G. G. Lipid Data Analyzer: unattended identification and quantitation of lipids in LC-MS data. *Bioinformatics* **2011**, *27*, 572–577.
- (⁴¹) Adams, K. J.; Pratt, B.; Bose, N.; Dubois, L. G.; St. John-Williams, L.; Perrott, K. M.; Ky, K.; Kapahi, P.; Sharma, V.; MacCoss, M. J., et al. Skyline for small molecules: a unifying software package for quantitative metabolomics. *Journal of proteome research* **2020**, *19*, 1447–1458.
- (⁴²) Silva, Á. A. R.; Bertolucci, V.; Scariot, P. P. M.; da Cruz, J. P.; Mendes, F. M. M.; de Oliveira, D. C.; Plumari, C. D.; Dos Reis, I. G. M.; Porcari, A. M.; Messias, L. H. D. Glycerophospholipids in Red Blood Cells Are Associated with Aerobic Performance in Young Swimmers. *Nutrients* **2024**, *16*, 765.
- (⁴³) FastAPI — fastapi.tiangolo.com, <https://fastapi.tiangolo.com/>, [Accessed 04-07-2025].
- (⁴⁴) Panzenböck, L. Development of an analysis strategy for glycosyl inositol phospho ceramides: the major sphingolipids on earth, English, Includes illustrations and diagrams. Contains abstract in German. Supervised by Gunda Köllensperger; Co-supervised by Evelyn Rampler., Master's thesis, Vienna: University of Vienna, Faculty of Chemistry, 2020, pp VI, 61.
- (⁴⁵) Harvey, D. J. Fragmentation of negative ions from carbohydrates: part 2. Fragmentation of high-mannose N-linked glycans. *Journal of the American Society for Mass Spectrometry* **2005**, *16*, 631–646.
- (⁴⁶) Deguchi, K.; Ito, H.; Takegawa, Y.; Shinji, N.; Nakagawa, H.; Nishimura, S.-I. Complementary structural information of positive-and negative-ion MS_n spectra of glycopeptides with neutral and sialylated N-glycans. *Rapid communications in mass spectrometry* **2006**, *20*, 741–746.
- (⁴⁷) Chao, H.-C.; McLuckey, S. A. Manipulation of ion types via gas-phase ion/ion chemistry for the structural characterization of the glycan moiety on gangliosides. *Analytical chemistry* **2021**, *93*, 15752–15760.
- (⁴⁸) White, J. B.; Trim, P. J.; Salagaras, T.; Long, A.; Psaltis, P. J.; Verjans, J. W.; Snel, M. F. Equivalent carbon number and interclass retention time conversion enhance lipid identification in untargeted clinical lipidomics. *Analytical Chemistry* **2022**, *94*, 3476–3484.
- (⁴⁹) Olshansky, G.; Giles, C.; Salim, A.; Meikle, P. J. Challenges and opportunities for prevention and removal of unwanted variation in lipidomic studies. *Progress in Lipid Research* **2022**, *87*, 101177.
- (⁵⁰) Raboy, B.; Sharon, G.; Parag, H.; Shochat, Y.; Kulka, R. Effect of stress on protein degradation: role of the ubiquitin system. *Acta biologica Hungarica* **1991**, *42*, 3–20.
- (⁵¹) Bar-Joseph, Z.; Gitter, A.; Simon, I. Studying and modelling dynamic biological processes using time-series gene expression data. *Nature Reviews Genetics* **2012**, *13*, 552–564.
- (⁵²) Zhang, R.; Zhu, Z.; Jia, W. Time-series lipidomics insights into the progressive characteristics of lipid constituents of fresh walnut during postharvest storage. *Journal of agricultural and food chemistry* **2021**, *69*, 13796–13809.

-
- (⁵³) Yu, L.; Zhou, C.; Fan, J.; Shanklin, J.; Xu, C. Mechanisms and functions of membrane lipid remodeling in plants. *The Plant Journal* **2021**, *107*, 37–53.
- (⁵⁴) Stefanovic, D.; Nikolic, N.; Kostic, L.; Todic, S.; Nikolic, M. Early leaf removal increases berry and wine phenolics in Cabernet Sauvignon grown in eastern Serbia. *Agronomy* **2021**, *11*, 238.
- (⁵⁵) Yue, X.-f. Effects of the severity and timing of basal leaf removal on the amino acids profiles of Sauvignon Blanc grapes and wines (vol 18, pg 2052, 2019). *Journal of Integrative Agriculture* **2021**, *20*, III–III.
- (⁵⁶) Vilanova, M.; Diago, M. P.; Genisheva, Z.; Oliveira, J. M.; Tardaguila, J. Early leaf removal impact on volatile composition of Tempranillo wines. *Journal of the Science of Food and Agriculture* **2012**, *92*, 935–942.
- (⁵⁷) Pearl, J.; Mackenzie, D., *The Book of Why: The New Science of Cause and Effect*; Basic Books: New York, 2018.
- (⁵⁸) Bach, L.; Faure, J.-D. Role of very-long-chain fatty acids in plant development, when chain length does matter. *Comptes rendus biologies* **2010**, *333*, 361–370.
- (⁵⁹) Raffaele, S.; Vailleau, F.; Leger, A.; Joubès, J.; Miersch, O.; Huard, C.; Blée, E.; Mongrand, S.; Domergue, F.; Roby, D. A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in Arabidopsis. *The Plant Cell* **2008**, *20*, 752–767.
- (⁶⁰) Griffiths, W. J.; Ogundare, M.; Williams, C. M.; Wang, Y. On the future of “omics”: lipidomics. *Journal of inherited metabolic disease* **2011**, *34*, 583–592.
- (⁶¹) Ambrosone, A.; Barbulova, A.; Cappetta, E.; Cillo, F.; De Palma, M.; Ruocco, M.; Pocsfalvi, G. Plant extracellular vesicles: Current landscape and future directions. *Plants* **2023**, *12*, 4141.

Appendix A

Tools and Software Used

A.1 Word Processing Software

This thesis was written in Overleaf based on a LaTeX template (Version 2.5, dated 27/08/2017) developed by Steve Gunn and Sunil Patel. The template is licensed under the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 license (CC BY-NC-SA 3.0): <http://creativecommons.org/licenses/by-nc-sa/3.0/>.

A.2 Bibliography Management

Zotero (Version 7.0.16) was used for reference management. The citation style was set according to university guidelines using the American Chemical Society (ACS) format. BibTeX citations were primarily retrieved via Google Scholar.

A.3 Data Analysis

Python scripts were written and executed in Visual Studio Code (Version 1.101.2) using Python 3.12.8 and Git 2.47.1 (64-bit). These scripts were developed for data quality assessment, processing, user interface, and statistical analysis. All required Python libraries and their versions are listed in Listing A.1.

Plots were primarily created using Microsoft Excel or custom Python scripts. Canva was additionally used to design figures, particularly for Chapters 1 and 2.

LISTING A.1: Python libraries and their versions used in the project.

```
pandas==2.2.3
matplotlib==3.10.1
numpy==1.26.4
seaborn==0.13.2
networkx==3.4.2
scipy==1.13.1
imageio==2.37.0
scikit-learn==1.6.1
statsmodels==0.14.4
fastapi==0.115.12
uvicorn==0.34.1
python-multipart==0.0.20
```

The following additional software tools were used:

- **Lipid Data Analyzer (LDA)** — Version 2.10.1 (downloaded on 10.12.2024)
- **ProteoWizard** — Version 3.0.25071
- **Skyline (64-bit)** — Version 24.1.0.199
- **ChemDraw** — Version 19.1
- **SCIEX OS** — Version 3.3.1.43
- **ClearCore2.Service** — Version 3.3.1.43

A.4 Use of AI Tools

ChatGPT (GPT-4o, Standard Model) was used to support formatting of listings, generation of tables and info boxes, structural improvement of the text, as well as refinement of figure captions. Grammar was checked via **Grammarly**. **Claude Sonnet 3.7** was used for verifying, debugging, restructuring, and correcting Python codes used in the project.

Appendix B

Normalization Table

TABLE B.1: Normalization table for all wine samples showing initial weight, dry weight (DW) percentile, calculated dry weight, and corresponding protein content in mg/mL.

Sample	Weight [mg]	DW_Percentile	DW [mg]	Protein_Content [mg/mL]
S1_GV_c_1	106,66	0,272	28,979	5,465
S1_GV_c_2	99,78	0,272	27,110	10,902
S1_GV_c_3	101,54	0,272	27,588	11,439
S1_GV_c_4_rep1	98,22	0,272	26,686	7,028
S1_GV_c_4_rep2	105,01	0,272	28,531	7,028
S1_GV_c_4_rep3	104,08	0,272	28,278	7,028
S1_GV_c_4_rep4	106,61	0,272	28,966	7,028
S1_GV_s_1	103,22	0,256	26,462	8,060
S1_GV_s_2	105,3	0,256	26,995	12,691
S1_GV_s_3	103,6	0,256	26,559	7,925
S1_GV_s_4	100,75	0,256	25,829	10,919
S1_R_c_1	102,51	0,312	32,019	7,508
S1_R_c_2	101,52	0,312	31,710	14,889
S1_R_c_3	102,54	0,312	32,028	14,713
S1_R_c_4	101,72	0,312	31,772	11,109
S1_R_s_1	103,7	0,290	30,095	14,210
S1_R_s_2	103,33	0,290	29,987	14,249
S1_R_s_3	99,91	0,290	28,995	19,854
S1_R_s_4	102,09	0,290	29,627	13,152
S2_GV_c_1	102,07	0,305	31,109	5,822
S2_GV_c_2	104,18	0,305	31,752	12,728
S2_GV_c_3	101,15	0,305	30,828	10,009
S2_GV_c_4	101,66	0,305	30,984	8,136
S2_GV_s_1	99,58	0,333	33,143	5,822
S2_GV_s_2	101,63	0,333	33,825	21,744
S2_GV_s_5	101,4	0,333	33,749	10,646
S2_GV_s_4	100,09	0,333	33,313	6,771

Sample	Weight [mg]	DW_Percentile	DW [mg]	Protein_Content [mg/mL]
S2_R_c_1	101,18	0,282	28,573	9,293
S2_R_c_2	100,95	0,282	28,508	19,743
S2_R_c_3	102,03	0,282	28,813	7,085
S2_R_c_4	103,39	0,282	29,197	27,259
S2_R_s_1	94,35	0,272	25,632	20,937
S2_R_s_2	100,73	0,272	27,365	17,724
S2_R_s_3_rep1	104,53	0,272	28,397	12,220
S2_R_s_3_rep2	100,5	0,272	27,302	12,220
S2_R_s_3_rep3	102,18	0,272	27,759	12,220
S2_R_s_3_rep4	99,53	0,272	27,039	12,220
S2_R_s_4	100,63	0,272	27,338	15,944
S4_GV_c_1	105,28	0,277	29,130	7,753
S4_GV_c_2	102,62	0,277	28,394	8,863
S4_GV_c_3	103,87	0,277	28,739	19,278
S4_GV_c_4	99,26	0,277	27,464	8,582
S4_GV_s_1	100,62	0,301	30,293	17,471
S4_GV_s_2	99,49	0,301	29,953	7,775
S4_GV_s_3	105,15	0,301	31,657	7,775
S4_GV_s_4	100,38	0,301	30,221	9,437
S4_R_c_1	101,74	0,304	30,979	17,660
S4_R_c_2	101,33	0,304	30,855	20,960
S4_R_c_3	100,52	0,304	30,608	17,576
S4_R_c_4	109,2	0,304	33,251	8,551
S4_R_s_1	105,83	0,270	28,528	7,201
S4_R_s_2	104,06	0,270	28,051	5,348
S4_R_s_3	101,58	0,270	27,383	5,181
S4_R_s_4	105,92	0,270	28,553	5,903
S5_GV_c_1	101,48	0,330	33,537	7,955
S5_GV_c_2	99,03	0,330	32,727	7,567
S5_GV_c_3	103,43	0,330	34,182	6,729
S5_GV_c_4	100,26	0,330	33,134	12,986
S5_GV_s_1	103,13	0,314	32,393	13,488
S5_GV_s_2	97,58	0,314	30,650	11755,760
S5_GV_s_3	100,48	0,314	31,561	7114,294
S5_GV_s_4	105,44	0,314	33,119	12346,016
S5_R_c_1	102,14	0,289	29,531	11862,160
S5_R_c_2	103	0,289	29,780	26033,814
S5_R_c_3	102,14	0,289	29,531	12728,065
S5_R_c_4	103,2	0,289	29,838	10799,447
S5_R_s_1	100,88	0,310	31,285	11106,279
S5_R_s_2	100	0,310	31,012	12418,402
S5_R_s_3	104,52	0,310	32,414	10799,447
S5_R_s_4	102,78	0,310	31,874	12418,402

Appendix C

GIPC ID Lists GV Sampling 1

TABLE C.1: GIPC ID List: Replicates of *S1_GV_c* with matching retention times in CID positive mode.

160325_S1_GV_c_1_pos_CID						
Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
A-NH-39:2;O3 A-NH-43:1;O2		1223,6599 1265,7477		16,43 16,36		B1_amine:184,069 Z0mH2O:650,602 Z0:668,614
A-NH-44:2;O3		1293,7635		28,29		Z0mH2O:678,638
A-OH-38:1;O4	1206,6722		13,45		IP:261,218 Z0:608,561	
A-OH-39:1;O4	1220,691		14,58		Z0mH2O:604,558 Z0:622,578	
A-OH-18:0;O3/22:0;O	1236,7221		16,23		IP:261,037 W-H2O(18:0;O3):282,282 W(18:0;O3):300,29 Z0mH2O:620,599 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,7053	1256,687	15,63	15,62	IP:261,031 W(18:1;O3):298,27 Z0mH2O:618,58 Z0:636,595	IP:283,019 B2_hydroxy:361,078 C3PO3_B1_459:459.049 621,101 Z0:658,552
A-OH-18:0;3O/23:0;O	1250,7373		16,74		IP:261,045 W(18:0;O3):300,293 Z0mH2O:634,601 Z0:652,625	
A-OH-41:1;O3	1232,7211		16,63		Z0mH2O:616,597 Z0:634,612	
A-OH-18:1;O3/23:0;O	1248,7198	1270,7071	16,34	16,35	IP:261,039 W-H2O(18:1;O3):280,272 W(18:1;O3):298,27 Z0mH2O:632,596 Z0:650,606	IP:283,159 B2_hydroxy:361,072 C3PO3_B1_459:459,052 621,104 Z0:672,561
A-OH-41:1;O4	1248,7209		28,08		IP:261,032 Z0:650,605	
A-OH-42:0;O4	1264,7484	1286,73	17,18	17,16	IP:261,034 Z0mH2O:648,625 Z0:666,632	IP:283,021 B2_hydroxy:361,078 C3PO3_B1_459:459,055
A-OH-18:1;O3/24:0	1246,7436		17,05		IP:261,035 W(18:1;O3):298,274	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7363	1284,7197	16,84	16,85	IP:261,036 W-H2O(18:1;O3):280,257 W(18:1;O3):298,272 Z0mH2O:646,613 Z0:664,623	IP:283,017 B2_hydroxy:361,09 C3PO3_B1_459:459,049 621,097 Z0:686,587
A-OH-43:0;O3		1284,7201		17,31		C3PO3_B1_459:459,055 621,104 667,645 Z0mH2O:668,648 669,648
A-OH-18:1;O3/25:0;O	1276,7538	1298,735	17,26	17,23	IP:261,034 W(18:1;O3):298,277 Z0mH2O:660,62 Z0:678,639	IP:283,024 B2_hydroxy:361,052 C3PO3_B1_459:459,052 621,105 Z0:700,602
A-OH-18:1;O3/26:0;O	1290,7668		17,54		IP:261,035 W-H2O(18:1;O3):280,263 W(18:1;O3):298,274 Z0mH2O:674,643 Z0:692,658	
A-OH-18:1;O3/26:1;O	1310,7171		14,1		184,071 W(18:1;O3):298,277 Z0:712,609	
160325_S1_GV_c_2_pos_CID						
A-NH-42:1;O2		1251,7303		15,52		Z0mH2O:636,59
A-NH-18:0;O3/25:1	1281,7738		17,19		W(18:0;O3):300,282 Z0mH2O:666,638 Z0:684,646	
A-NH-46:3;O2	1281,7738		17,19		IP:261,035 Z0mH2O:666,638 Z0:684,646	
A-OH-38:1;O4	1206,6729		13,36		IP:261,025 Z0mH2O:590,546 Z0:608,561	
A-OH-18:0;O3/22:0;O	1236,7159		16,16		IP:261,035 W-H2O(18:0;O3):282,282 Z0mH2O:620,598 Z0:638,605	
A-OH-18:1;O3/22:0;O	1234,7038	1256,6808	15,55	15,51	IP:261,034	IP:283,019

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,272 Z0mH2O:618,593 Z0:636,591	B2_hydroxy:361,072 C3PO3_B1_459:459,051 621,104 Z0:658,581
A-OH-18:0;O3/23:0;O	1250,7305		16,69		IP:261,037 W(18:0;O3):300,292 Z0mH2O:634,615 635,611 Z0:652,623	
A-OH-18:1;O3/23:0;O	1248,7187	1270,7017	16,26	16,3	IP:261,034 W-H2O(18:1;O3):280,265 W(18:1;O3):298,275 Z0mH2O:632,6 Z0:650,606	B2_hydroxy:361,086 C3PO3_B1_459:459,048 621,104 Z0:672,602
A-OH-18:0;O3/24:0;O	1264,7505		17,13		IP:261,05 W(18:0;O3):300,29 C3PO3_B1_437:437,062 Z0mH2O:648,635 Z0:666,635	
A-OH-18:1;O3/24:0	1246,7369		17,01		IP:261,035 W(18:1;O3):298,273 Z0:648,624	
A-OH-18:1;O3/24:0;O	1262,7361	1284,7168	16,78	16,8	IP:261,041 W-H2O(18:1;O3):280,262 W(18:1;O3):298,275 Z0mH2O:646,608 Z0:664,622	IP:283,013 B2_hydroxy:361,078 C3PO3_B1_459:459,052 621,104 Z0:686,59
A-OH-43:1;O4		1298,7292		17,22		B2_hydroxy:361,075 C3PO3_B1_459:459,048 621,1 Z0:700,609
A-OH-18:1/O3/25:0;O	1276,747		17,22		IP:261,036 W(18:1;O3):298,28 Z0mH2O:660,615 Z0:678,638	
A-OH-44:1;O4	1290,7596		17,54		165,09 IP:261,03 Z0:692,653	
160325_S1_GV_c_3_pos_CID						
A-NH-42:1;O2		1251,7233		15,58		Z0mH2O:636,589

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
						Z0:654,603
A-NH-18:1;O3/25:1	1279,7718		17,07		W(18:1;O3):298,286 Z0mH2O:664,62	
A-NH-44:1;O2		1279,7718		17,07		Z0mH2O:664,62
A-OH-18:0;O3/22:0;O	1236,7208		16,21		IP:261,034 W(18:0;O3):300,297 Z0mH2O:620,587 Z0:638,608	
A-OH-18:1;O3/22:0;O	1234,7037	1256,6819	15,63	15,64	IP:261,04 W-H2O(18:1;O3):280,274 W(18:1;O3):298,307 Z0mH2O:618,596 Z0:636,593	C3PO3_B1_459:459,05 621,099
A-OH-41:0;O3		1256,6819		15,64		C3PO3_B1_459:459,05 621,099
A-OH-18:1;O3/23:0;O	1248,7174		16,34		IP:261,037 W-H2O(18:1;O3):280,26 W(18:1;O3):298,271 Z0mH2O:632,587 Z0:650,612	
A-OH-42:1;O4		1284,7168		16,82	B2_hydroxy:361,071 C3PO3_B1_459:459,05 621,106 Z0:686,608	IP:283,02
A-OH-18:1;O3/24:0;O	1262,7345		16,83		IP:261,033 W-H2O(18:1;O3):280,266 W(18:1;O3):298,277 Z0:646,615 Z0:664,621	
A-OH-18:1;O3/25:0;O	1276,748		17,21		IP:261,036 W-H2O(18:1;O3):280,258 W(18:1;O3):298,27 C3PO3_B1_437:437,071 Z0mH2O:660,641 Z0:678,636	
160325_S1_GV_c_4_rep1_pos_CID						
A-NH-18:1;O3/24:1	1265,7434		16,36		W(18:1;O3):298,268 Z0mH2O:650,601	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:668,609	
A-NH-45:2;O3		1307,7851		17,53		Z0mH2O:692,6485 Z0:710,653
A-OH-39:1;O4	1220,6884		14,51		IP:261,034 Z0:622,58	
A-OH-18:0;O3/22:0;O	1236,7171		16,2		IP:261,037 W-H2O(18:0;O3):282,274 Z0mH2O:620,598 Z0:638,605	
A-OH-18:1;O3/22:0;O	1234,7036	1256,6858	15,62	15,63	IP:261,038 W-H2O(18:1;O3):280,303 W(18:1;O3):298,333 Z0mH2O:618,597 Z0:638,59	IP:283,02 B2_hydroxy:361,074 C3PO3_B1_459:459.048 621,103 Z0:658,565
A-OH-18:0;O3/23:0;O	1250,7297		16,75		IP:261,041 W-H2O(18:0;O3):282,277 C3PO3_B1_437:437,073 Z0mH2O:634,614 Z0:652,623	
A-OH-18:1;O3/23:0;O	1248,718	1270,7003	16,37	16,34	IP:261,038 W(18:1;O3):298,274 Z0:650,605	IP:283,015 B2_hydroxy:361,074 C3PO3_B1_459:459,05 621,099 Z0:672,594
A-OH-18:0;O3/24:0;O	1264,7475	1286,7259	17,18	17,15	IP:261,034 W(18:0;O3):300,287 Z0mH2O:648,627 Z0:666,634	B2_hydroxy:361,066 C3PO3_B1_459:459,051 621,103
A-OH-18:1;O3/24:0	1246,7341		17,04		IP:261,037 W(18:1;O3):298,273 C3PO3_B1_437:437,058 Z0:648,63	
A-OH-18:1;O3/24:0;O	1262,7328	1284,7147	16,82	16,83	IP:261,035 W-H2O(18:1;O3):280,264 W(18:1;O3):298,274 Z0mH2O:646,615 Z0:664,621	IP:283,009 B2_hydroxy:361,069 C3PO3_B1_459:459,051 621,103 Z0:686,593
A-OH-43:0;O4	1278,767		17,5		IP:261,039 Z0mH2O:662,644	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:680,648	
A-OH-18:1;O3/25:0;O	1276,7473	1298,7292	17,23	17,26	IP:261,038 W-H2O(18:1;O3):280,274 Z0mH2O:660,622 Z0:678,633	IP:283,018 B2_hydroxy:361,108 C3PO3_B1_459:459,048 621,103 Z0:700,62
A-OH-18:1;O3/26:0;O	1290,7634		17,52		IP:261,037 W-H2O(18:1;O3):280,253 Z0mH2O:674,653 Z0:692,656	
A-OH-18:1;O3/26:1;O	1310,6928		14,01		W(18:1;O3):298,273 Z0:712,61	
160325_S1_GV_c_4_rep2_pos_CID						
A-NH-18:1;O3/23:1	1251,7323		15,49		W-H2O(18:1;O3):280,263 W(18:1;O3):298,271 Z0mH2O:636,589	
A-OH-18:0;O3/22:0;O	1236,7188		16,16		IP:261,034 W-H2O(18:0;O3):282,277 Z0mH2O:620,594 Z0:638,604	
A-OH-18:1;O3/22:0;O	1234,7058	1256,685	15,53	15,52	IP:261,033 W-H2O(18:1;O3):280,262 W(18:1;O3):298,269 Z0mH2O:618,579 Z0:636,594	B2_hydroxy:361,072 C3PO3_B1_459:459,051 621,1 Z0:658,573
A-OH-18:0;O3/23:0;O	1250,7377		16,67		IP:261,032 W(18:0;O3):300,29 635,612 Z0mH2O:634,608 Z0:652,624	
A-OH-18:1;O3/23:0;O	1248,7223	1270,7031	16,26	16,27	IP:261,038 W-H2O(18:1;O3):280,266 W(18:1;O3):298,267 Z0mH2O:632,606 Z0:650,609	B2_hydroxy:361,066 C3PO3_B1_459:459,051 621,103 Z0:672,594
A-OH-18:0;O3/24:0;O	1264,7502	1286,7293	17,13	17,12	IP:261,033 W(18:0;O3):300,288 Z0mH2O:648,627	IP:283,016 B2_hydroxy:361,077 C3PO3_B1_459:459,054 621,104 Z0mH2O:670,61

