Glycomics and Lipidopics

Extended Abstract

Quantification of Lipids: Model, Reality, and Compromise

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Abstract:
Lipids are key molecules in various biological processes, thus their quantification is a crucial point in a lot of studies and should be taken into account in lipidomics development. This family is complex and presents a very large diversity of structures, so analyzing and quantifying all this diversity is a real challenge. In this review, the different techniques to analyze lipids will be presented: from nuclear magnetic resonance (NMR) to mass spectrometry (with and without chromatography) including universal detectors. First of all, the state of the art of quantification, with the definitions of terms and protocol standardization, will be presented with quantitative lipidomics in mind, and then technical considerations and limitations of analytical chemistry's tools, such as NMR, mass spectrometry and universal detectors, will be discussed, particularly in terms of absolute quantification.

Introduction:

Lipids represent a large and complex class of hydrophobic and amphipathic small molecules with a huge structural diversity (e.g., various combinations of fatty acyls and functional headgroups in phospholipids). They can be divided into eight basic groups according to the Lipid Mapsconsortium: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenollipids, saccharolipids, and polyketides . Changes in the level and/or in the composition of lipid species and/or classes occur after perturbation or during several physiological processes. Therefore, it is important to be able to profile the lipidome with a serious annotation and to determine the absolute or relative abundance of one, several, or all lipids present in the sample of interest. However, the chemical heterogeneity of lipids, the occurrence of many isomeric and isobaric species and the large concentration range over which lipids are found, preclude the measurement of complete lipidome profiles with a single analytical method and greatly hampers the quantification of this family. Each lipid class (family), especially complex lipids, such as sphingolipids or glycerophospholipids, present a large number of molecular species, which complicate their absolute quantification. Fortunately, some processes are available to quantify these molecules but with some limitations. The aim of this review is to go through the different proposed techniques: universal detectors such Corona-CAD (charged aerosol detector) and ELSD (evaporative light scattering detector), NMR (nuclear magnetic resonance), and MS (mass spectrometry) with or without chromatographic separation, and to discuss their advantages and limitations for lipids quantification. Quantitative analysis of lipids has been rapidly expanding in the two last decades concomitantly with the advances in technologies such as MS and NMR, and the number of published methods related to this is still growing. However, some cautions should be taken alongside the bioanalysis process to get an accurate result, especially when it comes to absolute quantification i.e., assigning an amount or concentration from the analytical response. Whatever the purpose of the study (-omics, toxicological, pharmaceutical, etc.), quantitative bioanalytical methods are critical for the interpretation of results, which underlines the need to have universal validation procedures. Thus, two workshops were held in 1990 and 2000 by regulatory agencies and scientific communities to harmonize validation procedures and to define the main parameters of validation with their criteria of acceptance. Several guidelines resulted from these workshops, where methodologies and acceptance criteria are detailed for each validation parameter. Indeed, they suggest using reference standards spiked in the same matrix of the intended studies to establish the calibration curve and quality control samples. These calibrators and quality control should be extracted with the same protocol as the studied samples and by using an internal standard, which should be the closest to the analytes. This last point is essential, especially when talking about absolute quantification. For lipids, we can distinguish two cases: the simple lipids; such as sterols and fatty acids with their derivatives, and the complex lipids; such as sphingolipids, glycerolipids, or glycerophospholipids. In the first case, pure analytical

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standards are available to prepare calibration curves. In the second case, hundreds of molecular species for each family can be detected for complex lipids in a biological sample. Today, 9856 species are listed on the Lipid Maps website glycerophospholipids and only about 80 analytical standards are commercially available. The same is true for sphingolipids: 4411 species can be found in the Lipid Maps website and very few species are available. Under these circumstances, we will not be able to obtain calibration curves for each molecular species, and as we know that detection is sensitive to the nature of the molecule (especially with fatty acids), the absolute quantification will not be possible. Furthermore, a full validation process should be performed prior to the assays, including the evaluation of selectivity, concentration range of the calibration curve, accuracy, precision, recovery, and sensitivity. Moreover, it is recommended that assays are validated routinely by performing a new extracted calibration curve and/or a qualitative control sample series every day. These validation steps could be very demanding when several analytes, with different physico-chemical properties, should be analyzed in the same run, involving tedious sample preparation and extraction steps. Moreover, these guidelines are mainly addressed to pharmaceutical and toxicological applications implying that they are mostly established for the quantification of exogenous compounds in biological matrices. In the case of lipidomic studies, where compounds of interest are endogenous, absolute quantification can be achieved via a standard addition method, using surrogate matrices, or using isotopic dilution when lipids are detected by mass spectrometry. However, lipid analytical standards are not commercially available in most cases meaning that only relative quantification, i.e., comparing the amount of the analyte to an analyte of reference, could be applied.

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