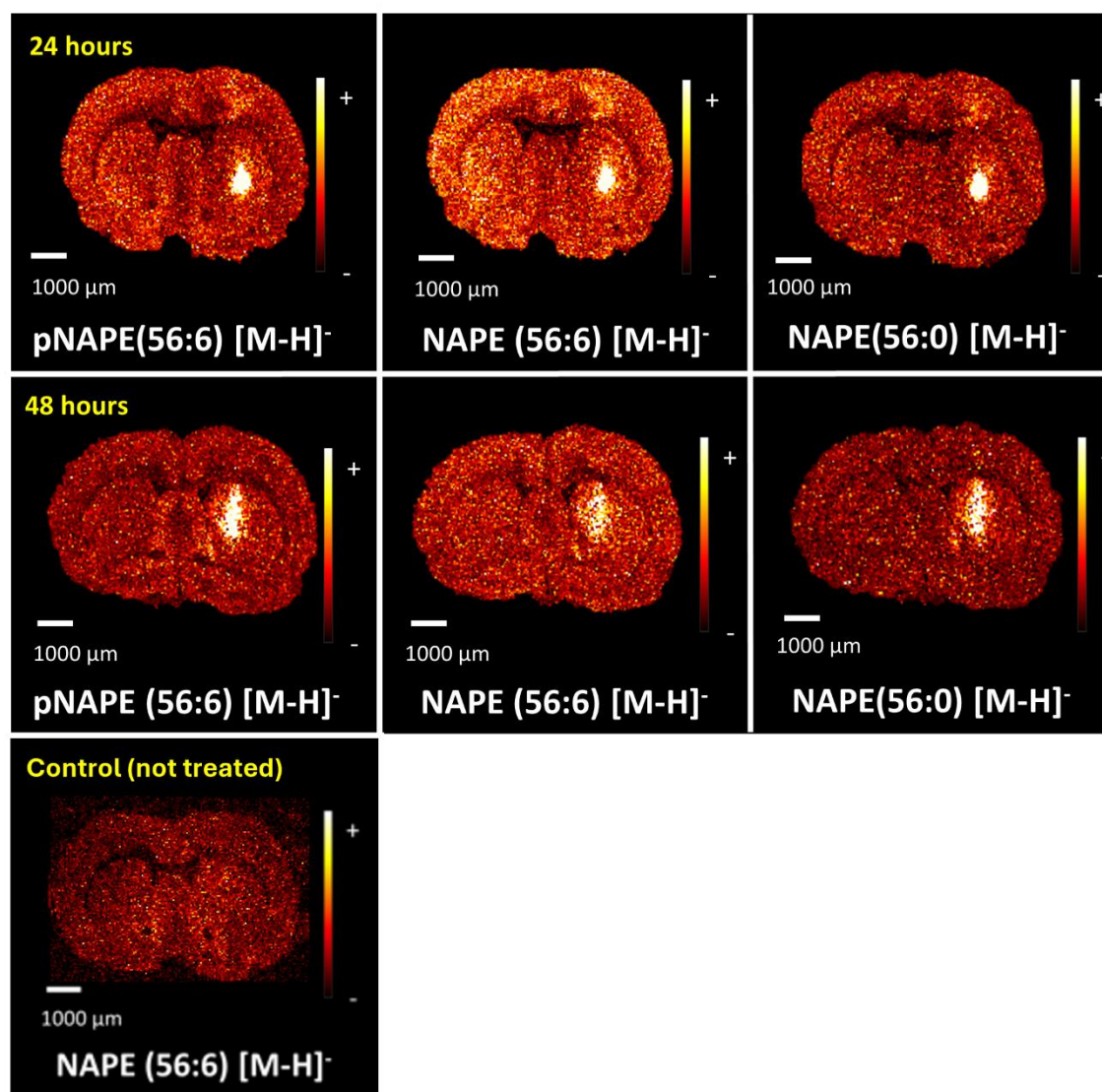


# Accumulation of bioactive lipid species in LPS-induced neuroinflammation models analysed with multi-modal mass spectrometry imaging



**Figure S1:** Single ion images obtained with DESI-MS. The segmentation maps were obtained with bisecting k-mean clustering to separate the region of inflammation (seen in blue/grey) from the rest of the brain structures (yellow/orange) and background substrate (red). Single ion images show the detection of N-Acylphosphatidylethanolamine (NAPE) lipid species with DESI Imaging in negative ion mode. pNAPE(56:6), NAPE(56:6) and NAPE(56:0) were detected mainly within the inflammation area after 24 hours and 48 hours of inducing the neuroinflammatory event with LPS.