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
A-OH-18:1;O3/24:0;O	1262,7372	1284,7157	16,78	16,8	IP:261,038 W-H2O(18:1;O3):280,266 W(18:1;O3):298,276 Z0mH2O:646,611 Z0:664,621	Z0:689,034 IP:283,024 B2_hydroxy:361,073 C3PO3_B1_459:459,046 621,104 Z0:686,601
A-OH-18:1;O3/24:0;O	1262,7383		17,41		IP:261,037 W-2H2O(18:1;O3):262,251 Z0:664,62	
A-OH-43:0;O4	1278,7634		17,48		IP:261,035 Z0mH2O:662,634 Z0:680,653	
A-OH-18:1;O3/25:0;O	1276,7483	1298,7322	17,22	17,25	IP:261,036 W(18:1;O3):298,302 C3PO3_B1_437:437,117 Z0mH2O:660,622 Z0:678,641	IP:283,015 B2_hydroxy:361,071 C3PO3_B1_459:459,048 621,1 Z0:700,632
A-OH-44:1;O4	1290,774		17,54		IP:261,039 Z0mH2O:674,639 Z0:692,653	
160325_S1_GV_c_4_rep3_pos_CID						
A-NH-45:1;O2		1298,7722		17,2	Z0mH2O:678,638	Z0:496,647
A-OH-18:0;O3/22:0;O	1236,7165		16,21		IP:261,04 W(18:0;O3):300,295 Z0mH2O:620,599 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,704	1256,6846	15,58	15,59	IP:261,034 W(18:1;O3):298,272 Z0mH2O:618,571 Z0:636,587	IP:283,018 B2_hydroxy:361,066 C3PO3_B1_459:459,052 621,104 Z0:658,566
A-OH-18:0;O3/23:0;O	1250,733		16,73		IP:261,035 W(18:0;O3):300,285 Z0mH2O:634,608 Z0:652,62	
A-OH-18:1;O3/23:0;O	1248,7194	1270,7002	16,31	16,29	IP:261,038 W-H2O(18:1;O3):280,26 W(18:1;O3):298,27 Z0mH2O:632,589	IP:283,016 B2_hydroxy:361,077 C3PO3_B1_459:459,055 621,104

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:650,606	Z0:672,583
A-OH-18:0;O3/24:0;O	1264,7522	1286,7259	17,17	17,15	IP:261,031 W(18:0;O3):300,286 C3PO3_B1_437:437,087 Z0mH2O:648,624	IP:283,018 B2_hydroxy:361,066 C3PO3_B1_459:459,055 621,104 Z0:688,606
A-OH-18:1;O3/24:0	1246,7335		17,04		IP:261,04 W(18:1;O3):298,271 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7354	1284,7155	16,81	16,83	IP:261,036 W(18:1;O3):298,272 Z0mH2O:646,612 Z0:664,622	IP:283,017 B2_hydroxy:361,087 C3PO3_B1_459:459,049 621,101 Z0:686,606
A-OH-18:1;O3/25:0;O	1276,7499		17,23		IP:261,034 W-H2O(18:1;O3):280,235 W(18:1;O3):298,275 Z0mH2O:660,623 Z0:678,638	
A-OH-18:1;O3/26:0;O	1290,7656		17,58		IP:261,037 W(18:1;O3):298,279 Z0mH2O:674,639 Z0:692,657	
160325_S1_GV_c_4_rep4_pos_CID						
A-NH-41:2;O2	1281,7736		17,11		Z0mH2O:666,635	Z0:684,643
A-NH-44:1;O2	1279,7575		17,39		Z0mH2O:664,622	
A-NH-46:1;O2	1307,798		17,52		Z0mH2O:692,653	Z0:710,646
A-OH-38:1;O4	1206,6694		13,3		Z0mH2O:590,55	Z0:608,557
A-OH-18:0;O3/22:0;O	1236,718		16,12		IP:261,033 W-H2O(18:0;O3):282,277 W(18:0;O3):300,282 Z0mH2O:620,614 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,7044	1256,685	15,5	15,48	IP:261,031 W-H2O(18:0;O3):280,269 W(18:1;O3):298,27 Z0mH2O:618,576 Z0:636,594	IP:283,02 B2_hydroxy:361,081 C3PO3_B1_459:459,052 621,104 Z0:658,562
A-OH-18:0;O3/23:0;O	1250,7303		16,66		IP:261,037	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:0;O3):300,285 Z0mH2O:634,616 635,612 Z0:652,621	
A-OH-18:1;O3/23:0;O	1248,7164	1270,7001	16,23	16,24	IP:261,029 W-H2O(18:1;O3):280,257 W(18:1;O3):298,274 Z0:650,606	IP:283,016 B2_hydroxy:361,061 C3PO3_B1_459:459,048 621,1
A-OH-18:0;O3/24:0;O	1264,7472	1286,7292	17,12	17,1	IP:261,041 W-H2O(18:0;O3):282,281 W(18:0;O3):300,28 Z0mH2O:648,631 Z0:666,635	B2_hydroxy:361,075 C3PO3_B1_459:459,048 621,1 Z0:699,617
A-OH-18:1;O3/24:0	1246,7315		16,94		IP:261,037 W(18:1;O3):298,271 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7343	1284,7155	16,75	16,72	IP:261,038 W-H2O(18:1;O3):280,265 W(18:1;O3):298,28 C3PO3_B1_437:437,066 Z0mH2O:646,612 Z0:664,622	IP:283,021 B2_hydroxy:361,072 C3PO3_B1_459:459,048 621,1 Z0:686,605
A-OH-18:1;O3/25:0;O	1276,7508	1298,7368	17,19	17,18	IP:261,034 W-H2O(18:1;O3):280,257 W(18:1;O3):298,27 Z0mH2O:660,63 Z0:678,634	B2_hydroxy:361,069 C3PO3_B1_459:459,051 621,1 Z0:700,625
A-OH-43:3;O3	1278,7243		16,05		Z0mH2O:662,598	Z0:680,615
A-OH-18:1;O3/26:0;O	1290,7821		17,49		IP:261,033 W-H2O(18:1;O3):280,264 Z0:692,654	

TABLE C.2: GIPC ID List: Replicates of *S1_GV_s* with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S1_GV_s_1_pos_CID						
A-OH-18:1;O3/20:0;O	1206,6745		13,31		IP:261,036 W(18:1;O3):298,275 Z0:608,56	
A-OH-18:0;O3/22:0;O	1236,7201		16,15		IP:261,041 W(18:0;O3):300,294 Z0mH2O:620,609 Z0:638,608	
A-OH-18:1;O3/22:0;O	1234,7079	1256,6917	15,51	15,48	IP:261,037 W-H2O(18:1;O3):280,263 W(18:1;O3):298,283 Z0mH2O:618,578 Z0:636,589	IP:283,017 B2_hydroxy:361,074 C3PO3_B1_459:459,051 621,096 Z0:658,561
A-OH-18:0;O3/23:0;O	1250,7328		16,68		IP:261,033 W-H2O(18:0;O3):282,273 W(18:0;O3):300,293 Z0mH2O:634,61 Z0:652,623	
A-OH-18:1;O3/23:0;O	1248,7217	1270,7092	16,25	16,24	IP:261,035 W-H2O(18:1;O3):280,261 W(18:1;O3):298,276 Z0mH2O:632,606 Z0:650,609	IP:283,02 B2_hydroxy:361,079 C3PO3_B1_459:459,053 621,103 Z0:672,594
A-OH-18:0;O3/24:0;O	1264,7513	1286,7442	17,14	17,12	IP:261,038 W-H2O(18:0;O3):282,286 W(18:0;O3):300,286 Z0mH2O:648,626	B2_hydroxy:361,07 C3PO3_B1_459:459,053 621,103 Z0:688,616
A-OH-18:0;O3/24:0;O	1264,7396		17,43		IP:261,043 W(18:0;O3):300,291 Z0mH2O:648,631 Z0:666,637	
A-OH-18:1;O3/24:0;O	1262,7381	1284,7203	16,76	16,81	IP:261,035 W-H2O(18:1;O3):280,263 W(18:1;O3):298,276 C3PO3_B1_437:437,071 Z0mH2O:646,611 Z0:664,625	IP:283,018 B2_hydroxy:361,09 C3PO3_B1_459:459,048 621,104 Z0:686,617
A-OH-18:1;O3/25:0	1260,76		17,36		IP:261,045	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,274 V_2(25:0):397,382 Z0:662,644	
A-OH-18:1;O3/25:0;O	1276,7548	1298,734	17,21	17,22	IP:261,037 W-H2O(18:1;O3):280,26 W(18:1;O3):298,275 Z0mH2O:660,621 Z0:678,64	IP:283,022 B2_hydroxy:361,09 C3PO3_B1_459:459,05 621,103 Z0:700,62
A-OH-44:1;O4	1290,7607		17,51		IP:261,033 Z0mH2O:674,649 Z0:692,656	
160325_S1_GV_s_2_pos_CID						
A-OH-18:1;O3/20:0;O	1206,666		13,46		IP:261,038 W-H2O(18:1;O3):280,259 W(18:1;O3):298,277 Z0mH2O:590,555 Z0:608,559	
A-OH-18:0;O3/22:0;O	1236,715	1258,6982	16,25	16,26	IP:261,035 W-H2O(18:0;O3):282,278 W(18:0;O3):300,288 Z0mH2O:620,592 Z0:638,603	C3PO3_B1_459:459,052 621,098
A-OH-18:1;O3/22:0;O	1234,7026	1256,683	15,64	15,65	IP:261,036 W-H2O(18:1;O3):280,257 W(18:1;O3):298,273 Z0mH2O:618,577 Z0:636,591	IP:283,016 B2_hydroxy:361,078 C3PO3_B1_459:459,052 621,105 Z0:658,567
A-OH-18:0;O3/23:0;O	1250,7314		16,75		IP:261,04 W-H2O(18:0;O3):282,275 W(18:0;O3):300,29 Z0mH2O:634,609 Z0:652,621	
A-OH-18:1;O3/23:0;O	1248,7172	1270,6974	16,35	16,33	IP:261,031 W-H2O(18:1;O3):280,26 W(18:1;O3):298,277 Z0mH2O:632,604 Z0:650,606	IP:283,014 B2_hydroxy:361,067 C3PO3_B1_459:459,052 C3PO3_B1_459:450,052 621,105 Z0:672,599
A-OH-18:0;O3/24:0;O	1264,7453		17,2		IP:261,046 W(18:0;O3):300,286	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:648,617 Z0:666,632	
A-OH-18:1;O3/24:0	1246,7362		17,04		IP:261,035 W-H2O(18:1;O3):280,261 W(18:1;O3):298,271 Z0:648,625	
A-OH-18:1;O3/24:0;O	1262,7342	1284,7172	16,84	16,86	IP:261,036 W-H2O(18:1;O3):280,257 W(18:1;O3):298,275 Z0mH2O:646,605 Z0:664,623	IP:283,01 B2_hydroxy:361,071 C3PO3_B1_459:459,05 621,102 Z0:686,607
A-OH-18:1;O3/25:0;O	1276,7511		17,26		IP:261,034 W-H2O(18:1;O3):280,267 W(18:1;O3):298,638 C3PO3_B1_437:437,075 Z0mH2O:660,612 Z0:678,635	
A-OH-44:1;O4	1290,7636		17,54		IP:261,033 Z0mH2O:674,643 Z0:692,654	
160325_S1_GV_s_3_pos_CID						
A-NH-43:1;O2		1265,75		16,43		Z0mH2O:650,606 Z0:668,622
A-NH-18:1;O2/25:0;O	1281,7701		17,2		W(18:1;O2):282,277 Z0:684,632	
A-OH-18:1;O3/20:0;O	1206,6734		13,44		IP:261,038 W-2H2O(18:1;O3):262,255 W(18:1;O3):298,269 Z0mH2O:590,544 Z0:608,558	
A-OH-18:0;O3/22:0;O	1236,7224		16,23		IP:261,035 W-H2O(18:0;O3):282,275 W(18:0;O3):300,288 Z0mH2O:620,6 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,7049	1256,683	15,62	15,65	IP:261,034 W-H2O(18:1;O3):280,263 W(18:1;O3):298,273 Z0mH2O:618,581	IP:283,016 B2_hydroxy:361,073 C3PO3_B1_459:459,052 621,105

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
A-OH-18:0;O3/23:0;O	1250,7407		16,74		Z0:636,592 IP:261,035 W-H2O(18:0;O3):282,278 W(18:0;O3):300,288 Z0mH2O:634,613 Z0:652,621	Z0:658,567
A-OH-18:1;O3/23:0;O	1248,72	1270,708	16,33	16,35	IP:261,039 W-H2O(18:1;O3):280,262 W(18:1;O3):298,273 Z0mH2O:632,6 Z0:650,607	B2_hydroxy:361,070 C3PO3_B1_459:459,052 621,105 Z0:672,588
A-OH-18:0;O3/24:0;O	1264,7503	1286,7284	17,16	17,17	IP:261,036 W-H2O(18:0;O3):282,276 W(18:0;O3):300,299 C3PO3_B1_437:437,07 Z0mH2O:648,629 Z0:666,64	IP:283,014 C3PO3_B1_459:459,052 621,101
A-OH-18:1;O3/24:0	1246,7446		17,03		IP:261,028 W-H2O(18:1;O3):280,261 W(18:1;O3):298,274 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7356	1284,7192	16,83	16,85	IP:261,036 W-H2O(18:1;O3):280,262 W(18:1;O3):298,275 Z0mH2O:646,605 Z0:664,623	IP:283,025 B2_hydroxy:361,073 C3PO3_B1_459:459,053 621,105 Z0:686,606
A-OH-18:1;O3/25:0;O	1276,751	1298,7305	17,23	17,22	IP:261,041 W-H2O(18:1;O3):280,257 W(18:1;O3):298,267 Z0mH2O:660,631 Z0:678,639	B2_hydroxy:361,070 C3PO3_B1_459:459,052 621,105 Z0mH2O:682,634 Z0:700,622
A-OH-18:1;O3/26:0;O	1290,7631		17,54		IP:261,037 W(18:1;O3):298,276 Z0mH2O:674,640 Z0:692,654	
160325_S1_GV_s_4_pos_CID						
A-NH-45;O21	1293,7809		17,21			Z0mH2O:678,638 Z0:696,647
A-OH-18:1;O3/20:0;O	1206,6729		13,38		IP:261,038 W-H2O(18:1;O3):280,271	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,269 Z0:608,558	
A-OH-40:0;O4			16,23	16,23	B2_hydroxy:361,075 C3PO3_B1_459:459,052 621,101 Z0:660,585	
A-OH-18:0;O3/22:0;O	1236,718		16,2		IP:261,038 W-H2O(18:0;O3):282,277 W(18:0;O3):300,29 Z0mH2O:620,599 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,7043	1256,6843	15,58	15,59	IP:261,038 W-H2O(18:1;O3):280,262 W(18:1;O3):298,275 Z0mH2O:618,583 Z0:636,591	IP:283,016 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,101 Z0:658,577
A-OH-18:0;O3/23:0;O	1250,7307		16,73		IP:261,035 W-H2O(18:0;O3):282,282 W(18:0;O3):300,29 C3PO3_B1_437:437,068 Z0mH2O:634,623 Z0:652,624	
A-OH-18:1;O3/23:0	1232,7213		16,6		W(18:1;O3):298,266 Z0:634,608	
A-OH-18:0;O3/23:0;O	1232,7307		16,73		IP:261,035 W-H2O(18:0;O3):280,282 W(18:0;O3):300,29 C3PO3_B1_437:437,068 Z0mH2O:634,623 Z0:652,624	
A-OH-18:1;O3/23:0;O	1248,7187	1270,699	16,29	16,32	IP:261,039 W-H2O(18:1;O3):280,26 W(18:1;O3):298,27 Z0mH2O:632,589 Z0:650,606	IP:283,016 B2_hydroxy:361,075 C3PO3_B1_459:459,049 621,101 Z0:672,595
A-OH-18:0;O3/24:0;O	1264,7483		17,41		IP:261,035 W-H2O(18:0;O3):282,28 W(18:0;O3):300,29 Z0mH2O:648,635	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:666,64	
A-OH-18:1;O3/24:0	1246,7372		17,06		W-H2O(18:1;O3):280,261 W(18:1;O3):298,274 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7368	1284,7167	16,81	16,84	IP:261,036 W(18:1;O3):298,275 Z0mH2O:646,612 Z0:664,622	IP:283,019 B2_hydroxy:361,07 C3PO3_B1_459:459,052 621,104 Z0:686,602
A-OH-18:1;O3/24:0;O	1262,7399		17,43		IP:261,04 W(18:1;O3):298,274 C3PO3_B1_437:437,071 Z0mH2O:646,612 Z0:664,622	
A-OH-18:1;O3/25:0;O	1276,7498		17,24		IP:261,036 W(18:1;O3):298,295 Z0mH2O:660,612 Z0:678,638	

