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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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Dear Dr Fredricks and Anonymous Referee,

Thank you for reviewing our manuscript on the separation of phytoplankton lipids by ultra-high performance supercritical fluid chromatography (UHPSFC). The aim of this paper is to introduce a new, and in our opinion very powerful and versatile, separation technology to the readers of Biogeosciences. It is not intended as "an attempt to characterize the lipids of marine phytoplankton", which is something we expressly stated in the main text (lines 235-237, line 299). As the manuscript title indicates, our focus is thus on the novel chromatographic aspects, and not on the subsequent detection or quantification of the lipids per se. For this there are a multitude of methods, instru-

ments and bioinformatics approaches available, and we routinely use state-of-the-art mass spectrometry to measure a wide variety of lipid classes at the lowest possible levels. However, we do not contend the referees' key point that the results of the phytoplankton screening are inconsistent and at times very poor. The reasons for this do not lie with the UHPSFC separation, but rather with the MS detection. All method development was done on a single UPC2-TQD system, specifically obtained to trial the use of this novel type of chromatography for a wide range of analytes. This MS was not purposefully set up and optimised for lipid analysis, nor does this specific build of detector have the best levels of sensitivity. The fact that the results did not necessarily reflect the lipidomes of the screened phytoplankton species, which are indeed well-established and familiar to us, was therefore not very surprising. As indicated, the purpose of measuring a limited number of phytoplankton extracts was to test the use of UHPSFC chromatography on real-life samples. Optimising the MS detection and establishing a sensitive and (as far as this is possible) quantitative method was unfortunately not possible at the time, although we do recognise the need for this. To this end, we have now installed a dedicated UHPSFC system for use with our highest-end MS systems. We are in the process of replicating the method presented in this paper, this time with a fully validated MS method, and will be in a position to present these results in the very near future. Based on the editorial decision we are happy for this to be in the form of an amended manuscript or a full (re)submission. Finally, we thank the referees for highlighting this specific issue with our results and thereby strengthening the resubmission of our work.

Specific comments:

* With regards to the galactolipid chromatography we want to point out that the peak shape, while not as sharp as for example the phospho- or betaine lipids, is still at least as good as that obtained in regular LC systems. Figure 1 may be somewhat misleading as the galactolipid (and betaine lipid) peaks presented there are from a complex mixture of different lipid species (lines 493-495), whereas those in the upper two panels

are SRM traces of individual phospholipid species. This figure will be updated to show the chromatography of both individual lipids and whole classes in natural samples.

* Although we did not observe any deleterious effect from the use of formic acid in the make-up solvent, we have now modified this to ammonium acetate to be on the safe side.

* The specified high flow rates are typical for UHPSFC and no negative impact on electrospray ionisation efficiency has been reported to date.

* Line 121: changed

* Full MS settings will be added to the revised manuscript.

* Finally, with regards to accurate quantification of phytoplankton lipids in LC-MS methods, we want to point out that this is possible for only a very small number of lipid species for which isotopically labelled reference standards are available. Methods that work around this problem are certainly feasible (i.e. Popendorf et al. 2013 Lipids; Brandsma et al. 2012 Biogeosciences), but these do not fully account for effects such as species/class-specific differences in ionisation efficiency or concentration-dependent ion suppression in the chromatography. It is our hope that more synthetic standards will become commercially available to support this type of work.

Yours sincerely,

Joost Brandsma

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