Brain Lipidomics

A MALDI mass spectrometry study and the application of related techniques

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Why Lipid?

High abundance in the brain: ~ 50% dry weight

Structural support: cytoplastic and organelle membranes, intracellular compartmentalization

Form functional membrane domains -- lipid rafts : functional regulation of membrane proteins

Altered levels of lipids in the brain are associated with several diseases such as Alzheimer's disease, neuronal ceroid-lipfuscinosis, and Niemann-Pick disease.

Mediate signal transduction (anandamides, PI, PIP2, etc.)

One of the mediators involved in drug abuse (C.J. Hillard 2005 review)

Three major categories of lipid in the brain:

Cholesterol:

Sphingolipids:

sphingomyelin (SM)
sulfatides (ST)
cerebrosides
gangliosides

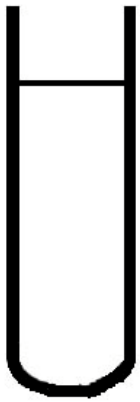
Glycerophospholipids:

phosphatidylcholines (PC)
phospshatidylethanolamines (PE)
phosphatidylinositols (PI)
phosphatidylserine (PS)

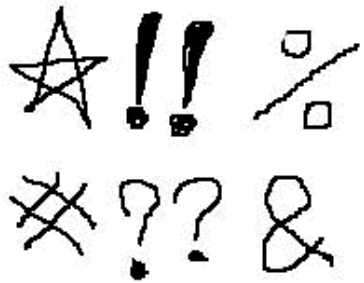
How was it usually done? Folch extraction:

(Rat) Brain

+

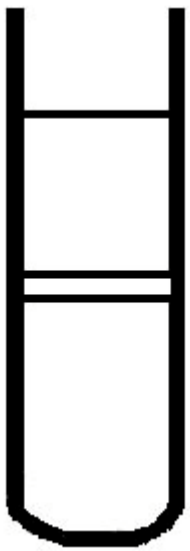


Chloroform + MeOH



Homogenization

Centrifugation
& handling



Aqueous layer
(gangliosides and
hydrophilic lipids)

Organic layer
(polar lipids & goodies)

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Lipid analysis after extraction:

Conventional ways: chromatography-based methods:

TLC, GC, HPLC etc.

Advantages: some degree of separation, easy to setup and operate

Recent advancements: mass spectrometry-based approach:

ESI-MS(/MS), LC-MS(/MS), MALDI-MS(/MS)

Advantages: accuracy, provide detailed species information: total fatty acid chain length, number of double-bonds, etc.

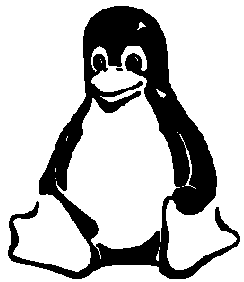
...but a common disadvantage in Folch extraction:

Lack of histological correlations

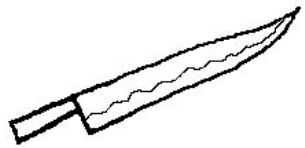
To address the lack of histological correlations:

1. Carefully dissect the regions of interest for Folch extraction
2. (Immuno-) Histochemistry stainings (gangliosides)
3. *In situ* MALDI-MS analysis:

(Rat) Brain

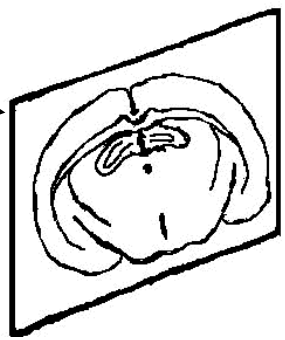


Snap-frozen

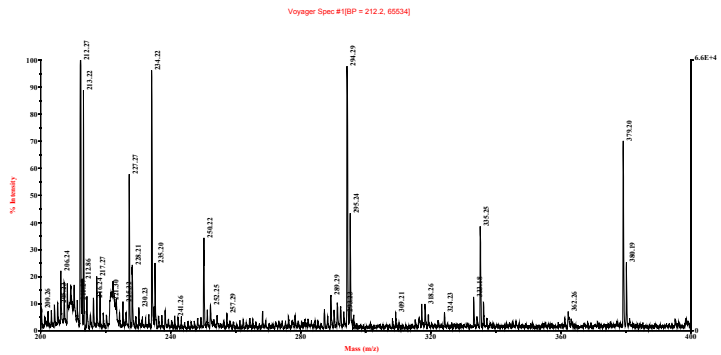


Cryosection

Stainless Steel target plate



Section collection, handling, & matrix deposition



MS or MS/MS analysis

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Cautions in handling the sections for *in situ* MALDI-MS analysis:

Freeze the brain/tissue ASAP.

Avoid low mass interference from various sources (OTC, blade oil, etc.)

Avoid "*in situ* extraction" process in the name of enhancing ion signals.

Reduce matrix spot size as much as practically possible to enhance histological resolution.

Results:

Whole brain extract vs. *in situ* analysis:

Positive Ion Mode MALDI-MS

Negative Ion Mode MALDI-MS

PC 34:1

Lipid species Acyl chain length : No. of double bond

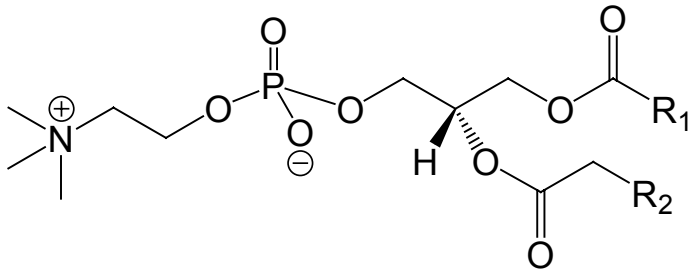
PC, PE, PI, and PS species: number corresponds to the total length and number of double bonds of both acyl chains.

SM and ST species: number corresponds to the length and number of double bonds of the acyl chain attached to the sphingosine base.

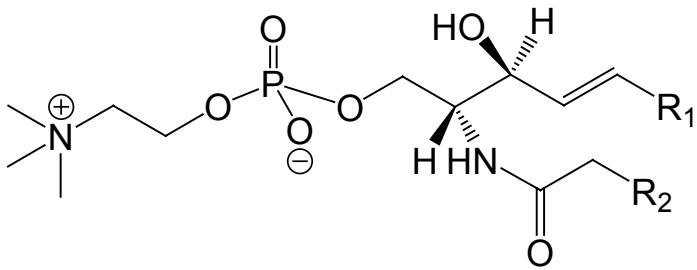
Positive Ion Mode MALDI-MS

Expected major lipid species:

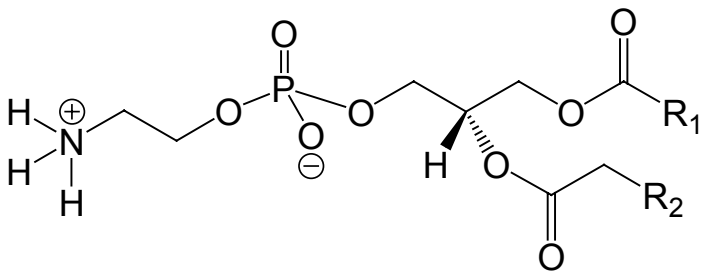
Phosphatidylcholine (PC)