Appendix D

GIPC ID Lists R Sampling 1

TABLE D.1: GIPC ID List: Replicates of *S1_R_c* with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S1_R_c_1_pos_CID						
A-NH-42:1;O2		1251,7357		15,49		Z0mH2O:636,588
A-NH-18:1;O3/26:1	1293,7673		17,18		W-H2O(18:1;O3):280,259 W(18:1;O3):298,274 Z0mH2O:678,635 Z0:696,633	
A-OH-18:0;O3/22:0;O	1236,7171		16,04		IP:261,03 W-H2O(18:0;O3):282,285 W(18:0;O3):300,288 Z0mH2O:620,593 Z0:638,607	
A-OH-18:1;O3/22:0;O	1234,7049	1256,6833	15,37	15,39	IP:261,039 W-H2O(18:1;O3):280,265 W(18:1;O3):298,278 Z0mH2O:618,596 Z0:636,588	IP:283,021 B2_hydroxy:361,073 C3PO3_B1_459:459,049 621,105 Z0:658,571
A-OH-18:1;O3/23:0;O	1248,7207	1270,701	16,16	16,18	IP:261,036 W(18:1;O3):298,28 Z0mH2O:632,604 Z0:650,607	IP:283,022 B2_hydroxy:361,073 C3PO3_B1_459:459,05 621,102 Z0:672,581
A-OH-42:0;O4		1286,7268		17,08		C3PO3_B1_459:459,049 621,101
A-OH-18:1;O3/24:0;O	1262,7356	1284,7159	16,71	16,73	IP:261,036 W-H2O(18:1;O3):280,263 W(18:1;O3):298,275 Z0mH2O:646,606 Z0:664,623	IP:283,023 B2_hydroxy:361,074 C3PO3_B1_459:459,05 621,102 Z0:686,608
A-OH-18:1;O3/25:0;O	1276,7495		17,14		IP:261,039 W-H2O(18:1;O3):280,27 W(18:1;O3):298,28 Z0mH2O:660,635 Z0:678,639	
A-OH-18:1;O3/26:0;O	1290,7588		17,49		IP:261,035 W(18:1;O3):298,274 C3PO3_B1_437:437,072 Z0mH2O:674,66 Z0:692,651	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S1_R_c_2_pos_CID						
A-OH-18:0;O3/22:0;O	1236,7184		16,09		IP:261,03 W-H2O(18:0;O3):282,275 W(18:0;O3):300,29 Z0mH2O:620,596 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,7034	1256,6827	15,41	15,44	IP:261,036 W-H2O(18:1;O3):280,26 W(18:1;O3):298,27 Z0mH2O:618,591 Z0:636,587	IP:283,011 B2_hydroxy:361,069 C3PO3_B1_459:459,052 621,101 Z0:658,57
A-OH-41:0;O4	1250,735		16,71		IP:261,033 Z0mH2O:634,616 Z0:652,62	
A-OH-18:1;O3/23:0;O	1248,7196	1270,6967	16,19	16,22	IP:261,039 W-H2O(18:1;O3):280,265 W(18:1;O3):298,275 C3PO3_B1_437:437,079 Z0mH2O:632,596 Z0:650,606	IP:283,014 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,105 Z0:672,588
A-OH-42:0;O4	1264,7496		17,17		IP:261,04 Z0mH2O:648,624 Z0:666,639	
A-OH-18:1;O3/24:0	1246,7408		16,96		IP:261,035 W-H2O(18:1;O3):280,259 W(18:1;O3):298,274 Z0:648,629	
A-OH-18:1;O3/24:0;O	1262,7344	1284,7147	16,73	16,75	IP:261,039 W-H2O(18:1;O3):280,262 W(18:1;O3):298,272 Z0mH2O:646,624 Z0:664,622	IP:283,017 B2_hydroxy:361,071 C3PO3_B1_459:459,053 621,101 Z0:686,599
A-OH-18:1;O3/25:0;O	1276,748	1298,7291	17,12	17,16	IP:261,035 W(18:1;O3):298,274 C3PO3_B1_437:437,071 Z0mH2O:660,627 Z0:678,638	IP:283,023 B2_hydroxy:361,069 C3PO3_B1_459:459,049 621,101 Z0:700,614
A-OH-18:1;O3/26:0;O	1290,7603		17,5		IP:261,04 W-H2O(18:1;O3):280,259 W(18:1;O3):298,274	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					C3PO3_B1_437:437,072 Z0mH2O:674,647 Z0:692,654	
160325_S1_R_c_3_pos_CID						
A-NH-42:1;O2		1251,7344		15,41		Z0mH2O:636,593 Z0:654,606
A-NH-43:1;O2		1265,7527		16,21		Z0mH2O:650,608 Z0:668,616
A-OH-18:1;O3/20:0;O	1206,6707		13,19		IP:261,034 W-H2O(t18:1):280,289 W(t18:1):298,275 Z0mH2O:590,542 Z0:608,56	
A-OH-18:0;O3/22:0;O	1236,7218		16,09		IP:261,036 W-H2O(t18:0):282,275 W(t18:0):300,288 Z0mH2O:620,593 Z0:638,604	
A-OH-18:1;O3/22:0;O	1234,7076	1256,6848	15,4	15,45	IP:261,034 W-H2O(t18:1):280,26 W(t18:1):298,278 Z0mH2O:618,585 Z0:636,593	IP:283,014 B2_hydroxy:361,076 C3PO3_B1_459:459.053 621,106 Z0:658,568
A-OH-18:1;O3/23:0;O	1248,7215	1270,7025	16,19	16,21	IP:261,039 W-H2O(t18:1):280,288 W(t18:1):298,265 C3PO3_B1_437:437,073 Z0mH2O:632,631 Z0:650,608	IP:283,014 B2_hydroxy:361,073 C3PO3_B1_459:459,05 621,102 Z0:672,589
A-OH-18:0;O3/24:0;O	1264,7508		17,04		IP:261,036 W-H2O(t18:0):282,273 W(t18:0):300,288 Z0mH2O:648,63 Z0:666,633	
A-OH-18:1;O3/24:0	1246,7374		16,96		W(t18:1):298,272 Z0:648,629	
A-OH-18:1;O3/24:0;O	1262,7368	1284,7147	16,73	16,72	IP:261,035 W-H2O(t18:1):280,266 W(t18:1):298,273 Z0mH2O:646,611	IP:283,018 B2_hydroxy:361,066 C3PO3_B1_459:459,051 621,103

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:664,621	Z0:686,601
A-OH-43:1;O4		1298,7264		17,19	IP:283,019 B2_hydroxy:361,076 C3PO3_B1_459:459,053 621,106 Z0:700,6	
A-OH-18:1;O3/25:0;O	1276,7547		17,17		IP:261,034 W(t18:1):298,314 Z0:678,64	
A-OH-44:1;O4	1290,7668		17,47		IP:261,036 Z0:692,656	
160325_S1_R_c_4_pos_CID						
A-OH-38:1;O4	1206,6742		13,1		Z0:608,558	
A-OH-18:0;O3/22:0;O	1236,7185		16,03		IP:261,035 W-H2O(18:0;O3):282,282 W(18:0;O3):300,29 Z0mH2O:620,602 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,704	1256,6862	15,32	15,34	IP:261,036 W-H2O(18:1;O3):280,26 W(18:1;O3):298,273 Z0mH2O:618,588 Z0:636,591	IP:283,019 B2_hydroxy:361,075 C3PO3_B1_459:459.052 621,101 Z0:658,578
A-OH-18:0;O3/23:0;O	1250,7277		16,61		IP:261,037 W(18:0;O3):300,29 C3PO3_B1_437:437,068 Z0mH2O:634,616 Z0:652,621	
A-OH-18:1;O3/23:0;O	1248,7197	1270,701	16,13	16,14	IP:261,036 W-H2O(18:1;O3):280,272 W(18:1;O3):298,285 C3PO3_B1_437:437,073 Z0mH2O:632,585 Z0:650,608	IP:283,016 B2_hydroxy:361,086 C2_hydroxy:379,078 C3PO3_B1_459:459.049 621,101 Z0:672,591
A-OH-18:0;O3/24:0;O	1264,7453		17,04		IP:261,036 W(18:0;O3):300,281 Z0mH2O:648,629 Z0:666,637	
A-OH-18:1;O3/24:0	1246,75		16,89		IP:261,04 W(18:1;O3):298,274	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:648,624	
A-OH-18:1;O3/24:0;O	1262,735	1284,7165	16,68	16,71	IP:261,036 W-H2O(18:1;O3):280,262 W(18:1;O3):298,275 Z0mH2O:646,617 Z0:664,623	IP:283,017 B2_hydroxy:361,068 C3PO3_B1_459:459.053 621,098 Z0:686,603
A-OH-42:2;O2		1250,7277		16,61		Z0mH2O:634,616 Z0:652,621
A-OH-18:1;O3/25:0;O	1276,7516	1298,7321	17,12	17,14	IP:261,036 W(18:1;O3):298,265 Z0mH2O:660,631 Z0:678,635	IP:283,014 B2_hydroxy:361,073 C3PO3_B1_459:459.052 621,106 Z0:700,611
A-OH-44:1;O4	1290,7677		17,47		IP:261,035 Z0mH2O:674,636 Z0:692,654	

TABLE D.2: GIPC ID List: Replicates of *S1_R_s* with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S1_R_s_1_pos_CID						
A-NH-42:1;O2		1251,7376		15,44		Z0mH2O:636,592 Z0:654,602
A-OH-40:1;O4		1256,6849		15,51	IP:283,012	B2_hydroxy:361,078 C3PO3_B1_459:459.053 621,105 Z0:658,582
A-OH-18:1;O3/22:0;O	1234,7069		15,52		IP:261,034 W-H2O(18:1;O3):280,263 W(18:1;O3):298,293 Z0mH2O:618,606 Z0:636,588	
A-OH-18:1;O3/23:0;O	1248,7218	1270,6998	16,27	16,3	IP:261,033 W-H2O(18:1;O3):280,262 W(18:1;O3):298,272 Z0mH2O:632,597 Z0:650,611	C3PO3_B1_459:459,043 621,102 Z0:672,573
A-OH-18:0;O3/24:0;O	1264,7519		17,14		IP:261,031 W(18:0;O3):300,293 Z0mH2O:648,625 Z0:666,637	
A-OH-18:1;O3/24:0;O	1262,7392	1284,7199	16,77	16,76	IP:261,034 W-H2O(18:1;O3):280,26 W(18:1;O3):298,27 Z0mH2O:646,661 Z0:664,623	IP:283,019 B2_hydroxy:361,078 C3PO3_B1_459:459,052 621,105 Z0:686,618
A-OH-18:1;O3/25:0;O	1276,7491	1298,7374	17,16	17,27	IP:261,035 W(18:1;O3):298,272 C3PO3_B1_437:437,072 Z0mH2O:660,639 Z0:678,635	B2_hydroxy:361,078 C3PO3_B1_459:459,052 621,101 Z0:700,618
A-OH-44:1;O4	1290,7596		17,55			Z0:692,655
160325_S1_R_s_2_pos_CID						
A-NH-45:1;O2		1293,7851		17,12		Z0mH2O:678,635 Z0:696,651
A-OH-18:0;O3/22:0;O	1236,7152		16,07		IP:261,04 W-H2O(18:0;O3):282,275 W(18:0;O3):300,29	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:620,607 Z0:638,607	
A-OH-18:1;O3/22:0;O	1234,7042	1256,6844	15,42	15,46	IP:261,048 W-H2O(18:1;O3):280,263 W(18:1;O3):298,275 Z0mH2O:618,585 Z0:636,592	IP:283,016 B2_hydroxy:361,078 C3PO3_B1_459:459.052 621,105 Z0:658,578
A-OH-18:0;O3/23:0;O	1250,7398		16,6		W(18:0;O3):300,282 Z0mH2O:634,605 Z0:652,621	
A-OH-41:1;O4		1270,697		16,21	IP:283,016	B2_hydroxy:361,075 C3PO3_B1_459:459.052 621,101 Z0:672,596
A-OH-18:0;O3/24:0;O	1264,7518		17,06		IP:261,037 W-H2O(18:0;O3):282,28 W(18:0;O3):300,285 C3PO3_B1_437:437,069 Z0mH2O:648,628 Z0:666,636	
A-OH-18:1;O3/24:0;O	1262,7363	1284,7177	16,73	16,75	IP:261,036 W-H2O(18:1;O3):280,252 W(18:1;O3):298,27 Z0mH2O:646,62 Z0:664,622	IP:283,019 B2_hydroxy:361,073 C3PO3_B1_459:459,049 621,105 Z0:686,595
A-OH-43:1;O4	1276,7491	1298,7319	17,25	17,24	IP:261,035 Z0:678,635	IP:283,236 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,101
A-OH-44:1;O4	1290,7703		17,51		IP:261,037 W(18:1;O3):298,274 Z0mH2O:674,636 Z0:692,654	
160325_S1_R_s_3_pos_CID						
A-OH-18:0;O3/22:0;O	1236,7195		16,11		IP:261,037 W-H2O(18:0;O3):282,282 W(18:0;O3):300,277 Z0mH2O:620,57 Z0:638,602	
A-OH-18:1;O3/22:0;O	1234,7032	1256,6817	15,49	15,49	IP:261,036	IP:283,019

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W-H2O(18:1;O3):280,262 W(18:1;O3):298,275 Z0mH2O:618,569 Z0:636,587	B2_hydroxy:361,067 C3PO3_B1_459:459.049 621,105 Z0:658,574
A-OH-18:1;O3/23:0;O	1248,7213	1270,7018	16,22	16,25	IP:261,036 W-H2O(18:1;O3):280,267 W(18:1;O3):298,28 Z0mH2O:632,589 Z0:650,606	IP:283,016 B2_hydroxy:361,075 C3PO3_B1_459:459,049 621,101 Z0:672,596
A-OH-18:0;O3/24:0;O	1264,746		17,1		IP:261,039 W(18:0;O3):300,278 Z0mH2O:648,625 Z0:666,632	
A-OH-18:1;O3/24:0	1246,738		17		IP:261,035 W(18:1;O3):298,271 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7349	1284,7139	16,76	16,75	IP:261,039 W-H2O(18:1;O3):280,265 W(18:1;O3):298,272 Z0mH2O:646,605 Z0:664,623	IP:283,02 B2_hydroxy:361,076 C3PO3_B1_459:459,05 621,105 Z0:686,602
A-OH-18:1;O3/25:0;O	1276,7478	1298,7288	17,19	17,19	IP:261,037 W(18:1;O3):298,276 C3PO3_B1_437:437,065 Z0mH2O:660,639 Z0:678,639	IP:283,014 B2_hydroxy:361,072 C3PO3_B1_459:459,052 621,105 Z0:700,622
A-OH-44:1;O4	1290,7753		17,55		IP:261,032 Z0:692,649	
160325_S1_R_s_4_pos_CID						
A-OH-18:1;O3/21:0;O	1220,6845		14,39		W(18:1;O3):298,272 Z0:622,575	
A-OH-40:0;O4	1236,7174		16,09		IP:261,033 Z0mH2O:620,596 Z0:638,607	
A-OH-40:1;O4		1256,685		15,47	IP:283,022	B2_hydroxy:361,07 C3PO3_B1_459:459.05 621,102 Z0:658,572
A-OH-18:1;O3/22:0;O	1234,705		15,46		IP:261,034	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W-H ₂ O(18:1;O ₃):280,263 W(18:1;O ₃):298,273 Z0mH ₂ O:618,574 Z0:636,593	
A-OH-18:1;O ₃ /23:0;O	1248,7202	1270,6952	16,23	16,25	IP:261,039 W-H ₂ O(18:1;O ₃):280,258 W(18:1;O ₃):298,268 Z0mH ₂ O:632,601 Z0:650,603	IP:283,012 B2_hydroxy:361,073 C3PO ₃ _B1_459:459,047 621,098 Z0:672,6
A-OH-42:0;O ₄	1264,7493		17,1		IP:261,041 Z0mH ₂ O:648,622 Z0:666,637	
A-OH-42:0;O ₄	1264,7528		17,37		IP:261,045 Z0mH ₂ O:648,625 Z0:666,637	
A-OH-18:1;O ₃ /24:0;O	1262,7357	1284,7178	16,75	16,77	IP:261,037 W-H ₂ O(18:1;O ₃):280,255 W(18:1;O ₃):298,276 Z0mH ₂ O:646,603 Z0:664,62	IP:283,04 B2_hydroxy:361,074 C3PO ₃ _B1_459:459,051 621,102 Z0mH ₂ O:668,661 Z0:686,596
A-OH-18:1;O ₃ /25:0;O	1276,752	1298,7308	17,17	17,17	IP:261,037 W(18:1;O ₃):298,278 Z0mH ₂ O:660,628 Z0:678,636	IP:283,017 B2_hydroxy:361,073 C3PO ₃ _B1_459:459,053 621,102 Z0:700,627
A-OH-18:1;O ₃ /26:0;O	1290,7681		17,52		IP:261,033 W(18:1;O ₃):298,277 C3PO ₃ _B1_437:437,064 Z0mH ₂ O:674,665 Z0:692,656	

Appendix E

GIPC ID Lists GV Sampling 2

TABLE E.1: GIPC ID List: Replicates of S2_GV_c with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S2_GV_c_1_pos_CID						
A-NH-45:1;O2		1293,784		17,03	Z0mH2O:678,635	Z0:696,644
A-NH-18:1;O3/27:1	1307,7825		17,44		Z0mH2O:692,651 Z0:710,671	
A-OH-38:1;O4	1206,6789		12,97		IP:261,035 Z0:608,562	
A-OH-18:0;O3/22:0;O	1236,7161		15,95		IP:261,033 W-H2O(18:0;O3):282,275 W(18:0;O3):300,293 Z0mH2O:620,592 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,706	1256,6889	15,23	15,25	IP:261,039 W-H2O(18:1;O3):280,267 W(18:1;O3):298,27 Z0mH2O:618,577 Z0:636,591	IP:283,011 B2_hydroxy:361,075 C3PO3_B1_459:459.046 621,101 Z0:658,563
A-OH-18:0;O3/23:0;O	1250,7327		16,53		IP:261,038 W(18:0;O3):300,29 C3PO3_B1_437:437,069 Z0mH2O:634,616 Z0:652,625	
A-OH-41:1;O3	1232,7298		16,34			Z0:634,612
A.OH-18:1;O3/23:0;O	1248,7204	1270,7047	16,05	16,07	IP:261,039 W-H2O(18:1;O3):280,262 W(18:1;O3):298,275 Z0mH2O:632,633 Z0:650,61	IP:283,021 B2_hydroxy:361,073 C3PO3_B1_459:459,049 621,101 Z0:672,592
A-OH-18:1;O3/23:0;O	1248,7261		16,48		IP:261,04 W(18:1;O3):298,271 Z0mH2O:632,596 Z0:650,606	
A-OH-42:0;O4	1264,7468	1286,7247	16,99	17	IP:261,039 Z0mH2O:648,628 Z0:666,639	IP:283,029 B2_hydroxy:361,078 C3PO3_B1_459:459,049 621,101 Z0mH2O:670,649 Z0:686,607
A-OH-18:1;O3/24:0	1246,741		16,85		IP:261,044	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W-H2O(18:1;O3):280,264 W(18:1;O3):298,274 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7358	1284,7162	16,67	16,64	IP:261,039 W-H2O(18:1;O3):280,262 W(18:1;O3):298,275 Z0mH2O:646,617 Z0:664,623	IP:283,01 B2_hydroxy:361,084 C3PO3_B1_459:459,053 621,108 Z0:686,606
A-OH-18:1;O3/24:0;O	1262,7386		17,3		IP:261,035 W-H2O(18:1;O3):280,261 W(18:1;O3):298,276 Z0mH2O:646,612 Z0:664,626	
A-OH-18:1;O3/25:0	1260,7517		17,28		IP:261,035 W-H2O(18:1;O3):280,268 W(18:1;O3):298,269 V_2(25:0):397,387 Z0:662,646	
A-OH-18:1;O3/25:0;O	1276,7497	1298,7333	17,07	17,11	IP:261,034 W(18:1;O3):298,275 Z0mH2O:660,62 Z0:678,639	IP:283,019 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,101 Z0:700,614
A-OH-18:1;O3/26:0;O	1290,7593		17,45		IP:261,043 W(18:1;O3):298,27 Z0mH2O:674,667 Z0:692,651	
160325_S2_GV_c_2_pos_CID						
A-NH-43:1;O2		1265,7494		16,07	Z0mH2O:650,611	Z0:668,619
A-OH-18:0;O3/22:0;O	1236,7223		15,9		IP:261,035 W-H2O(18:0;O3):282,28 W(18:0;O3):300,29 Z0mH2O:620,607 Z0:638,607	
A-OH-40:1;O4		1256,6838		15,21	IP:283,017	B2_hydroxy:361,09 C3PO3_B1_459:459.049 621,102 Z0:658,567
A-OH-18:1;O3/22:0;O	1234,705		15,21		IP:261,037 W-H2O(18:1;O3):280,26	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,278 Z0mH2O:618,577 Z0:636,592	
A-OH-18:0;O3/23:0;O	1250,7332		16,54		IP:261,04 W(18:0;O3):300,306 Z0mH2O:634,617 Z0:652,625	
A-OH-18:1;O3/23:0;O	1248,7226		16,05		IP:261,033 W-H2O(18:1;O3):280,264 W(18:1;O3):298,274 Z0mH2O:632,593 Z0:650,611	
A-OH-18:0;O3/24:0;O	1264,7515		17,01		IP:261,036 W-H2O(18:0;O3):282,276 W(18:0;O3):300,291 Z0mH2O:648,632 Z0:666,64	
A-OH-18:1;O3/24:0;O	1262,7372	1284,7198	16,63	16,64	IP:261,039 W-H2O(18:1;O3):280,26 W(18:1;O3):298,275 Z0mH2O:646,624 Z0:664,623	IP:283,02 B2_hydroxy:361,082 C3PO3_B1_459:459,053 621,105 Z0:686,591
A-OH-18:1;O3/24:0;O	1262,742		17,29		IP:261,038 W(18:1;O3):298,274 Z0:664,623	
A-OH-18:1;O3/25:0;O	1276,7493	1298,7327	17,03	17,07	IP:261,038 W-H2O(18:1;O3):280,264 W(18:1;O3):298,277 Z0mH2O:660,612 Z0:678,639	IP:283,016 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,101 Z0:700,618
A-OH-44:1;O4	1290,7638		17,45		IP:261,038 Z0mH2O:674,659 Z0:692,654	
160325_S2_GV_c_3_pos_CID						
A-NH-18:1;O3/24:1	1265,7354		16,18		W(18:1;O3):298,269 Z0mH2O:650,603 Z0:668,611	
A-OH-18:1;O3/20:0;O	1206,6703		13,02		IP:261,033 W-H2O(18:1;O3):280,267 W(18:1;O3):298,272	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:590,548 Z0:608,559	
A-OH-18:0;O3/22:0;O	1236,7221		15,99		IP:261,038 W(18:0;O3):300,285 Z0mH2O:620,589 Z0:638,611	
A-OH-18:1;O3/22:0;O	1234,7049	1256,6858	15,25	15,27	IP:261,034 W-H2O(18:1;O3):280,265 W(18:1;O3):298,281 Z0mH2O:618,578 Z0:636,589	IP:283,017 B2_hydroxy:361,07 C3PO3_B1_459:459.053 621,106 Z0:658,571
A-OH-18:1;O3/23:0;O	1248,7216	1270,7065	16,1	16,14	IP:261,034 W-H2O(18:1;O3):280,29 W(18:1;O3):298,273 C3PO3_B1_437:437,064 Z0mH2O:632,59 Z0:650,607	B2_hydroxy:361,073 C3PO3_B1_459:459.056 621,102 Z0:672,589
A-OH-18:0;O3/24:0;O	1264,7519	1286,724	17,02	17,05	IP:261,028 W-H2O(18:0;O3):282,28 W(18:0;O3):300,283 Z0mH2O:648,632 Z0:666,636	IP:283,012 C3PO3_B1_459:459.052 621,105 Z0:688,623
A-OH-18:1;O3/24:0;O	1262,737	1284,7168	16,66	16,68	IP:261,037 W-H2O(18:1;O3):280,268 W(18:1;O3):298,27 Z0mH2O:646,614 Z0:664,624	IP:283,022 B2_hydroxy:361,078 C3PO3_B1_459:459.05 621,102 Z0mH2O:668,623 Z0:686,607
A-OH-18:1;O3/24:0;O	1262,7357		17,3		IP:261,038 W(18:1;O3):298,272 Z0:664,623	
A-OH-18:1;O3/25:0;O	1276,7535	1298,7278	17,1	17,12	IP:261,033 W(18:1;O3):298,269 C3PO3_B1_437:437,066 Z0mH2O:660,628 Z0:678,636	IP:283,024 B2_hydroxy:361,073 C3PO3_B1_459:459,049 621,105 Z0:700,603
A-OH-44:1;O4	1290,7679		17,44		IP:261,038 C3PO3_B1_437:437,066 Z0mH2O:674,655	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:692,65	
160325_S2_GV_c_4_pos_CID						
A-OH-18:0;O3/22:0;O	1236,714		15,96		IP:261,036 W-H2O(18:0;O3):282,276 W(18:0;O3):300,288 Z0mH2O:620,597 Z0:638,607	
A-OH-18:1;O3/22:0;O	1234,705	1256,6864	15,23	15,24	IP:261,036 W-H2O(18:1;O3):280,26 W(18:1;O3):298,275 Z0mH2O:618,573 Z0:636,591	IP:283,024 B2_hydroxy:361,075 C3PO3_B1_459:459.049 621,101 Z0:658,578
A-OH-18:1;O3/23:0	1232,7208		16,39		W(18:1;O3):298,274 Z0:634,609	
A-OH-18:1;O3/23:0;O	1248,717	1270,7012	16,07	16,09	IP:261,039 W-H2O(18:1;O3):280,26 W(18:1;O3):298,27 C3PO3_B1_437:437,079 Z0mH2O:632,601 Z0:650,611	IP:283,019 B2_hydroxy:361,084 C3PO3_B1_459:459.053 621,102 Z0:672,581
A-OH-18:0;O3/24:0;O	1264,7519	1286,7363	17,02	17,03	IP:261,028 W-H2O(18:0;O3):282,28 W(18:0;O3):300,283 Z0mH2O:648,632 Z0:666,636	IP:283,014 C3PO3_B1_459:459,046 621,102
A-OH-18:1;O3/24:0;O	1262,7368	1284,7171	16,61	16,64	IP:261,036 W-H2O(18:1;O3):280,26 W(18:1;O3):298,273 Z0mH2O:646,598 Z0:664,623	IP:283,02 B2_hydroxy:361,085 C3PO3_B1_459:459,05 621,109 Z0:686,603
A-OH-43:1;O4		1298,7302		17,08		IP:283,017 B2_hydroxy:361,073 C3PO3_B1_459:459,053 621,102 Z0:700,638
A-OH-44:1;O4	1290,7652		17,41		IP:261,035 C3PO3_B1_437:437,075 Z0mH2O:674,648 Z0:692,659	
A-OH-44:2;O2	1278,7345		15,83		Z0mH2O:662,616	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:680,616	

