

Supplementary Information

Biomarkers for multiple sclerosis determined by metabolomic profiling using coupled UHPLC-MS

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Figure 1 PCA plot of raw data in MPP for plasma analysis of multiple sclerosis (blue), neuropathic pain (grey), multiple sclerosis and neuropathic pain (brown), control group (red) and QC injections (green). The principle component is plotted on the X-axis and represents 17% of data variation. Component 2 on the Y-axis represents 11% of the variation and component 3 on the Z-axis 6% of the variation.

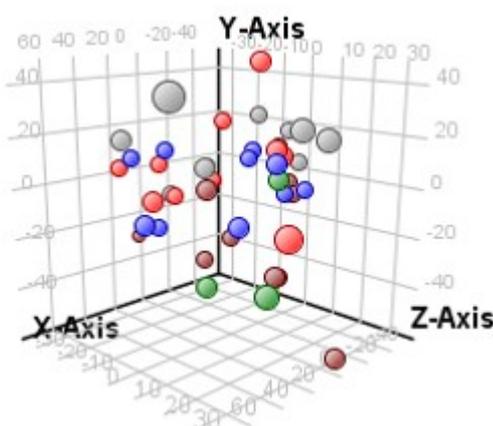


Figure 2 PCA plot of re-processed data in MPP for plasma analysis of multiple sclerosis (blue), neuropathic pain (grey), multiple sclerosis and neuropathic pain (brown), control group (red) and QC injections (green). The principle component is plotted on the X-axis and represents 27% of data variation. Component 2 on the Y-axis represents 13% of the variation and component 3 on the Z-axis 7% of the variation.

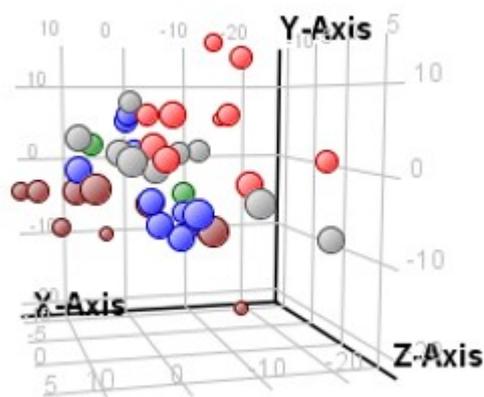


Figure 3 An example of a missed integration in 7/10 samples in Profinder of compound m/z 805.0323.