Putative assignment	Chemical formula [M]	Accurate m/z value [M-H] <sup>-</sup>	Detected m/z [M-H] <sup>-</sup>	Δ Mass (ppm)
NAPE(52:0)	C <sub>57</sub> H <sub>112</sub> NO <sub>9</sub> P	984.7996	984.7728	27.21
NAPE(54:4)	C <sub>59</sub> H <sub>108</sub> NO <sub>9</sub> P	1004.7686	1004.8004	31.64
pNAPE(56:6)*	C <sub>61</sub> H <sub>108</sub> NO <sub>8</sub> P	1012.7734	1012.8035	29.72
NAPE (56:6)*	C <sub>61</sub> H <sub>108</sub> NO <sub>9</sub> P	1028.7683	1028.7957	26.63
NAPE(56:4)	C <sub>61</sub> H <sub>112</sub> NO <sub>9</sub> P	1032.7996	1032.8158	15.68
NAPE(56:0)*	C <sub>61</sub> H <sub>120</sub> NO <sub>9</sub> P	1040.8622	1040.8512	10.56
NAPE(58:6)	C <sub>63</sub> H <sub>111</sub> NO <sub>9</sub> P	1056.7996	1056.8457	43.62
*Annotation based on MS/MS analysis. The rest of the assignment was based on accurate masses.				

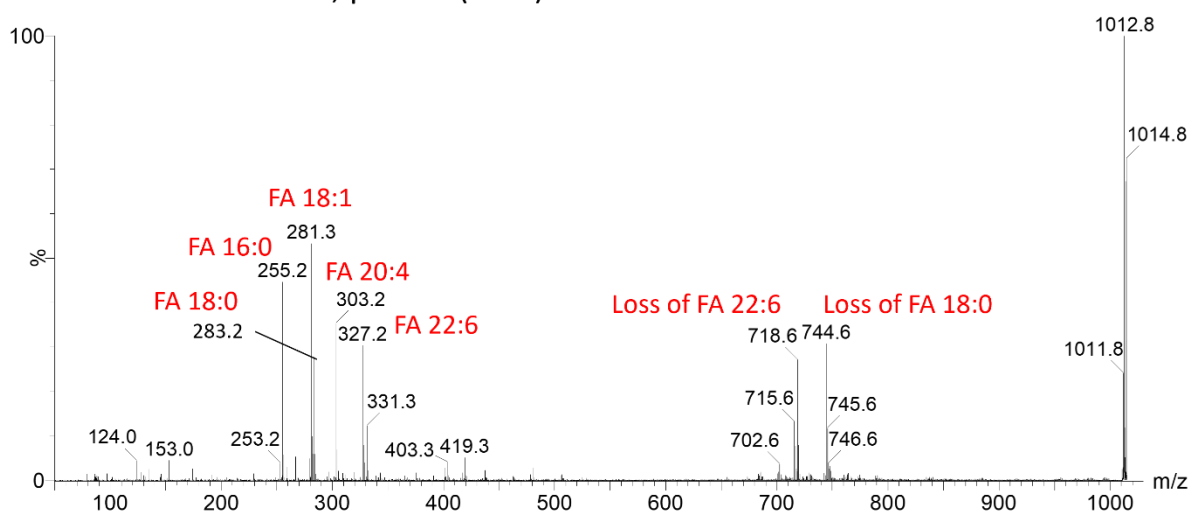
**Table S1** List of NAPE lipid species detected as deprotonated [M-H]<sup>-</sup> molecules with DESI-MSI.

