LC-SICRIT®-HRMS Analysis of Non-Polar Lipids featuring Shimadzu Nexera LC and 9030 LC-MS QToF

Summary

In this set of measurements, we demonstrate the detection capabilities of 8 different Triacyl-glycerides of varying length and saturation with LC-SICRIT®-HRMS. Here we show how specific disadvantages of the currently used methods can be addressed effectively with our source, particularly when analyzing the non-polar lipids like those belonging to the TAG lipid class.

Introduction

Though lipids and lipid-like structures are known to be an integral part of the inherent metabolism of living creatures, it was thought that these compounds may not be as crucial as other small molecule metabolites of interest in the field of metabolomics. However, in recent years, the data has shown otherwise, showing that lipids play an integral role in cell signaling, in the overall function of the immune system, and in overall health of the brain particularly when it comes to oxidized lipids and the relation to global inflammation. These new findings are some of the many reasons the study of lipidomics has risen in popularity within metabolomics and corresponding fields.

The typical analysis for lipids is HPLC-ESI-HRMS, which has become the standard within the metabolomics community. However, there are still a few disadvantages that surface from this method pertaining to ion suppression, multiple charge states, and increased in-source fragmentation. Additionally, the range of ionization that can be obtained does not fully cover both the non-polar and highly polar lipids in a single method. Here, we propose a new method utilizing the SICRIT® ionization source that can combat some of these lingering issues. With the SICRIT® technology we can softly ionize a dynamic range of components, while introducing only singly charged species to the detector. Due to these qualities of our ionization technology we are able to minimize or completely eliminate the disadvantages present in the ESI method such as suppression effects, in-source fragmentation, and multiple charged ions.

Experimental Setup

The SICRIT® technology enables flexible coupling of a GC or LC to any LC-MS. Here, the SICRIT® LC-Module was interfaced to a Shimadzu 9030 LC-MS with the accompanying SC-30 control unit (Fig 1).

Fig 1: Experimental Set-up for the SICRIT® LC-Module, coupling the Shimadzu LC-40 to the Shimadzu 9030 LC-MS QToF

The SICRIT® LC-Module was set to 500 °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 HRMS can be found in the tables below, however, it should be mentioned that the module as also equipped with a flowmeter to ensure that the nebulizer gas flow (3.0 L/min) and the heating gas flow (4.0 L/min) were held at a constant flow (Table 1&2).

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For the LC method, an Agilent InfinityLab Poroshell C-18 column was used with an organic solvent set-up of isopropanol (Solvent A) and methanol (Solvent B). A linear gradient over 45 minutes with a flow rate of 0.4 mL/min was used to ensure the elution of all components (Table 1).

LC - Settings		
	Instrument	Shimadzu LC-40
	SETTINGS	CONDITIONS
	Column	
	Type	InfinityLab Poroshell 120 EC-C18 50mm x 2.1mm, 2,7µm
	Guard Column	Phenomenex
	Column Oven Temp.	30°C
	Injection	
	Total Injection Volume (in µL)	5
	Injection Programm	Standard
	Set Injection Volume (in µL)	5
	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 1: Parameters for the Shimadzu LC-40, including the Solvent Gradient and run-times of the method.

The MS had a set scan range of 50-1200 m/z, where the method was run in both positive and negative mode (Table 2).

The mixed samples were prepared from two different standards (Standard GLC-406 and Standard GLC-437), where the count and concentration of analytes differed (Table 3). These samples were diluted with methanol to 333 ppm and 500 ppm, respectively. The qualitative runs were comprised of four consecutive runs with the parameters and methods mentioned above.

MS - Settings		
	Instrument	Shimadzu 9030 QTOF
	Ion Source	SICRIT / LC-Module
	MS Tuning Settings	
	Last Tuning	14.03.2023
	Last Mass Calibration	14.03.2023
	Tuning File	Tuning_14032023
	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
	CID Gas Pressure	150 kPa
	Scan Conditions	
	Scan Mode	Full Scan
	Scan Range	50-1200 m/z
	Polarity	+ / -
	ID	off
	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 2: Parameters for the Shimadzu 9030 HRMS, including the SICRIT® LC-Module settings.