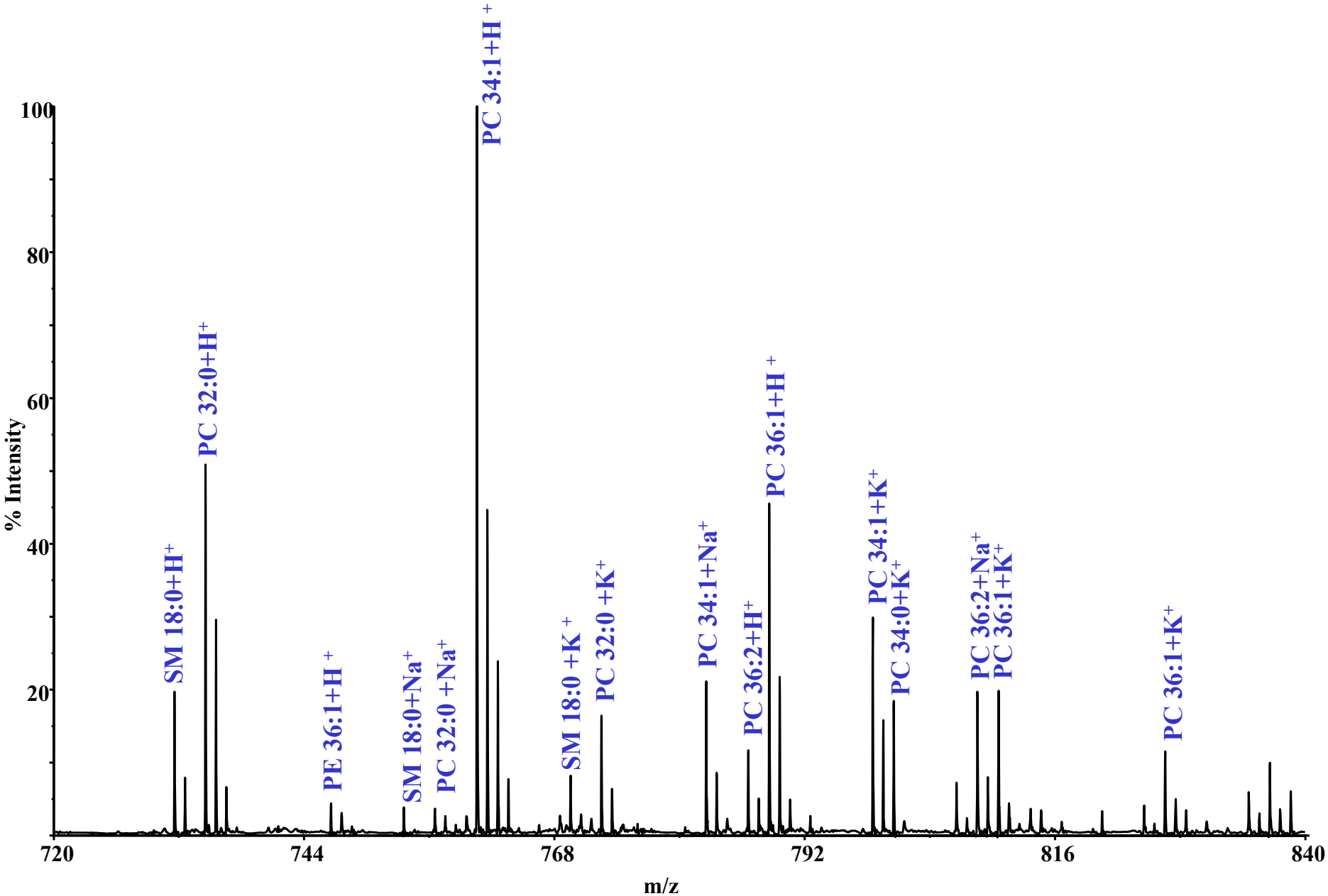
Sphingomyelin (SM)



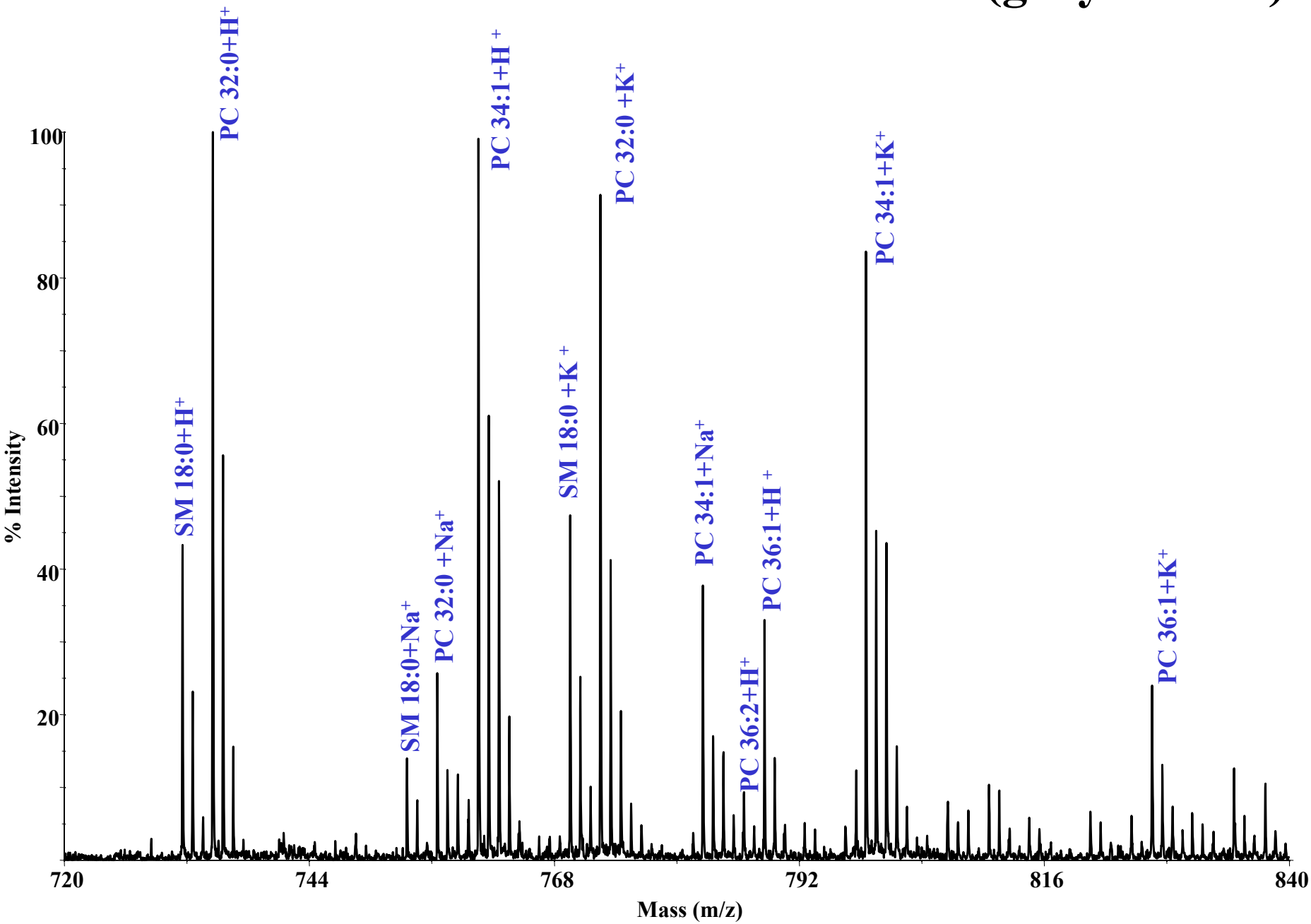
Phosphatidylethanolamine (PE)



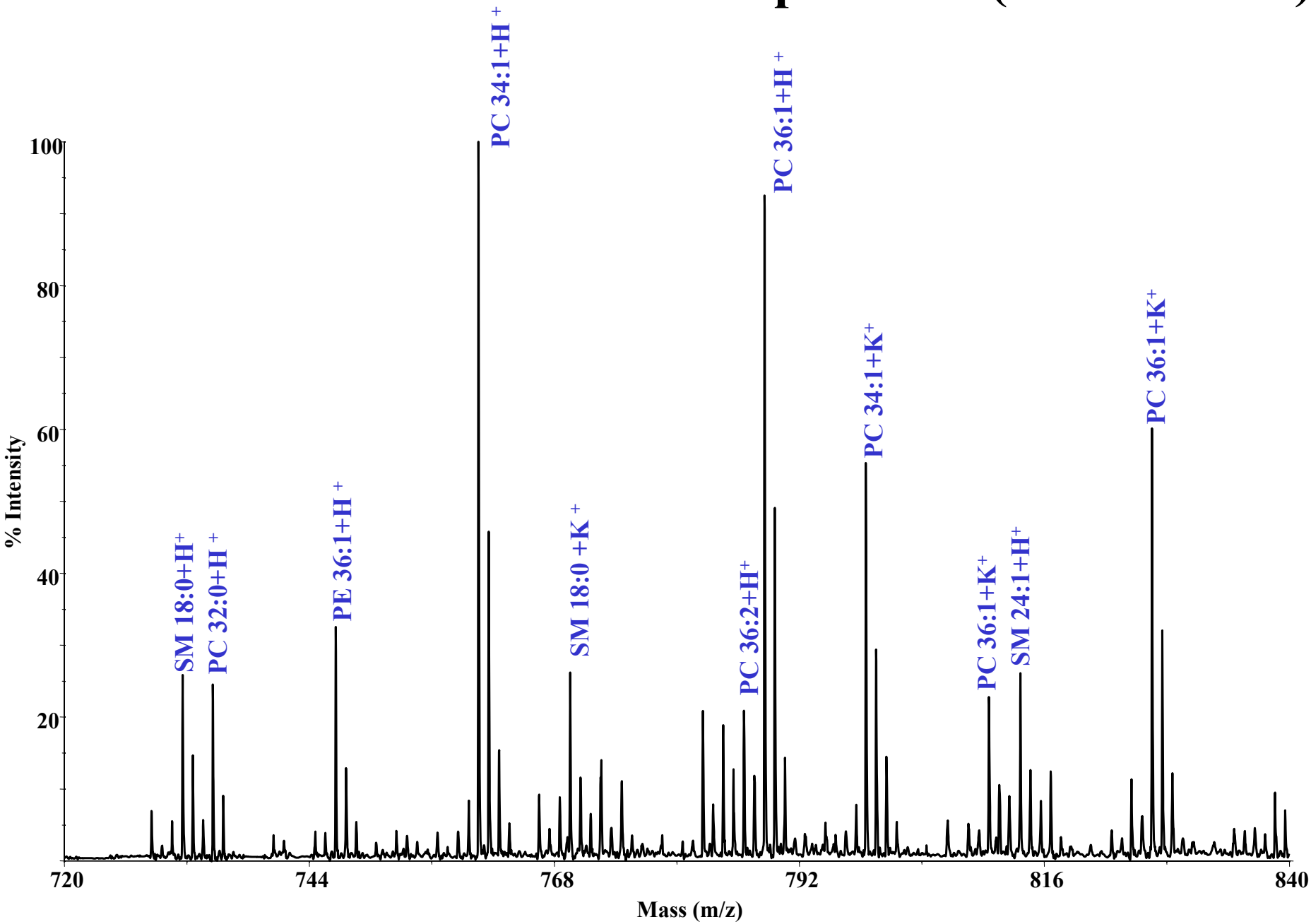
Whole rat brain extract



Cerebellar cortex (gray matter)



