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LETTER TO THE EDITOR

Response to Letter to the Editor regarding "A quantitative and comprehensive method to analyze human milk oligosaccharide structures in the urine and feces of infants"

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To the Editor:
We appreciate the opportunity to clarify and expand on our publication further. Human milk oligosaccharides have become an important area of research, primarily because of the pioneering efforts of several researchers and, particularly, of Professor Kunz. This publication [1] is one in a string of manuscripts that were submitted around the same time summarizing our efforts in measuring human milk oligosaccharides rapidly in several biological fluids. The paper published in this journal is best considered along with two other manuscripts in this area. With this in mind, we respond to the comments in each point.

Points 1 and 2. Reproducibility and precision for the MALDI/MS was illustrated in this paper but the ESI LC/MS was not rigorously shown. We have published an additional paper [2] that shows the reproducibility and the repeatability of the LC/MS method using both isotopic labeling and peak areas. We show very good correlation between isotopic labeling and peak areas and now use primarily chromatographic peak areas for quantitation.

We have also published a recent comprehensive paper [3] that shows high repeatability of LC/MS for oligosaccharides in terms of retention times, chromatographic peak area, and total ion abundances for the same sample injected nearly 30 times over a 3-d period.

Point 3. We are very familiar with ion suppression in oligosaccharide analysis as demonstrated by an early paper on ion suppression on MALDI [4], where we show that native sialylated species are suppressed by neutral species in the positive mode by nearly a factor of 10, whereas in the negative mode sialylated species suppress neutral species. Similar suppression is observed in the ESI; however, when LC is performed, the components are separated and much less suppression occurs. In the earlier paper, we show that when the sialylated species and the neutral species are separated, their MS responses are very similar.

Point 4. The water content is an issue with every biological fluid, whether it is serum, spinal fluid, saliva, or milk. It is an issue that everyone in this research needs to deal with carefully by freezing the sample as soon as possible and keeping the samples in −80 °C freezers in sealed containers. This is, in our opinion, standard operating procedure for nearly all laboratories working in this area, and we felt no need to comment on it further.

Point 5. We agree that more descriptions should have been provided. However, taking into account the recommended length of the manuscript, we felt compelled to leave out

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some of these points. In hindsight, it would have been prudent to include these descriptions in a supplementary information section.

Finally, the term “rapid high-throughput” was perhaps an overenthusiastic use of the term, but we have made significant progress in this area. We now perform the preparation on 96-well plates and have removed the Folch extraction and one solid phase extraction. We can process several hundred samples in a day to prepare for MS analysis. The limiting step is now the LC/MS, as it is in other “omics” methods.

We hope that our article will stimulate further work on the analysis of human milk oligosaccharides.

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