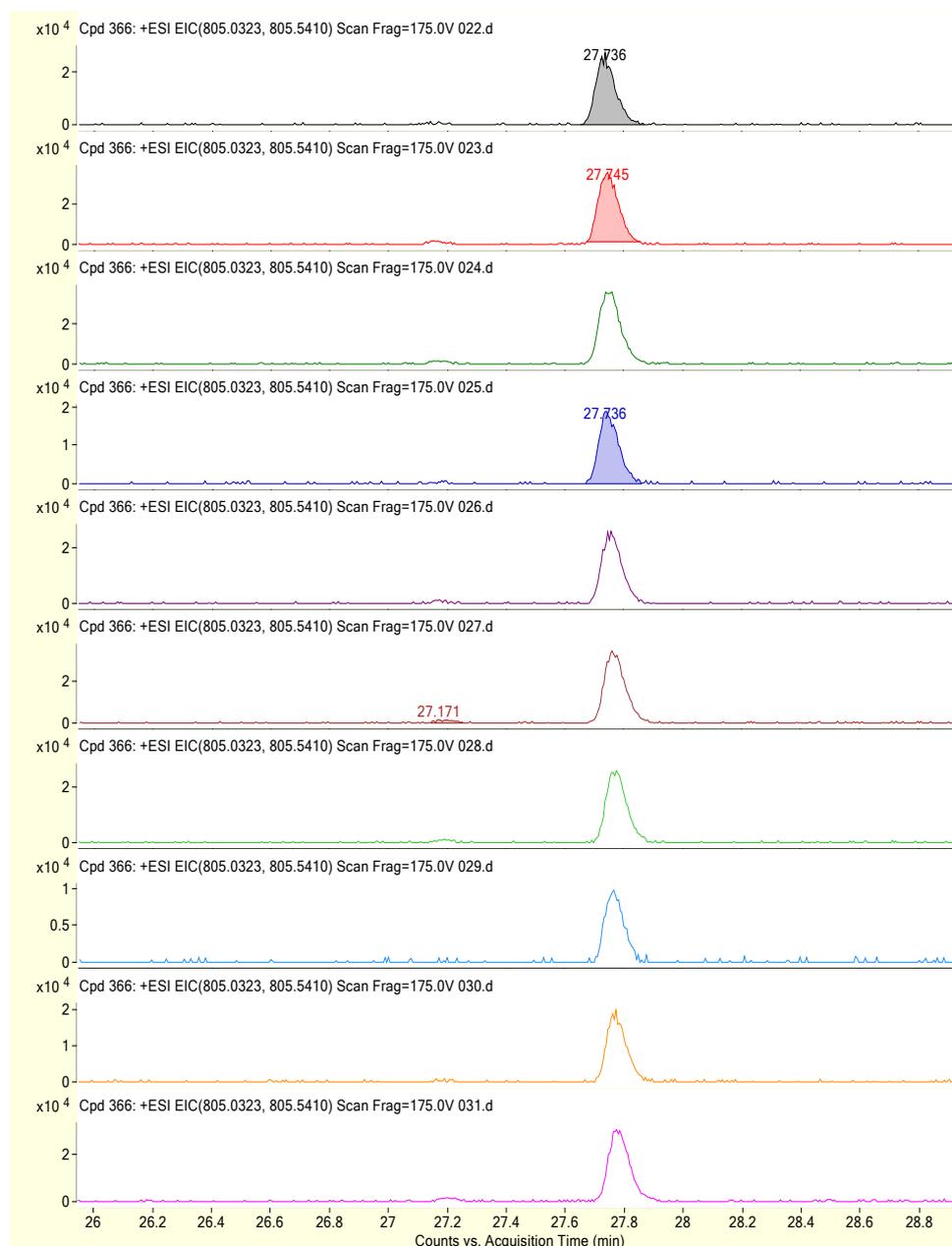


Figure 4 EIC of m/z 300.2892 in control group sample showing the presence of two compounds with that m/z.

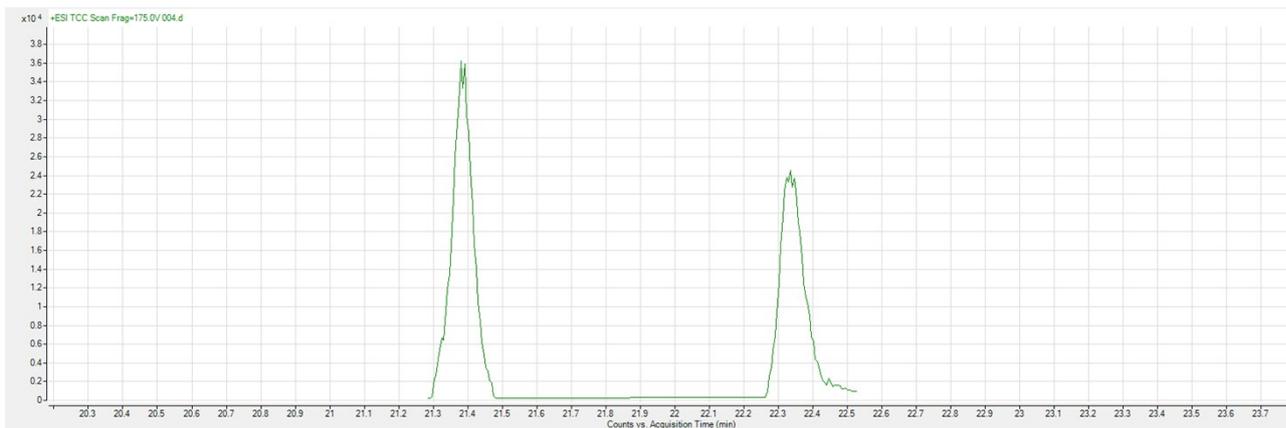


Figure 5. Isotope fit for formula C₁₈H₃₇NO₂ eluting at 21.3min in control group sample