TABLE E.2: GIPC ID List: Replicates of S2_GV_s with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S2_GV_s_1_pos_CID						
A-NH-18:1;O3/26:1	1293,7676		17,11		W(18:1;O3):298,273 Z0mH2O:678,637 Z0:696,647	
A-NH-45:1;O2		1293,7831		17,18		Z0mH2O:678,638 Z0:696,651
A-OH-18:1;O3/20:0;O	1206,6717		13,11		IP:261,04 W-H2O(18:1;O3):280,266 Z0:608,561	
A-OH-18:1;O3/21:0;O	1220,6849		14,27		IP:261,037 W-H2O(18:1;O3):280,256 W(18:1;O3):298,269 Z0:622,578	
A-OH-18:0;O3/22:0;O	1236,716		16,05		IP:261,035 W-H2O(18:0;O3):282,272 W(18:0;O3):300,287 Z0mH2O:620,595 Z0:638,606	
A-OH-40:1;O3	1218,7092		15,75		Z0:620,591	
A-OH-18:1;O3/22:0;O	1234,7029		15,36		IP:261,034 W-H2O(18:1;O3):280,26 W(18:1;O3):298,27 Z0mH2O:618,583 Z0:636,59	
A-OH-18:1;O3/23:0	1232,7241		16,48		W(18:1;O3):298,268 Z0:634,611	
A-OH-41:1;O4		1270,6944		16,16		IP:283,018 B2_hydroxy:361,069 C3PO3_B1_459:459,049 621,104 Z0:672,58
A-OH-18:0;O3/24:0;O	1264,7504		17,06		IP:261,037 W-H2O(18:0;O3):282,277 W(18:0;O3):300,287 Z0mH2O:648,631 Z0:666,635	
A-OH-18:1;O3/24:0	1246,737		16,92		IP:261,032 W-H2O(18:1;O3):280,261	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,271 C3PO3_B1_437:437,09 Z0:648,624	
A-OH-18:1;O3/24:0;O	1262,7329	1284,7137	16,7	16,71	IP:261,036 W-H2O(18:1;O3):280,262 W(18:1;O3):298,275 Z0mH2O:646,612 Z0:664,622	IP:283,016 B2_hydroxy:361,072 C3PO3_B1_459:459,048 621,1 Z0:686,602
A-OH-18:1;O3/24:0;O	1262,7368		17,25		IP:261,035 W-H2O(18:1;O3):280,258 V_2(24:0;O):299,296 Z0:664,621	
A-OH-18:1;O3/25:0;O	1276,7518	1298,7282	17,09	17,14	IP:261,035 W-H2O(18:1;O3):280,261 W(18:1;O3):298,271 Z0mH2O:660,626 Z0:678,638	IP:283,016 B2_hydroxy:361,072 C3PO3_B1_459:459,048 621,104 Z0:700,632
A-OH-43:3;O3		1278,722		15,92		Z0mH2O:662,604 Z0:680,615
160325_S2_GV_s_2_pos_CID						
A-NH-18:1;O3/26:1	1293,7633		17,11		Z0mH2O:678,637	
A-OH-38:1;O4	1206,6725		13,15		Z0:608,558	
A-OH-18:0;O3/22:0;O	1236,7173		16,04		IP:261,04 W-H2O(18:0;O3):282,285 W(18:0;O3):300,285 Z0mH2O:620,589 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,7029	1256,6792	15,37	15,35	IP:261,039 W-H2O(18:1;O3):280,265 W(18:1;O3):298,273 Z0mH2O:618,577 Z0:636,592	IP:283,021 B2_hydroxy:361,07 C3PO3_B1_459:459,049 621,102 Z0:658,578
A-OH-18:1;O3/23:0	1232,7241		16,48		W(18:1;O3):298,274 Z0:634,612	
A-OH-18:1;O3/23:0;O	1248,7204	1270,7069	16,16	16,16	IP:261,039 W-H2O(18:1;O3):280,265 W(18:1;O3):298,268 C3PO3_B1_437:437,054 Z0mH2O:632,586	IP:283,021 B2_hydroxy:361,073 C3PO3_B1_459:459,049 621,101 Z0:672,592

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
A-OH-18:0;O3/24:0;O	1264,7466		17,08		Z0:650,607 IP:261,035 W-H2O(18:0;O3):282,278 W(18:0;O3):300,285 Z0mH2O:648,625 Z0:666,64	
A-OH-18:1;O3/24:0	1246,7466		17,08		IP:261,042 W-H2O(18:1;O3):280,264 W(18:1;O3):298,269 Z0:648,625	
A-OH-18:1;O3/24:0;O	1262,7353	1284,7174	16,69	16,71	IP:261,036 W-H2O(18:1;O3):280,26 W(18:1;O3):298,272 Z0mH2O:646,613 Z0:664,623	IP:283,012 B2_hydroxy:361,076 C3PO3_B1_459:459,053 621,105 Z0:686,649
A-OH-18:1;O3/25:0;O	1276,7525	1298,7321	17,08	17,11	IP:261,035 W-H2O(18:1;O3):280,266 W(18:1;O3):298,269 Z0mH2O:660,628 Z0:678,635	IP:283,016 B2_hydroxy:361,078 C3PO3_B1_459:459,049 621,102 Z0:700,611
A-OH-44:1;O4	1290,7675		17,55		B1_hydroxy:163,145 IP:261,035 Z0:692,651	
160325_S2_GV_s_4_pos_CID						
A-OH-18:1;O3/20:0;O	1206,674		13,04		IP:261,035 W(18:1;O3):298,266 Z0mH2O:590,561 Z0:608,558	
A-OH-18:0/22:0;O	1236,7154		15,97		IP:261,035 W-H2O(18:0;O3):282,275 W(18:0;O3):300,29 Z0mH2O:620,596 Z0:638,606	
A-OH-18:1;O3/22:0	1218,7165		15,75		W-H2O(18:1;O3):280,261 W(18:1;O3):298,269 Z0:620,593	
A-OH-18:1;O3/22:0;O	1234,7052	1256,6867	15,27	15,25	IP:261,039 W-H2O(18:1;O3):280,257 W(18:1;O3):298,277 Z0mH2O:618,576	IP:283,018 B2_hydroxy:361,075 C3PO3_B1_459:459,049 621,104

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:636,591	Z0:658,574
A-OH-18:;O3/23:0;O	1250,7305		16,57		IP:261,035 W-H2O(18:0;O3):282,277 W(18:0;O3):300,287 Z0mH2O:634,612 Z0:652,624	
A-OH-18:1;O3/23:0	1232,7241		16,48		IP:261,042 W(18:1;O3):298,276 Z0:634,616	
A-OH-18:1;O3/23:0;O	1248,7186	1270,6972	16,1	16,12	IP:261,034 W-H2O(18:1;O3):280,302 W(18:1;O3):298,262 C3PO3_B1_437:437,063 Z0mH2O:632,604 Z0:650,607	B2_hydroxy:361,075 C3PO3_B1_459:459,052 621,101 Z0:672,557
A-OH-18:0;O3/24:0;O	1264,7483	1286,7244	17,12	17,04	IP:261,032 W-H2O(18:0;O3):282,277 W(18:0;O3):300,285 Z0mH2O:648,628 Z0:666,631	IP:283,266 B2_hydroxy:361,078 C3PO3_B1_459:459,048 621,1 Z0:688,621
A-OH-18:1;O3/24:0;O	1262,7341	1284,7137	16,65	16,68	IP:261,036 W-H2O(18:1;O3):280,265 W(18:1;O3):298,267 Z0mH2O:646,62 Z0:664,618	IP:283,025 B2_hydroxy:361,082 C3PO3_B1_459:459,053 621,097 Z0:686,598
A-OH-18:1;O3/25:0;O	1276,7476		17,1		IP:261,032 W-H2O(18:1;O3):280,267 W(18:1;O3):298,27 Z0mH2O:660,648 Z0:678,639	
160325_S2_GV_s_5_pos_CID						
A-NH-42:1;O2		1251,735		15,17		Z0mH2O:636,59
A-OH-18:1;O3/21:0;O	1220,687		14,22		IP:261,037 W-H2O(18:1;O3):280,261 W(18:1;O3):298,276 Z0mH2O:604,588 Z0:622,578	
A-OH-18:0;O3/22:0;O	1236,7187		15,99		IP:261,035 W-H2O(18:0;O3):282,277 W(18:0;O3):300,287	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:620,595 Z0:638,609	
A-OH-18:1;O3/22:0	1218,7069		15,73		IP:261,033 W-H2O(18:1;O3):280,261 W(18:1;O3):298,274 Z0:620,599	
A-OH-18:1;O3/22:0;O	1234,7048	1256,6863	15,3	15,31	IP:261,038 W(18:1;O3):298,275 Z0mH2O:618,583 Z0:636,591	IP:283,028 B2_hydroxy:361,078 C3PO3_B1_459:459,052 621,104 Z0:658,566
A-OH-18:0;O3/23:0;O	1250,7361		16,57		IP:261,037 W(18:0;O3):300,29 Z0mH2O:634,612 Z0:652,624	
A-OH-18:1;O3/23:0	1232,7249		16,41		IP:261,035 W-H2O(18:1;O3):280,263 W(18:1;O3):298,271 Z0:634,615	
A-OH-18:1;O3/23:0;O	1248,7184	1270,7004	16,11	16,12	IP:261,039 W-H2O(18:1;O3):280,718 W(18:1;O3):298,26 Z0mH2O:632,596 Z0:650,606	IP:283,018 B2_hydroxy:361,080 C3PO3_B1_459:459,052 621,104 Z0:672,572
A-OH-18:0;O3/24:0;O	1264,7481	1286,7304	17,06	17,05	IP:261,036 W-H2O(18:0;O3):282,288 W(18:0;O3):300,298 Z0mH2O:648,628 Z0:666,639	IP:283,018 B2_hydroxy:361,075 C3PO3_B1_459:459,045 621,104 Z0:688,625
A-OH-18:1;O3/24:0	1246,7358		16,92		IP:261,044 W(18:1;O3):298,274 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7352	1284,7182	16,66	16,68	IP:261,038 W(18:1;O3):298,28 Z0mH2O:646,612 Z0:664,622	IP:283,015 B2_hydroxy:361,062 C3PO3_B1_459:459,055 621,108 Z0mH2O:668,644 Z0:686,594
A-OH-18:1;O3/25:0	1260,7536		17,29		IP:261,025 W(18:1;O3):298,276	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					V_2(25:0):397,381 Z0:662,641	
A-OH-18:1;O3/25:0;O	1276,752	1298,7279	17,11	17,11	IP:261,036 W-H2O(18:1;O3):280,267 W(18:1;O3):298,27 Z0mH2O:660,615 Z0:678,638	IP:283,016 B2_hydroxy:361,072 C3PO3_B1_459:459,048 621,1 Z0:700,617
A-OH-44:1;O4	1290,7634		17,47		IP:261,032 Z0:692,654	

Appendix F

GIPC ID Lists R Sampling 2

TABLE F.1: GIPC ID List: Replicates of S2_R_c with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S2_R_c_1_pos_CID						
A-OH-18:0;O3/22:0;O	1236,7189		15,74		IP:261,037 W-H2O(18:0;O3):282,272 W(18:0;O3):300,292 Z0mH2O:620,584 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,7013		14,97		IP:261,036 W-H2O(18:1;O3):280,265 W(18:1;O3):298,275 Z0mH2O:618,580 Z0:636,591	
A-OH-18:1;O3/23:0;O	1248,717		15,89		IP:261,038 W-H2O(18:1;O3):280,267 W(18:1;O3):298,272 Z0mH2O:632,614 Z0:650,602	
A-OH-18:0;O3/24:0;O	1264,7495		16,92		IP:261,037 W-H2O(18:0;O3):282,287 W(18:0;O3):300,292 C3PO3_B1_437:437,071 Z0mH2O:648,624 Z0:666,635	
A-OH-42:1;O4		1284,715		16,52		IP:283,007 B2_hydroxy:361,079 C3PO3_B1_459:459,046 621,105 Z0:686,598
A-OH-43:1;O4		1298,73		16,97		IP:283,021 B2_hydroxy:361,074 C3PO3_B1_459:459,048 621,1 Z0:700,629
A-OH-18:1;O3/25:0;O	1276,7428		16,98		IP:261,037 Z0mH2O:660,634 Z0:678,638	
A-OH-18:1;O3/26:0;O	1290,7676		17,32		IP:261,037 Z0:692,646	
160325_S2_R_c_2_pos_CID						

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
A-NH-42:1;O2		1251,7352		15,03		Z0mH2O:636,591 Z0:654,612
A-OH-40:0;O4	1236,7171		15,76		IP:261,04 Z0mH2O:620,599 Z0:638,61	
A-OH-18:1;O3/22:0;O	1234,7048	1256,6843	14,93	14,99	IP:261,036 W-H2O(18:1;O3):280,262 W(18:1;O3):298,272 Z0mH2O:618,573 Z0:636,591	IP:283,021 B2_hydroxy:361,072 C3PO3_B1_459:459,052 621,101 Z0:658,577
A-OH-18:1;O3/23:0;O	1248,7199	1270,7045	15,9	15,91	IP:261,036 W-H2O(18:1;O3):280,257 W(18:1;O3):298,277 Z0mH2O:632,581 Z0:650,61	IP:283,014 B2_hydroxy:361,072 C3PO3_B1_459:459,046 621,104 Z0:672,58
A-OH-18:0;O3/24:0;O	1264,743		16,84		IP:261,035 W-H2O(18:0;O3):282,292 W(18:0;O3):300,287 C3PO3_B1_437:437,059 Z0mH2O:648,631 Z0:666,639	
A-OH-18:1;O3/24:0;O	1262,735	1284,7161	16,49	16,5	IP:261,038 W-H2O(18:1;O3):280,262 W(18:1;O3):298,275 Z0mH2O:646,612 Z0:664,622	IP:283,025 B2_hydroxy:361,072 C3PO3_B1_459:459,053 621,101 Z0:686,602
A-OH-18:1;O3/25:0;O	1276,7498	1298,7331	16,97	16,98	IP:261,035 W(18:1;O3):298,276 C3PO3_B1_437:437,071 Z0mH2O:660,630 Z0:678,638	IP:283,021 B2_hydroxy:361,077 C2_hydroxy:379,083 C3PO3_B1_459:459,051 621,104 Z0:700,617
A-OH-18:1;O3/26:0;O	1290,7598		17,31		IP:261,037 W(18:1;O3):298,274 Z0:692,65	
160325_S2_R_c_3_pos_CID						
A-NH-43:1;O2		1265,7506		15,93		Z0mH2O:650,608 Z0:668,62
A-NH-44:1;O2		1279,7663		16,58		Z0mH2O:664,624

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
						Z0:682,635
A-NH-45:1;O2		1293,7822		16,98		Z0mH2O:678,644 Z0:696,638
A-OH-18:1;O3/20:0;O	1206,6793		12,77		IP:261,038 W-H2O(18:1;O3):280,264 W(18:1;O3):298,277 Z0mH2O:590,513 Z0:608,563	
A-OH-18:1;O3/21:0;O	1220,6968		14,02		IP:261,038 W(18:1;O3):298,274 Z0:622,579	
A-OH-18:0;O3/22:0;O	1236,7264		15,8		IP:261,036 W-H2O(18:0;O3):282,276 Z0mH2O:620,597 Z0:638,608	
A-OH-40:1;O4		1256,6843		15,04		IP:283,019 B2_hydroxy:361,071 C3PO3_B1_459:459,05 621,106 Z0:658,572
A-OH-18:1;O3/22:0;O	1234,7062		15,01		IP:261,032 W-H2O(18:1;O3):280,263 W(18:1;O3):298,278 Z0mH2O:618,582 Z0:636,593	
A-OH-18:1;O3/23:0;O	1248,7241	1270,7009	15,91	15,92	IP:261,037 W-H2O(18:1;O3):280,258 W(18:1;O3):298,273 Z0mH2O:632,596 Z0:650,605	IP:283,019 B2_hydroxy:361,079 C3PO3_B1_459:459,05 621,102 Z0:672,589
A-OH-18:0;O3/24:0;O	1264,7505	1286,7341	16,93	16,92	IP:261,038 W-H2O(18:0;O3):282,278 W(18:0;O3):300,288 Z0mH2O:648,626 Z0:666,637	B2_hydroxy:361,07 C3PO3_B1_459:459,047 621,106
A-OH-18:1;O3/24:0	1246,7421		16,77		IP:261,036 W(18:1;O3):298,275 Z0:648,63	
A-OH-18:1;O3/24:0;O	1262,7385	1284,7168	16,5	16,53	IP:261,037 W-H2O(18:1;O3):280,263	IP:283,018 B2_hydroxy:361,069

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,273 Z0mH2O:646,607 Z0:664,625	C3PO3_B1_459:459,054 621,103 Z0:686,636
A-OH-18:1;O3/25:0;O	1276,752	1298,7349	16,99	16,99	IP:261,036 W-H2O(18:1;O3):280,257 W(18:1;O3):298,272 Z0mH2O:660,63 Z0:678,644	IP:283,014 B2_hydroxy:361,076 C3PO3_B1_459:459,053 621,102 Z0:700,635
A-OH-18:1;O3/26:0;O	1290,7699		17,37		IP:261,036 W-H2O(18:1;O3):280,262 W(18:1;O3):298,272 Z0mH2O:674,645 Z0:692,656	
160325_S2_R_c_4_pos_CID						
A-NH-42:1;O2		1251,734		15,14		Z0mH2O:636,594
A-OH-t18:0/h22:0	1236,7203		15,75		IP:261,04 W-H2O(18:0;O3):282,277 W(18:0;O3):300,292 Z0mH2O:620,592 Z0:638,609	
A-OH-40:1;O4		1256,6869		14,96		IP:283,018 B2_hydroxy:361,078 C3PO3_B1_459:459.052 621,104 Z0:658,574
A-OH-18:1;O3/22:0;O	1234,7047		14,95		IP:261,042 W(18:1;O3):298,272 Z0mH2O:618,584 Z0:636,595	
A-OH-18:1;O3/23:0;O	1248,7213	1270,7022	15,93	15,89	IP:261,036 W(18:1;O3):298,298 Z0mH2O:632,603 Z0:650,607	IP:283,019 B2_hydroxy:361,097 C3PO3_B1_459:459.049 621,101 Z0:672,599
A-OH-18:0;O3/24:0;O	1264,7506	1286,7291	16,89	17	IP:261,034 W-H2O(18:0;O3):282,273 W(18:0;O3):300,288 Z0:666,636	B2_hydroxy:361,072 C3PO3_B1_459:459,048 621,104
A-OH-18:1;O3/24:0	1246,7397		16,75		IP:261,03 W-H2O(18:1;O3):280,261	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,266 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7376	1284,7178	16,5	16,5	IP:261,034 W-H2O(18:1;O3):280,267 W(18:1;O3):298,27 Z0mH2O:646,616 Z0:664,622	IP:283,02 B2_hydroxy:361,073 C2_hydroxy:379,09 C3PO3_B1_459:459,056 621,105 Z0:686,591
A-OH-18:1;O3/25:0;O	1276,7515	1298,7409	16,97	17,01	IP:261,034 W(18:1;O3):298,277 Z0mH2O:660,65 Z0:678,634	IP:283,018 B2_hydroxy:361,069 C3PO3_B1_459:459,048 621,107 Z0:700,605
A-OH-18:1;O3/26:0;O	1290,7584		17,32		IP:261,04 W(18:1;O3):298,271 Z0mH2O:674,638 Z0:692,658	