Results and Discussion

Our first observation was that we could effectively ionize all compounds of interest, which were separated and identified through chromatography. This in itself is a new advancement in the SICRIT® technology, which is inherently a gas phase technique, however, when the source is coupled with SICRIT® LC-Module we can ionize species well above the 1000 Da mark. Furthermore, we maintained our soft ionization with minimal fragmentation to the point where compounds could be identified through an MS1 scan match in the Shimadzu compound identification software (Table 3).

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GLC-406 (% Conc.)	GLC-437 (% Conc.)	Name	Num. Double Bonds (per chain)	m/z	Found RT
9	16.67	Tri-a-linolenin [M+H]+	3	873.697	4.37
21	16.67	Trilinolein [M+H]+	2	879.744	8.33
61	16.67	Triolein [M+NH4]+	1	902.817	16.60
1	0	Trieicosenoin [M+NH4]+	1	986.911	24.65
1	0	Trierucin [M+NH4]+	1	1071.005	32.73
4	16.67	Tripalmitin [M+NH4]+	0	824.770	15.12
2	16.67	Tristearin [M+NH4]+	0	908.864	24.30
1	16.67	Triarachidin [M+NH4]+	0	992.958	32.64

Table 3: The unique Triglycerides with percent concentration within each standard, the prominent ionization specie found and the corresponding retention time.

An example of this can be observed with Triarachidin, where the main ionization species ($[M+NH4]^+$) results in a side-by-side match at the MS1 level with the software (Fig 3). The reason this finding is significant and impressive is that the Shimadzu software was able to accurately predict the correct compound at the MS1 level. This is noteworthy due to the compound's highly non-polar nature and the length of its aliphatic chain. These factors can result in low abundances of ionized species and higher levels of fragmentation, making it challenging to identify the compound in an MS1 scan using conventional ionization methods. However, our technology can provide a clean MS1 scan of three separate ion species with minimal fragmentation even with some of the most difficult Triglycerides.

A secondary observation we found was that three main ionization species ($[M+H]^+$, $[M+NH4]^+$, $[M+Na]^+$) are prevalent in different ratios depending on the level of unsaturation for each compound of interest. Essentially, the more unsaturated a compound's aliphatic chain was (2-3 double bonds per chain), the more likely the prevalent adduct present would be the $[M+H]^+$ adduct, where as if the level of unsaturation was lower (0-1 double bonds per chain), the $[M+NH4]^+$ specie became more prevalent (Table 3). What makes this pattern remarkable is that we can determine certain aspects of the structure immediately from the MS1, making overall identification potentially more efficient, especially in the event of coelution. For untargeted lipidomics this could serve as an additional layer of structural understanding, and coupled with a computational prediction technique it count fill in the gaps where a software may struggle to identify. An example of this difference in ionization ratios is apparent when comparing Tri-alpha-linolenin and Triolein, where the dominant species are $[M+H]^+$ and $[M+NH4]^+$, respectively (Fig 4).

With a wider array of compounds we can further confirm these findings and potentially find the nuisances within the ratios themselves and how they correlate to the structural information.