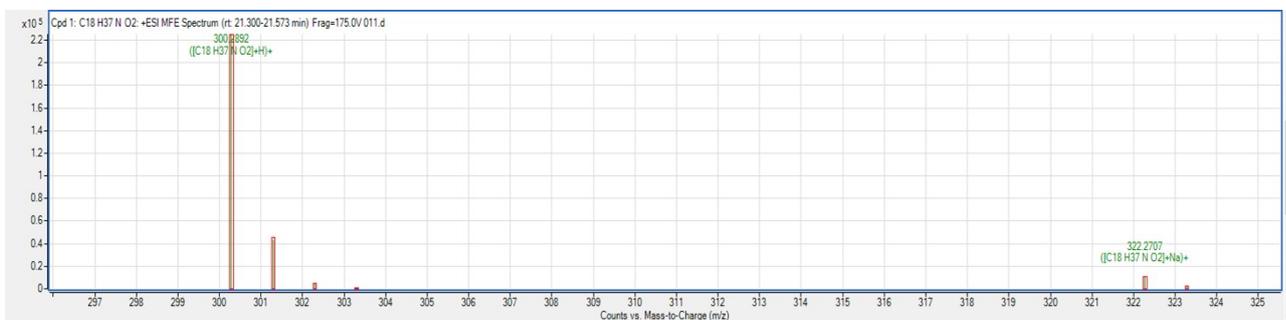


Figure 6. EIC's of m/z 300.2892 in control sample and sphingosine spiked control sample showing peak alignment for peak eluting at 21.4min.

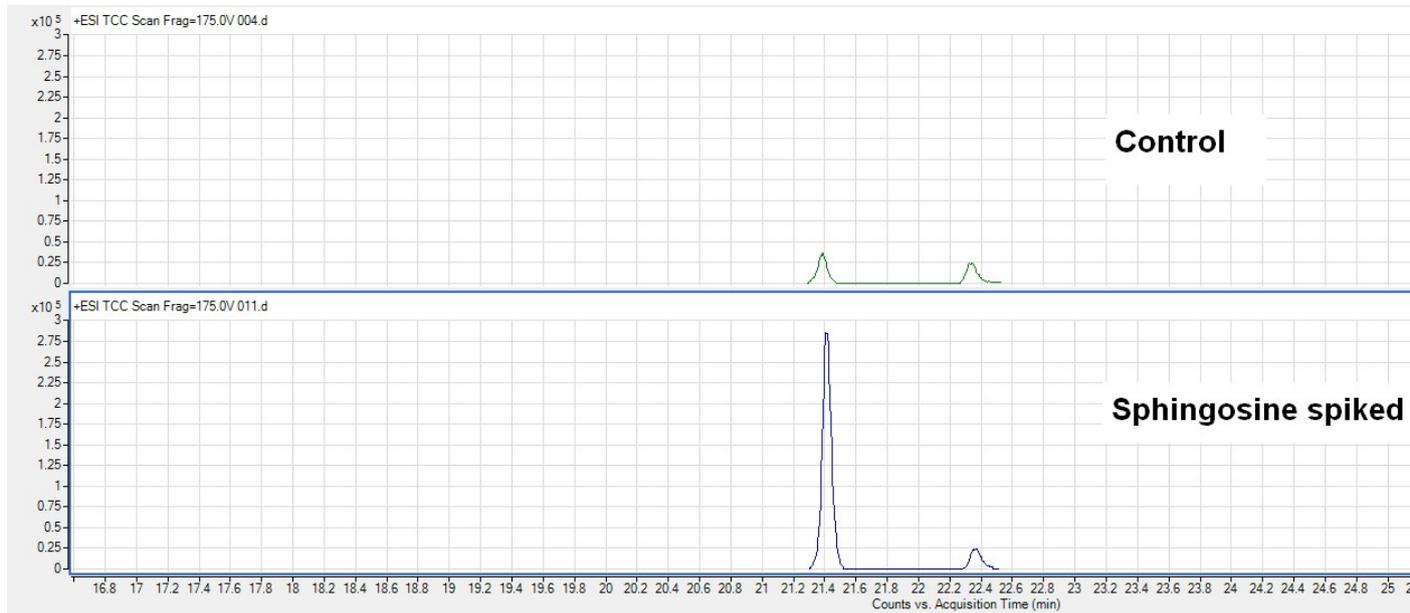


Figure 7. Fragmentation pattern for m/z 300.2892 in control group sample.

