Interactive comment on “Technical Note: Rapid Normal-phase Separation of Phytoplankton Lipids by Ultra-High Performance Supercritical Fluid Chromatography (UHPSFC)” by J. Brandsma et al.

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Received and published: 18 February 2016


The authors present a technical note wherein they attempt to characterize the lipids of marine phytoplankton, along with phytoplankton community samples collected in Antarctic waters during a phytoplankton bloom. The novelty here is that the method employs ultra-high performance supercritical fluid chromatography (UHPSFC) coupled to a triple quadrupole mass spec. This underutilized branch of chromatography affords a high resolution normal-phase type separation with highly specific detection of lipids of the triple quadrupole mass spec. My overall impression is that the method has great potential, but as presented here there are sufficient major deficiencies that render the method unfit for purpose as it presented, that is, a method for analyzing the lipids of phytoplankton, and therefore I must recommend rejecting the manuscript.

In detail; general comments: The authors give no quantitative response factors or limits of detection for any of the lipids observed in spite of authentic standards being obtained which would allow them to easily do this. This in itself is not an insurmountable problem, the authors could easily quantify standards and add details to this note, but this knowledge would perhaps have alerted the authors to the bigger problem that is the crux of my review: the inadequate response of the glycolipids MGDG, DGDG and SQDG, rendering them unobserved in several phytoplankton cultures! Phytoplankton are by definition organisms capable of photosynthesis. Table 2 describes two phytoplankton as no MGDG or SQDG observed, and all others except the synechocystis as having relatively low amounts. The photosynthetic membranes are well known to contain MGDG,DGDG,SQDG and PG (Wada and Murata 1998) and there are numerous references in the literature that quantify the lipids of these organisms in detail (e.g. Abidi et al, Plant Physiology 2015: Van Mooy and Fredricks, GCA 2010) and show absolutely, that MGDG and SQDG the most abundant lipids in photosynthetic membranes. Since glycolipids are the bulk components of photosynthetic membranes a method published to examine phytoplankton surely must be able to sufficiently detect glycolipids?? Without any quantitative data it is impossible to know for sure, but there are several clues to suggest that the glycolipid response is very low, apart from not observing it in several well-characterized cultured organisms: the noisy baseline in the chromatograms and the peak shape is very poor – I’m not convinced it is due to poor chromatography as it told in the text– in my experience normal phase chromatography of glycolipids gives good peak shapes. I can think of several possible causes / remedies for this; the glycolipids are analyzed as their ammonium adducts, with the high flow-rate and large volume of make-up solvent containing a surprising 1% formic acid (more commonly
0.1% formic or 1% acetic), perhaps ammonium adducts are not the major ions of the glycolipids? Sodium adducts can be a problem even when ammonium is present in optimum conditions, did the authors look at the speciation of analyte/adduct ions? Neutral loss scans on a triple quadrupole mass spec are the least sensitive type of scan since both Q1 and Q3 are required to scan in tandem, and so sufficient scan time (0.5 seconds on our Thermo TSQ) is essential to obtain satisfactory sensitivity – the authors do not present sufficient analytical detail to comment fully on this, was this optimized? Presumably so, since the response PE and PG are more than satisfactory. I have no personal experience of SFC but it seems like the flow rates are very high for an electrospray method – typically flow rates are reduced to ‘concentrate’ the analyte yet 0.45 mL/min is added post-column, perhaps reducing this would yield further sensitivity?

Specific detailed comments:

Line # 121: Institution, not Institute.

Using both acetate (ammonium acetate in co-solvent) and formate (formic acid in the make-up) would lead to a confusion of anion-adducts... presumably PA is observed as both formate and acetate?

Capital L for litres/liters is easier to read than l I think.

Table 2 has very little meaning. Presumably this is based on peak area or peak height? Even a relative comparison of such data where the data is present from positive and negative ion mode and parent scan and neutral loss scan is impossible with no quantitative element! Of course PC is the most abundant – PC gives a very strong response since the 184 ion is often 1:1 abundance compared to the precursor ion! Consequently, the view of the membrane lipid profile is completely and unacceptably skewed by the lack of any consideration of quantitative response. Popendorf et al, (Lipids 2013), is a useful reference here, for example, MGDG is 2 orders of magnitude less sensitive that PC, under the given conditions.

The chromatograms in figure 1 would be better presented with some indication of absolute abundance. It would be useful to see chromatograms of the cultures and environmental samples too.

Section 2.3: No details are given for the MS settings; gas flows, temperatures, etc which would be useful to reproduce this method.

Conclusion: I suspect that further method development would increase the sensitivity of the glyco- and other lipids to a sufficient degree. However, given the method development that is required, and consequent re-analysis of standards and samples I feel that “major revisions” does not adequately cover the necessary complete re-do of the work and so must sadly recommend rejection of this note. I have spent a great deal of time considering my response. I hope that by not making this review anonymous the authors will know and appreciate my intimate knowledge of phytoplankton lipids. I also hope my comments prove useful to the authors and I would be happy to correspond privately over the fine details. I look forward to seeing a sensitive, quantitative SFC method in the future and would be pleased to review such a manuscript.