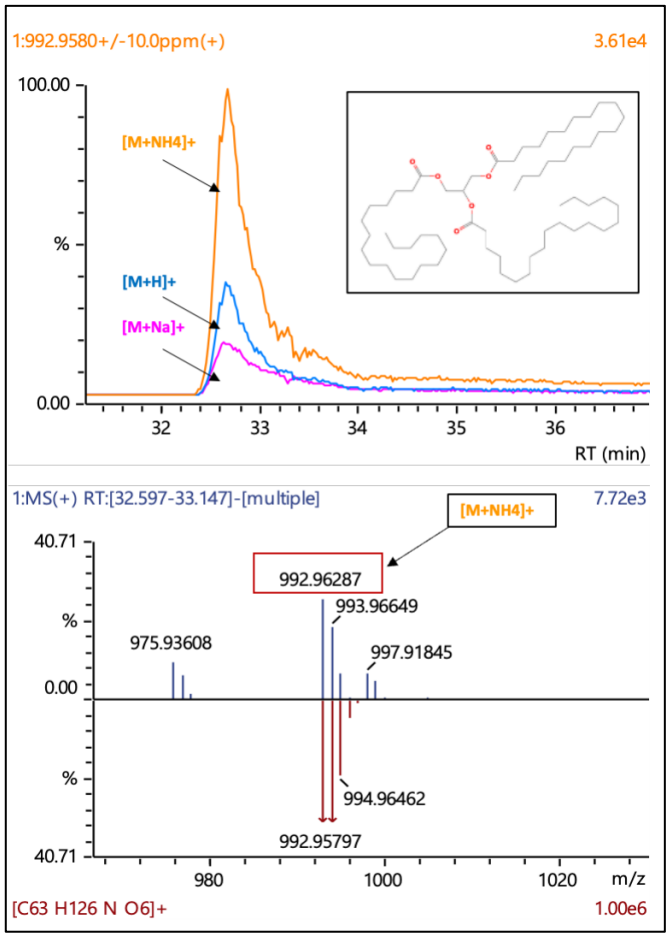

Fig 3: The top chromatogram provides different ionization species ratios for the Triarachidin, including the structure to the right. The bottom stem plot shows the MS1 spectra for the compound (blue) and the library match in the Shimadzu software (red), where the match is on the ammonia adduct.

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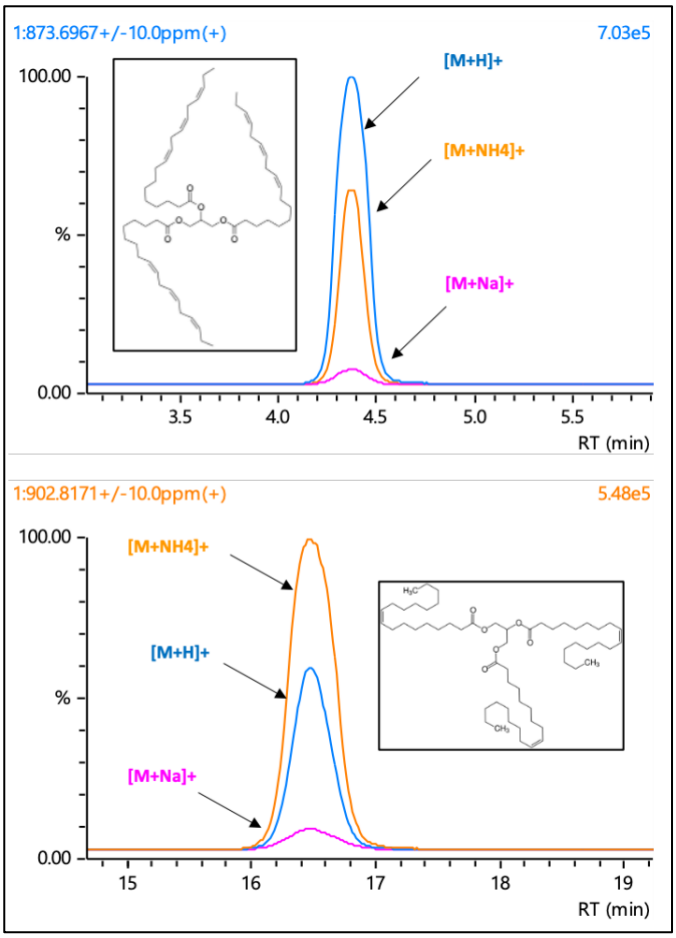

Fig 4: The top chromatogram provides the ionization specie ratios for Tri-alpha-linolenin, where the bottom chromatogram provides the specie ratios for Triolein.

Conclusion

The presented data provides a new field where the SICRIT® ionization source is able to perform well, overcoming the disadvantages of the standard ionization methods, leading to consistent ionization with minimal fragmentation of non-polar lipids, even with masses spanning from 800 Da to over 1000 Da. Furthermore, due to the consistent ionization and minimal ion species formed, we could begin to identify structural patterns simply from the dominant ion specie present at the MS1 level.

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References

[1] Shimadzu application news 04-AD-0240-EN

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