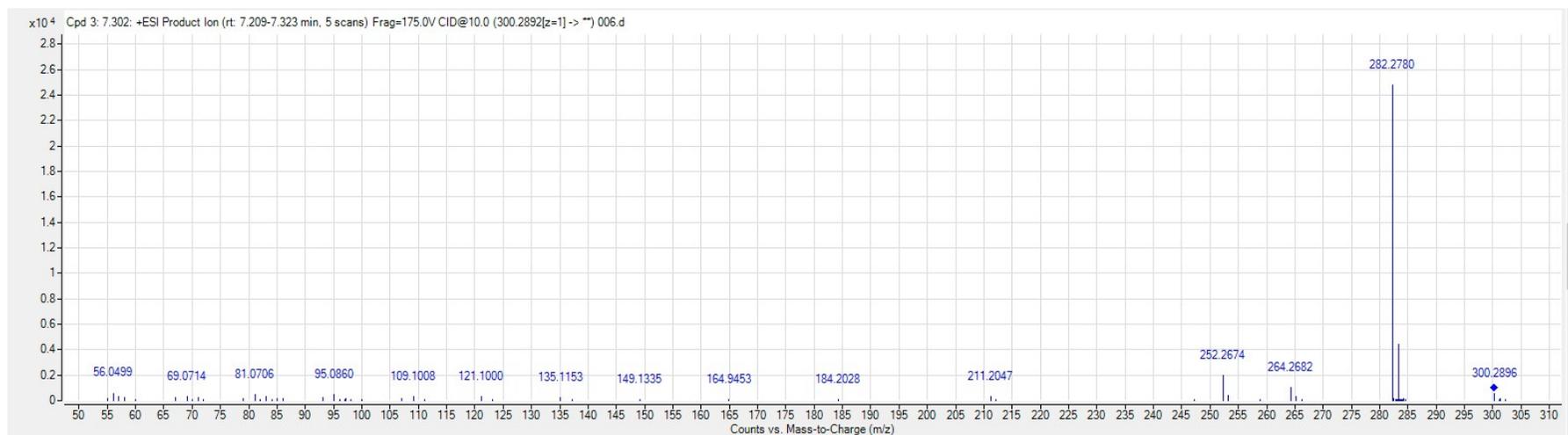


Figure 8. Fragmentation pattern for m/z 300.2892 in sphingosine standard.

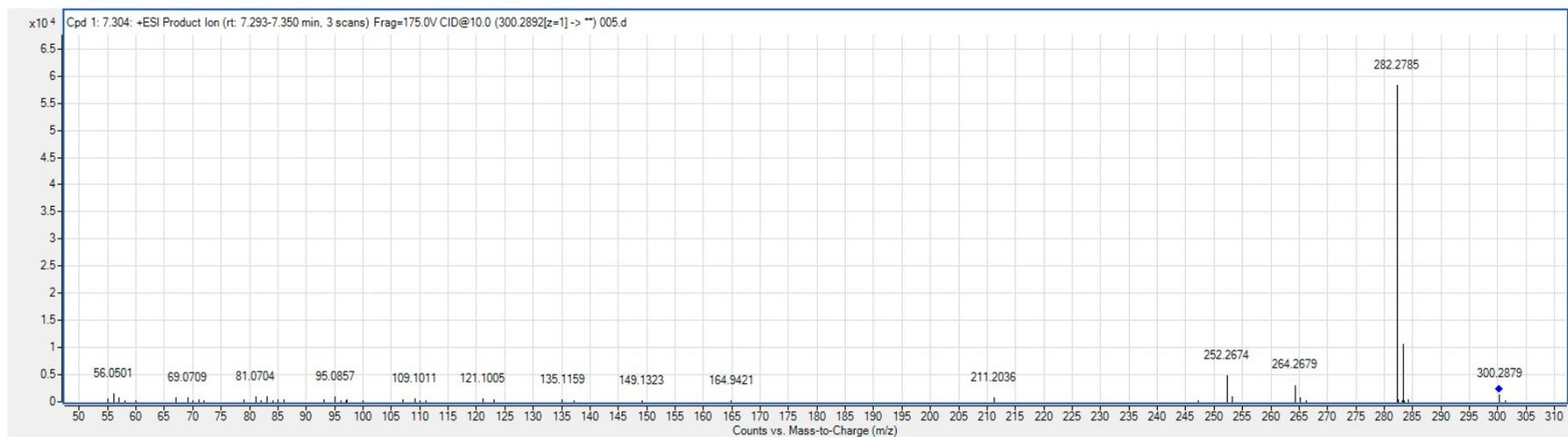


Figure 9 Abundance of sphingosine in the multiple sclerosis, neuropathic pain, multiple sclerosis with neuropathic pain and control group.

