Evaluation of the Quantification Capabilities of Untargeted Lipidomics Approaches

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Both single-point and multipoint calibration result in reasonably accurate quantitation of lipid molecular species in LC-MS-based lipidomics experiments. Across the multiple lipid classes, multipoint calibration appeared to be slightly more accurate. The right selection of calibration and internal standards can have a substantial effect on the accuracy of the results.

Introduction

Mass spectrometry has become the gold standard in performing untargeted lipidomics analysis. It allows the simultaneous quantitation of hundreds of molecular species in biological samples, providing unique insights into the roles of lipids in health and disease. Single-point calibration based on the known amounts of internal standards is used in many studies, especially in those using shotgun (*i.e.*, in the absence of liquid chromatography) MS analysis, to calculate the concentrations of lipid analytes in samples. However, questions remain about the accuracy of these calculations as compared to the traditional interpolation in calibration curves prepared with authentic standards which is widely used in analytical chemistry, as well as the suitability of single-point calibration in LC-MS studies, where the internal standards do not coelute with most of the endogenous analytes.

The objective of this study is to assess the accuracy of quantitation of lipids in human plasma by LC-MS using either single-point or multipoint calibration curves with authentic or surrogate lipid standards. The SRM 1950 Metabolites in Human Plasma material (NIST) was used for validation of the methods used and for comparison of the values obtained with known published values.[1,2]

Experimental Conditions

The workflow of the study is summarized in Figure 1. After addition of a mixture of deuterated lipid standards, three 50-microliter replicate aliquots of plasma, as well as a series of dilutions of calibrators, were extracted using a methyl-tert-butyl ether-based protocol.[3] Extracts were analyzed by reversed-phase HPLC, coupled with high-resolution MS in both polarities and data-dependent MS/MS, on a Q Exactive mass spectrometer (Thermo Fisher Scientific). Lipostar software (2.1.9, Molecular Discovery) was used to process the data, including identification of lipid molecular species using the LIPID MAPS database

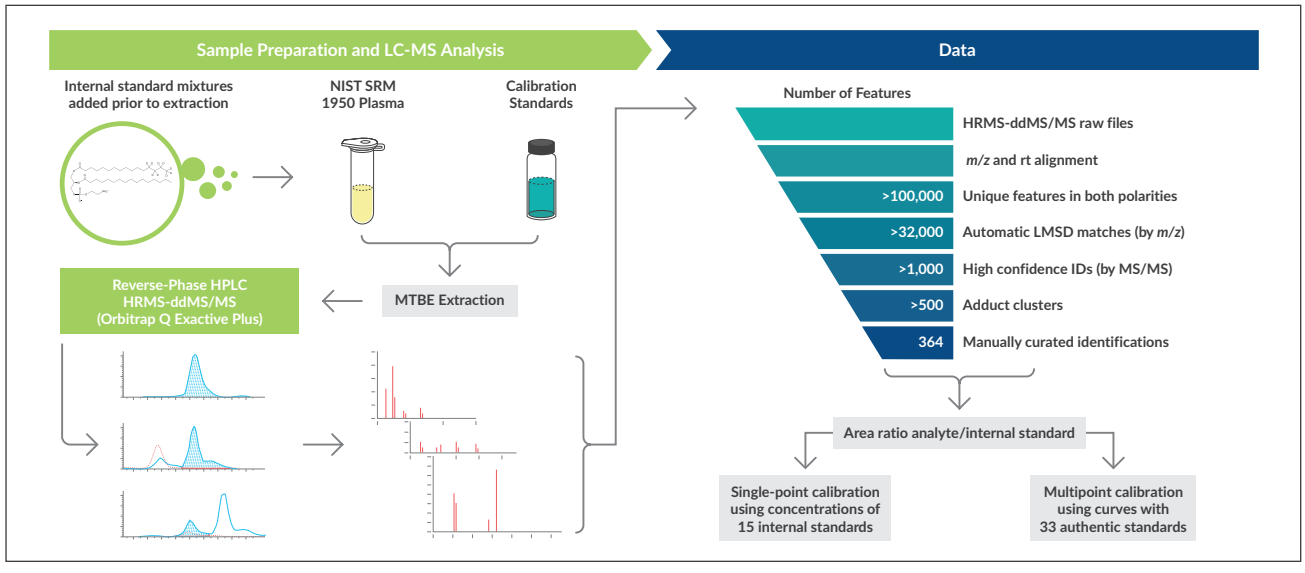

FIGURE 1: Schematic summary of the workflow followed in this study.