<b>m/z</b>	<b>Putative assignment</b>
960.779	N/A
984.7728	NAPE(52:0)
986.7772	N/A
988.8022	N/A
1000.7559	N/A
1004.8004	NAPE(54:4)
1010.7631	N/A
1012.8035	pNAPE(56:6)
1014.8142	N/A
1016.8459	N/A
1028.7957	NAPE (56:6)
1032.8158	NAPE(56:4)
1038.8235	N/A
1040.8512	NAPE(56:0)
1042.848	N/A
1044.8661	N/A
1056.8457	NAPE(58:6)

**Table S2:** m/z values co-localised to the inflammation regions with Person correlation analysis.

MS/MS

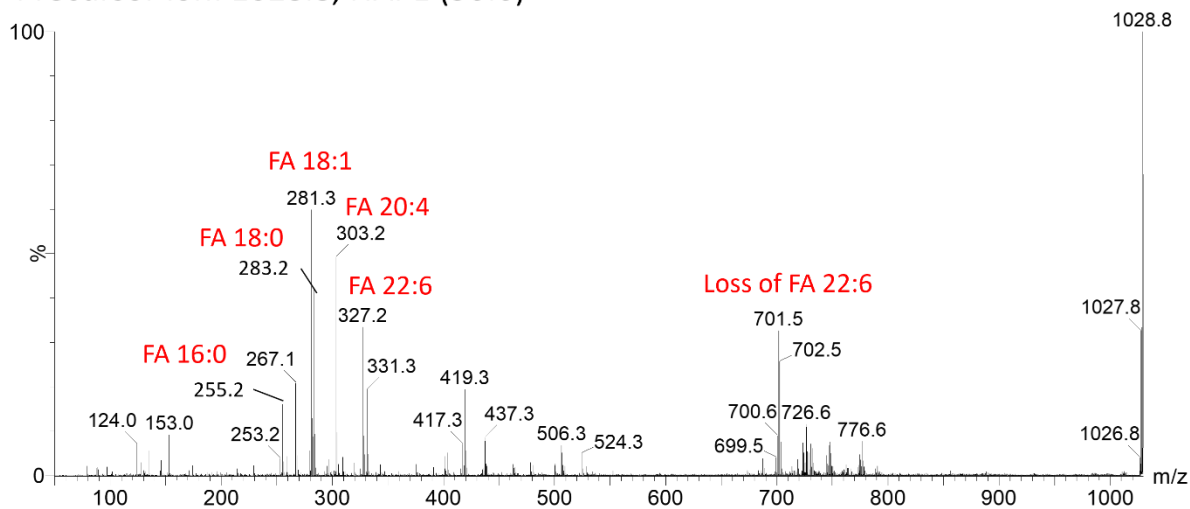
Precursor ion: 1012.8, pNAPE (56:6)



**Figure S2:** Combined MS/MS spectrum of pNAPE(56:6) acquired with DESI across the inflammation region of a brain section.

MS/MS

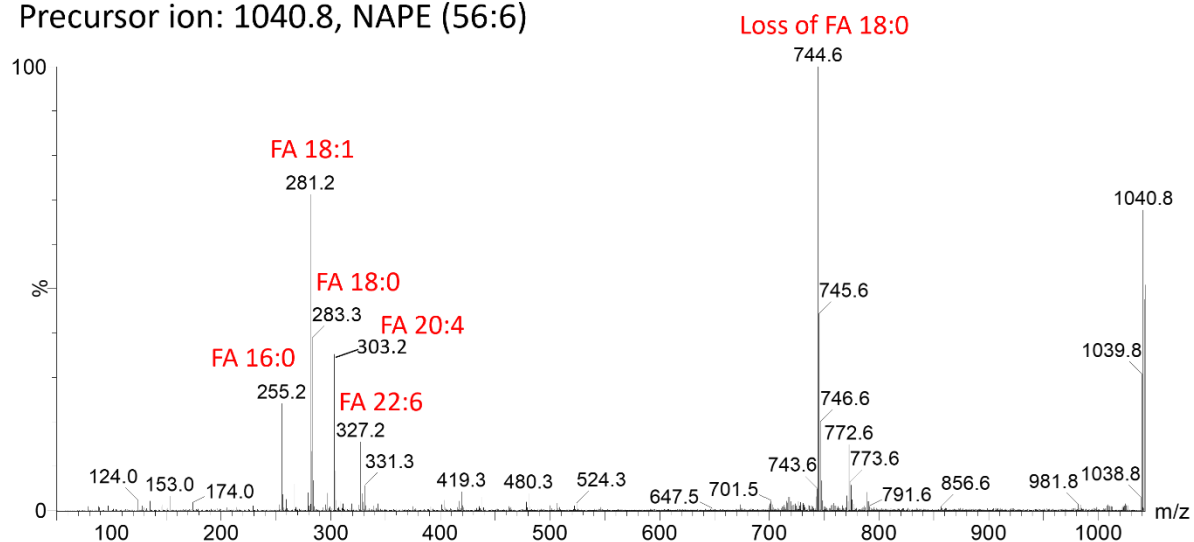
Precursor ion: 1028.8, NAPE (56:6)