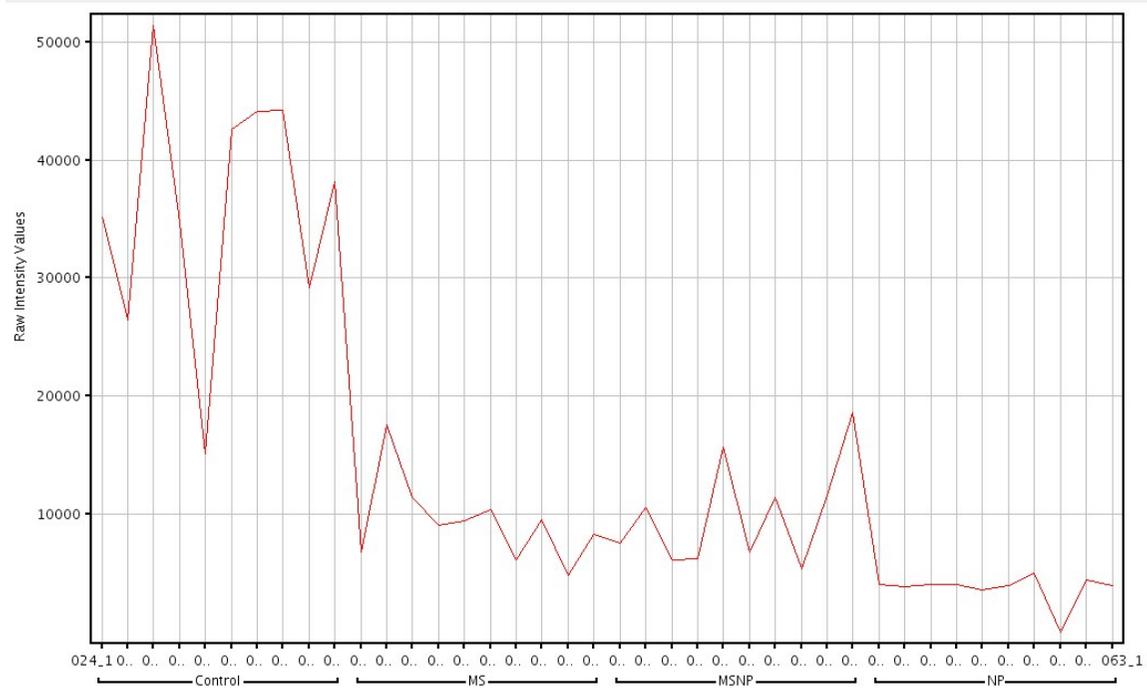


Figure 10. EIC's of m/z 302.30536 in dihydrospingosine, control sample and spiked control sample.

