Lipidomics Decoded: Targeted Assignment of Polar Lipids using the SICRIT® LC-Module

Introduction

Polar lipids play a pivotal role in lipidomics due to their significance in cellular structure, signaling pathways, and physiological functions. Unlike traditional neutral lipids, polar lipids encompass a diverse array of compounds such as phospholipids, glycolipids, and sphingolipids, which are crucial for cellular membranes, energy metabolism, and cellular communication. Their importance lies in their dynamic roles in cellular processes, making them essential targets for lipidomic analysis.

Polar lipids offer unique insights into cellular biology and disease pathology, making them indispensable in lipidomics research. Their diverse structures and functions necessitate specialized analytical techniques for comprehensive characterization. Liquid chromatography (LC) coupled with mass spectrometry (MS) has emerged as a powerful method for polar lipid analysis. LC separates polar lipids based on their hydrophilic interactions, while MS enables their detection and quantification based on their mass-to-charge ratios. LC-MS boasts high sensitivity, selectivity, and accuracy, rendering it ideal for scrutinizing complex matrices harboring diverse chemical constituents. Typically, LC-MS analysis incorporates ionization sources such as electrospray ionization (ESI) for lipid analysis. ESI involves the formation of charged droplets via the application of a high voltage to a liquid stream. As these droplets evaporate, analyte molecules become ionized, generating ions amenable to MS analysis.

This is where we propose an ionization technique that can act in part as ESI, while also able to employ mechanisms similar to APCI and APPI, bridging any gap between the different analyte classes, and provide a new perspective on LC ionization, but also can be seamlessly incorporated into this standard pipeline. Here we present SICRIT® LC-Module, which takes the advantages of the pre-existing SICRIT® ionization source, conventionally a gas-phase ionization technique, and applies it to an LC method, allowing for soft ionization of both polar and non-polar compounds. This means that only a single ionization source is required to cover the broad spectrum of all major polar lipid classes. In this study, we utilize a HRMS coupled to the SICRIT® LC-Module to analyze a variety of polar lipids with a standard ESI polar lipids method. This is an expansion upon a previous application looking at the more non-polar lipids, such as TAGs. Additionally, we also conduct a DDA MS2 analysis using the LabSolution Insight Assign functionality to determine if our MS2 is compatible with the online search and fragment analysis.

Image 1: Instrumental setup for this study

Sample Preparation & Analysis Conditions

Overall sample preparation was minimal. The Avanti Polar Splash Standard was measured with the pure dilutions (Table 1) and with a 1:10 dilution in methanol.

Compound	Standard	Absolute amount / ng
15:0-18:1(d7) PC	Polar Standard	80
15:0-18:1(d7) PE	Polar Standard	2.5
15:0-18:1(d7) PA	Polar Standard	3.5
18:1(d7) LPC	Polar Standard	12.5
18:1(d7) LPE	Polar Standard	2.5
18:1(d9) SM	Polar Standard	15

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18:1(d7) Chol Ester	Polar Standard	175
18:1(d7) MG	Polar Standard	1
15:0-18:1(d7) DG	Polar Standard	5
15:0-18:1(d7)-15:0 TG	Polar Standard	27.5
15:0-18:1(d7) PS	Polar Standard	2.5
15:0-18:1(d7) PI	Polar Standard	5
15:0-18:1(d7) PG	Polar Standard	15

Table 1: List of compounds and absolute amounts

Here, a Shimadzu LC-40 was interfaced to a Shimadzu 9030 LC-MS via the LC-Module and SICRIT® Ion source with accompanying SC-30 Control unit (Image 1). The SICRIT® LC-Module was set to 400 °C with the SC-30 parameters for voltage set to 1600 V and the frequency set to 15000 Hz. The settings for the various flows pertaining to the LC and MS can be found in the tables below (Table 2 & 3).

LC - Settings		
	Instrument	Shimadzu LC-40
	SETTINGS	CONDITIONS
	Column	
	Type	Zorbax Eclipse Plus C18, 50mm x 2.1 mm, 1.8 µm
	Guard Column	Phenomenex
	Column Oven Temp.	30°C
	Injection	
	Total Injection Volume (in µL)	5
	Injection Programm	Standard
	Set Injection Volume (in µL)	5
	Autosampler Temp	20 °C
	Solvents	
	Flow (mL/min)	0,4
	Solvent A	Isopropanol
	Solvent B	Methanol
	Solvent Gradient	
	Time	% Solvent B
	0.00	92.0
	10.00	92.0
	20.00	83.0
	21.00	65.0
	31.00	65.0
	32.00	50.0
	40.00	48.0
	40.01	92.0
	45.00	92.0
	Total Runtime (min)	45.00