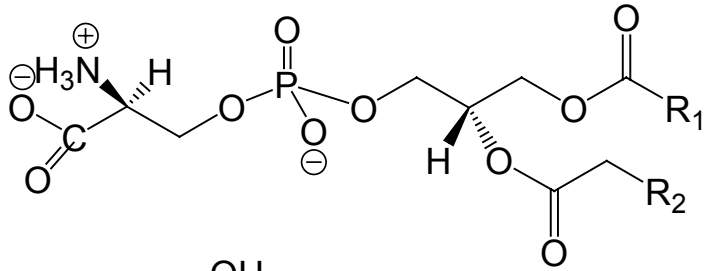
Cerebellar peduncle (white matter)



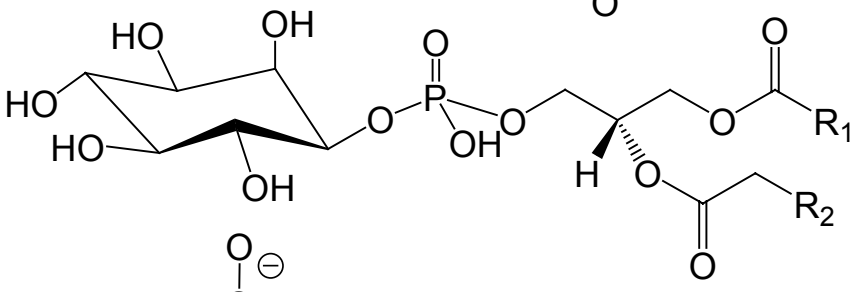
Negative Ion Mode MALDI-MS

Expected major lipid species:

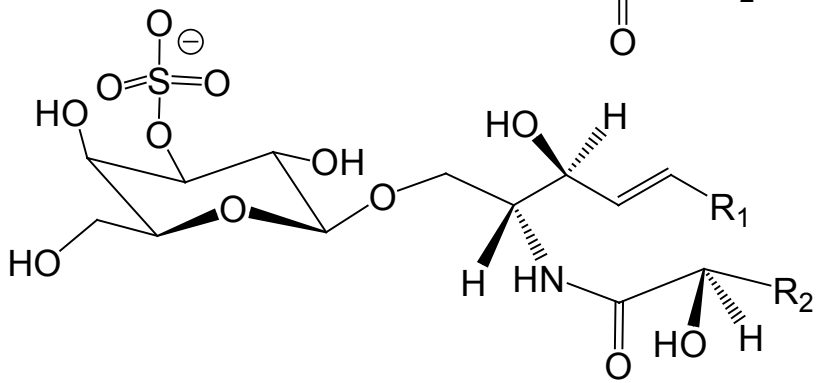
Phosphatidylserine (PS)



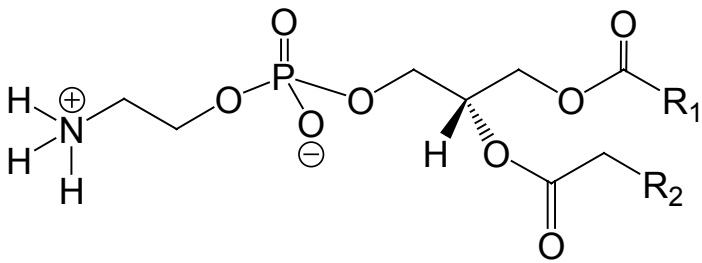
Phosphatidylinositol (PI)



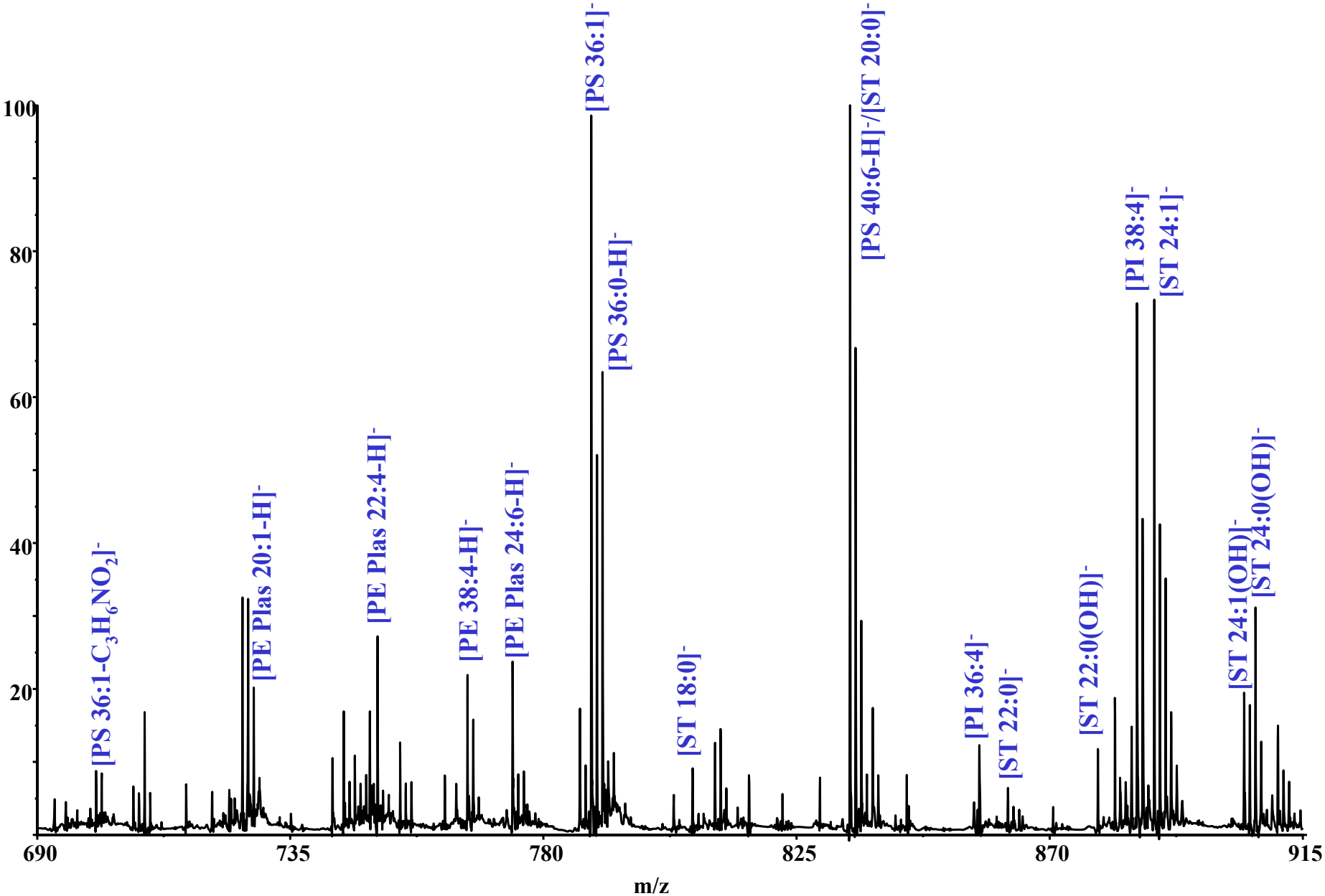
Sulfatide (hydroxylated; ST)