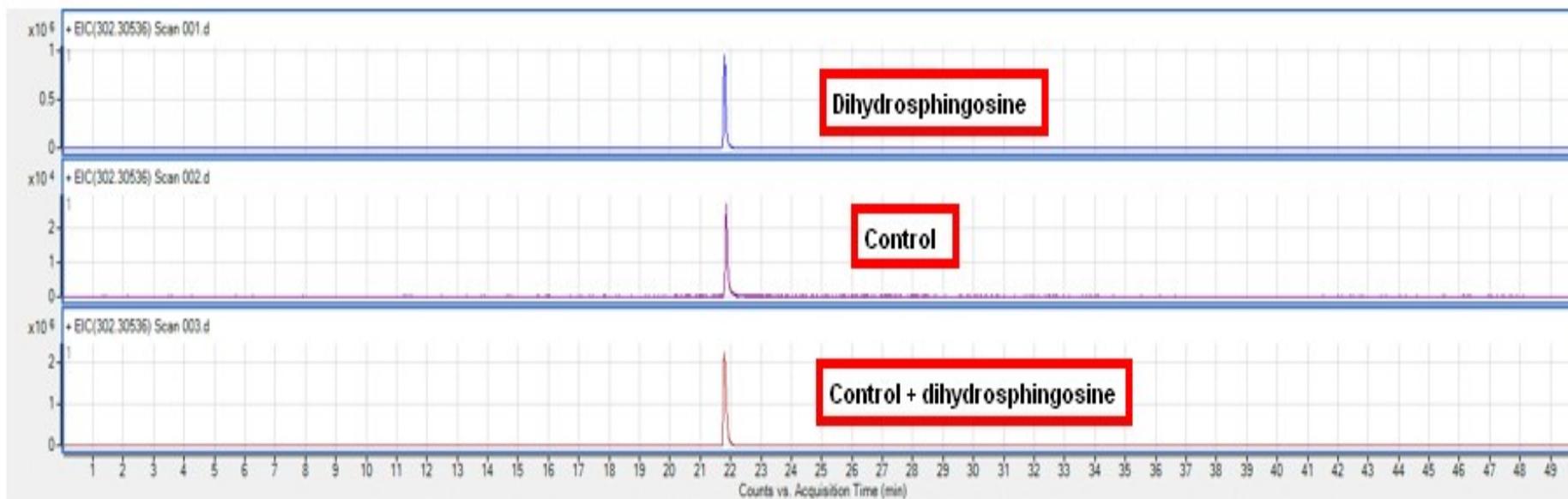


Figure 11. Fragmentation pattern for m/z 302.30536 in control and dihydrosphingosine standard sample.

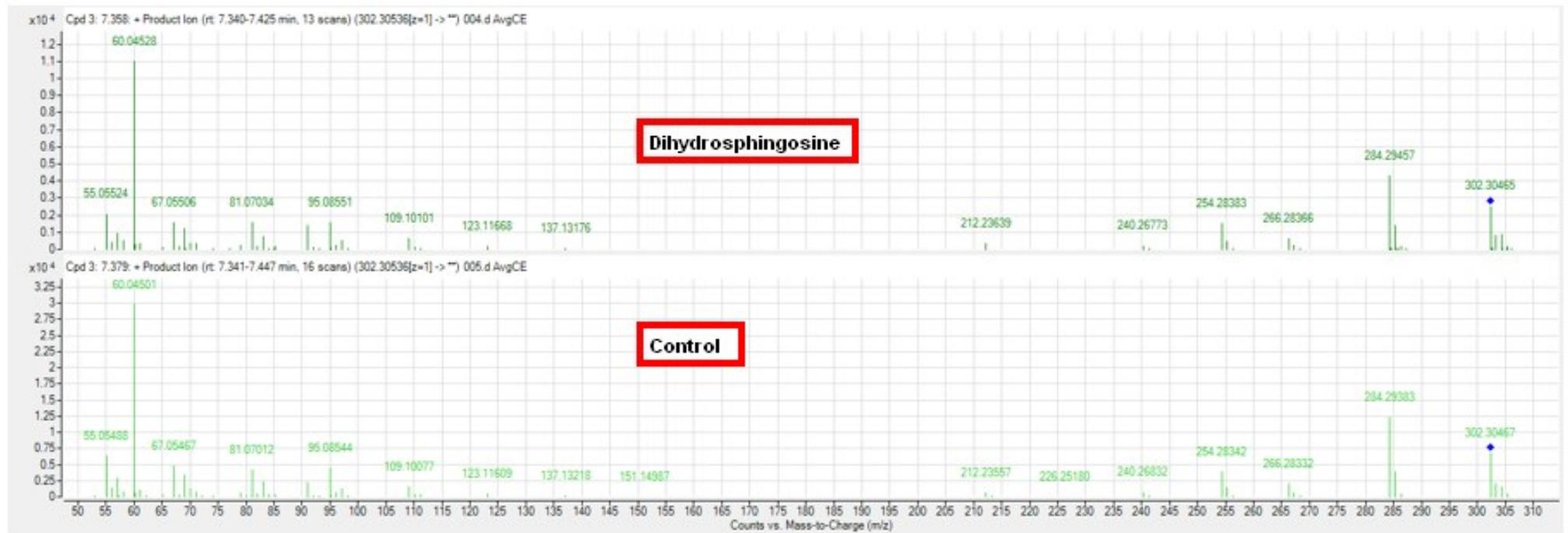


Figure 12. Sphingosine peak area for large sample set of control (blue), multiple sclerosis (red) and multiple sclerosis with neuropathic pain (green) groups.