Table 2: LC Parameters

MS - Settings		
	Instrument	Shimadzu 9030 QTOF
	Ion Source	SICRIT / LC-Module
	MS Tuning Settings	
	Last Tuning	changing
	Last Mass Calibration	changing
	Tuning File	changing
	Inlet / Source Conditions	
	Interface Voltage	off
	Detector Voltage	0,2 kV
	Nebulizing Gas Flow	0,5 L/min (set to 3,0)
	Drying Gas Flow	off
	Heating Gas Flow	3,0 L/min (set to 4,0)
	Desolvation Line Temp.	250 °C
	Heat Block Temp.	250 °C
	Interface Temp.	off
	CID Gas Pressure	150 kPa
	Scan Conditions	
	Scan Mode	DDA
	Scan Range	50-1200 m/z
	Polarity	+ / -
	CE	35.0 (+/- 17)
	Event Time	0.043s
	ID	off
	Q1 Resolution	10.0 (Center)
	Event Time	0.100 sec
	Save Profile	no
	Threshold	low
	SICRIT Conditions	
	Voltage	1600 Vpp
	Frequency	15000 Hz
	Modul Temp	500 °C
	Humidifier	no

Table 3: MS & SICRIT Parameters

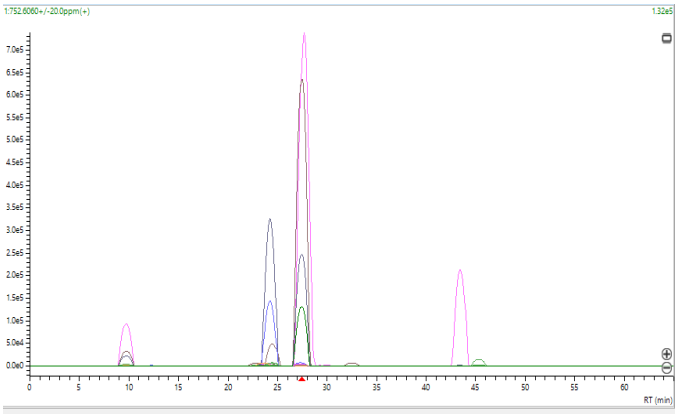

Figure 1: Overlapping EICs from the positive ion mode measurements of the polar lipids

Results from Analysis of Standard Samples

From this initial study, we wanted to be able to determine how and if we can fully ionize all these compounds in positive ion mode and how the ionization changes using the pure standard. What we see is that the common ionizations that are typically observed such as [M]+, [M+H]+, [M+NH4]+, and loss of water are all found (Figure 1).

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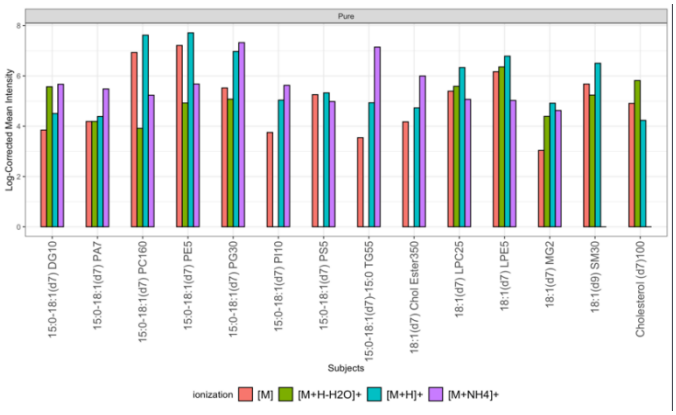

Figure 2: Ionization species found for all the analytes in the pure standard, the 1:10 standards, along with a spiked brain standard of these deuterated compounds

The next step in analysis was translating the EICs (Figure 1) data into understanding how the MS1 appears, even with co-elution of multiple components and if an MS2 collection was even possible and if it was possible, how could we leverage the online search and annotation capabilities to get a proper match from the Shimadzu software. By analyzing the MS1 and MS2 we were able to effectively identify the component from the MS1, extract the DDA data provided and then use the Assign module within LabSolutions Insight to tentatively assign the compound through an online search of PubChem and ChemSpider using only a chemical formula or monoisotopic mass within 10 ppm, while validating the resulting compound returned through fragmentation analysis of the MS2. The provided chemical formula can come from a targeted approach, where it is known, or through an untargeted means, where the formula can be predicted with the monoisotopic mass within the LabSolution software itself. The workflow for this can be seen below (Scheme 1). We were able to successfully identify every valid MS2 through this LabSolution Analysis technique, which further expands what we are capable of doing with the SICRIT technology, where we can effectively validate the compound without an in-house library. Instead, we can take full advantage of large databases to tentatively identify fragment patters, which allows for us to apply this technique to any compound with an LC-MSMS spectra within PubChem or ChemSpider.

