Analytica Chimica Acta 929 (2016) 31–38

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Quantification of monosialogangliosides in human plasma through chemical derivatization for signal enhancement in LC–ESI-MS

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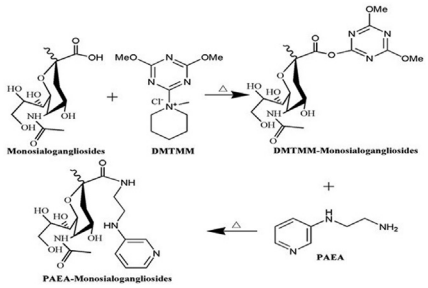
HIGHLIGHTS

- A UPLC/MS/MS method for analyzing monosialogangliosides GM1, GM2, and GM3 in human plasma was developed and validated.
- PAEA&DMTMM-based derivatization greatly improved the sensitivity.
- The method was applied to measure GM1, GM2, and GM3 in the plasma from the patients with GM3 synthase deficiency.
- GM3 was detected, for the first time, at a significant amount in the patients with GM3 synthase deficiency.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 March 2016
Accepted 18 April 2016
Available online 28 April 2016

Keywords:
Gangliosides
Mass spectrometry
Derivatization
Signal enhancement
GM3 synthase deficiency
Neurological disorders

ABSTRACT

Gangliosides are found in abundance in the central nervous system of vertebrates. Their metabolic disruption and dysfunction are associated with various neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. In order to improve our understanding of the etiology of these diseases, analytical ganglioside assays with sufficient specificity and sensitivity in relevant biological matrices are required. In the present work we have developed and validated a reverse-phase ultra-performance liquid chromatography (UPLC)/tandem mass spectrometry (MS) method for determining monosialogangliosides GM1, GM2, and GM3 present in human plasma. Compared with our previous method, this method enhanced, by 15 fold, MS responses of the analytes by employing 2-(2-Pyridilamino)-ethylamine (PAEA) & 4-(4, 6-Dimethoxy-1, 3, 5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM)-based derivatization. The analytes and internal standards were derivatized with PAEA&DMTMM after extraction from plasma using a protein precipitation procedure. They were then purified using liquid–liquid partitioning. When the samples were then analyzed by UPLC-MS/MS with a multiple reaction monitoring (MRM) mode, we achieved superior sensitivity and specificity. This method was evaluated for extraction recovery, calibration linearity, precision, accuracy, and lower limit of quantification (LLOQ). The validated method was successfully applied to monitor monosialoganglioside levels in the plasma from patients

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http://dx.doi.org/10.1016/j.aca.2016.04.043
0003-2670/

with GM3 synthase deficiency. With significantly increased sensitivity, we have, for the first time, detected a significant amount of GM3 in the affected patients.

1. Introduction

Gangliosides are a large subfamily of glycosphingolipids that are abundant in the central nervous system of vertebrates [1]. Except for the presence of sialic acid residues, they are similar to other glycosphingolipid species, as they possess hydrophilic carbohydrate moieties attached to ceramide backbones (shown in Fig. 1). Gangliosides are generally classified and named according to the degree of sialylation and the monosaccharide makeup of the carbohydrate moieties. In addition to the diversity of their carbohydrate moieties, the presence of long alkyl chain fatty acids with variable chain length and saturation degree in the ceramide portion generates an extra degree of heterogeneity of their structures [2], thus, explaining their physiological versatility in regulation of the central nervous system of living organisms.

Gangliosides have been found to be ubiquitous components which are predominantly localized on the outer leaflet of the plasma membrane along with other integral membrane components, such as trans-membrane proteins, sphingomyelins, and cholesterols. These components form characteristic regions known as glycolipid-enriched microdomains or lipid rafts on the mammalian cell membrane, which play a fundamental role in maintenance and organization of the integration and dynamic behavior of the membrane lipid bilayer scaffold [3]. Moreover, gangliosides are involved in cell proliferation, differentiation, migration, and adhesion by functional interaction with the extracellular domain of receptors, including epidermal growth factor receptor (EGFR) [4], platelet-derived growth factor receptor [5–8], fibroblast growth factor receptor [9], Trk receptor [10–12], and insulin receptors [13]. Disruption of gangliosides has been found to contribute to the pathogenesis of certain tyrosine kinase receptor-mediated disorders [14].