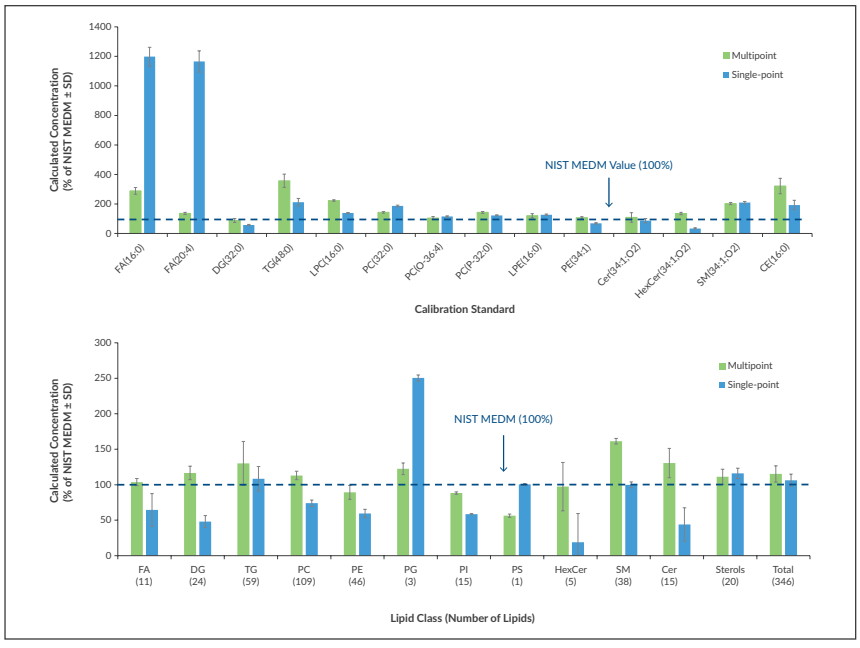

FIGURE 2: Calculated concentrations in NIST SRM 1950 plasma of the analytes for which authentic standards were used (top panel), or of all molecular species of lipids quantified in this study (bottom panel), using single-point or multipoint calibration, as compared with the published median of the means (MEDM).

TABLE I: Published and calculated concentrations (ng/µL) of the sums of all glycerophosphatidylchlines in NIST reference plasma using multipoint calibration with three different curves.

PC Subclass (Number of Molecular Species)	Published MEDM	Curve Used for Multipoint Calculation		
		PC(16:0/16:0)	PC(O-16:0/20:4)	PC(P-16:0/16:0)
Diacylglycerophosphocholines (18)	502.9	588.7	1334.9	1052.6
1-Alkyl,2-acylglycerophosphocholines (6)	31.6	39.9	56.5	61.4
1-(1Z-Alkenyl),2-acylglycerophosphocholines (6)	20.7	22.5	35.9	36.1
Relative Error (Δ%)	N/A	17.3	105.89	92.67

and integration of the peak areas of all analytes and their standards across several lipid classes.[4] The area ratios analyte/internal standard were used to calculate the lipid concentrations, both by interpolation in the calibration curves with the authentic or surrogate standards, and by single-point calibration using the signals of the corresponding internal standards in each sample (at least one deuterated standard for each lipid class listed at the bottom panel of Figure 2).

Results

Although several hundred lipids were identified in the reference plasma preparation, only 364 molecular species for which there were published data were manually curated and considered for quantitation in order to compare the two calculation procedures. Figure 2 shows the calculated values, compared to each other and to the previously published values for the NIST reference plasma. With some discrepancies, especially for some individual molecular species, both calculation methods were reasonably precise, as well as reasonably accurate when compared with the published data, especially considering that the median of the means (MEDM) takes into consideration a broad range of reported concentrations in different laboratories using different LC-MS methods. Across all analytes in the different lipid classes, the use of multipoint calibration curves resulted in better overall accuracy.

The effect of which calibration and internal standards are used in the accuracy of the calculations was also evaluated using three different subclasses of phosphatidylcholines (diacyl, alkylacyl, and alkenylacyl) for which authentic calibration standards were used. The results are shown in Table I. All measurements were within the same order of magnitude expected from the MEDM, but more accurate results were obtained when using the calibration curve for PC(16:0/16:0). Since all three curves were built using the same internal standard, PC(16:0-d_9/16:0), this suggests that the right combination of calibration and internal standards would have a noticeable effect on the accuracy of the calculations for different types of lipids, although these results are preliminary, and a bigger variety of calibration and deuterated internal standards would be required to perform an exhaustive investigation of these effects. ■

References

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