Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1146 (2007) 164–185
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Liquid chromatography with dual parallel mass spectrometry and ^{31}P nuclear magnetic resonance spectroscopy for analysis of sphingomyelin and dihydrosphingomyelin II. Bovine milk sphingolipids

Wm. Craig Byrdwell [a,*], Richard H. Perry [b]

[a] *USDA, ARS, BHNRC, FCL, Beltsville, MD 20904, USA*
[b] *Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA*

Received 24 October 2006; received in revised form 25 January 2007; accepted 29 January 2007
Available online 3 February 2007

Abstract

Liquid chromatography coupled to atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) mass spectrometry (MS), in parallel, was used for simultaneous detection of bovine milk sphingolipids (BMS). APCI-MS mass spectra exhibited mostly ceramide-like fragment ions, $[\text{Cer-H}_2\text{O} + \text{H}]^+$ and $[\text{Cer-2H}_2\text{O} + \text{H}]^+$, which were used to identify individual molecular species of BMS according to fatty acyl chain length:degree of unsaturation and long-chain base (LCB). ESI-MS was used to confirm the molecular weights of BMS species. Both sphingomyelin (SM) and dihydrosphingomyelin (DSM) molecular species were identified, with DSM species constituting 20% of BMS. Approximately 56 to 58% of DSM species contained a *d*16:0 LCB, while 34 to 37% contained a *d*18:0 LCB. Approximately 26 to 30% of SM species contained a *d*16:1 LCB, while 57 to 60% contained a *d*18:1 LCB. BMS species contained both odd and even carbon chain lengths. The most abundant DSM species contained a *d*16:0 LCB with a 22:0, 23:0 or 24:0 fatty acyl chain, while the most abundant SM species contained a *d*18:1 LCB with a 16:0 or 23:0 fatty acyl chain. ^{31}P NMR spectroscopy was used to conclusively confirm that DSM is a dietary component in BMS.

Keywords: Sphingomyelin; Dihydrosphingomyelin; Sphingolipids; Phospholipids; Mass spectrometry; APCI-MS; ESI-MS; Atmospheric pressure chemical ionization; Electrospray ionization

1. Introduction

It has been well demonstrated that sphingomyelin (SM) acts as the substrate for *sphingomyelinase*, to produce the potent cell signaling molecule ceramide, in a process referred to as the Sphingomyelin Cycle [1–3]. Ceramide, the product of the Sphingomyelin Cycle, has been implicated in initiating cellular signaling processes from apoptosis to lipid transport to ion channel operation and many others, as evidenced by numerous studies using synthetic ceramide [4–13]. A few studies have also been done on dihydroceramide that demonstrated that it is often much less active in the same signaling pathways in which ceramide is so active [14–17]. The lack of the 4,5 *trans* double bond in dihydroceramide may give it the opposite behavior from its closely related structural analog ceramide in many cellular systems. However, this general rule is not universally true [18].

Studies have shown that the *sphingomyelinase* acts on dihydrosphingomyelin (DSM) just as efficiently (perhaps even more so) as it does on SM [19]. Thus, dihydroceramide, the often inactive signaling molecule, is produced with equal efficiency as ceramide, the active signaling molecule, by treatment of sphingolipids (SLs), which may contain SM or DSM, with *sphingomyelinase*. This sets up the potential for a competitive activation/inhibition system, in which the relative amounts of DSM and SM determine the amount of activation of cellular systems by action of *sphingomyelinase* on a mixture of SLs to produce either ceramide or dihydroceramide. Thus, it is impor-

* Corresponding author at: Food Composition Laboratory, USDA, ARS, BHNRC, Beltsville, MD 20705, USA. Tel.: +1 301 504 9357; fax: +1 301 504 8314.
E-mail address: Byrdwell@ba.ars.usda.gov (Wm.C. Byrdwell).
URL: http://www.byrdwell.com (Wm.C. Byrdwell).

0021-9673/$ – see front matter.
doi:10.1016/j.chroma.2007.01.108