Genetic defects in the ganglioside biosynthesis pathway may be devastating. GM3 synthase deficiency (GSD) is a newly identified neurological disorder that has been prevalently found in the Amish population in the United States [15–17]. Although the pathological mechanism remains to be understood, the condition is severe. It is characterized by infantile onset of severe irritability, failure to thrive, developmental stagnation, cortical blindness, profound intellectual disability and intractable seizures.

In addition, mounting evidence has illustrated that the abnormal ganglioside profile is implicated in the development of various neurodegenerative syndromes, such as Parkinson's disease [18,19] and Alzheimer's disease [20].

A variety of assays have been developed for the measurement or profiling of different gangliosides in animal tissues, culture cells, and cerebrospinal fluid using thin layer chromatography interfaced with densitometric [21] or immunochemical detection [22], high performance liquid chromatography (HPLC) [23,24], supercritical fluid chromatography [25], and enzyme-linked immune-sorbent assay [26]. However, these methods are, in general, limited by large sample requirements, laborious sample preparation, and poor assay sensitivity. The technological advancement of mass spectrometry interfaced with the HPLC system with electrospray ionization (ESI) source technology has given rise to greater specificity and sensitivity of ganglioside assays from biological matrices. Previously, Gu et al. established a method for simultaneous quantification of GM1 and GM2 gangliosides in human cerebrospinal fluid using reverse phase LC/MS [27]. Sorensen et al. reported a liquid chromatographic approach with tandem mass spectrometry for the quantification of gangliosides GD3 and GM3 in bovine milk and infant formula [28].

Recently, we developed a reverse-phase UPLC-MS/MS method for the determination of gangliosides GM2, GM3, GD2, and GD3 in human plasma [29]. This method has been applied to monitor the levels of these gangliosides in plasma samples from patients with GSD. However, insufficient sensitivity of this method has been one of the major obstacles in measuring the plasma levels of monosialogangliosides in patients with GSD. In the present work, we have developed and validated a new UPLC-MS/MS method with enhanced sensitivity for the measurement of three monosialoganglioside species (GM1, GM2, and GM3) in human plasma using PAEA&DMTMM-based derivatization. This method employed reverse phase UPLC for chromatographic separation and tandem mass spectrometry in the MRM mode for enhanced detection, sensitivity, and specificity. The ESI-MS responses of the three monosialogangliosides were enhanced over 15 fold following PAEA&DMTMM-based derivatization. We have successfully applied this method to the determination of monosialoganglioside levels in human plasma from GSD patients, carriers and normal adults.

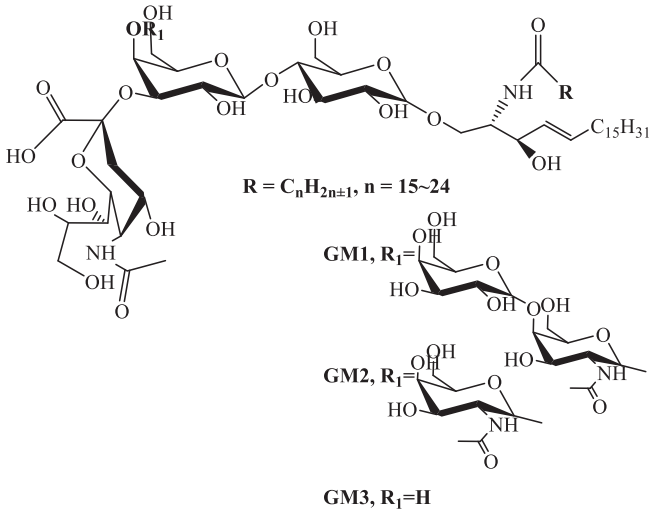

Fig. 1. Chemical structures of monosialogangliosides.

2. Experimental

2.1. Materials

Derivatization reagents4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) and 2-(2-Pyridilamino) ethylamine (PAEA) were purchased from Sigma Aldrich (St. Louis, MO) and Santa Cruz (Dallas, TX), respectively. Calibration standards GM1, GM2, and GM3 were obtained from EMD Chemicals (Billerica, MA) and Avanti lipids (Alabaster, AL). Internal standards (ISs) N-omega-CD3-Octadecanoyl monosialogangliosidesGM1 ($^2D_3$-GM1),