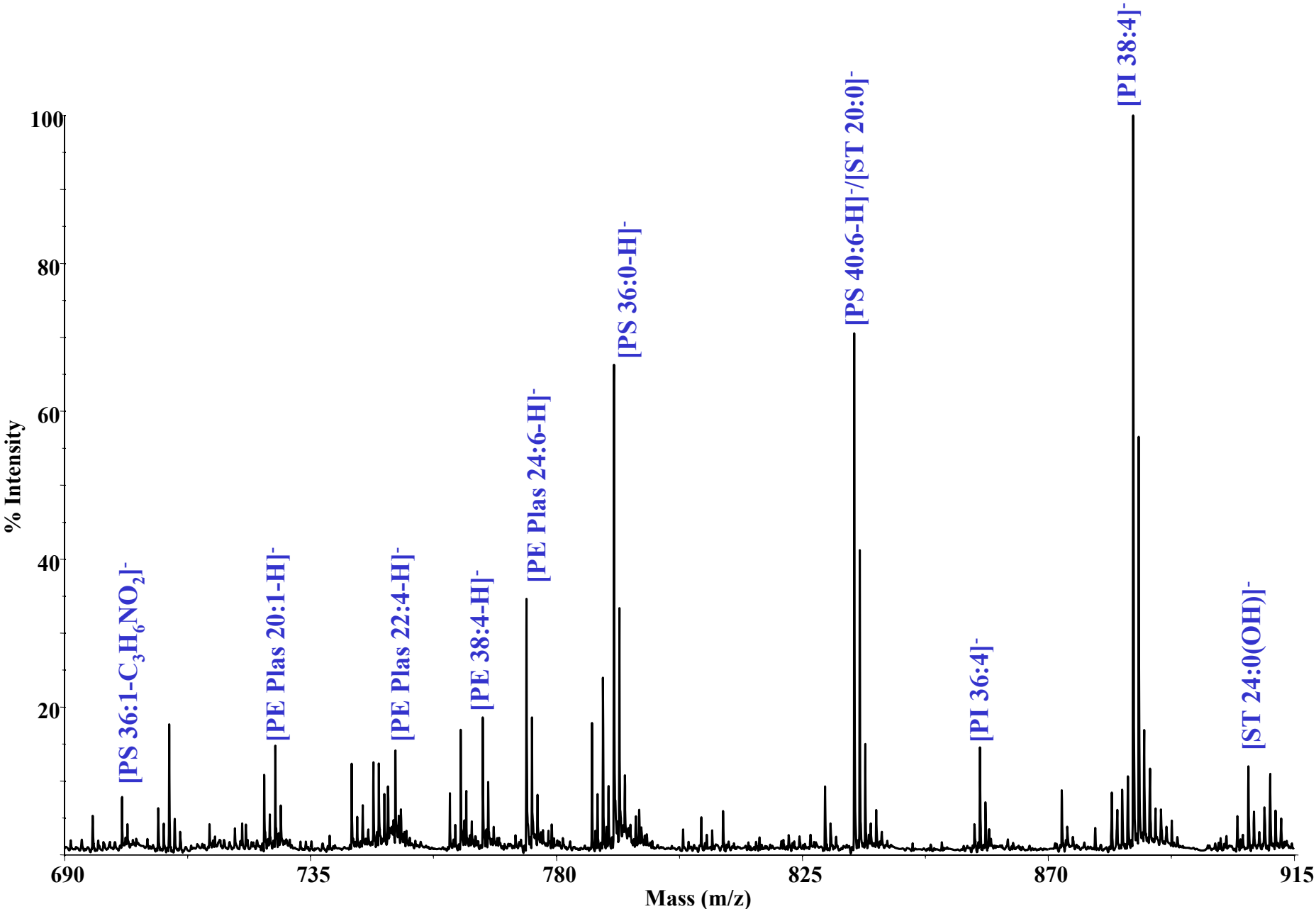
Phosphatidylethanolamine (PE)



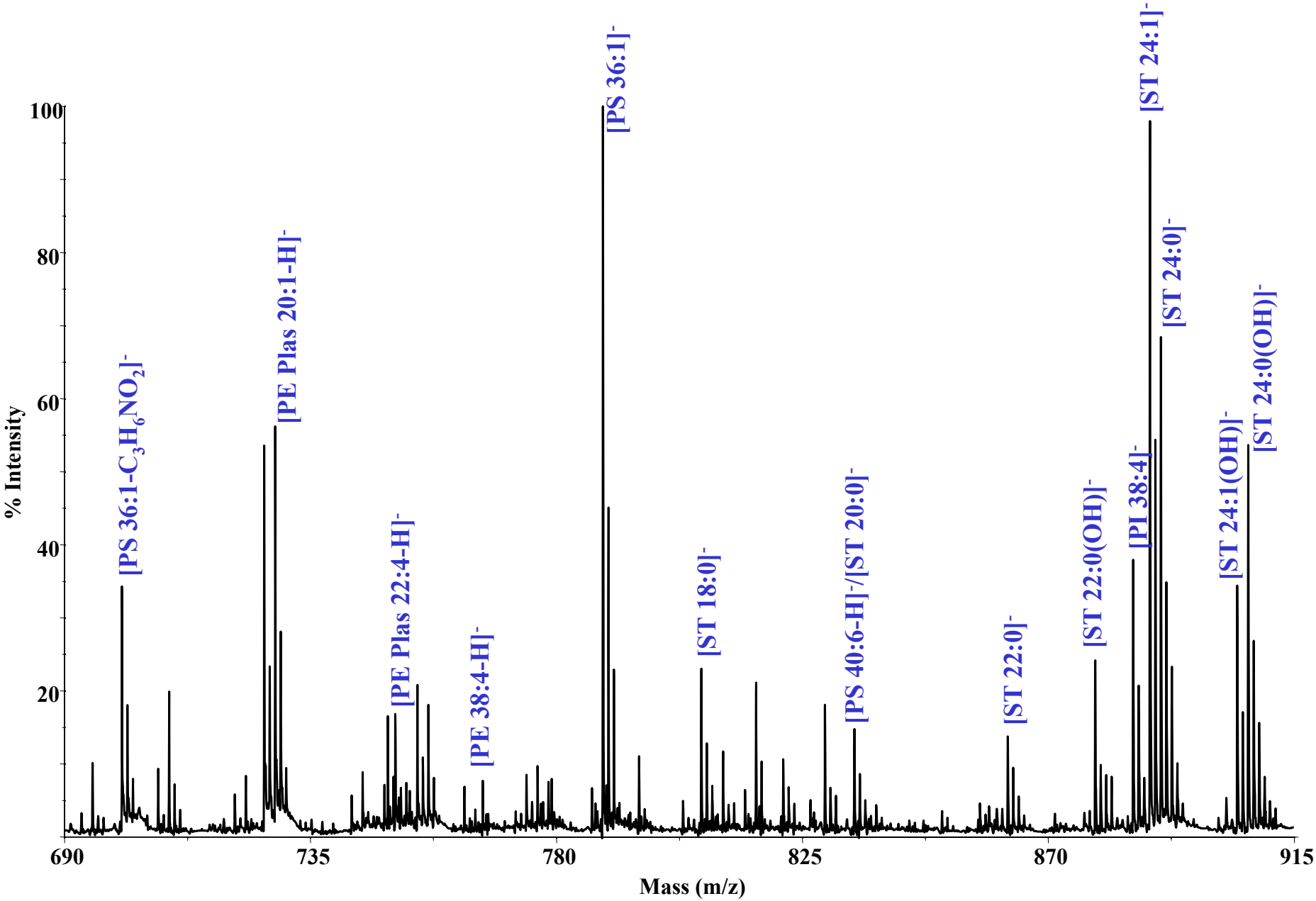
Whole rat brain extract



Cerebellar cortex (gray matter)



Cerebellar peduncle (white matter)



"……Mass does not equal structure. Mass spectrometric studies could not resolve the structures of the lipids!....."

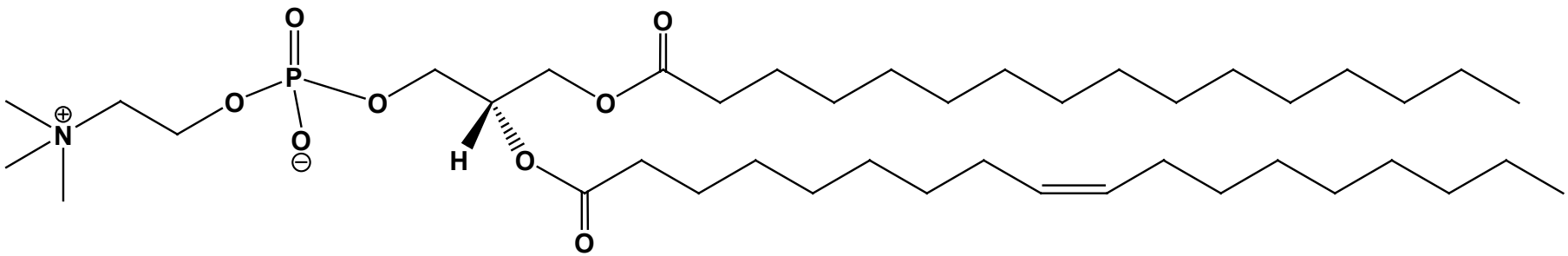
Comment from the editorial office
Lipid Research

(#&$%*!.....Really, eh?!)

See: Pulfer and Murphy, Mass Spectrometry Reviews 2003.