TABLE F.2: GIPC ID List: Replicates of S2_R_s with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S2_R_s_1_pos_CID						
A-NH-42:1;O2		1251,734		15,4		Z0mH2O:636,588
A-OH-18:0;O3/22:0;O	1236,7197		15,9		IP:261,043 W-H2O(18:0;O3):282,31 W(18:0;O3):300,293 Z0mH2O:620,597 Z0:638,608	
A-OH-40:1;O4		1256,6888		15,19		IP:283,017 B2_hydroxy:361,07 C3PO3_B1_459:459,047 621,102 Z0:658,579
A-OH-18:1;O3/22:0;O	1234,7073		15,15		IP:261,039 W-H2O(18:1;O3):280,265 W(18:1;O3):298,273 Z0mH2O:618,593 Z0:636,589	
A-OH-18:0;O3/23:0;O	1250,7321		16,49		IP:261,035 Z0mH2O:634,617 Z0:652,625	
A-OH-18:1;O3/23:0;O	1248,7233	1270,703	16,04	16,03	IP:261,034 W-H2O(18:1;O3):280,26 W(18:1;O3):298,275 Z0mH2O:632,59 Z0:650,608	IP:283,024 B2_hydroxy:361,07 C3PO3_B1_459:459,053 621,106 Z0:672,581
A-OH-18:0;O3/24:0;O	1264,7477		16,99		IP:261,032 W(18:0;O3):300,312 Z0mH2O:648,625 Z0:666,637	
A-OH-t18:1;O3/24:0	1246,7413		16,83		W(18:1;O3):298,272 Z0:648,63	
A-OH-18:1;O3/24:0;O	1262,7375	1284,72	16,58	16,6	IP:261,032 W-H2O(18:1;O3):280,26 W(18:1;O3):298,273 Z0mH2O:646,621 Z0:664,624	IP:283,015 B2_hydroxy:361,071 C3PO3_B1_459:459,056 621,106 Z0:686,6
A-OH-18:1;O3/25:0;O	1276,753	1298,7349	17,02	17,03	IP:261,039 W(18:1;O3):298,275	IP:283,019 B2_hydroxy:361,076

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:660,651 Z0:678,64	C3PO3_B1_459:459,05 621,102 Z0:700,619
A-OH-44:1;O4	1290,7683		17,37		IP:261,04 Z0mH2O:674,649 Z0:692,652	
160325_S2_R_s_2_pos_CID						
A-OH-18:0;O3/22:0;O	1236,72		15,83		IP:261,042 W-H2O(18:0;O3):282,285 W(18:0;O3):300,285 Z0mH2O:620,595 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,705	1256,6867	15,09	15,11	IP:261,036 W-H2O(18:1;O3):280,269 W(18:1;O3):298,275 Z0mH2O:618,576 Z0:636,591	IP:283,016 B2_hydroxy:361,075 C3PO3_B1_459:459,049 621,101 Z0:658,566
A-OH-18:0;O3/23:0;O	1250,7357		16,47		Z0mH2O:634,612 Z0:652,624	
A-OH-18:1;O3/23:0;O	1248,7181	1270,699	16,32	16,04	IP:261,042 C3PO3_B1_437:437,056 Z0mH2O:632,566 Z0:650,602	IP:283,018 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,104 Z0:672,584
A-OH-18:0;O3/24:0;O	1264,7446		16,95		IP:261,032 W(18:0;O3):282,277 W(18:0;O3):300,285 Z0mH2O:648,625 Z0:666,635	
A-OH-t18:1;O3/24:0	1246,7353		16,83		IP:261,042 W-H2O(18:1;O3):280,268 W(t18:1):298,276 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7363	1284,717	16,57	16,55	IP:261,033 W(18:1;O3):298,28 Z0mH2O:646,606 Z0:664,622	IP:283,015 B2_hydroxy:361,067 C3PO3_B1_459:459,046 621,101 Z0:686,606
A-OH-18:1;O3/25:0;O	1276,7487	1298,7243	16,99	17,05	IP:261,035 W-H2O(18:1;O3):280,263	IP:283,018 B2_hydroxy:361,078

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,271 C3PO3_B1_437:437,068 Z0mH2O:660,627 Z0:678,635	C3PO3_B1_459:459,052 621,101 Z0:700,614
A-OH-44:1;O4	1290,7612		17,55		IP:261,042 Z0:692,654	
160325_S2_R_s_3_rep1_pos_CID						
A-OH-40:1;O4		1256,6929		15,11		IP:283,016 B2_hydroxy:361,07 C3PO3_B1_459:459,049 621,101
A-OH-18:1;O3/22:0;O	1234,7083		15,09		IP:261,041 W-H2O(18:1;O3):280,27 W(18:1;O3):298,27 Z0mH2O:618,589 Z0:636,596	
A-OH-18:1;O3/23:0;O	1248,7194		16,04		IP:261,04 W-H2O(18:1;O3):280,264 W(18:1;O3):298,274 Z0mH2O:632,601 Z0:650,604	
A-OH-18:1;O3/24:0;O	1262,7375	1284,7192	16,58	16,56	IP:261,034 W(18:1;O3):298,273 Z0mH2O:646,587 Z0:664,623	IP:283,024 B2_hydroxy:361,078 C2_hydroxy:379,319 C3PO3_B1_459:459,053 621,102
A-OH-q43:1		1298,7284		17,04		C3PO3_B1_459:459,046 621,105
160325_S2_R_s_3_rep2_pos_CID						
A-OH-38:1;O4	1206,674		12,88		Z0:608,562	
A-OH-18:0;O3/22:0;O	1236,7171		15,87		IP:261,03 W-H2O(18:0;O3):282,277 W(18:0;O3):300,288 Z0mH2O:620,592 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,7038	1256,6828	15,08	15,07	IP:261,038 W-H2O(18:1;O3):280,27 W(18:1;O3):298,28 Z0mH2O:618,573	IP:283,014 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,105

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:636,591	Z0:658,563
A-OH-18:1;O3/23:0;O	1248,7187	1270,6992	16,02	16,02	IP:261,039 W(18:1;O3):298,278 Z0mH2O:632,598 Z0:650,607	IP:283,019 B2_hydroxy:361,07 C2_hydroxy:379,084 C3PO3_B1_459:459,046 621,101 Z0:672,581
A-OH-18:0;O3/24:0;O	1264,7444		16,93		IP:261,035 W-H2O(18:0;O3):282,272 W(18:0;O3):300,29 Z0mH2O:648,624 Z0:666,636	
A-OH-18:1;O3/24:0;O	1262,735	1284,7176	16,58	16,6	IP:261,034 W-H2O(18:1;O3):280,26 W(18:1;O3):298,277 Z0mH2O:646,618 Z0:664,623	IP:283,016 B2_hydroxy:361,072 C3PO3_B1_459:459,052 621,105 Z0:686,603
A-OH-18:1;O3/25:0;O	1276,753	1298,7319	16,99	17,09	IP:261,042 W(18:1;O3):298,274 Z0mH2O:660,612 Z0:678,639	IP:283,016 B2_hydroxy:361,075 C3PO3_B1_459:459,049 621,101 Z0:700,622
A-OH-44:1;O4	1290,7662		17,36		IP:261,038 Z0:692,659	
160325_S2_R_s_3_rep3_pos_CID						
A-NH-18:0;O3/25:1	1281,7699		16,94		W(18:0;O3):300,288 Z0:634,644	
A-OH-18:1;O3/22:0;O	1236,7213		15,81		IP:261,035 W-H2O(18:0;O3):282,277 W(18:0;O3):300,288 Z0mH2O:620,592 Z0:638,606	
A-OH-40:1;O4		1256,6836		14,99		IP:283,014 B2_hydroxy:361,07 C3PO3_B1_459:459,055 621,101 Z0:658,578
A-OH-18:1;O3/23:0;O	1248,7216	1270,7016	15,95	15,94	IP:261,032 W-H2O(18:1;O3):280,265 W(18:1;O3):298,275	IP:283,019 B2_hydroxy:361,075 C3PO3_B1_459:459,049

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:632,601 Z0:650,607	621,101 Z0:672,584
A-OH-18:0;O3/24:0;O	1264,7436		16,88		IP:261,035 W-H2O(18:0;O3):282,275 W(18:0;O3):300,287 Z0mH2O:648,625 Z0:666,636	
A-OH-18:1;O3/24:0	1246,7409		16,76		IP:261,03 W(18:1;O3):298,274 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7342	1284,7164	16,53	16,54	IP:261,039 W-H2O(18:1;O3):280,265 W(18:1;O3):298,273 Z0mH2O:646,606 Z0:664,623	IP:283,027 B2_hydroxy:361,076 C3PO3_B1_459:459,05 621,105 Z0:686,603
A-OH-18:1;O3/25:0;O	1276,75	1298,7299	16,99	17,02	IP:261,034 W-H2O(18:1;O3):280,26 W(18:1;O3):298,275 Z0mH2O:660,648 Z0:678,639	IP:283,019 B2_hydroxy:361,075 C3PO3_B1_459:459,046 621,101 Z0mH2O:682,627 Z0:700,622
A-OH-18:1;O3/26:0;O	1290,765		17,36		IP:261,03 W(18:1;O3):298,274 Z0:692,655	
160325_S2_R_s_3_rep4_pos_CID						
A-NH-42:1;O2		1251,7349		15,01		Z0mH2O:636,596 Z0:654,606
A_NH-43:1;O2		1265,7493		15,96		Z0mH2O:650,608 Z0:668,612
A-OH-18:1;O3/22:0;O	1236,7216		15,79		IP:261,036 W-H2O(18:0;O3):282,275 W(18:0;O3):300,291 Z0mH2O:620,608 Z0:638,607	
A-OH-40:1;O4	1234,7065	1256,6891	15,03	15,06	IP:261,037 W-H2O(18:1;O3):280,258 W(18:1;O3):298,273 Z0mH2O:618,585 Z0:636,589	IP:283,022 B2_hydroxy:361,076 C3PO3_B1_459:459,05 621,106 Z0:658,568
A-OH-18:1;O3/23:0;O	1248,726	1270,6974	15,98	15,97	IP:261,032	IP:283,017

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W-H2O(18:1;O3):280,261 W(18:1;O3):298,278 Z0mH2O:632,591 Z0:650,608	B2_hydroxy:361,073 C3PO3_B1_459:459,05 621,106 Z0:672,574
A-OH-18:0;O3/24:0;O	1264,7529	1286,7304	16,89	16,91	IP:261,038 W-H2O(18:0;O3):282,278 W(18:0;O3):300,298 Z0mH2O:648,629 Z0:666,641	C3PO3_B1_459:459,056 621,102
A-OH-18:1;O3/24:0	1246,7413		16,78		IP:261,03 W(18:1;O3):298,277 Z0:648,633	
A-OH-18:1;O3/24:0;O	1262,7395	1284,7206	16,54	16,56	IP:261,03 W-H2O(18:1;O3):280,275 Z0mH2O:646,61 Z0:664,623	IP:283,02 B2_hydroxy:361,071 C3PO3_B1_459:459,05 621,109 Z0:686,607
A-OH-18:1;O3/25:0;O	1276,7518	1298,7387	17,01	17,01	IP:261,039 W-H2O(18:1;O3):280,263 W(18:1;O3):298,282 Z0mH2O:660,629 Z0:678,64	IP:283,019 B2_hydroxy:361,078 C3PO3_B1_459:459,053 621,102 Z0:700,619
A-OH-18:1;O3/26:0;O	1290,7594		17,36		IP:261,038 W-H2O(18:1;O3):280,264 Z0mH2O:674,641 Z0:692,655	
160325_S2_R_s_4_pos_CID						
A-OH-18:0;O3/22:0;O	1236,7174		15,82		IP:261,035 W(18:0;O3):300,293 Z0mH2O:620,596 Z0:638,610	
A-OH-40:1;O4	1234,7044	1256,6843	15,04	15,03	IP:261,034 W(18:1;O3):298,272 Z0mH2O:618,566 Z0:636,591	IP:283,019 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,101 Z0:658,578
A-OH-18:1;O3/23:0;O	1248,7199	1270,6978	15,95	15,99	IP:261,034 W(18:1;O3):298,272 Z0mH2O:632,6 Z0:650,606	IP:283,014 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,105

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
						Z0:672,588
A-OH-18:0;O3/24:0;O	1264,7449		16,91		IP:261,036 W(18:0;O3):300,291 C3PO3_B1_437:437,069 Z0mH2O:648,629 Z0:666,640	
A-OH-18:1;O3/24:0	1246,7392		16,78		W-H2O(18:1;O3):280,266 W(18:1;O3):298,259 Z0mH2O:630,617 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7343	1284,7153	16,55	16,56	Z0mH2O:646,613 Z0:664,623	IP:283,02 B2_hydroxy:361,076 C3PO3_B1_459:459,05 621,101 Z0mH2O:668,645 Z0:686,591
A-OH-18:1;O3/25:0;O	1276,7487	1298,7262	17,01	17,04	IP:261,041 W-H2O(18:1;O3):280,26 W(18:1;O3):298,295 Z0mH2O:660,62 Z0:678,638	IP:283,018 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,101 Z0:700,622
A-OH-18:1;O3/26:0;O	1290,7711		17,34		IP:261,035 W(18:1;O3):298,284 Z0mH2O:674,651 Z0:692,658	

Appendix G

GIPC ID Lists GV Sampling 4

TABLE G.1: GIPC ID List: Replicates of *S4_GV_c* with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S4_GV_c_1_pos_CID						
A-OH-38:1;O4	1206,6789		12,97		Z0mH2O:590,554 Z0:608,561	
A-OH-18:0;O3/22:0;O	1236,7156		15,65		IP:261,039 W-H2O(18:0;O3):282,272 W(18:0;O3):300,284 Z0mH2O:620,594 Z0:638,608	
A-OH-18:1;O3/22:0;O	1234,702	1256,682	14,78	14,78	IP:261,033 W(18:1;O3):298,277 Z0mH2O:618,579 Z0:636,59	IP:283,023 B2_hydroxy:361,072 C3PO3_B1_459:459,051 621,1 Z0:658,565
A-OH-18:0;O3/23:0;O	1250,7311		16,31		IP:261,034 W-H2O(18:0;O3):282,272 W(18:0;O3):300,289 Z0mH2O:634,615 Z0:652,62	
A-OH-41:1;O3	1232,7214		16,13		IP:261,032 Z0:634,611	
A-OH-18:1;O3/23:0;O	1248,7181	1270,7039	15,8	15,81	IP:261,036 W-H2O(18:1;O3):280,262 W(18:1;O3):298,269 Z0mH2O:632,592 Z0:650,606	IP:283,018 B2_hydroxy:361,069 C3PO3_B1_459:459,048 621,1 Z0:672,579
A-OH-18:0;O3/24:0;O	1264,7456	1286,727	16,86	16,88	IP:261,033 W-H2O(18:0;O3):282,283 W(18:0;O3):300,298 Z0mH2O:648,622 Z0:666,638	IP:283,02 B2_hydroxy:361,08 C3PO3_B1_459:459,051 621,103 Z0:686,62
A-OH-18:1;O3/24:0	1246,7353		16,67		IP:261,037 W-H2O(18:1;O3):280,26 W(18:1;O3):298,271 Z0:648,627	
A-OH-18:1;O3/24:0;O	1262,7333	1284,7158	16,42	16,44	IP:261,036 W(18:1;O3):298,274 Z0mH2O:646,604 Z0:664,621	IP:283,021 B2_hydroxy:361,075 C3PO3_B1_459:459,052 621,1

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
						Z0:686,593
A-OH-18:1;O3/25:0;O	1276,748	1298,7285	16,92	16,91	IP:261,036 W-H2O(18:1;O3):280,259 W(18:1;O3):298,277 Z0mH2O:660,607 Z0:678,633	IP:283,018 B2_hydroxy:361,071 C3PO3_B1_459:459,048 621,103 Z0:700,55
160325_S4_GV_c_2_pos_CID						
A-NH-18:0;O3/25:1	1281,7698		16,81		Z0mH2O:666,639 Z0:684,639	
A-OH-38:1;O4	1206,672		12,55		IP:261,039 Z0:608,56	
A-OH-18:0;O3/22:0;O	1236,7211		15,62		IP:261,006 W(18:0;O3):300,292 Z0mH2O:620,595 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,7047	1256,6782	14,73	14,72	IP:261,038 W-H2O(18:1;O3):280,267 W(18:1;O3):298,277 Z0mH2O:618,583 Z0:636,591	IP:283,021 B2_hydroxy:361,069 C2_hydroxy:379,098 C3PO3_B1_459:459,052 621,101
A-OH-18:1;O3/23:0;O	1248,7195	1270,6933	15,73	15,74	IP:261,107 W-H2O(18:1;O3):280,269 W(18:1;O3):298,3 Z0mH2O:632,592 Z0:650,602	IP:283,016 C2_hydroxy:379,089 C3PO3_B1_459:459,052 621,104 Z0:672,587
A-OH-18:0;O3/24:0;O	1264,7482		16,76		IP:261,037 W(18:0;O3):300,282 Z0mH2O:648,627 Z0:666,635	
A-OH-18:1;O3/24:0	1246,7437		16,67		IP:261,035 W(18:1;O3):298,271 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7348	1284,7182	16,38	16,4	IP:261,033 W-H2O(18:1;O3):280,259 W(18:1;O3):298,277 Z0mH2O:646,604 Z0:664,621	IP:283,018 B2_hydroxy:361,072 C3PO3_B1_459:459,051 621,1 Z0:686,609
A-OH-18:1;O3/25:0;O	1276,7459	1298,7301	16,87	16,87	IP:261,032 W-H2O(18:1;O3):280,266 W(18:1;O3):298,273	IP:283,006 B2_hydroxy:361,066 C3PO3_B1_459:459,048

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:660,623 Z0:678,638	621,104 Z0:700,609
160325_S4_GV_c_3_pos_CID						
A-OH-38:1;O4	1206,6665		12,59		IP:261,037 Z0mH2O:590,553 Z0:608,56	
A-OH-18:1;O3/21:0;O	1220,6821		13,69		W(18:1;O3):298,271 Z0:622,577	
A-OH-18:0;O3/22:0;O	1236,7147		15,61		IP:261,035 W-H2O(18:0;O3):282,272 Z0mH2O:620,591 Z0:638,609	
A-OH-18:1;O3/22:0;O	1234,7018	1256,6841	14,74	14,72	IP:261,033 W-H2O(18:1;O3):280,257 W(18:1;O3):298,274 Z0mH2O:618,575 Z0:636,59	IP:283,016 B2_hydroxy:361,075 C3PO3_B1_459:459,051 621,101 Z0:658,58
A-OH-18:1;O3/23:0;O	1248,7174	1270,7026	15,73	15,77	IP:261,038 W-H2O(18:1;O3):280,267 W(18:1;O3):298,262 C3PO3_B1_437:437,099 Z0mH2O:632,591 Z0:650,605	IP:283,018 B2_hydroxy:361,069 C3PO3_B1_459:459,048 621,1 Z0:672,564
A-OH-18:0;O3/24:0;O	1264,745		16,83		IP:261,037 W(18:0;O3):300,287 Z0mH2O:648,627 Z0:666,638	
A-OH-18:1;O3/24:0;O	1262,735		16,39		IP:261,038 W-H2O(18:1;O3):280,264 W(18:1;O3):298,277 Z0mH2O:646,611 Z0:664,621	
A-OH-18:1;O3/25:0;O	1276,7458	1298,7251	16,87	16,88	IP:261,037 W(18:1;O3):298,273 Z0mH2O:660,626 Z0:678,637	IP:283,021 B2_hydroxy:361,069 C3PO3_B1_459:459,051 621,1 Z0:700,617
A-OH-18:1;O3/25:0;O	1290,7614		17,28		IP:261,037 W(18:1;O3):298,263	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:674,657 Z0:692,653	
160325_S4_GV_c_4_pos_CID						
A-NH-18:1;O3/25:0	1281,769		16,81		Z0mH2O:666,639 Z0:684,647	
A-OH-18:1;O3/20:0;O	1206,6702		12,54		IP:261,035 W-H2O(18:1;O3):280,268 W(18:1;O3):298,271 Z0mH2O:590,554 Z0:608,557	
A-OH-18:1;O3/22:0;O	1234,7041	1256,6857	14,78	14,77	IP:261,041 W-H2O(18:1;O3):280,257 W(18:1;O3):298,27 Z0mH2O:618,572 Z0:636,59	IP:283,018 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,104 Z0:658,581
A-OH-18:0;O3/23:0;O	1250,7292		16,31		IP:261,032 W(18:0;O3):300,287 Z0mH2O:634,615 Z0:652,624	
A-OH-18:1;O3/23:0	1232,7244		16,14		IP:261,035 W(18:1;O3):298,281 Z0mH2O:616,603 Z0:634,611	
A-OH-18:1;O3/23:0;O		1270,699		15,77	IP:283,021 B2_hydroxy:361,072 C3PO3_B1_459:459,052 621,1 Z0:672,587	
A-OH-18:0;O3/24:0;O	1264,7453	1286,7321	16,82	16,85	IP:261,038 W-H2O(18:0;O3):282,276 W(18:0;O3):300,306 Z0mH2O:648,627 Z0:666,635	B2_hydroxy:361,072 C3PO3_B1_459:459,048 621,107
A-OH-18:1;O3/24:0	1246,737		16,65		IP:261,037 W(18:1;O3):298,273 Z0:668,627	
A-OH-18:1;O3/24:0;O	1262,7339	1284,7133	16,39	16,41	IP:261,036 W-H2O(18:1;O3):280,264 W(18:1;O3):298,275 Z0mH2O:646,612	IP:283,022 B2_hydroxy:361,067 C3PO3_B1_459:459,046 621,1

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:664,622	Z0:686,598
A-OH-18:1;O3/25:0;O	1276,7449	1298,729	16,9	16,87	IP:261,037 W-H2O(18:1;O3):280,266 W(18:1;O3):298,273 Z0mH2O:660,626 Z0:678,638	IP:283,018 B2_hydroxy:361,077 C3PO3_B1_459:459,051 621,103 Z0:700,62
A-OH-18:1;O3/26:0;O	1290,7686		17,28		IP:261,037 W(18:1;O3):298,263 Z0mH2O:674,646 Z0:692,653	

