Dual Parallel Mass Spectrometry (LC1/MS2 and LC2/MS2) for Lipid and Vitamin D Analysis

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Introduction

Atmospheric pressure chemical ionization (APCI) mass spectrometry (MS) and electrospray ionization (ESI) MS are complementary techniques that provide different types of information for lipids such as triacylglycerols (TAGs), phospholipids, and fat-soluble vitamins. Since no one technique is by itself ideal, we routinely employ two mass spectrometers in parallel to provide APCI-MS and ESI-MS, MS/MS and MS^n data. Dual parallel mass spectrometers have been attached to the same chromatographic system to provide two or more types of information from components in the same column effluent, referred to as an LC1/MS2 approach. Alternatively, two chromatographic systems have been attached in a column-switching configuration to perform a total lipid analysis of both polar and non-polar lipids on normal-phase (NP) and reversed-phase (RP) systems, respectively, with detection by two different mass spectrometers simultaneously operated with different ionization modes. This is referred to as an LC2/MS2 arrangement. An LC1/MS2 approach has also been used for vitamin D analysis in foods, which allows accurate quantification using APCI-MS in SIM mode with parallel acquisition of APCI-MS for qualitative monitoring of interfering species.

Materials and Methods

Triacylglycerols were separated by RP-HPLC using two Inertsil ODS-2 columns in series (25 cm x 4.6 mm). Elution was performed using a gradient of dichloromethane in acetonitrile, typically from 65%/35% ACN/DCM to 35%/65% ACN/DCM. Detection was done using an evaporative light scattering detector (ELSD) in conjunction with mass spectrometry. The HPLC system consisted of a P4000 quaternary pump with membrane degasser, an AS3500 autosampler, and a Varex MKIII ELSD. For the LC1/MS2 analysis of triacylglylcerols, flow at 0.7 mL/min was split using two consecutive tees such that 0.4 mL/min was directed to the ELSD, while 0.30 mL/min was split equally to go to two mass spectrometers, in parallel.

The two mass spectrometers currently in use are a TSQ700 tandem sector quadrupole instrument (ThermoElectron Corp., San Jose, CA, USA) and an LCQ Deca XP ion trap instrument (ThermoElectron Corp., San Jose, CA).

Results and Discussion

Our first report of dual parallel mass spectrometry was an example of LC1/MS2 for analysis of phospholipids, reported in 1998 (1). We reported the analysis of sphingomyelin (SM) and dihydrosphingomyelin (DSM) in bovine brain. DSM differs from SM by only two mass units, and the two closely related species are not usually chromatographically resolved. Therefore, dihydrosphingomyelin has gone largely unreported in many cellular systems. The complementary nature of APCI-MS and ESI-MS (and ESI-MS/MS) allowed us to obtain both intact protonated molecules (by ESI-MS) and structurally informative fragments (APCI-MS) to definitively elucidate the structures of SM and DSM species present, and to show that commercially available bovine brain SM contained 12 to 14% dihydrosphingomyelin.

We later applied the LC1/MS2 approach to analysis of TAGs in canola oil and triacylglycerol oxidation products (TAGOX) (2). Although it may seem counterintuitive that large neutral molecules like triacylglycerols could be ionized by ESI, addition of ammonium formate as an electrolyte produced abundant intact ammonium adducts. The adducts formed by ESI-MS allowed the molecular weights of canola oil TAGs to be determined, while the fragments provided by APCI-MS allowed assignment of the specific fatty acyl chains, and can be used for their regiospecific localization (*sn*-1,3 versus *sn*-2 position). We showed that ESI-MS/MS spectra obtained from ammoniated parent ions were very similar to APCI-MS spectra. The difference between APCI-MS and ESI-MS was highlighted in the analysis of TAGOX, where ESI formed intact adducts even of high molecular weight oligomers formed by crosslinked TAGOX. ESI-MS was very efficient at forming ammonium adducts, while APCI-MS provided fragments that allowed the identities of oxidized functional groups to be identified as aldehydes, epoxides, etc. Chain-shortened core aldehydes were also identified.

Recently, we used an LC1/MS2 approach to revisit the problem of the under-reporting of dihydrosphingomyelin (3,4). We used both APCI-MS and ESI-MS, in parallel, combined with 31P-NMR to conclusively demonstrate that the sphingolipids in bovine milk are composed of at least 16% DSM, so milk constitutes a dietary source of DSM.

We now routinely apply an LC1/MS2 approach to analysis of vitamin D in food extracts. APCI-MS operated in

selected ion monitoring (SIM) mode is very effective for quantification of vitamin D from a variety of sources, but it intentionally overlooks ions that are not directly associated with the analyte. Thus, the presence of interfering species is often not apparent. We use full-scan APCI-MS on an ion trap instrument to obtain qualitative structural information for coeluting species while at the same time performing quantification of the analyte using APCI-MS in SIM mode on another instrument.

One single analysis for identification of all polar and non-polar lipids has long been a goal of the field of lipidomics. However, polar components (phospholipids, etc.) are analyzed using normal-phase chromatography, while non-polar lipids (TAGs, ceramides, sterols, etc.) are analyzed using reversed-phase chromatography, and these two systems are fundamentally incompatible. We demonstrated a column-switching approach (Fig. 1) in which the bolus of non-polar lipids that were unretained by NP-HPLC were redirected to a RP-HPLC system and separated using a C18 column while the polar lipids were separated on the NP amine column (5).

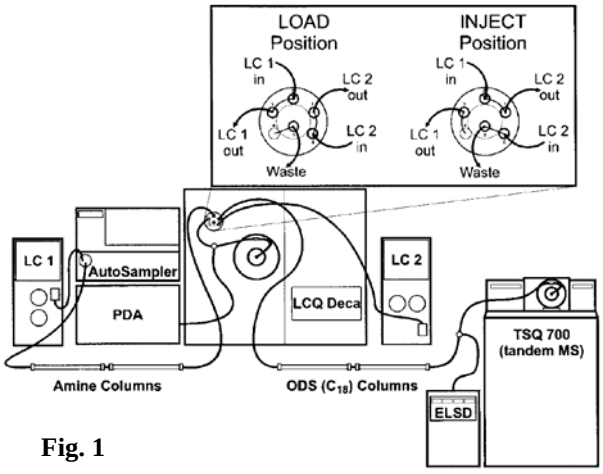

Fig. 1

Since this used two HPLC systems and two MS systems, it was referred to as an LC2/MS2 configuration. Polar lipids were analyzed by ESI-MS, MS/MS and MS^3, while neutral lipids were detected using APCI-MS and MS/MS. Dual parallel MS techniques have recently been reviewed (6,7).

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