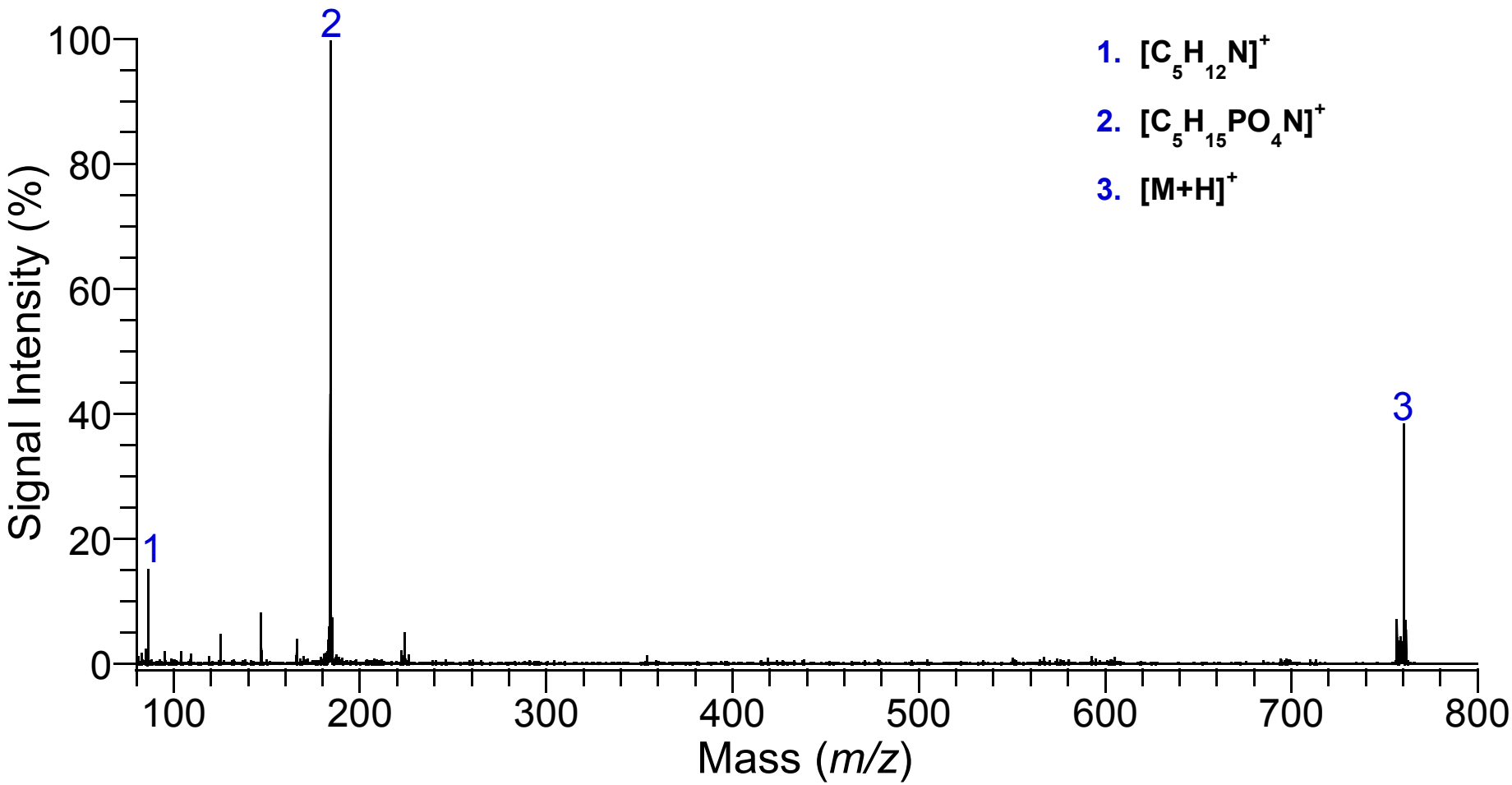
Positive Ion mode:

Phosphatidylcholine 34:1
PC 34:1, $[M+H]^+$ = 760.58

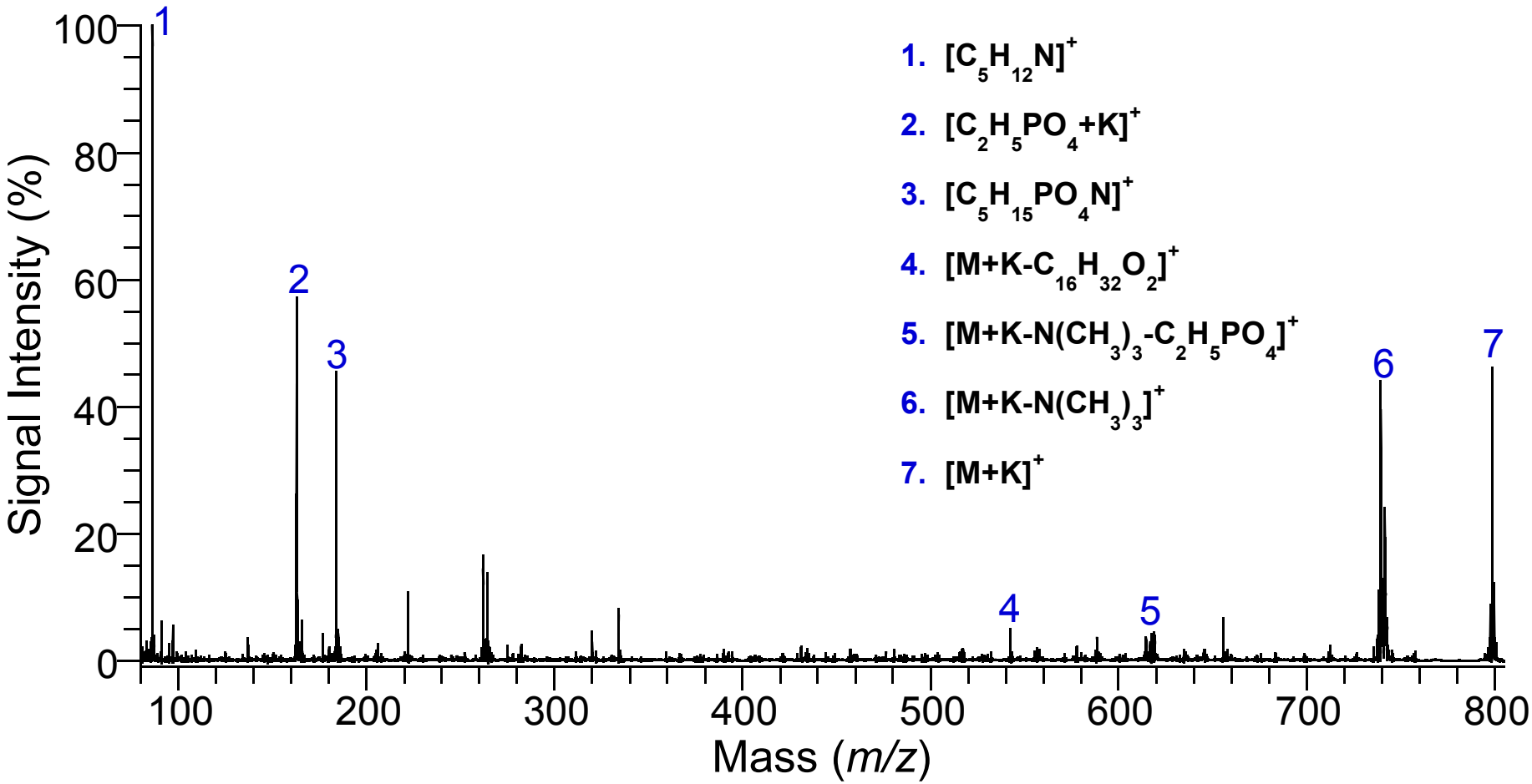


Positive Ion MS/MS of PC 34:1 from rat cerebellum

MS/MS of $[PC\ 34:1+H]^+$

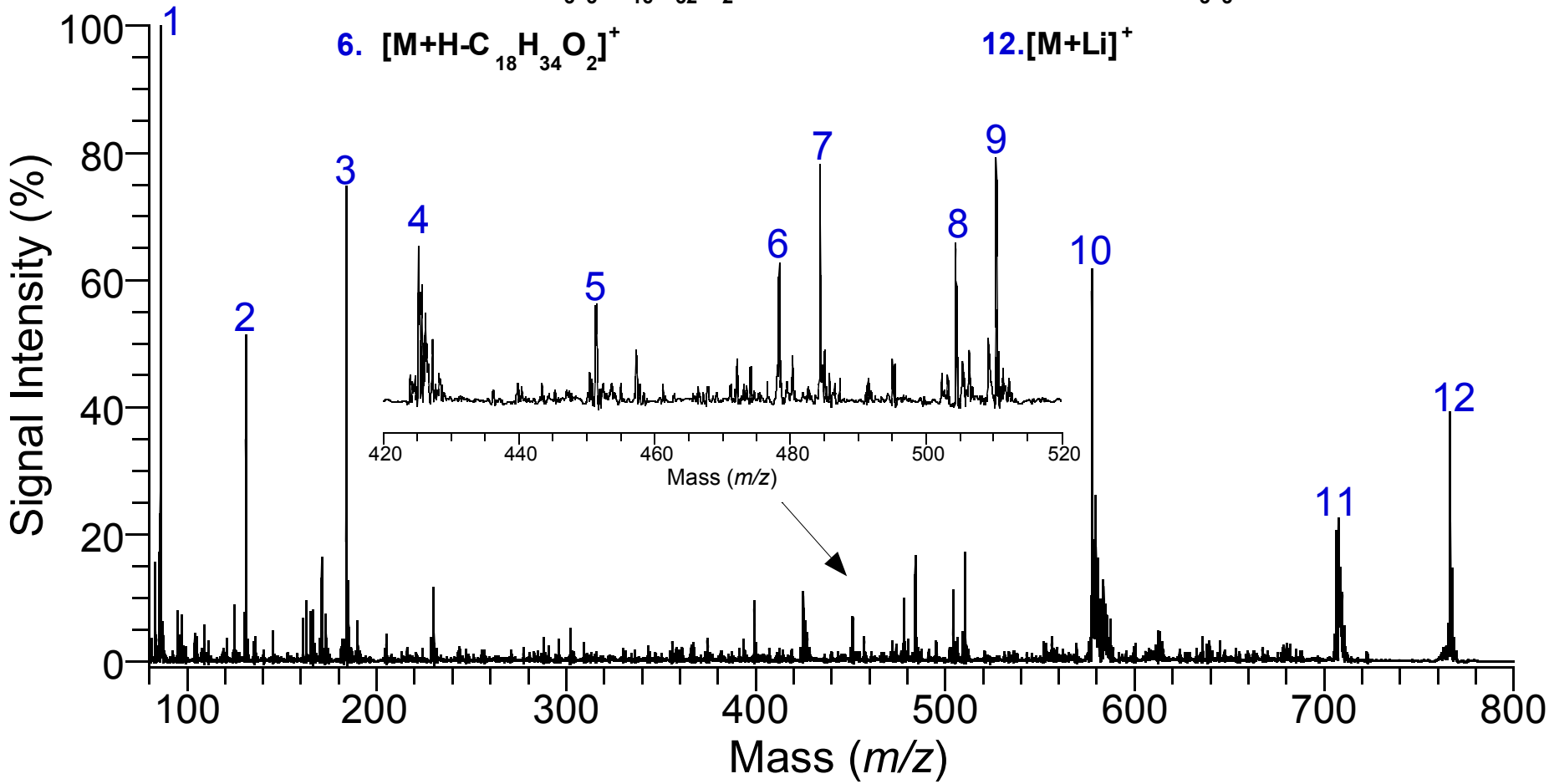


MS/MS of $[PC\ 34:1+K]^+$



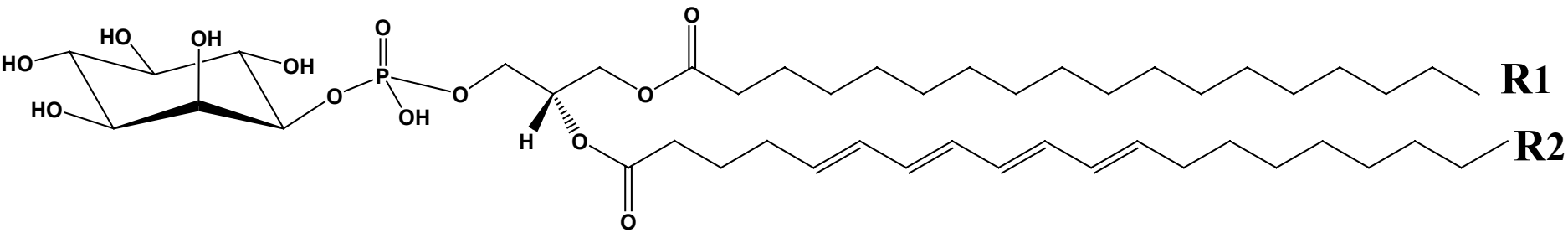
MS/MS of $[PC\ 34:1+Li]^+$

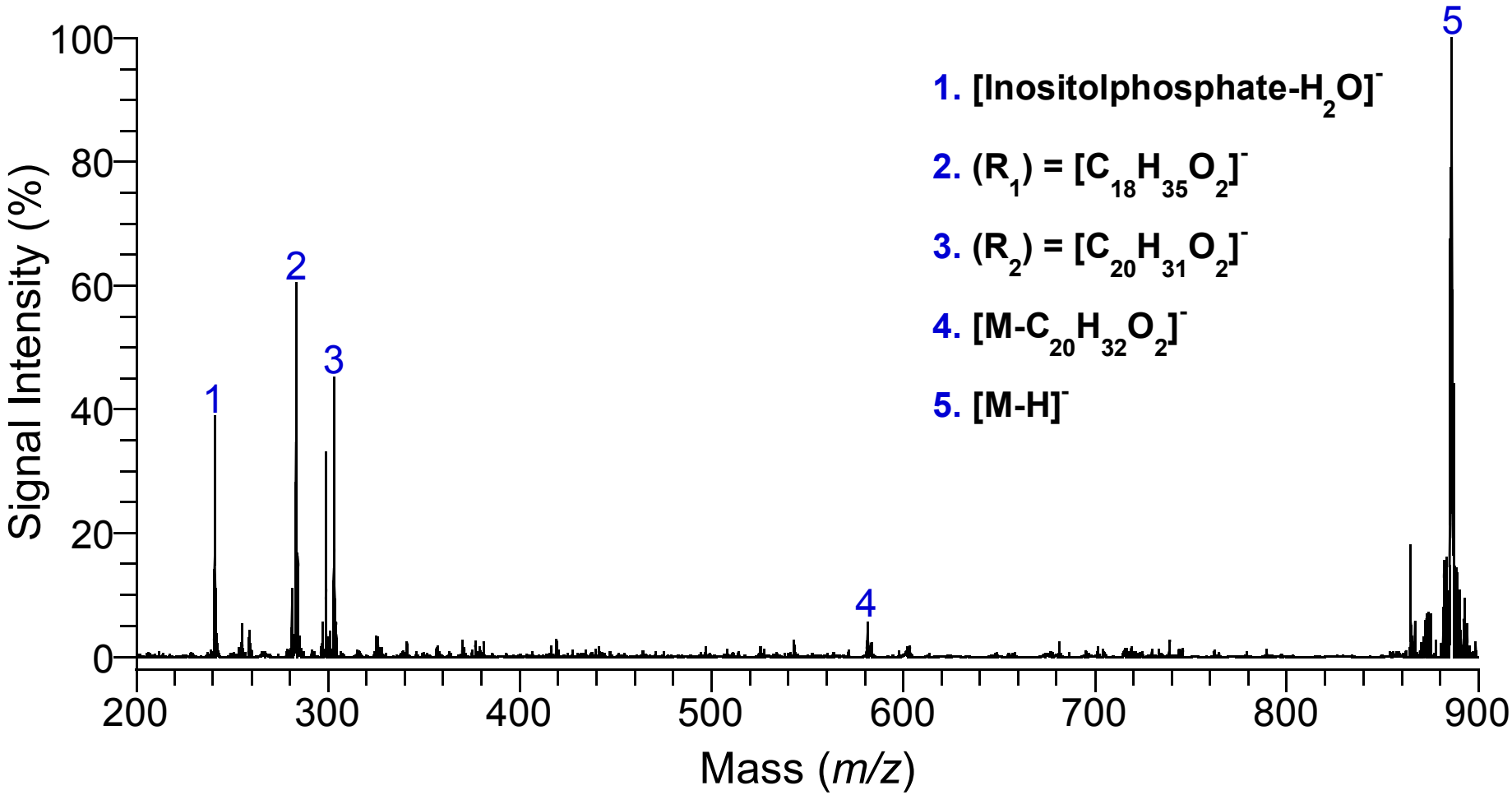
1. $[C_5H_{12}N]^+$
2. $[C_2H_5PO_4+Li]^+$
3. $[C_5H_{15}PO_4N]^+$
4. $[M+Li-N(CH_3)_3-C_{18}H_{34}O_2]^+$
5. $[M+Li-N(CH_3)_3-C_{16}H_{32}O_2]^+$
6. $[M+H-C_{18}H_{34}O_2]^+$
7. $[M+Li-C_{18}H_{34}O_2]^+$
8. $[M+H-C_{16}H_{32}O_2]^+$
9. $[M+Li-C_{16}H_{32}O_2]^+$
10. $[M+H-N(CH_3)_3-C_2H_5PO_4]^+$
11. $[M+Li-N(CH_3)_3]^+$
12. $[M+Li]^+$