TABLE G.2: GIPC ID List: Replicates of *S4_GV_s* with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S4_GV_s_1_pos_CID						
A-OH-18:1;O3/21:0;O	1220,6822		13,77		IP:261,033 W-H2O(18:1;O3):280,288 W(18:1;O3):298,284 Z0:622,578	
A-OH-18:0;O3/22:0;O	1236,7189		15,7		IP:261,037 W-H2O(18:0;O3):282,279 W(18:0;O3):300,294 Z0mH2O:620,59 Z0:638,604	
A-OH-18:1;O3/22:0;O	1234,7024	1256,6821	14,86	14,89	IP:261,038 W(18:1;O3):298,262 Z0mH2O:618,579 Z0:636,59	IP:283,015 B2_hydroxy:361,074 C3PO3_B1_459:459,051 621,103 Z0:658,554
A-OH-18:1;O3/23:0	1232,7257		16,19		W(18:1;O3):298,268 Z0:634,615	
A-OH-18:1;O3/23:0;O	1248,718	1270,7016	15,87	15,84	IP:261,036 W(18:1;O3):298,277 Z0mH2O:632,595 Z0:650,609	IP:283,018 B2_hydroxy:361,077 C3PO3_B1_459:459,051 621,106 Z0:672,589
A-OH-18:0;O3/24:0;O	1264,7489		16,84		IP:261,033 W-H2O(18:0;O3):282,29 W(18:0;O3):300,288 Z0mH2O:648,627 Z0:666,642	
A-OH-18:1;O3/24:0	1246,7365		16,71		IP:261,034 W(18:1;O3):298,266 Z0:648,631	
A-OH-18:1;O3/24:0;O	1262,7329	1284,7152	16,46	16,47	IP:261,033 W-H2O(18:1;O3):280,262 W(18:1;O3):298,269 Z0mH2O:646,619 Z0:664,622	IP:283,022 B2_hydroxy:361,078 C3PO3_B1_459:459,049 621,1 Z0:686,617
A-OH-18:1;O3/25:0;O	1276,7477	1298,7304	16,94	16,98	IP:261,031 W-H2O(18:1;O3):280,262 W(18:1;O3):298,272	IP:283,015 B2_hydroxy:361,074 C3PO3_B1_459:459,047

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:678,637	621,103 Z0:700,627
160325_S4_GV_s_2_pos_CID						
A-NH-43:1;O2		1265,7493		15,78		Z0mH2O:650,609 Z0:668,606
A-OH-18:0;O3/22:0;O	1236,7171		15,7		IP:261,032 W(18:0;O3):300,284 Z0mH2O:620,595 Z0:638,605	
A-OH-18:1;O3/22:0;O	1234,707	1256,6828	14,9	14,88	IP:261,036 W-H2O(18:1;O3):280,262 W(18:1;O3):298,274 Z0mH2O:618,568 Z0:636,594	IP:283,018 B2_hydroxy:361,077 C3PO3_B1_459:459,051 621,108 Z0:658,573
A-OH-18:1;O3/23:0;O	1248,7196	1270,6959	15,84	15,86	IP:261,166 W-H2O(18:1;O3):280,267 W(18:1;O3):298,272 Z0mH2O:632,588 Z0:650,605	IP:283,018 B2_hydroxy:361,072 C3PO3_B1_459:459,052 621,101 Z0:672,583
A-OH-18:0;O3/24:0;O	1264,7428		17,01		Z0mH2O:648,623 Z0:666,638	
A-OH-18:1;O3/24:0	1246,7361		16,72		IP:261,027 W(18:1;O3):298,268 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,736	1284,7162	16,46	16,47	IP:261,038 W(18:1;O3):298,274 Z0mH2O:646,608 Z0:664,621	IP:283,019 B2_hydroxy:361,072 C3PO3_B1_459:459,052 621,107 Z0:686,605
A-OH-18:1;O3/25:0;O	1276,7513	1298,7317	16,93	16,99	IP:261,038 W-H2O(18:1;O3):280,269 W(18:1;O3):298,269 Z0mH2O:660,63 Z0:678,638	IP:283,021 B2_hydroxy:361,069 C3PO3_B1_459:459,048 621,1 Z0:700,64
A-OH-18:1;O3/26:0;O	1290,7661		17,33		IP:261,035 W(18:1;O3):298,271 Z0mH2O:674,636 Z0:692,65	
160325_S4_GV_s_3_pos_CID						
A-OH-18:1;O3/21:0;O	1220,6957		13,71		IP:261,04	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W-H2O(18:1;O3):280,256 W(18:1;O3):298,279 Z0mH2O:604,565 Z0:622,574	
A-OH-18:0;O3/22:0;O	1236,7128		15,66		IP:261,037 W-H2O(18:0;O3):282,275 W(18:0;O3):300,292 Z0mH2O:620,58 Z0:638,605	
A-OH-18:1;O3/22:0;O	1234,7028	1256,6852	14,78	14,75	IP:261,034 W-H2O(18:1;O3):280,267 W(18:1;O3):298,277 Z0mH2O:618,558 Z0:636,587	IP:283,018 B2_hydroxy:361,072 C3PO3_B1_459:459,038 621,097 Z0:658,566
A-OH-18:0;O3/23:0;O	1250,7302		16,31		IP:261,034 W-H2O(18:0;O3):282,279 W(18:0;O3):300,287 Z0mH2O:634,607 Z0:652,623	
A-OH-18:1;O3/23:0;O	1248,7203	1270,6953	15,77	15,78	IP:261,036 W-H2O(18:1;O3):280,279 W(18:1;O3):298,272 C3PO3_B1_437:437,065 Z0mH2O:632,588 Z0:650,601	IP:283,016 B2_hydroxy:361,069 C3PO3_B1_459:459,048 621,104 Z0:672,591
A-OH-18:0;O3/24:0;O	1264,7428	1286,7278	16,85	16,85	IP:261,033 W(18:0;O3):300,293 Z0mH2O:648,024 Z0:666,639	B2_hydroxy:361,063 C3PO3_B1_459:459,051 621,104 Z0:688,629
A-OH-18:1;O3/24:0	1246,7382		16,67		IP:261,03 W(18:1;O3):298,283 Z0:648,627	
A-OH-18:1;O3/24:0;O	1262,7338	1284,7147	16,42	16,43	IP:261,038 W(18:1;O3):298,269 Z0mH2O:646,604 Z0:664,622	IP:283,014 B2_hydroxy:361,07 C3PO3_B1_459:459,052 621,101 Z0:686,602
A-OH-18:1;O3/25:0;O	1276,749	1298,7321	16,91	16,94	IP:261,029 W-H2O(18:1;O3):280,262 W(18:1;O3):298,269	IP:283,018 B2_hydroxy:361,071 C3PO3_B1_459:459,051

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:660,645 Z0:678,637	621,103 Z0:700,62
160325_S4_GV_s_4_pos_CID						
A-NH-45:2;O3		1307,7846		17,3		Z0mH2O:692,65 Z0:710,662
A-OH-18:0;O3/22:0;O	1236,7206		15,67		IP:261,035 W-H2O(18:0;O3):282,277 Z0mH2O:620,598 Z0:638,606	
A-OH-18:1;O3/22:0;O	1234,7054	1256,6887	14,81	14,86	IP:261,041 W-H2O(18:1;O3):280,26 W(18:1;O3):298,275 Z0mH2O:618,573 Z0:636,591	IP:283,013 B2_hydroxy:361,075 C3PO3_B1_459:459.048 621,104 Z0:658,569
A-OH-18:0;O3/23:0;O	1250,7328		16,33		IP:261,032 W(18:0;O3):300,287 Z0mH2O:634,608 Z0:652,62	
A-OH-18:1;O3/23:0;O	1248,7189	1270,7008	15,77	15,82	IP:261,036 W(18:1;O3):298,272 Z0mH2O:632,611 Z0:650,61	IP:283,021 B2_hydroxy:361,075 C3PO3_B1_459:459.052 621,104 Z0:672,591
A-OH-18:0;O3/24:0;O	1264,749	1286,7283	16,86	16,87	IP:261,036 W-H2O(18:0;O3):282,283 W(18:0;O3):300,296 C3PO3_B1_437:437,081 Z0mH2O:648,024 Z0:666,631	IP:283,013 B2_hydroxy:361,074 C3PO3_B1_459:459.045 621,104
A-OH-18:1;O3/24:0	1246,7382		16,68		W(18:1;O3):298,271 Z0mH2O:630,623 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7352	1284,7182	16,42	16,44	IP:261,038 W-H2O(18:1;O3):280,267 Z0mH2O:646,616 Z0:664,622	IP:283,017 B2_hydroxy:361,07 C3PO3_B1_459:459.046 621,101 Z0:686,606
A-OH-18:1;O3/25:0;O	1276,7521	1298,7317	16,92	16,92	IP:261,038 W-H2O(18:1;O3):280,269 W(18:1;O3):298,272	IP:283,013 B2_hydroxy:361,072 C3PO3_B1_459:459.048

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:660,657 Z0:678,638	621,1 Z0:700,621
A-OH-18:1;O3/26:0;O	1290,7629	1312,7398	17,32	17,3	IP:261,034 W(18:1;O3):298,276 Z0mH2O:674,619 Z0:692,653	IP:283,003 B2_hydroxy:361,072 C3PO3_B1_459:459.051 621,104 Z0:714,633

Appendix H

GIPC ID Lists R Sampling 4

TABLE H.1: GIPC ID List: Replicates of S4_R_c with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S4_R_c_1_pos_CID						
A-NH-44:1;O2		1279,7659		16,35		Z0mH2O:664,623 Z0:682,635
A-OH-18:1;O3/20:0;O	1206,6744		12,45		IP:261,04 W(18:1;O3):298,279 Z0mH2O:590,566 Z0:608,559	
A-OH-18:0;O3/22:0;O	1236,7211		15,51		IP:261,038 W-H2O(18:0;O3):282,275 W(18:0;O3):300,306 Z0mH2O:620,593 Z0:638,611	
A-OH-18:1;O3/22:0;O	1234,7077	1256,6893	14,7	14,65	IP:261,044 W-H2O(18:1;O3):280,26 W(18:1;O3):298,684 Z0mH2O:618,581 Z0:636,592	IP:283,016 B2_hydroxy:361,081 C3PO3_B1_459:459.052 621,105 Z0:658,571
A-OH-18:0;O3/23:0;O	1250,7406		16,31		Z0mH2O:634,616 Z0:652,621	
A-OH-18:1;O3/23:0;O	1248,7213	1270,7012	15,69	15,7	IP:261,037 W-H2O(18:1;O3):280,26 W(18:1;O3):298,273 Z0mH2O:632,602 Z0:650,608	IP:283,002 B2_hydroxy:361,073 C3PO3_B1_459:459.056 621,106 Z0:672,597
A-OH-18:0;O3/24:0;O	1264,7504	1286,7339	16,77	16,83	IP:261,033 W-H2O(18:0;O3):282,28 W(18:0;O3):300,288 Z0mH2O:648,626 Z0:666,636	B2_hydroxy:361,073 C3PO3_B1_459:459.053 621,102
A-OH-18:1;O3/24:0	1246,7421		16,63		IP:261,026 W(18:1;O3):298,277 Z0:648,629	
A-OH-18:1;O3/24:0;O	1262,739	1284,7169	16,35	16,35	IP:261,039 W-H2O(18:1;O3):280,255 W(18:1;O3):298,275 Z0mH2O:646,617 Z0:664,623	IP:283,019 B2_hydroxy:361,073 C3PO3_B1_459:459.049 621,102 Z0:686,603
A-OH-18:1;O3/25:0;O	1276,751	1298,7337	16,85	16,84	IP:261,035	IP:283,021

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,272 Z0mH2O:660,62 Z0:678,639	B2_hydroxy:361,081 C3PO3_B1_459:459.053 621,102 Z0:700,615
A-OH-44:1;O4	1290,7787		17,23		IP:261,038 Z0:692,655	
160325_S4_R_c_2_pos_CID						
A-OH-18:1;O3/21:0;O	1220,6873		13,48		IP:261,044 W(18:1;O3):298,273 C3PO3_B1_437:437,067 Z0mH2O:604,556 Z0:622,573	
A-OH-18:0;O3/22:0;O	1236,713		15,47		IP:261,039 W-H2O(18:0;O3):282,279 W(18:0;O3):300,292 Z0mH2O:620,598 Z0:638,604	
A-OH-18:1;O3/22:0;O	1234,7021	1256,6847	14,59	14,54	IP:261,033 W-H2O(18:1;O3):280,257 W(18:1;O3):298,279 Z0mH2O:618,579 Z0:636,59	IP:283,018 B2_hydroxy:361,074 C3PO3_B1_459:459,051 621,103 Z0:658,568
A-OH-18:0;O3/23:0;O	1250,7296		16,35		Z0mH2O:634,611 Z0:652,619	
A-OH-18:1;O3/23:0;O	1248,7157	1270,6952	15,63	15,61	IP:261,036 W-H2O(18:1;O3):280,259 W(18:1;O3):298,272 Z0mH2O:632,591 Z0:650,605	IP:283,016 B2_hydroxy:361,072 C3PO3_B1_459:459,051 621,103 Z0:672,594
A-OH-18:1;O3/24:0	1246,7375		16,56		W(18:1;O3):298,278 Z0:648,626	
A-OH-18:1;O3/24:0;O	1262,7326	1284,7192	16,33	16,31	IP:261,038 W-H2O(18:1;O3):280,262 W(18:1;O3):298,269 Z0mH2O:646,611 Z0:664,621	IP:283,017 B2_hydroxy:361,114 C3PO3_B1_459:459,049 621,104 Z0:686,594
A-OH-18:1;O3/25:0;O	1276,7503	1298,7306	16,83	16,79	IP:261,039 Z0mH2O:660,62 Z0:678,632	IP:283,018 B2_hydroxy:361,074 C3PO3_B1_459:459,051 621,103

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
						Z0:700,624
A-OH-18:1;O3/26:0;O	1290,7601		17,19		IP:261,034 W(18:1;O3):298,293 Z0:692,652	
160325_S4_R_c_3_pos_CID						
A-NH-43:1;O2		1265,755		15,69	Z0mH2O:650,609	Z0:668,614
A-OH-18:0;O3/22:0;O	1236,7182		15,46		IP:261,04 W-H2O(18:0;O3):282,275 W(18:0;O3):300,287 Z0mH2O:620,599 Z0:638,605	
A-OH-18:1;O3/22:0;O		1256,6863		14,58	IP:283,013 B2_hydroxy:361,075 C3PO3_B1_459:459,052 621,108 Z0:658,566	
A-OH-18:0;O3/23:0;O	1250,7376		16,23		IP:261,037 W(18:0;O3):300,287 Z0mH2O:634,597 Z0:652,624	
A-OH-18:1;O3/23:0;O	1248,7207	1270,707	15,63	15,65	IP:261,036 W-H2O(18:1;O3):280,282 W(18:1;O3):298,275 Z0mH2O:632,603 Z0:650,609	IP:283,016 B2_hydroxy:361,072 C3PO3_B1_459:459,055 621,105 Z0:672,576
A-OH-42:0;O4	1264,749	1286,7248	16,7	16,73	IP:261,032 W(18:0;O3):300,285 Z0mH2O:648,624 Z0:666,635	IP:283,018 C3PO3_B1_459:459,048 621,104
A-OH-18:1;O3/24:0	1246,7386		16,57		IP:261,246 W(18:1;O3):298,276 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7383	1284,7131	16,29	16,31	IP:261,038 W-H2O(18:1;O3):280,26 W(18:1;O3):298,275 Z0mH2O:646,609 Z0:664,622	IP:283,016 B2_hydroxy:361,075 C3PO3_B1_459:459,049 621,105 Z0:686,606
A-OH-18:1;O3/25:0;O	1276,752	1298,7308	16,83	16,87	IP:261,035 Z0mH2O:660,623 Z0:678,638	IP:283,018 B2_hydroxy:361,055 C3PO3_B1_459:459,052

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
						621,104 Z0:700,614
160325_S4_R_c_4_pos_CID						
A-NH-18:1;O3/24:1		1265,7386		15,53	Z0mH2O:650,609	Z0:668,617
A-NH-18:0;O3/25:1		1281,7688		16,7	Z0mH2O:666,635	Z0:684,647
A-NH-45:1;O2		1293,7826		16,84	Z0mH2O:678,637	
A-OH-18:1;O3/21:0;O	1220,6895		13,5		IP:261,037 W(18:1;O3):298,207 Z0:622,577	
A-OH-18:0;O3/22:0;O	1236,7178		15,47		IP:261,04 W-H2O(18:0;O3):282,28 W(18:0;O3):300,292 Z0mH2O:620,632 Z0:638,609	
A-OH-18:1;O3/22:0;O	1234,7046	1256,6875	14,56	14,61	IP:261,036 W-H2O(18:1;O3):280,265 W(18:1;O3):298,272 Z0mH2O:618,58 Z0:636,591	IP:283,023 B2_hydroxy:361,069 C3PO3_B1_459:459,048 621,104 Z0:658,577
A-OH-18:1;O3/23:0	1232,725		16,02		W(18:1;O3):298,268 Z0:634,611	
A-OH-18:1;O3/23:0;O	1248,72	1270,7022	15,64	15,63	IP:261,043 W(18:1;O3):298,28 Z0mH2O:632,599 Z0:650,606	IP:283,018 B2_hydroxy:361,078 C3PO3_B1_459:459,052 621,104 Z0:672,606
A-OH-42:0;O4	1264,746		16,73		IP:261,038 Z0mH2O:648,624 Z0:666,631	
A-OH-18:1;O3/24:0;O	1262,7354	1284,7157	16,35	16,31	IP:261,041 W-H2O(18:1;O3):280,262 W(18:1;O3):298,272 C3PO3_B1_437:437,069 Z0mH2O:646,604 Z0:664,622	IP:283,019 B2_hydroxy:361,081 C3PO3_B1_459:459,049 621,101 Z0:686,606
A-OH-18:1;O3/25:0;O	1276,7492	1298,7423	16,83	16,78	IP:261,035 W-H2O(18:1;O3):280,266 W(18:1;O3):298,276 Z0mH2O:660,637 Z0:678,637	IP:283,018 B2_hydroxy:361,072 C2_hydroxy:379,278 C3PO3_B1_459:459,054 621,103

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
						Z0:700,62
A-OH-44:1;O4	1290,7615		17,2		IP:261,039 Z0:692,649	

TABLE H.2: GIPC ID List: Replicates of S4_R_s with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S4_R_s_1_pos_CID						
A-OH-18:1;O3/20:0;O	1206,6735		12,54		IP:261,032 W-H2O(18:1;O3):280,261 W(18:1;O3):298,256 Z0mH2O:590,55 Z0:608,561	
A-OH-39:1;O4	1220,6917		13,65		IP:261,037 Z0mH2O:604,561 Z0:622,574	
A-OH-18:0;O3/22:0;O	1236,7202	1258,7	15,63	15,8	IP:261,037 W-H2O(18:0;O3):282,277 W(18:0;O3):300,285 Z0mH2O:620,594 Z0:638,605	C3PO3_B1_459:459,858
A-OH-40:1;O3	1218,7146		15,31		Z0:620,595	
A-OH-18:1;O3/22:0;O	1234,7049	1256,6847	14,77	14,78	IP:261,033 W-H2O(18:1;O3):280,274 W(18:1;O3):298,274 Z0mH2O:618,579 Z0:636,59	IP:283,023 B2_hydroxy:361,08 C3PO3_B1_459:459,051 621,104 Z0:658,577
A-OH-18:0;O3/23:0;O	1250,7335		16,35		IP:261,034 W-H2O(18:0;O3):282,277 W(18:0;O3):300,284 Z0mH2O:634,615 Z0:652,619	
A-OH-18:1;O3/23:0	1232,7182		16,13		W(18:1;O3):298,278 Z0:634,611	
A-OH-18:1;O3/23:0;O	1248,7194	1270,7041	15,78	15,77	IP:261,038 W-H2O(18:1;O3):280,267 W(18:1;O3):298,264 Z0mH2O:632,594 Z0:650,605	IP:283,018 B2_hydroxy:361,074 C3PO3_B1_459:459,048 621,103 Z0:672,555
A-OH-18:0;O3/24:0;O	1264,7497		16,81		IP:261,038 W(18:0;O3):300,288 Z0mH2O:648,625 Z0:666,634	
A-OH-t18:1;O3/24:0	1246,7357		16,7		IP:261,037 W-H2O(18:1;O3):280,268	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,271 Z0:648,627	
A-OH-18:1;O3/24:0;O	1262,7343	1284,717	16,45	16,42	IP:261,036 W-H2O(18:1;O3):280,264 W(18:1;O3):298,28 Z0mH2O:646,608 Z0:664,621	IP:283,009 B2_hydroxy:361,075 C3PO3_B1_459:459,055 621,104 Z0:686,613
A-OH-18:1;O3/25:0	1260,7488		17,07		IP:261,034 W(18:1;O3):298,273 Z0:662,645	
A-OH-18:1;O3/25:0;O		1298,7295		16,89		IP:283,021 B2_hydroxy:361,072 C3PO3_B1_459:459,048 621,104 Z0:700,621
A-OH-44:1;O4	1290,7641		17,29		IP:261,037 Z0mH2O:674,63 Z0:692,653	
160325_S4_R_s_2_pos_CID						
A-NH-43:1;O2	1265,7494		15,63			Z0mH2O:650,61 Z0:668,622
A-NH-18:0;O3/25:1	1281,7672		16,79		W(18:0;O3):300,285 Z0mH2O:666,635 Z0:684,647	
A-NH-44:1;O2	1279,7721		16,73			Z0mH2O:664,621 Z0:682,641
A-OH-18:1;O3/20:0;O	1206,6765		12,43		IP:261,035 W-H2O(18:1;O3):280,266 C3PO3_B1_437:437,09 Z0mH2O:590,554 Z0:608,561	
A-OH-18:1;O3/21:0;O	1220,6876		13,52		IP:261,033 W-H2O(18:1;O3):280,259 W(18:1;O3):298,271 Z0mH2O:604,561 Z0:622,578	
A-OH-18:0;O3/22:0;O	1236,7197		15,51		IP:261,033 W-H2O(18:0;O3):282,282 W(18:0;O3):300,288 Z0mH2O:620,592	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:638,61	
A-OH-18:1;O3/22:0	1218,708		15,19		IP:261,04 W-2H2O(18:1;O3):262,257 W(18:1;O3):298,274 Z0:620,595	
A-OH-18:1;O3/22:0;O	1234,7056	1256,6856	14,64	14,63	IP:261,036 W-H2O(18:1;O3):280,266 W(18:1;O3):298,272 Z0mH2O:618,569 Z0:636,591	IP:283,019 B2_hydroxy:361,072 C3PO3_B1_459:459,052 621,101 Z0:658,577
A-OH-18:0;O3/23:0;O	1250,7301		16,25		IP:261,037 W(18:0;O3):300,285 Z0mH2O:634,616 Z0:652,624	
A-OH-18:1;O3/23:0	1232,7223		16,05		IP:261,035 W-H2O(18:1;O3):280,254 W(18:1;O3):298,274 Z0:634,612	
A-OH-18:1;O3/23:0;O	1248,7223	1270,7033	15,66	15,66	IP:261,041 W-H2O(18:1;O3):280,265 W(18:1;O3):298,27 Z0mH2O:632,592 Z0:650,61	IP:283,021 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,105 Z0:672,576
A-OH-18:0;O3/24:0;O		1286,7272		16,81		IP:283,019 B2_hydroxy:361,075 C3PO3_B1_459:459,049 621,101 Z0:688,622
A-OH-t18:1;O3/24:0	1246,7393		16,62		IP:261,04 W(18:1;O3):298,271 Z0:648,632	
A-OH-18:1;O3/24:0;O	1262,7368	1284,7185	16,32	16,33	IP:261,039 W(18:1;O3):298,27 Z0mH2O:646,62 Z0:664,622	IP:283,022 B2_hydroxy:361,079 C3PO3_B1_459:459,049 621,105 Z0:686,602
A-OH-18:1;O3/25:0	1260,7557		17,02		IP:261,03 W(18:1;O3):298,276 Z0:662,646	
A-OH-18:1;O3/25:0;O	1276,7478	1298,7313	16,84	16,86	IP:261,03	IP:283,021

