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Dual Parallel Mass Spectrometers for Analysis of Sphingolipid, Glycerophospholipid and Plasmalogen Molecular Species

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Analysis of phospholipids was performed using a liquid chromatographic separation with two mass spectrometers in parallel providing electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) data simultaneously from a triple quadrupole instrument and a single quadrupole instrument, respectively. The output from UV-Vis and evaporative light scattering detectors were also acquired by the two mass spectrometers, respectively, for four detectors overall. This arrangement was used to identify and calculate area percents for molecular species of dihydrosphingomyelin (DHS) and sphingomyelin (SPM) in commercially available bovine brain SPM, in human plasma extract and in porcine lens extract. Molecular species of phosphatidylethanolamine and its plasmalogen, and phosphatidylcholine and its plasmalogen were identified and semi-quantitative analysis performed. Commercially available bovine brain SPM was found to contain 11.5% DHS and 88.5% SPM. The only DHS molecular species identified in human plasma was 16:0-DHS, at or below 1% of the sphingolipid content. Porcine lens membranes were found to contain 14.4% DHS and 85.6% SPM. Other findings reported here include: (1) phospholipids were found to undergo dimerization in the electrospray source, giving masses representing combinations of species present. (2) Triacylglycerols gave usable mass spectra under electrospray ionization conditions, as well as under APCI-MS conditions. (3) Triacylglycerols gave ammonium adducts as base peaks in their APCI mass spectra, which reduced fragmentation and increased the proportions of molecular ions. (4) Mass spectra were obtained for phospholipids which underwent both protonation and sodium adduct formation in different chromatographic runs. © 1998 John Wiley & Sons, Ltd. This paper was prepared under the auspices of the US Government and it is therefore not subject to copyright in the US.

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Dihydrosphingomyelins (DHSs), which differ from sphingomyelins (SPMs) only by the absence of 4,5-unsaturation in the sphingoid backbone, are difficult to analyze because they usually occur chromatographically overlapped and indistinguishable from sphingomyelins. In mixtures, identification of some DHS species has been previously reported using mass spectrometry (MS),[1] but only saturated molecular species of DHS could be identified. Without prior chromatographic separation, only DHS species with no unsaturation in the acyl chains have unique masses, which are two mass units higher than sphingomyelins. Reversed-phase (RP) high performance liquid chromatography (HPLC) has been used successfully for separation of molecular species of SPMs (and coeluting DHSs).[2] However, differentiation of DHSs from SPMs required that the fractions be collected, and then different analyses were performed after derivatization to identify (i) the fatty acyl chain, and (ii) the sphingosine base. This method was very effective at elucidating the identities of the species, but was labor-intensive and time consuming. A similar RP-HPLC separation, but which utilized chemical ionization (CI) MS instead of chemical analysis of the sphingolipids, identified many SPM and DHS species, but the data still resulted in some ambiguities between SPM and DHS species.[3] And again, the method utilized fraction collection followed by analysis.

As technology has advanced, online liquid chromatography/mass spectrometric analysis has been employed. Thermospray mass spectrometry following the RP-HPLC separation of sphingomyelin did accomplish the identification of three minor DHS components in bovine brain SPM.[4] This represented a significant advance in the analysis of sphingolipids, but, since this was a RP-HPLC separation, prior phospholipid class separation was still required before molecular species could be separated and identified. A more recent study[5] also demonstrated the application of RP-HPLC/ thermospray MS to sphingomyelins, but in this report no data were given for dihydrosphingomyelins. Fast atom bombardment (mostly of unseparated mixtures), plasmaspray and other ionization methods have also been applied to phospholipids. Many of these applications have been recently reviewed,[6,7] and are not discussed here since they shed no light on the differentiation of DHS from SPM.

One of the most common interface/ionization sources for LC/MS analysis of phospholipids, including sphingomyelins, has become electrospray ionization (ESI).[1,8–10] Because most common phospholipids are naturally charged, they are particularly amenable to ESI-MS. However, most studies employing ESI-MS have either focused on unseparated mixtures, or have not included sphingomyelin in the samples. Thus, overall in the literature, there is a paucity of data which demonstrate the separation and identification of molecular species of DHSs differentiated from SPMs. We recently reported[11] the application of an HPLC separation using an amine column, adapted from a previous method,[12]

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