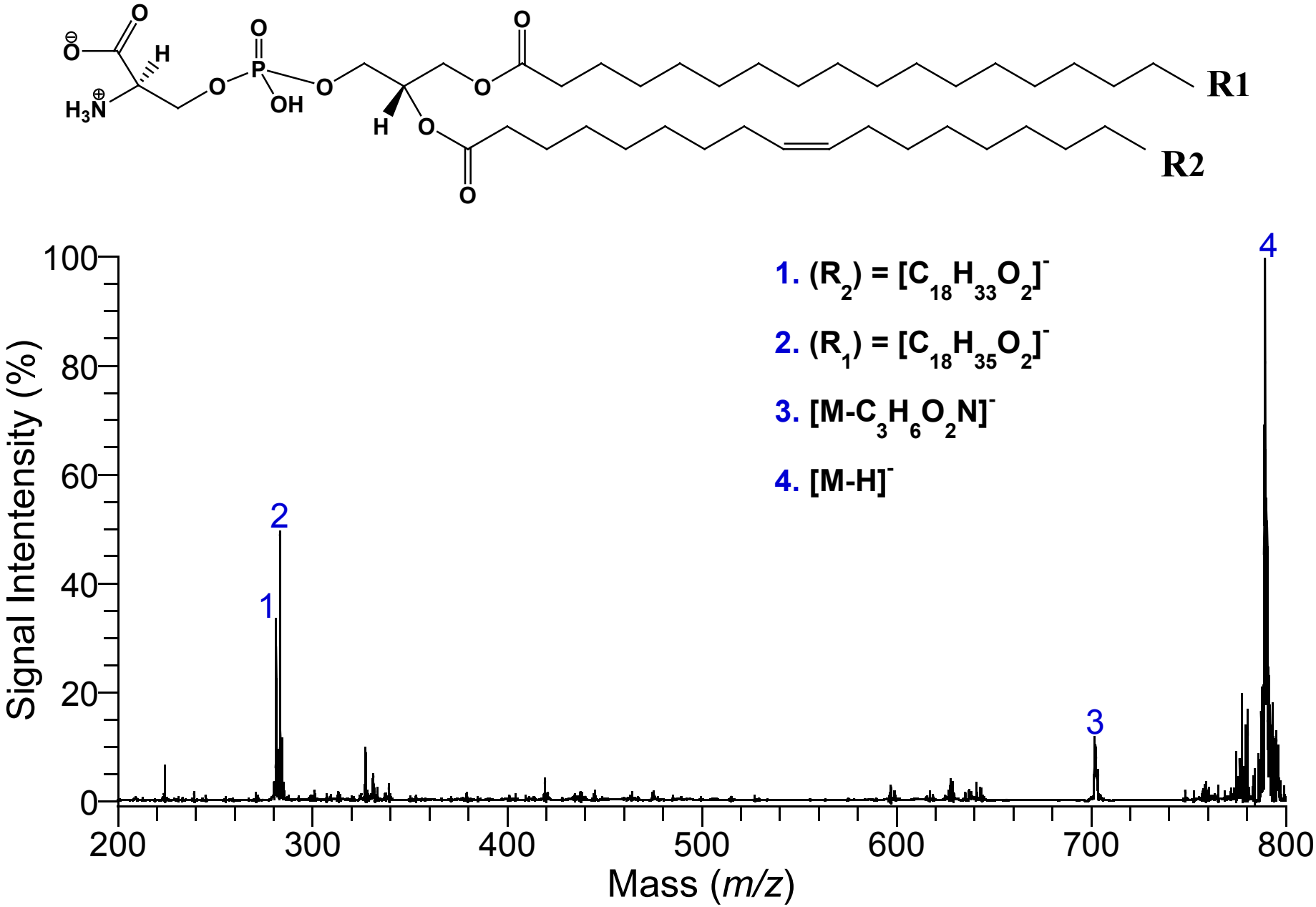
Negative Ion MS/MS of PI, PS, and ST species from rat cerebellum

18:0-20:4 Phosphatidylinositol (PI 38:4)

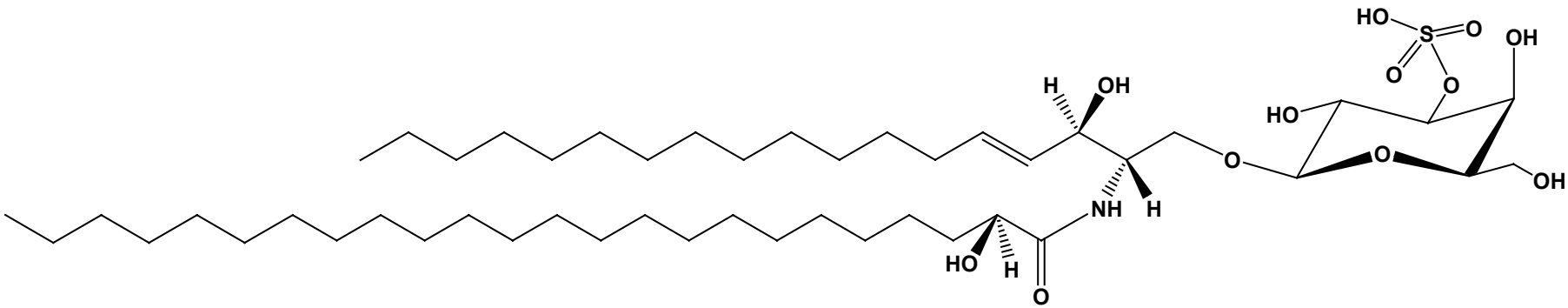


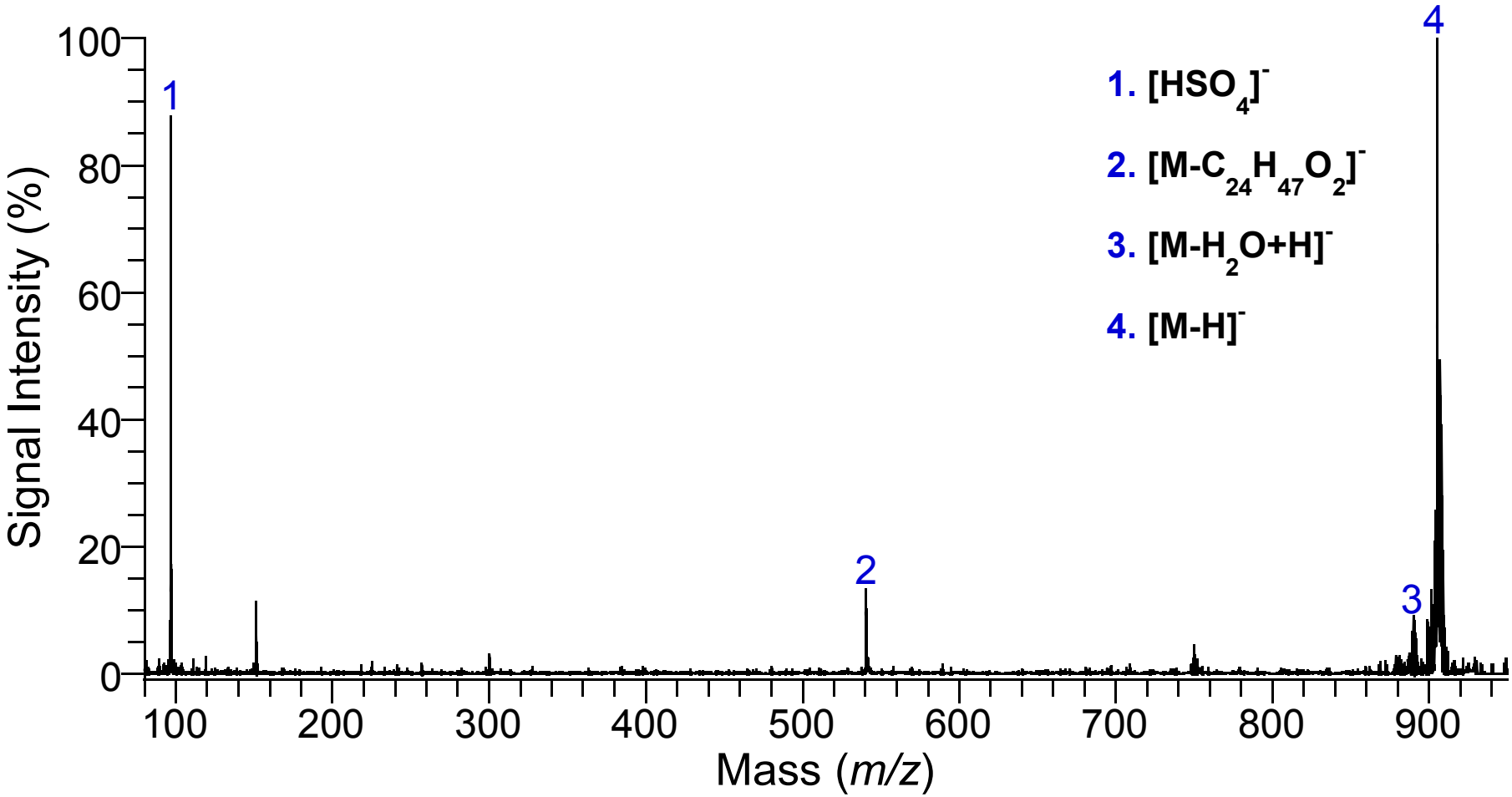


18:0-18:1 Phosphatidylserine (PS 36:1)