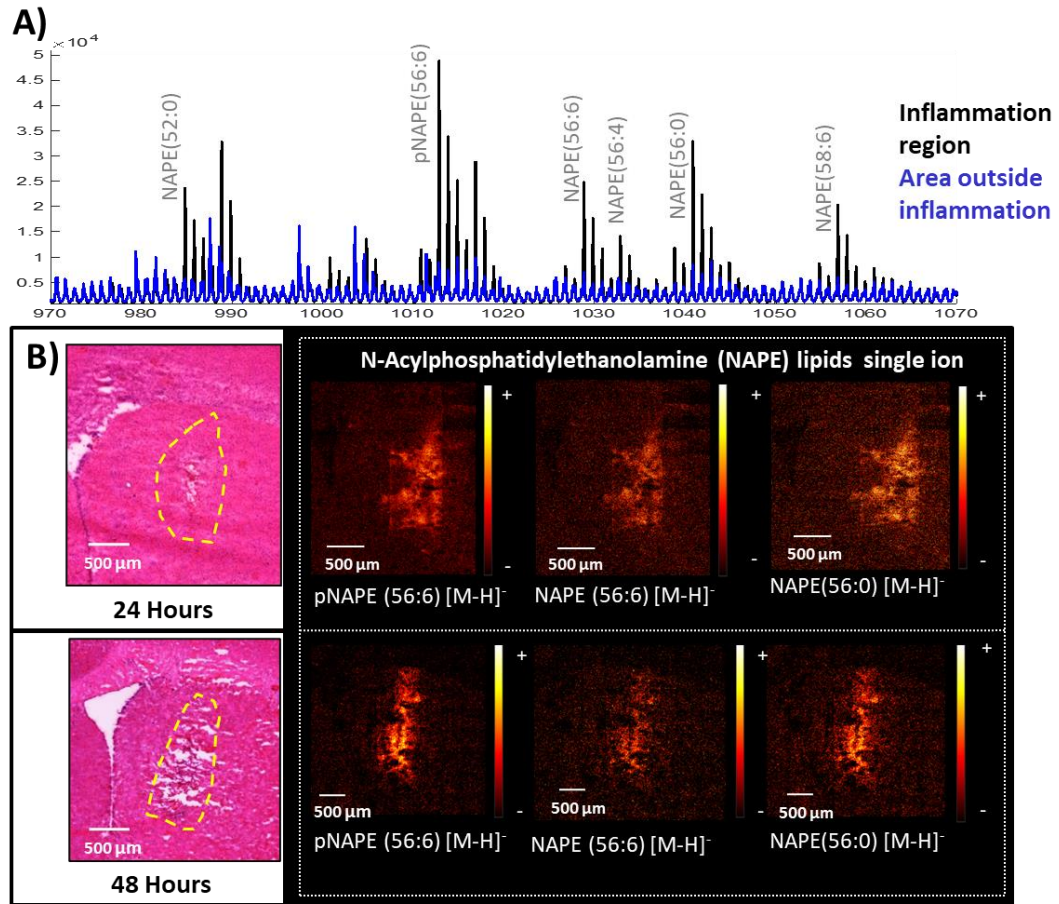
**Figure S3:** Combined MS/MS spectrum of NAPE(56:6) acquired with DESI across the inflammation region of a brain section.

# MS/MS

Precursor ion: 1040.8, NAPE (56:6)