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,27 Z0mH2O:660,627 Z0:678,638	B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,104 Z0:700,629
A-OH-18:1;O3/26:0;O	1290,7676		17,22	B1_hydroxy:163,149	IP:261,032 W(18:1;O3):298,274 Z0mH2O:674,648 Z0:692,657	
160325_S4_R_s_3_pos_CID						
A-OH-18:1;O3/20:0;O	1206,6744		12,47		IP:261,037 W(18:1;O3):298,271 Z0mH2O:590,54 Z0:608,561	
A-OH-18:1;O3/21:0;O	1220,6953		13,62		IP:261,033 W(18:1;O3):298,289 Z0:622,579	
A-OH-18:0;O3/22:0;O	1236,7198		15,54		IP:261,037 W-H2O(18:0;O3):282,287 W(18:0;O3):300,287 Z0mH2O:620,584 Z0:638,606	
A-OH-18:1;O3/22:0;O		1256,6843		14,69		IP:283,016 B2_hydroxy:361,067 C3PO3_B1_459:459,049 621,105 Z0:658,578
A-OH-18:0;O3/23:0;O	1250,7335		16,31		IP:261,035 W-H2O(18:0;O3):282,275 W(18:0;O3):300,295 C3PO3_B1_437:437,065 Z0mH2O:634,611 Z0:652,624	
A-OH-18:1;O3/23:0	1232,7257		16,05		IP:261,04 W(18:1;O3):298,274 Z0:634,612	
A-OH-18:1;O3/23:0;O	1248,7222	1270,7074	15,72	15,69	IP:261,038 W-H2O(18:1;O3):280,257 W(18:1;O3):298,267 Z0mH2O:632,588	IP:283,019 B2_hydroxy:361,069 C3PO3_B1_459:459,052 621,101

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
A-OH-t18:1;O3/24:0	1246,7386		16,64		Z0:650,609 IP:261,037 W-H2O(18:1;O3):280,261 W(18:1;O3):298,284 Z0:648,627	Z0:672,587
A-OH-18:1;O3/24:0;O	1262,7367	1284,7182	16,4	16,35	IP:261,036 W-H2O(18:1;O3):280,261 W(18:1;O3):298,272 Z0mH2O:646,601 Z0:664,622	IP:283,024 B2_hydroxy:361,076 C3PO3_B1_459:459,052 621,1 Z0:686,601
A-OH-18:1;O3/25:0;O	1276,7514	1298,7392	16,87	16,85	IP:261,037 W-H2O(18:1;O3):280,261 W(18:1;O3):298,274 Z0mH2O:660,63 Z0:678,634	IP:283,021 B2_hydroxy:361,072 C3PO3_B1_459:459,052 621,104 Z0:700,614
A-OH-18:1;O3/26:0;O	1290,7606		17,33		IP:261,037 Z0:692,657	
160325_S4_R_s_4_pos_CID						
A-OH-18:1;O3/20:0;O	1206,6686		12,48		IP:261,037 W(18:1;O3):298,283 Z0mH2O:590,545 Z0:608,556	
A-OH-18:1;O3/21:0;O	1220,6799		13,62		IP:261,037 W(18:1;O3):298,273 Z0:622,573	
A-OH-18:0;O3/22:0;O	1236,7155		15,56		IP:261,037 W-H2O(18:0;O3):282,279 W(18:0;O3):300,289 Z0mH2O:620,59 Z0:638,604	
A-OH-18:1;O3/22:0;O	1234,7015	1256,6817	14,7	14,72	IP:261,035 W-H2O(18:1;O3):280,261 W(18:1;O3):298,269 Z0mH2O:618,579 Z0:636,59	IP:283,015 B2_hydroxy:361,071 C3PO3_B1_459:459,051 621,103 Z0:658,572
A-OH-18:0;O3/23:0	1232,7227		16,06		IP:261,032 W-H2O(18:1;O3):280,258 W(18:1;O3):298,27 Z0:634,614	
A-OH-18:0;O3/23:0;O	1250,7335		16,31		IP:261,035	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W-H ₂ O(18:0;O ₃):282,275 W(18:0;O ₃):300,295 C ₃ PO ₃ _B1_437:437,065 Z0mH ₂ O:634,611 Z0:652,624	
A-OH-18:1;O ₃ /23:0;O	1248,7165	1270,6999	15,72	15,73	IP:261,035 W(18:1;O ₃):298,279 Z0mH ₂ O:632,595 Z0:650,605	IP:283,02 B2_hydroxy:361,069 C ₃ PO ₃ _B1_459:459,048 621,099 Z0:672,59
A-OH-18:0;O ₃ /24:0;O	1264,7438		16,82		IP:261,037 W-H ₂ O(18:0;O ₃):282,281 W(18:0;O ₃):300,287 Z0mH ₂ O:648,622 Z0:666,634	
A-OH-t18:1;O ₃ /24:0	1246,7383		16,6		IP:261,032 W-H ₂ O(18:1;O ₃):280,263 W(18:1;O ₃):298,273 Z0:648,63	
A-OH-18:1;O ₃ /24:0;O	1262,7335	1284,7146	16,39	16,37	IP:261,038 W-H ₂ O(18:1;O ₃):280,261 W(18:1;O ₃):298,276 Z0mH ₂ O:646,611 Z0:664,62	IP:283,021 B2_hydroxy:361,097 C ₃ PO ₃ _B1_459:459,048 621,099 Z0:686,62
A-OH-18:1;O ₃ /25:0	1260,75		17,08		IP:261,039 W(18:1;O ₃):298,27 Z0:662,64	
A-OH-18:1;O ₃ /25:0;O	1276,7473	1298,7314	16,86	16,85	IP:261,034 W-H ₂ O(18:1;O ₃):280,275 W(18:1;O ₃):298,27 Z0mH ₂ O:660,621 Z0:678,64	IP:283,013 B2_hydroxy:361,076 C ₃ PO ₃ _B1_459:459,05 621,102 Z0:700,619
A-OH-44:1;O ₄	1290,7578		17,24		IP:261,034 Z0:692,656	

Appendix I

GIPC ID Lists GV Sampling 5

TABLE I.1: GIPC ID List: Replicates of S5_GV_c with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S5_GV_c_1_pos_CID						
A-OH-18:1;O3/20:0;O	1206,6733		12,26		IP:261,033 W(18:1;O3):298,267 Z0mH2O:590,555 Z0:608,562	
A-OH-18:1;O3/21:0;O	1220,6906		13,34		IP:261,033 W(18:1;O3):298,271 Z0mH2O:604,558 Z0:622,574	
A-OH-18:0;O3/22:0;O	1236,7208	1258,6977	15,31	15,43	IP:261,038 W-H2O(18:0;O3):282,282 W(18:0;O3):300,293 Z0mH2O:620,596 Z0:638,606	C3PO3_B1_459:459,049 Z0:660,582
A-OH-18:1;O3/22:0	1218,7087		14,96		IP:261,033 W(18:1;O3):298,274 Z0:620,596	
A-OH-18:1;O3/22:0;O	1234,7042	1256,6845	14,36	14,4	IP:261,034 W-H2O(18:1;O3):280,26 W(18:1;O3):298,272 Z0mH2O:618,578 Z0:636,591	IP:283,019 B2_hydroxy:361,075 C3PO3_B1_459:459.052 621,101 Z0:658,574
A-OH-18:0;O3/23:0;O	1250,7282		16,12		IP:261,033 Z0mH2O:634,608 Z0:652,621	
A-OH-18:1;O3/23:0	1232,7227		15,9		IP:261,033 W(18:1;O3):298,271 Z0:634,612	
A.OH-18:1;O3/23:0;O	1248,7193	1270,6968	15,46	15,46	IP:261,034 W(18:1;O3):298,323 Z0mH2O:632,604 Z0:650,606	IP:283,019 B2_hydroxy:361,075 C3PO3_B1_459:459,049 621,101 Z0:672,595
A-OH-t18:0/h24:0	1264,7464		16,62		IP:261,035 W-H2O(18:0;O3):282,275 W(18:0;O3):300,298 Z0mH2O:648,628 Z0:666,636	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
A-OH-18:1;O3/24:0	1246,74	1268,7189	16,5	16,48	IP:261,033 W(18:1;O3):298,271 Z0:648,628	IP:283,014 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,101 Z0mH2O:652,613
A-OH-18:1;O3/24:0;O	1262,7353	1284,716	16,24	16,21	IP:261,036 W-H2O(18:1;O3):280,262 W(18:1;O3):298,272 Z0mH2O:646,609 Z0:664,622	IP:283,02 B2_hydroxy:361,107 C3PO3_B1_459:459,053 621,101 Z0:686,622
A-OH-18:1;O3/25:0;O	1276,7466	1298,7331	16,74	16,76	IP:261,035 W(18:1;O3):298,271 Z0mH2O:660,612 Z0:678,639	B2_hydroxy:361,067 C3PO3_B1_459:459,049 621,105 Z0:700,63
160325_S5_GV_c_3_pos_CID						
A-NH-18:1;O3/23:1	1251,7212		14,65		W(18:1;O3):298,274 Z0mH2O:636,591 Z0:654,597	
A-NH-18:0;O3/25:1	1281,7687		16,72		Z0mH2O:666,639 Z0:684,651	
A-NH-44:2;O2	1277,7644		16,99		Z0mH2O:662,642 Z0:680,654	
A-OH-18:1;O3/21:0;O	1220,6879		13,35		IP:261,04 W(18:1;O3):298,256 Z0mH2O:604,587 Z0:622,574	
A-OH-18:0;O3/22:0;O	1236,7209		15,34		IP:261,033 W-H2O(18:0;O3):282,272 W(18:0;O3):300,287 Z0mH2O:620,591 Z0:638,609	
A-OH-18:1;O3/22:0	1218,711		15,02		IP:261,035 W-H2O(18:1;O3):280,259 W(18:1;O3):298,271 Z0:620,595	
A-OH-18:1;O3/22:0;O	1234,7055	1256,6854	14,95	14,45	IP:261,035 W(18:1;O3):298,271 Z0mH2O:618,576 Z0:636,591	IP:283,016 B2_hydroxy:361,078 C3PO3_B1_459:459,049 621,105

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
						Z0:658,578
A-OH-18:0;O3/23:0;O	1250,7368		16,13		IP:261,04 Z0mH2O:634,612 Z0:652,624	
A-OH-18:1;O3/23:0	1232,7233		15,91		IP:261,03 W(18:1;O3):298,274 Z0:634,612	
A.OH-18:1;O3/23:0;O	1248,7166	1270,6991	15,5	15,49	IP:261,036 W(18:1;O3):298,267 Z0mH2O:632,593 Z0:650,602	IP:283,016 B2_hydroxy:361,07 C3PO3_B1_459:459,049 621,101 Z0:672,588
A-OH-t18:0/h24:0	1264,7419	1286,7314	16,75	16,65	IP:261,037 W-H2O(18:0;O3):282,277 W(18:0;O3):300,267 Z0mH2O:648,624 Z0:666,635	C3PO3_B1_459:459,049 621,104
A-OH-18:1;O3/24:0	1246,737	1268,7258	16,51	16,53	IP:261,038 W-H2O(18:1;O3):280,257 W(18:1;O3):298,275 Z0:648,624	C3PO3_B1_459:459,052 621,101 Z0mH2O:652,632
A-OH-18:1;O3/24:0;O	1262,7355	1284,7204	16,27	16,23	IP:261,038 W(18:1;O3):298,272 Z0mH2O:646,619 Z0:664,622	IP:283,012 B2_hydroxy:361,064 C3PO3_B1_459:459,046 621,1 Z0:686,625
A-OH-18:1;O3/25:0	1260,7531		16,95		IP:261,037 W(18:1;O3):298,274 Z0:662,642	
A-OH-18:1;O3/25:0;O	1276,7481		16,76		IP:261,037 W(18:1;O3):298,266 Z0mH2O:660,615 Z0:678,638	
160325_S5_GV_c_4_pos_CID						
A-NH-18:0;O3/23:1	1253,7316		15,28		W(18:0;O3):300,29 Z0mH2O:638,601 Z0:656,609	
A-OH-18:1;O3/20:0;O	1206,6707		12,23		IP:261,032 W-H2O(18:1;O3):280,261 W(18:1;O3):298,271	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:608,561	
A-OH-18:1;O3/21:0;O	1220,6916		13,34		IP:261,037 W-H2O(18:1;O3):280,266 W(18:1;O3):298,296 Z0mH2O:604,554 Z0:622,577	
A-OH-18:0;O3/22:0;O	1236,7153		15,3		IP:261,032 W-H2O(18:0;O3):282,275 W(18:0;O3):300,285 Z0mH2O:620,595 Z0:638,605	
A-OH-18:1;O3/22:0	1218,7016		14,97		IP:261,035 W(18:1;O3):298,273 Z0:620,595	
A-OH-18:1;O3/22:0;O	1234,7022	1256,6819	14,4	14,4	IP:261,036 W(18:1;O3):298,269 Z0mH2O:618,576 Z0:636,59	IP:283,016 B2_hydroxy:361,075 C3PO3_B1_459:459.048 621,104 Z0:658,581
A-OH-18:0;O3/23:0;O	1250,7315		16,05		IP:261,03 W(18:0;O3):300,285 Z0mH2O:634,608 Z0:652,62	
A.OH-18:1;O3/23:0;O	1248,7159	1270,6959	15,47	15,47	IP:261,043 W-H2O(18:1;O3):280,267 W(18:1;O3):298,269 Z0mH2O:632,581 Z0:650,605	IP:283,016 B2_hydroxy:361,069 C3PO3_B1_459:459,048 621,096 Z0:672,591
A-OH-t18:0/h24:0	1264,7451		16,66		IP:261,032 W(18:0;O3):300,287 C3PO3_B1_437:437,074 Z0mH2O:648,627 Z0:666,634	
A-OH-18:1;O3/24:0	1246,7388		16,51		IP:261,037 W-H2O(18:1;O3):280,263 W(18:1;O3):298,273 Z0:648,626	
A-OH-18:1;O3/24:0;O	1262,7316	1284,7131	16,23	16,2	IP:261,038 W-H2O(18:1;O3):280,262 W(18:1;O3):298,272	IP:283,019 B2_hydroxy:361,067 C3PO3_B1_459:459,055

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:646,611 Z0:664,621	621,096 Z0:686,593
A-OH-18:1;O3/25:0;O	1276,7497	1298,7416	16,74	16,74	IP:261,032 W(18:1;O3):298,276 Z0:678,637	C3PO3_B1_459:459,048 621,103 Z0:700,605

TABLE I.2: GIPC ID List: Replicates of S5_GV_s with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S5_GV_s_1_pos_CID						
A-OH-18:1;O3/20:0;O	1206,6645		12,3		W(18:1;O3):298,274 Z0:608,557	
A-OH-39:1;O4	1220,6864		13,43		IP:261,035 C3PO3_B1_437:437,068 Z0mH2O:604,568 Z0:622,573	
A-OH-18:0;O3/22:0;O	1236,7147		15,41		IP:261,034 W-H2O(18:0;O3):282,277 W(18:0;O3):300,284 Z0mH2O:620,601 Z0:638,604	
A-OH-18:1;O3/22:0	1218,7037		15,06		IP:261,034 W(18:1;O3):298,273 Z0:620,594	
A-OH-t18:0/h24:0	1264,7471		16,68		IP:261,039 W-H2O(18:0;O3):282,279 W(18:0;O3):300,294 C3PO3_B1_437:437,064 Z0mH2O:648,63 Z0:666,637	
A-OH-18:1;O3/24:0	1246,732		16,56		IP:261,039 W(18:1;O3):298,275 Z0:648,626	
A-OH-18:1;O3/24:0;O	1262,7305	1284,7098	16,25	16,28	IP:261,04 W(18:1;O3):298,274 Z0mH2O:646,611 Z0:664,618	IP:283,018 B2_hydroxy:361,077 C3PO3_B1_459:459,048 621,103 Z0:686,605
A-OH-18:1;O3/25:0	1260,7528		16,99		IP:261,02 W(18:1;O3):298,273 Z0:662,64	
A-OH-18:1;O3/25:0;O		1298,7291		16,75		C3PO3_B1_459:459,045 621,103 Z0:700,616
160325_S5_GV_s_2_pos_CID						
A-NH-18:1;O3/23:1	1251,7183		14,37		Z0mH2O:636,591 Z0:654,59	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
A-NH-18:0;O3/25:1	1281,7662		16,71		W(18:0;O3):300,292 Z0mH2O:666,639 Z0:684,651	
A-NH-18:0;O3/25:1	1279,7699		16,65		Z0mH2O:664,618	
A-OH-38:1;O4	1206,6788		12,44		Z0:608,561	
A-OH-39:1;O4	1220,6872	1242,6753	13,4	13,35	IP:261,035 W-H2O(18:1;O3):280,264 W(18:1;O3):298,269 Z0mH2O:604,566 Z0:622,574	C3PO3_B1_459:459,045
A-OH-18:0;O3/22:0;O	1236,7161		15,41		IP:261,035 W-H2O(18:0;O3):282,277 W(18:0;O3):300,282 Z0mH2O:620,596 Z0:638,606	
A-OH-18:1;O3/22:0	1218,7062		15,09		IP:261,032 W(18:1;O3):298,274 Z0:620,595	
A-OH-18:1;O3/22:0;O	1234,7018	1256,679	14,49	15,09	IP:261,036 W-H2O(18:1;O3):280,264 W(18:1;O3):298,267 Z0mH2O:618,583 Z0:636,591	IP:283,013 C3PO3_B1_459:459,043 621,101
A-OH-18:0;O3/23:0;O	1250,7269		16,21		IP:261,035 W(18:0;O3):300,285 Z0mH2O:634,603 Z0:652,624	
A-OH-18:1;O3/23:0	1232,7172		15,98		IP:261,035 W(18:1;O3):298,274 Z0:634,612	
A-OH-t18:0/h24:0	1264,7471		16,68		IP:261,039 W-H2O(18:0;O3):282,279 W(18:0;O3):300,294 C3PO3_B1_437:437,064 Z0mH2O:648,63 Z0:666,637	
A-OH-18:1;O3/23:0;O	1248,7148	1270,6927	15,57	15,55	IP:261,038 W-H2O(18:1;O3):280,262 W(18:1;O3):298,272 Z0mH2O:648,581	IP:282,999 B2_hydroxy:361,069 C3PO3_B1_459:459,049 621,101