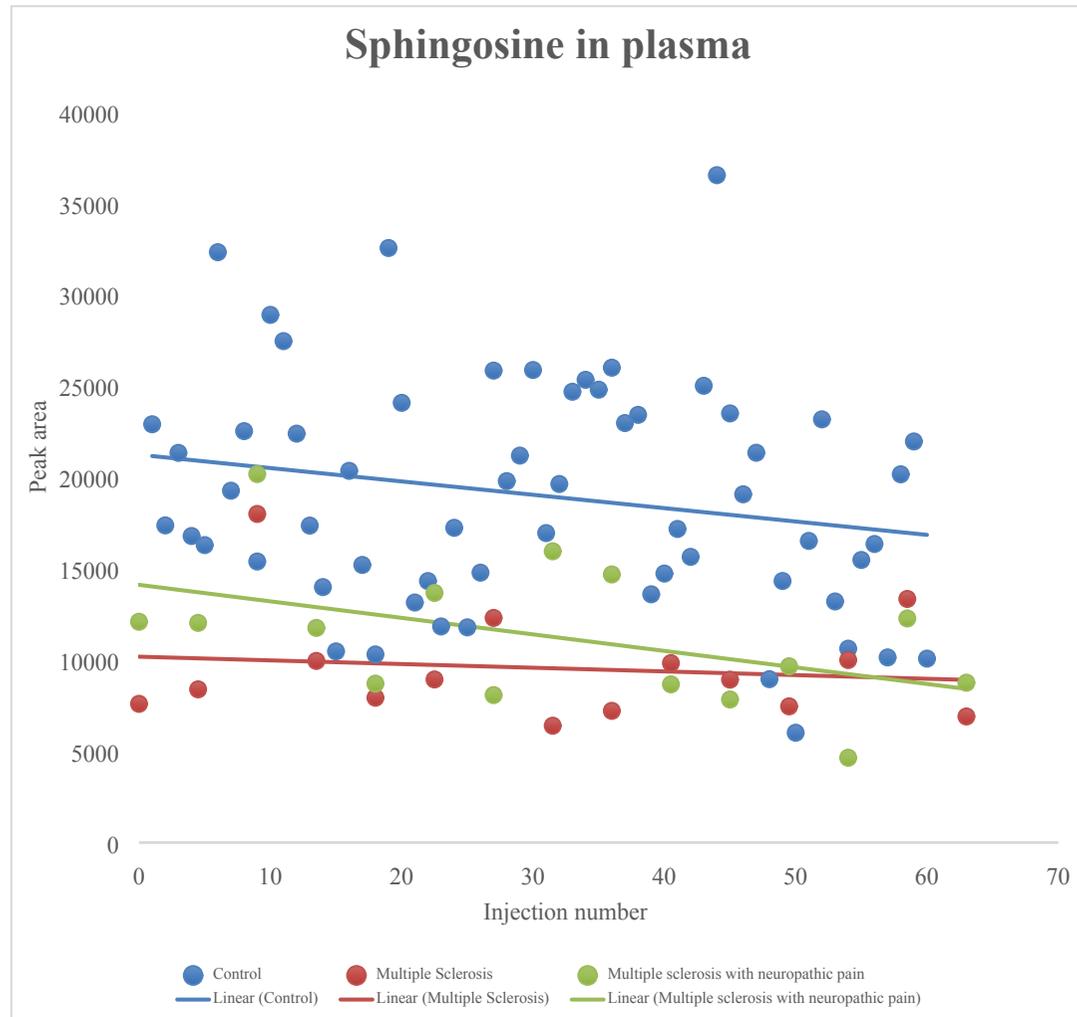


Table: Multiple sclerosis LC-MS conditions

HPLC-MS					
Instrument	6530 Q-TOF				
Column	C ₁₈ 1.8um 2.1X100mm				
Oven (°C)	35				
Pump	Mobile Phase A	Water 0.1% formic acid			
	Mobile Phase B	Acetonitrile 0.1% formic acid			
	Flow (ml/min)	0.5			
	Isocratic/Gradient	Gradient			
		Time/min	%A	%B	
		0.00	98	2	
		1.00	98	2	
		30.00	0	100	
		35.00	0	100	
	35.1.10	98	2		
	40.00	98	2		
	Runtime (min)	40			
Injector	Volume (ul)	10			
Detector	Wavelength Reference	N/A			
MS	QTOF/QQQ	QTOF/QQQ	QTOF	Mode	positive
	Source Duel jet stream Electrospray	Gas temp (°C)	300	Gas flow (l/min)	8
		Sheath gas temp (°C)	350	Sheath gas flow (l/min)	10
		Nebuliser pressure (psig)	35		
		VCap (V)	3750	Fragmentor (v)	175
		Nozzle Voltage (V)	1000		
		Mass range	100-1700	Acquisition rate (Scans/s)	3