Conclusion

What we have been able to show through this simple LC-HRMSMS study is that by using the SICRIT Ionization technique, you can conduct successful analysis of a wide range of polar lipids. Additionally, you can obtain a usable MS2 spectra that can be used for either targeted or untargeted analysis. This is shown using the Assign functionality in LabSolution, which uses an untargeted online search approach to match the produced MS2 to the compound, when provided minimal information (precursor mass or proposed formula). This allows us to fully streamline both our targeted and untargeted approaches for lipidomics or metabolomics analysis, similar to standard ionization techniques used in most pipelines.

Acknowledgement

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References

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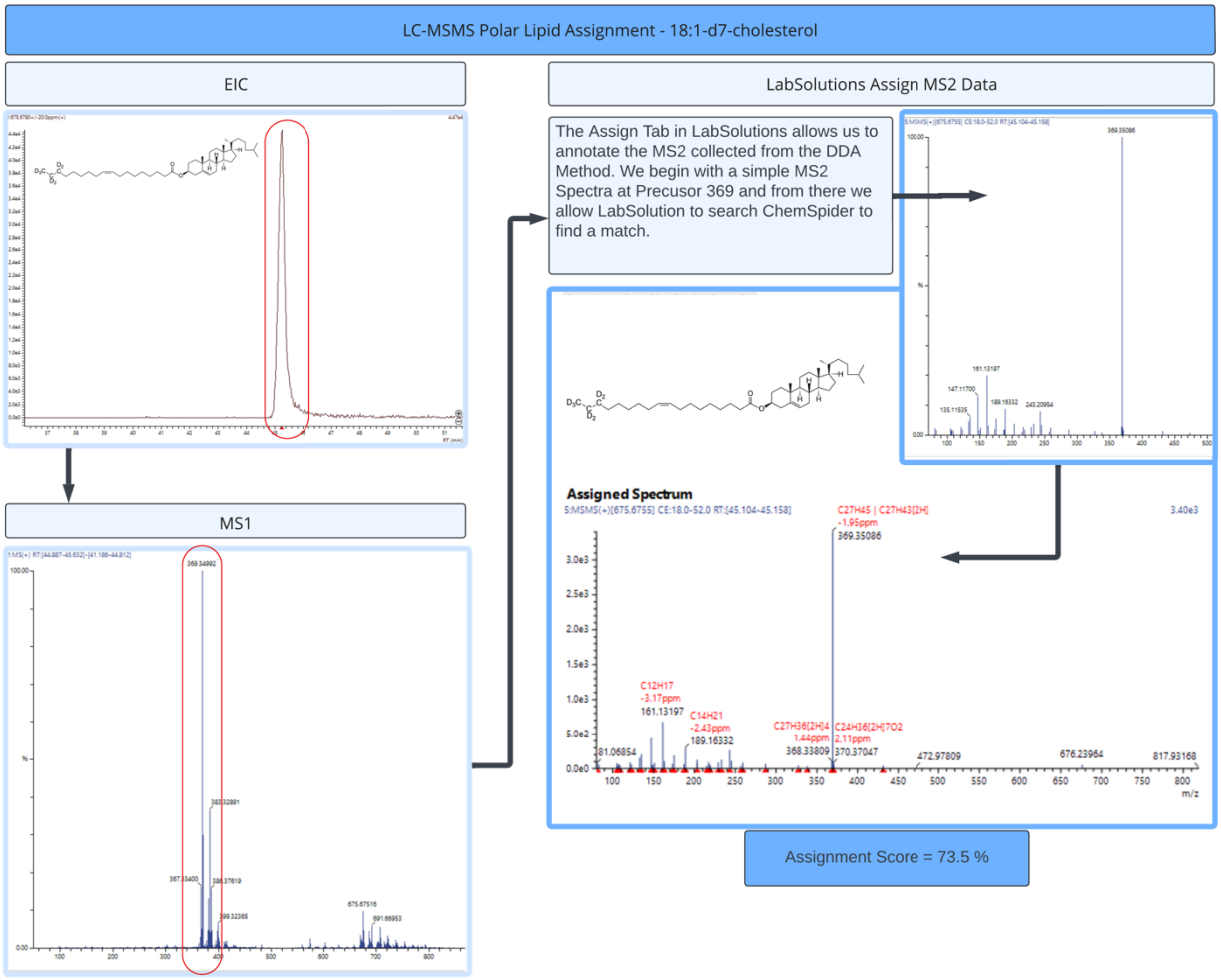

Scheme 1: Exemplary workflow and Data for MS2 assignment using Lab solutions assign function.

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