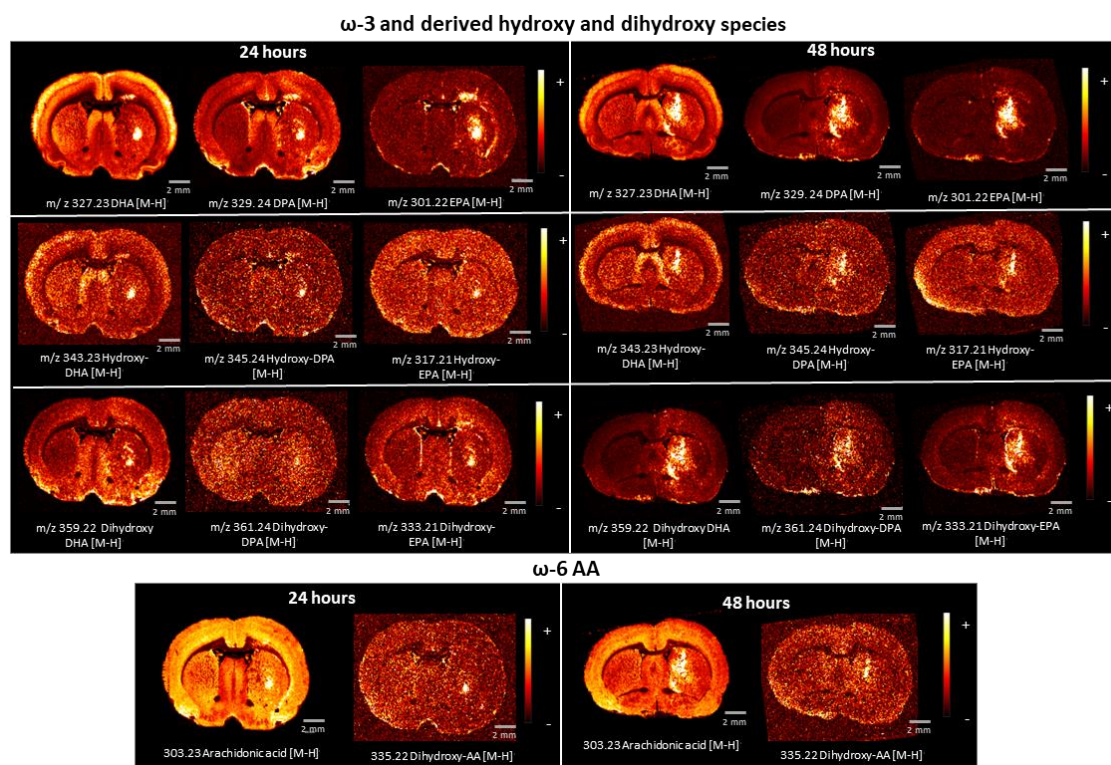
**Figure S4:** Combined MS/MS spectrum of NAPE(56:0) acquired with DESI across the inflammation region of a brain section.



**Figure S5:** Overlaid Spectra acquired with TOF-SIMS, the black spectrum was extracted from the inflammation region whereas the blue spectrum was extracted from a region without inflammation. Both areas have the same number of pixels for comparison purposes. When comparing the spectra, the main chemical differences can be observed within the mass range  $m/z$  970 to  $m/z$  1100. Here, N-Acylphosphatidylethanolamine (NAPE) lipid species have been identified. B) Single ion images of selected N-acylphosphatidylethanolamines (NAPEs), accumulated mainly within the region of inflammation after 24 hours and 48 hours after LPS treatment. Microscopy images from consecutive sections from the same brains stained with H&E are also shown as a reference, with the inflammation region highlighted in yellow dotted areas.

PUFA species					
Putative assignment	Chemical formula [M]	Accurate m/z value [M-H] <sup>-</sup>	Detected m/z [M-H] <sup>-</sup>	Δ Mass (ppm)	Lipid Maps ID
DHA	C <sub>22</sub> H <sub>32</sub> O <sub>2</sub>	327.2330	327.2362	9.78	LMFA01030185
DPA	C <sub>22</sub> H <sub>34</sub> O <sub>2</sub>	329.2486	329.2494	2.43	LMFA04000044
EPA	C <sub>20</sub> H <sub>30</sub> O <sub>2</sub>	301.2173	301.2225	17.26	LMFA01030759
Arachidonic acid	C <sub>20</sub> H <sub>32</sub> O <sub>2</sub>	303.2330	303.2373	14.18	LMFA01030001
Derivatives					
Putative assignment	Chemical formula [M]	Accurate m/z value [M-H] <sup>-</sup>	Detected m/z [M-H] <sup>-</sup>	Δ Mass (ppm)	
Hydroxy-DHA	C <sub>22</sub> H <sub>32</sub> O <sub>3</sub>	343.2279	343.2350	20.69	
Hydroxy-DPA	C <sub>22</sub> H <sub>34</sub> O <sub>3</sub>	345.2430	345.2573	4.14	
Hydroxy-EPA	C <sub>20</sub> H <sub>30</sub> O <sub>3</sub>	317.2117	317.2158	12.93	
Dihydroxy-DHA	C <sub>22</sub> H <sub>32</sub> O <sub>4</sub>	359.2228	359.2248	5.57	
Dihydroxy-DPA	C <sub>22</sub> H <sub>34</sub> O <sub>4</sub>	361.2384	361.2421	10.24	
Dihydroxy-EPA	C <sub>20</sub> H <sub>30</sub> O <sub>4</sub>	333.2066	333.2130	19.21	
Dihydroxy-AA	C <sub>20</sub> H <sub>32</sub> O <sub>4</sub>	335.2222	335.2295	21.78	