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:650,606	Z0:672,588
A-OH-18:0;O3/24:0;O	1264,7466		16,72		IP:261,035 W(18:0;O3):300,287 Z0mH2O:648,624 Z0:666,639	
A-OH-18:1;O3/24:0	1246,7331	1268,7164	16,57	16,59	IP:261,037 W(18:1;O3):298,273 Z0:648,628	C3PO3_B1_459:459,049 621,101 Z0mH2O:652,628
A-OH-18:1;O3/24:0;O	1262,734	1284,7118	16,28	16,27	IP:261,038 W-H2O(18:1;O3):280,26 W(18:1;O3):298,277 Z0mH2O:646,612 Z0:664,618	IP:283,016 B2_hydroxy:361,075 C3PO3_B1_459:459,049 621,104 Z0:686,564
A-OH-18:1;O3/25:0;O	1276,7462	1298,725	16,79	16,76	IP:261,035 W(18:1;O3):298,268 Z0mH2O:660,634 Z0:678,641	B2_hydroxy:361,072 C3PO3_B1_459:459,051 621,1
160325_S5_GV_s_3_pos_CID						
A-OH-18:1;O3/20:0;O	1206,6645		12,3		W(18:1;O3):298,274 Z0:608,557	
A-OH-39:1;O4	1220,6864		13,43		IP:261,035 C3PO3_B1_437:437,068 Z0mH2O:604,568 Z0:622,573	
A-OH-18:0;O3/22:0;O	1236,7147		15,41		IP:261,034 W-H2O(18:0;O3):282,277 W(18:0;O3):300,284 Z0mH2O:620,601 Z0:638,604	
A-OH-18:1;O3/22:0	1218,7037		15,06		IP:261,034 W(18:1;O3):298,273 Z0:620,594	
A-OH-t18:0/h24:0	1264,7471		16,68		IP:261,039 W-H2O(18:0;O3):282,279 W(18:0;O3):300,294 C3PO3_B1_437:437,064 Z0mH2O:648,63 Z0:666,637	
A-OH-18:1;O3/24:0	1246,732		16,56		IP:261,039 W(18:1;O3):298,275	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:648,626	
A-OH-18:1;O3/24:0;O	1262,7305	1284,7098	16,25	16,28	IP:261,04 W(18:1;O3):298,274 Z0mH2O:646,611 Z0:664,618	IP:283,018 B2_hydroxy:361,077 C3PO3_B1_459:459,048 621,103 Z0:686,605
A-OH-18:1;O3/25:0	1260,7528		16,99		IP:261,02 W(18:1;O3):298,273 Z0:662,64	
A-OH-18:1;O3/25:0;O		1298,7291		16,75		C3PO3_B1_459:459,045 621,103 Z0:700,616
160325_S5_GV_s_4_pos_CID						
A-NH-18:1;O3/23:1	1251,7203		14,7		W(18:1;O3):298,271 Z0mH2O:636,59	
A-OH-38:1;O4	1206,6688		12,28		IP:261,038 Z0:608,558	
A-OH-39:1;O4	1220,6841		13,4		IP:261,037 Z0mH2O:604,558 Z0:622,574	
A-OH-18:0;O3/22:0;O	1236,7181		15,4		IP:261,037 W(18:0;O3):300,292 C3PO3_B1_437:437,059 Z0mH2O:620,592 Z0:638,609	
A-OH-18:1;O3/22:0	1218,7064	1240,6846	15,07	15,08	IP:261,037 W(18:1;O3):298,271 Z0:620,595	C3PO3_B1_459:459,052 621,104
A-OH-18:1;O3/22:0;O	1234,7032	1256,6858	14,52	14,51	IP:261,036 W(18:1;O3):298,27 Z0mH2O:618,572 Z0:636,587	IP:283,019 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,104 Z0:658,566
A-OH-18:0;O3/23:0;O	1250,7313		16,17		IP:261,033 W(18:0;O3):300,29 C3PO3_B1_437:437,068 Z0mH2O:634,612 Z0:652,62	
A-OH-18:1;O3/23:0	1232,7232		15,95		IP:261,04 W-H2O(18:1;O3):280,254	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,281 Z0:634,608	
A-OH-18:1;O3/23:0;O	1248,7183	1270,6985	15,58	15,56	IP:261,031 W(t18:1):298,277 Z0mH2O:648,595 Z0:650,605	IP:283,013 B2_hydroxy:361,069 C3PO3_B1_459:459,055 621,104 Z0mH2O:654,593 Z0:672,598
A-OH-18:0;O3/24:0;O	1264,7571	1286,7272	16,85	16,77	IP:261,037 W(18:0;O3):300,29 C3PO3_B1_437:437,086 Z0mH2O:648,627 Z0:666,635	IP:283,011 C3PO3_B1_459:459,048 621,1
A-OH-18:1;O3/24:0	1246,7371	1268,72	16,55	16,53	IP:261,035 W-H2O(18:1;O3):280,258 W(18:1;O3):298,271 Z0:648,627	IP:283,021 B2_hydroxy:361,072 C3PO3_B1_459:459,048 621,104 Z0mH2O:652,616
A-OH-18:1;O3/24:0;O	1262,7329	1284,7159	16,24	16,27	IP:261,036 W-H2O(18:1;O3):280,264 Z0mH2O:646,616 Z0:664,621	IP:283,024 B2_hydroxy:361,067 C3PO3_B1_459:459,049 621,1 Z0:686,605
A-OH-18:1;O3/25:0	1260,7475		16,98		IP:261,032 W(18:1;O3):298,271 Z0mH2O:644,635 Z0:662,641	

Appendix J

GIPC ID Lists R Sampling 5

TABLE J.1: GIPC ID List: Replicates of S5_R_c with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S5_R_c_1_pos_CID						
A-OH-18:1;O3/20:0;O	1206,6753		12,11		IP:261,04 W(18:1;O3):298,271 Z0mH2O:590,54 Z0:608,557	
A-OH-18:1;O3/21:0;O	1220,6875		13,25		IP:261,037 W(18:1;O3):298,271 Z0mH2O:604,572 Z0:622,574	
A-OH-18:0;O3/22:0;O	1236,7145		15,24		IP:261,035 W-H2O(18:0;O3):282,272 W(18:0;O3):300,287 Z0mH2O:620,595 Z0:638,609	
A-OH-18:1;O3/22:0	1218,7105		14,85		IP:261,042 W(18:1;O3):298,268 Z0:620,595	
A-OH-18:1;O3/22:0;O	1234,7033	1256,6855	14,33	14,32	IP:261,041 W-H2O(18:1;O3):280,262 W(18:1;O3):298,275 Z0mH2O:618,587 Z0:636,591	IP:283,013 B2_hydroxy:361,072 C3PO3_B1_459:459,055 621,1 Z0:658,577
A-OH-18:1;O3/23:0;O	1248,7196	1270,6976	15,39	15,41	IP:261,036 W-H2O(18:1;O3):280,259 W(18:1;O3):298,272 Z0mH2O:632,599 Z0:650,605	IP:283,018 B2_hydroxy:361,072 C3PO3_B1_459:459,048 621,104 Z0:672,583
A-OH-18:1;O3/24:0	1246,7345		16,43		IP:261,032 W(18:1;O3):298,273 Z0:648,627	
A-OH-18:1;O3/24:0;O	1262,7329	1284,7141	16,18	16,17	IP:261,034 W-H2O(18:1;O3):280,264 W(18:1;O3):298,277 Z0mH2O:646,612 Z0:664,622	IP:283,017 B2_hydroxy:361,073 C3PO3_B1_459:459,043 621,104 Z0:686,644
A-OH-18:1;O3/25:0;O	1276,7456	1298,7331	16,72	16,73	IP:261,039 W(18:1;O3):298,286 Z0:678,634	B2_hydroxy:361,066 C3PO3_B1_459:459,048 621,1

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
						Z0:700,617
160325_S5_R_c_2_pos_CID						
A-NH-44:1;O2		1279,7652		16,14		Z0mH2O:664,622
A-OH-18:1;O3/21:0;O	1220,6881		13,23		IP:261,035 W-H2O(18:1;O3):280,261 W(18:1;O3):298,279 Z0mH2O:604,569 Z0:622,578	
A-OH-18:0;O3/22:0;O	1236,7216		15,25		IP:261,038 W-H2O(18:0;O3):282,275 W(18:0;O3):300,288 Z0mH2O:620,589 Z0:638,606	
A-OH-18:1;O3/22:0	1218,7046		14,86		IP:261,037 W(18:1;O3):298,274 Z0:620,595	
A-OH-18:1;O3/22:0;O	1234,7054	1256,6851	14,34	14,35	IP:261,039 W-H2O(18:1;O3):280,27 W(18:1;O3):298,262 Z0mH2O:618,581 Z0:636,592	IP:283,016 B2_hydroxy:361,072 C3PO3_B1_459:459,052 621,105 Z0:658,57
A-OH-41:0;O4	1250,737		16,11		IP:261,035 Z0mH2O:634,612 Z0:652,624	
A-OH-18:1;O3/23:0	1232,7249		15,82		IP:261,035 W-H2O(18:1;O3):280,266 W(18:1;O3):298,276 Z0:634,612	
A-OH-18:1;O3/23:0;O	1248,7211	1270,7056	15,39	15,38	IP:261,036 W-H2O(18:1;O3):280,262 W(18:1;O3):298,272 Z0mH2O:632,580 Z0:650,606	IP:283,016 B2_hydroxy:361,075 C3PO3_B1_459:459,049 621,101 Z0:672,591
A-OH-18:1;O3/24:0	1246,7392		16,43		IP:261,025 W-H2O(18:1;O3):280,258 W(18:1;O3):298,276 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7364	1284,7186	16,18	16,17	IP:261,031 W-H2O(18:1;O3):280,265 W(18:1;O3):298,267	IP:283,018 B2_hydroxy:361,075 C3PO3_B1_459:459,055

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:646,609 Z0:664,622	621,108 Z0:686,602
A-OH-18:1;O3/25:0;O	1276,7516	1298,7237	16,71	16,69	IP:261,042 W(18:1;O3):298,271 Z0mH2O:660,626 Z0:678,634	IP:283,009 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,104
160325_S5_R_c_3_pos_CID						
A-NH-18:1;O3/24:1	1265,7374		15,39		W(18:1;O3):298,273 Z0mH2O:650,608 Z0:668,612	
A-OH-18:0;O3/22:0;O	1236,7259		15,15		W-H2O(18:0;O3):282,279 W(18:0;O3):300,327 Z0mH2O:620,594 Z0:638,604	
A-OH-18:1;O3/22:0	1218,7071		14,76		W(18:1;O3):298,273 Z0:620,593	
A-OH-18:1;O3/22:0;O	1234,7035	1256,681	14,2	14,25	IP:261,035 W-H2O(18:1;O3):280,409 W(18:1;O3):298,269 Z0mH2O:618,57 Z0:636,593	IP:283,016 B2_hydroxy:361,077 C3PO3_B1_459:459,048 621,1 Z0:658,576
A-OH-18:1;O3/23:0;O	1248,7145		15,33		IP:261,033 W-H2O(18:1;O3):280,257 W(18:1;O3):298,269 C3PO3_B1_437:437,068 Z0mH2O:632,608 Z0:650,605	
A-OH-18:0;O3/24:0;O	1264,7486	1286,7307	16,62	16,62	IP:261,037 Z0mH2O:648,623 Z0:666,638	C3PO3_B1_459:459,048 621,103
A-OH-18:1;O3/24:0	1246,7401		16,44		W-H2O(18:1;O3):280,26 W(18:1;O3):298,278 Z0:648,622	
A-OH-18:1;O3/24:0;O	1262,7327	1284,7128	16,15	16,12	IP:261,036 W-H2O(18:1;O3):280,254 W(18:1;O3):298,272 Z0mH2O:646,611 Z0:664,621	IP:283,018 B2_hydroxy:361,074 C3PO3_B1_459:459,047 621,099 Z0:686,604
A-OH-43:1;O4	1276,7417	1298,7269	16,76	16,69	IP:261,044 Z0mH2O:660,64	IP:283,206 B2_hydroxy:361,069

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:678,633	C3PO3_B1_459:459,048 621,103 Z0:700,612
160325_S5_R_c_4_pos_CID						
A-NH-18:0;O3/23:1	1253,7258		15,17		W(18:0;O3):300,29 Z0mH2O:638,608 Z0:656,605	
A-OH-18:1;O3/20:0;O	1206,6736		12,08		IP:261,039 W(18:1;O3):298,276 Z0mH2O:590,55 Z0:608,56	
A-OH-18:1;O3/21:0;O	1220,6829		13,16		IP:261,032 W(18:1;O3):298,258 Z0mH2O:604,568 Z0:622,573	
A-OH-18:0;O3/22:0;O	1236,7195		15,19		IP:261,038 W-H2O(18:0;O3):282,275 W(18:0;O3):300,292 Z0mH2O:620,591 Z0:638,609	
A-OH-18:1;O3/22:0	1218,7078		14,8		IP:261,028 W(18:1;O3):298,271 Z0mH2O:5602,597 Z0:620,595	
A-OH-18:1;O3/22:0;O	1234,7032	1256,6822	14,28	14,27	IP:261,031 W-H2O(18:1;O3):280,257 W(18:1;O3):298,27 Z0mH2O:618,576 Z0:636,594	IP:283,016 B2_hydroxy:361,069 C3PO3_B1_459:459,051 621,1 Z0:658,577
A-OH-41:0;O4	1250,7331		16,07		IP:261,037 Z0mH2O:634,615 Z0:652,623	
A-OH-18:1;O3/23:0	1232,7249		15,79		IP:261,032 W-H2O(18:1;O3):280,268 W(18:1;O3):298,273 Z0:634,611	
A-OH-18:1;O3/23:0;O	1248,7178	1270,7025	15,35	15,32	IP:261,034 W-H2O(18:1;O3):280,267 W(18:1;O3):298,272 Z0mH2O:632,6	IP:283,018 B2_hydroxy:361,077 C3PO3_B1_459:459,051 621,104

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:650,606	Z0:672,587
A-OH-18:0;O3/24:0;O	1264,7472		16,57		IP:261,037 W-H2O(18:0;O3):282,279 W(18:0;O3):300,287 Z0mH2O:648,623 Z0:666,634	
A-OH-18:1;O3/24:0	1246,7395		16,46		IP:261,037 W(18:1;O3):298,271 Z0:648,63	
A-OH-18:1;O3/24:0;O	1262,7361	1284,7173	16,14	16,11	IP:261,036 W(18:1;O3):298,274 Z0mH2O:646,6 Z0:664,621	IP:283,018 B2_hydroxy:361,072 C3PO3_B1_459:459,048 621,107 Z0:686,597
A-OH-18:1;O3/25:0;O	1276,749	1298,73	16,68	16,65	IP:261,035 W-H2O(18:1;O3):280,266 Z0mH2O:660,626 Z0:678,634	IP:283,015 B2_hydroxy:361,099 C3PO3_B1_459:459,051 621,103 Z0:700,604
A-OH-44:1;O4	1290,7617		17,11		IP:261,035 Z0:692,653	

TABLE J.2: GIPC ID List: Replicates of S5_R_s with matching retention times in CID positive mode.

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
160325_S5_R_s_1_pos_CID						
A-OH-18:1;O3/22:0;O	1234,7045	1256,6887	14,39	14,37	IP:261,033 W-H2O(18:1;O3):280,257 W(18:1;O3):298,269 Z0mH2O:618,573 Z0:636,592	C3PO3_B1_459:459,05 621,106
A-OH-18:1;O3/23:0;O		1270,7031		15,44		C3PO3_B1_459:459,046 621,105
A-OH-18:0;O3/24:0;O	1264,7547		16,63		IP:261,035 W(18:0;O3):300,29 Z0mH2O:648,625 Z0:666,636	
A-OH-42:1;O3	1246,7396		16,53		Z0:648,629	
A-OH-18:1;O3/24:0;O	1262,733	1284,7142	16,2	16,21	IP:261,039 W(18:1;O3):298,27 Z0mH2O:646,602 Z0:664,619	IP:283,011 B2_hydroxy:361,067 C3PO3_B1_459:459,049 621,101 Z0:686,595
A-OH-18:1;O3/25:0;O	1276,7518	1298,7293	16,7	16,71	IP:261,035 Z0:678,639	IPmH2O:265,006 B2_hydroxy:361,072 C3PO3_B1_459:459,049 621,101
160325_S5_R_s_2_pos_CID						
A-OH-18:1;O3/20:0;O	1206,6756		12,16		IP:261,04 W(18:1;O3):298,277 Z0mH2O:590,551 Z0:608,558	
A-OH-18:1;O3/21:0;O	1220,6865		13,25		IP:261,035 W-H2O(18:1;O3):280,263 W(18:1;O3):298,274 Z0mH2O:604,566 Z0:622,578	
A-OH-18:0;O3/22:0;O	1236,7192		15,26		IP:261,037 W-H2O(18:0;O3):282,275 W(18:0;O3):300,29 Z0mH2O:620,592 Z0:638,606	
A-OH-18:1;O3/22:0	1218,7066		14,88		W-H2O(18:1;O3):280,263	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					W(18:1;O3):298,271 Z0:620,595	
A-OH-18:1;O3/22:0;O	1234,705	1256,6848	14,35	14,35	IP:261,043 W-H2O(18:1;O3):280,263 W(18:1;O3):298,272 Z0mH2O:618,595 Z0:636,587	IP:283,019 B2_hydroxy:361,075 C3PO3_B1_459:459,052 621,101 Z0:658,574
A-OH-41:0;O4	1250,7326		16,1		IP:261,037 Z0mH2O:634,612 Z0:652,624	
A-OH-18:1;O3/23:0	1232,7291		15,83		IP:261,04 W(18:1;O3):298,274 Z0:634,616	
A-OH-18:1;O3/23:0;O	1248,7214	1270,7014	15,41	15,41	IP:261,036 W-H2O(18:1;O3):280,265 W(18:1;O3):298,285 Z0mH2O:632,596 Z0:650,606	IP:283,021 B2_hydroxy:361,078 C3PO3_B1_459:459,052 621,108 Z0:672,591
A-OH-18:0;O3/24:0;O	1264,7473		16,59		IP:261,035 W(18:0;O3):300,287 C3PO3_B1_437:437,068 Z0mH2O:648,624 Z0:666,639	
A-OH-18:1;O3/24:0	1246,7379		16,44		IP:261,035 W(18:1;O3):298,266 Z0:648,628	
A-OH-18:1;O3/24:0;O	1262,7357	1284,7162	16,13	16,15	IP:261,034 W-H2O(18:1;O3):280,264 W(18:1;O3):298,267 Z0mH2O:646,628 Z0:664,622	IP:283,022 B2_hydroxy:361,076 C3PO3_B1_459:459,052 621,105 Z0:686,603
A-OH-18:1;O3/25:0;O	1276,749	1298,739	16,72	16,68	IP:261,037 W(18:1;O3):298,276 Z0mH2O:660,634 Z0:678,635	IP:283,013 B2_hydroxy:361,078 C3PO3_B1_459:459,052 621,104 Z0:700,621
160325_S5_R_s_3_pos_CID						
A-OH-18:1;O3/21:0;O	1220,6904		13,27		IP:261,035 Z0:622,575	
A-OH-18:0;O3/22:0;O	1236,7201		15,24		W(18:0;O3):300,293	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0mH2O:620,607 Z0:638,606	
A-OH-18:1;O3/22:0	1218,7006		14,89		W(18:1;O3):298,274 Z0:620,596	
A-OH-18:1;O3/22:0;O	1234,7039	1256,684	14,3	14,32	IP:261,029 W-H2O(18:1;O3):280,263 W(18:1;O3):298,268 Z0mH2O:618,585 Z0:636,588	IP:283,214 B2_hydroxy:361,075 C3PO3_B1_459:459,052 621,101 Z0:658,608
A-OH-18:1;O3/23:0	1232,7203		15,82		W(18:1;O3):298,271 Z0:634,609	
A-OH-18:1;O3/23:0;O	1248,717	1270,6979	15,42	15,39	IP:261,035 W-H2O(18:1;O3):280,259 W(18:1;O3):298,271 Z0mH2O:632,596 Z0:650,607	IP:283,021 B2_hydroxy:361,073 C3PO3_B1_459:459,052 621,101
A-OH-18:0;O3/24:0;O	1264,745		16,72		B1_hydroxy:163,059 Z0mH2O:648,628 Z0:666,636	
A-OH-18:1;O3/24:0	1246,7361		16,44		IP:261,035 W(18:1;O3):298,276 Z0:648,632	
A-OH-18:1;O3/24:0;O	1262,7345	1284,7162	16,19	16,17	IP:261,036 W(18:1;O3):298,276 Z0mH2O:646,605 Z0:664,619	IP:283,021 B2_hydroxy:361,069 C3PO3_B1_459:459,049 621,101 Z0:686,61
A-OH-43:1;O4	1276,7472		16,73		IP:261,033 Z0:678,638	
160325_S5_R_s_4_pos_CID						
A-NH-18:1;O3/26:1	1293,7659		16,75		W(18:1;O3):298,271 Z0mH2O:678,638	
A-OH-18:1;O3/21:0;O	1220,6841		13,28		IP:261,035 W(18:1;O3):298,276 Z0mH2O:604,569 Z0:622,578	
A-OH-18:0;O3/22:0;O	1236,7169		15,29		IP:261,04 W-H2O(18:0;O3):282,277 W(18:0;O3):300,282 Z0mH2O:620,588	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:638,606	
A-OH-18:1;O3/22:0	1218,7065		14,9		W-H2O(18:1;O3):280,259 W(18:1;O3):298,271 Z0:620,595	
A-OH-18:1;O3/22:0;O	1234,7022	1256,6871	14,37	14,33	IP:261,036 W-H2O(18:1;O3):280,248 W(18:1;O3):298,29 Z0mH2O:618,595 Z0:636,591	IP:283,018 B2_hydroxy:361,072 C3PO3_B1_459:459,051 621,104 Z0:658,569
A-OH-18:1;O3/23:0	1232,7246		15,82		W-H2O(18:1;O3):280,258 W(18:1;O3):298,281 Z0:634,612	
A-OH-18:1;O3/23:0;O	1248,7183	1270,7009	15,45	15,46	IP:261,024 W(18:1;O3):298,27 Z0mH2O:632,6 Z0:650,602	IP:283,016 B2_hydroxy:361,075 C2_hydroxy:379,092 C3PO3_B1_459:459,052 621,101 Z0:672,587
A-OH-18:0;O3/24:0;O	1264,7468	1286,7277	16,61	16,69	IP:261,037 W-H2O(18:0;O3):282,282 W(18:0;O3):300,29 Z0mH2O:648,628 Z0:666,635	C3PO3_B1_459:459,051 621,1
A-OH-18:1;O3/24:0	1246,739		16,46		IP:261,037 W-H2O(18:1;O3):280,266 W(18:1;O3):298,271 Z0:648,632	
A-OH-18:1;O3/24:0;O	1262,7354	1284,7165	16,21	16,18	IP:261,036 W-H2O(18:1;O3):280,264 W(18:1;O3):298,27 Z0mH2O:646,619 Z0:664,622	IP:283,017 B2_hydroxy:361,067 C3PO3_B1_459:459,049 621,101 Z0:686,625
A-OH-18:1;O3/25:0;O	1276,7504	1298,7335	16,74	16,71	IP:261,037 W-H2O(18:1;O3):280,266 Z0mH2O:660,634 Z0:678,642	IP:283,016 B2_hydroxy:361,075 C3PO3_B1_459:459,048 621,1 Z0:700,617
A-OH-44:1;O4	1290,7715		17,14		IP:261,035 Z0mH2O:674,654	

Continued on next page

Continued from previous page

Composition	m/z [M+H] ⁺	m/z [M+Na] ⁺	Rt_pos [min]	Rt_pos_+Na [min]	Fragments MS2 (+H)	Fragments MS2 (+Na)
					Z0:692,657	