Response to Letter to the Editor regarding “Lactose does not interfere with the analysis of sialic acids as their 1,2-diamino-4,5-methylenedioxybenzene derivatives”

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Dear Sir,

First, let us say that we fully endorse the purification step developed by Martin et al. [1]. We have used this procedure on several occasions; it is rapid, simple and does not result in any loss of analyte. It is useful when it is necessary to isolate sialic acids from interfering matrix components.

Nevertheless, we remain unconvinced that lactose interferes with the 1,2-diamino-4,5-methylenedioxybenzene assay of sialic acids. In our work reported in 2010 [2], we did not observe a positive bias in the spiking experiments of lactose-containing products. In our 2011 article [3], in Figs. 3 and 4 the sialic acid levels measured in various lactoses by the 1,2-diamino-4,5-methylenedioxybenzene assay or by high-performance anion-exchange chromatography with pulsed amperometric detection of free sialic acid and sialyl-lactose give the same result for total sialic acid (note that samples 2, 4 and 5 were all from Sigma). Thus, either lactose does not interfere, or it interferes to the same extent in two assays based on different analytical principles, which seems unlikely. We have also analysed a growing up milk using our method [2], and using the method of Martin et al. [1]. The analyses were performed in duplicate on six different days. The results (expressed in milligrams per 100 g) were as follows: for *N*-acetylneuraminic acid (Neu5Ac),

mean 105 and standard deviation 1.44 (Spichtig et al. [2]), and mean 105 and standard deviation 2.05 (Martin et al. [1]); for *N*-glycolylneuraminic acid (Neu5Gc), mean 2.74 and standard deviation 0.057 (Spichtig et al. [2]), and mean 2.73 and standard deviation 0.037 (Martin et al. [1]). Statistical analyses using the *t* test and *F* test demonstrate that neither the means nor the variances are significantly different. We believe this should be sufficient evidence to demonstrate that lactose does not interfere with the analysis.

During our work we observed a shoulder on the Neu5Ac peak when using sulphuric acid for hydrolysis. This did not occur when formic acid was used, and it was effectively removed when the purification step was applied to sulphuric acid hydrolysates. We wonder if there may be some link between this observation and the observed bias in their laboratory. We would also encourage Martin et al. to pass their lactose through an ion-exchange resin prior to spiking to see if they still observe such a bias.

Finally, to respond to the question of the fetuin concentrations used in the work reported in our 2011 publication, the fetuin concentration ranged from 205 to 217 mg/L and the lactose concentration was 10 g/L.

References

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