**Table S3:** Assignment of PUFA species and their derivatives

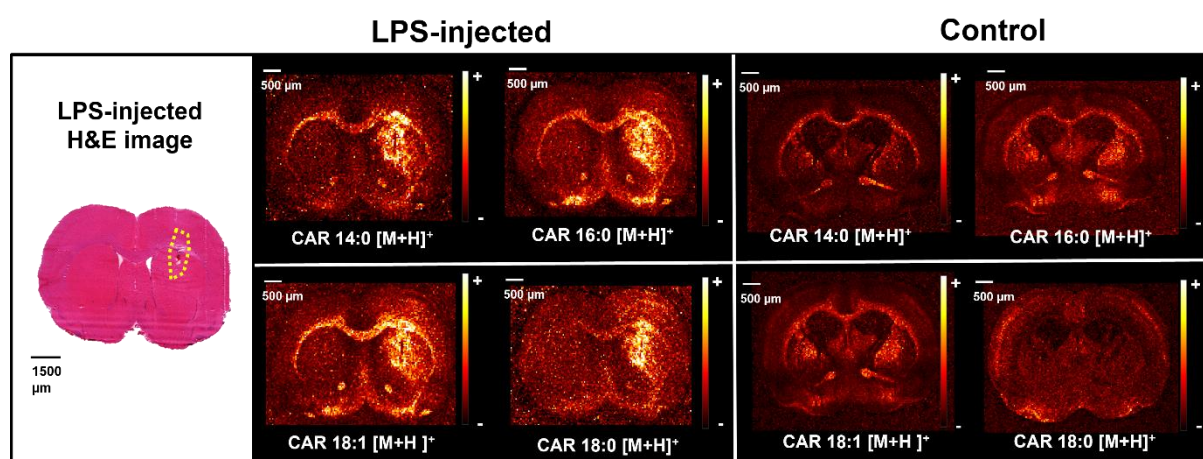


**Figure S6:** Accumulation of PUFAs and their hydroxy and Dihydroxy derivatives in brains injected with LPS.



Putative assignment	Chemical formula	Accurate m/z value	Detected m/z	$\Delta$ Mass (ppm)	Lipid Maps ID
	[M]	[M+H] <sup>+</sup>	[M+H] <sup>+</sup>		
CAR(14:0)	C <sub>21</sub> H <sub>41</sub> NO <sub>4</sub>	372.3108	372.3132	6.44	LMFA07070102
CAR(16:0)	C <sub>23</sub> H <sub>45</sub> NO <sub>4</sub>	400.3421	400.3453	7.99	LMFA07070098
CAR(18:1)	C <sub>25</sub> H <sub>47</sub> NO <sub>4</sub>	426.3578	426.3614	8.44	LMFA07070096 LMFA07070063
CAR(18:0)	C <sub>25</sub> H <sub>49</sub> NO <sub>4</sub>	428.3734	428.3719	3.50	LMFA07070051

**Table S4:** Assignment of Acylcarnitine species and their derivatives



**Figure S7:** Accumulation of Fatty acyl carnitines surrounding the inflammation region detected with DESI-MS. (Right) H&E microscopy image showing the inflammation region. (Left) Single ion images showing the detection of tetradecanoylcarnitine (CAR 14:0) at m/z 372.31, palmitoylcarnitine (CAR 16:0) at m/z 400.34, elaidic carnitine or O-oleoylcarnitine (CAR 18:1) at m/z 426.35 and stearoylcarnitine (CAR 18:0) at m/z 428.37.