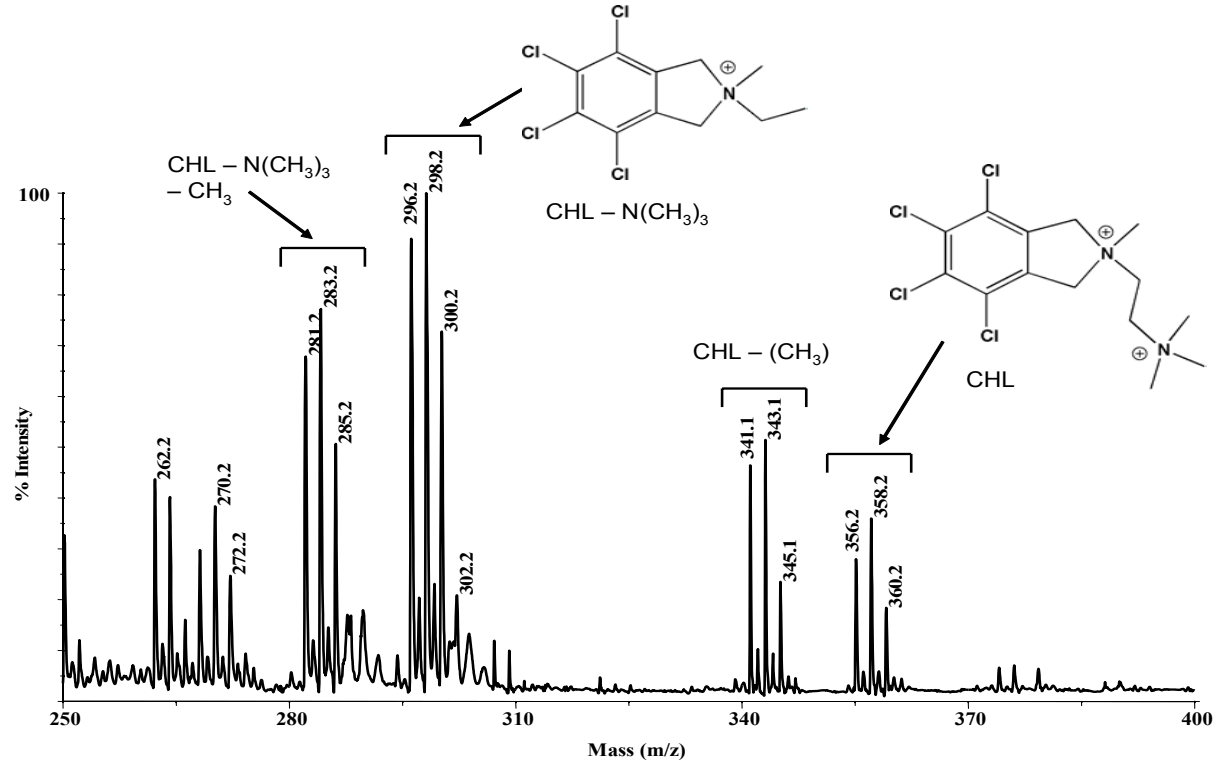


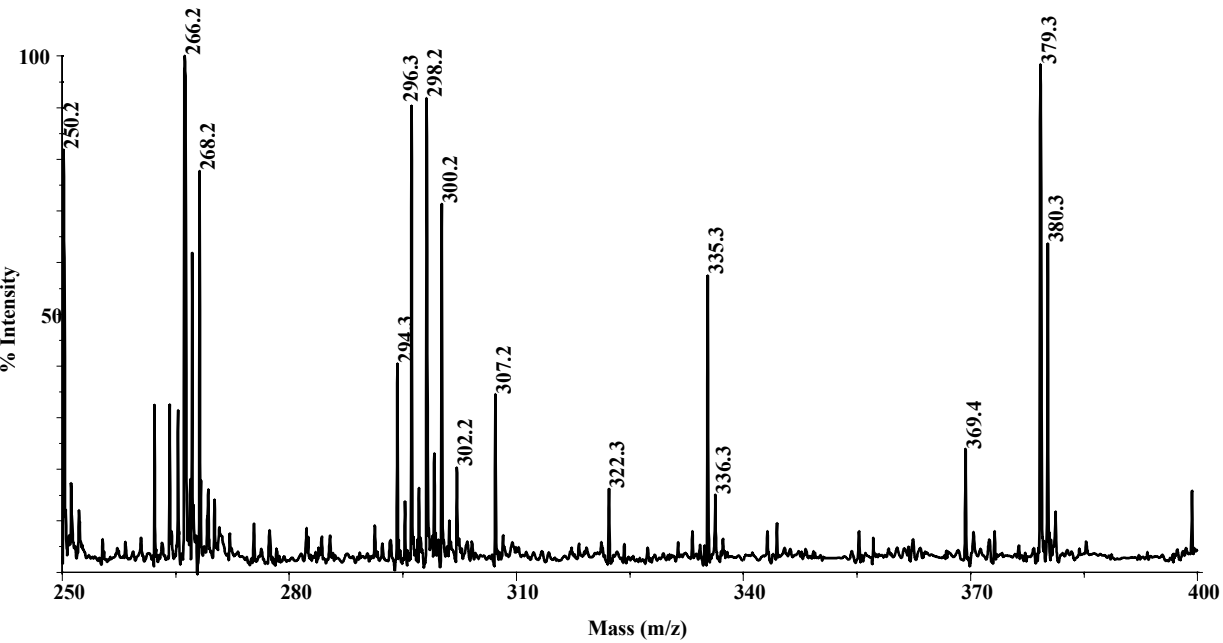
24:0(OH) Sulfatide





What else can we do with this technique?

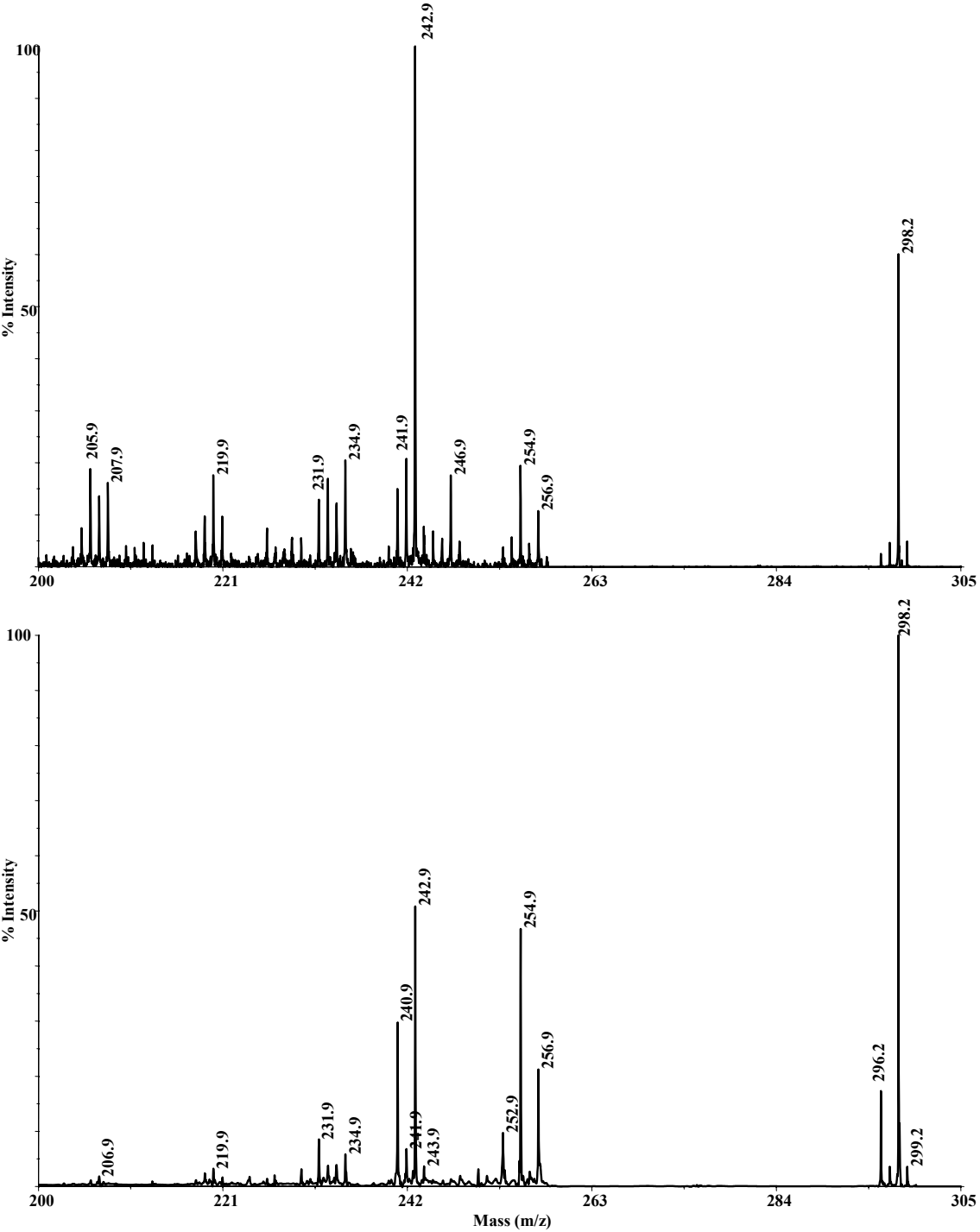




Drug localization and distribution:

Top:
Chlorisondamine (CHL) drug standard

Bottom:
Rat ventral tegmental area (VTA), 1week after injecting 20 µg freebase CHL into nucleus accumbens



MALDI-MS/MS of m/z 298.2:

Top: drug standard

Bottom: Rat ventral tegmental area (VTA), 1week after injecting 20 µg freebase CHL into nucleus accumbens

Conclusions:

Mass spectral lipid profiles obtained from rat cerebellum were in qualitative agreement with traditional lipid analysis techniques for most lipid classes.

MALDI-MS can rapidly analyze the samples with little sample consumption and preparation compared to the traditional extraction or immunohistochemistry methods.

MALDI-MS/MS can successfully carry out structure-based lipid species identification.

With small changes in matrix selection and instrument tuning, MALDI-MS(/MS) can be used to analyze peptide and protein *in situ*.

Application in drug distribution, tissue imaging, and related studies.

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