Review of LOM-11-10-0140

Part 1 – General comments (sent to author)

This manuscript describes the use of size exclusion chromatography to measure DON concentrations and then compares it to more traditional approaches. The method looks very interesting and it would be wonderful if it could be applied to marine systems but I fear not. The writing is a bit confusing in places so I suggest improvements below. I also found the apparently random use of different sample sizes curious, which I again comment on below. This reads like a study that evolved haphazardly as it went along, rather than starting with a well thought out plan.

Part 2 – Detailed comments (sent to author)

1. The title needs to be revised as it currently uses a lot of words to say very little. I also recall my advisor putting a big red line through words like novel – the reader should decide if something is novel or not. How about – Comparison of methods to measure dissolved organic nitrogen concentrations in freshwater systems: size exclusion chromatography versus high temperature oxidation……or something like that.
2. Page 3, line 43 – I think few analysts would call 2.5 hours per sample fast. Presumably samples can be staggered or run in batches. I think indicating an average number of samples that can be run in 8 hours (or some other time unit) would be more useful to the reader.
3. Page 5, line 56 – Specify that this method is for freshwater systems.
4. Page 7, line 33 – Why acidify and sparge for a TDN analysis? If this if for the DOC side of things, than state that.
5. Page 7, line 60 – Some discussion is needed on the principles behind SEC. What molecular size ranges are targeted or removed? Discuss how polarity factors in.
6. Page 9, line 1 – How did the authors "know" the concentration of their natural substances? As they note later – they didn't. Text needs to be revised accordingly.
7. Page 9, line 51 – The volumes of water used to rinse the filters seem excessive to me. Are these filters prone to contamination? If yes, why were they used?
8. Page 10, line 8 – Only one natural sample was tested. This strikes me as very odd for a method trial. I would have loved to see several natural samples with different characteristics used.
9. Page 10, line 25 – Why were model substances tested at 2 mg N/L while the natural sample was only 1.5 mg N/L.
10. Page 10, line 29 – Why was NH_4^+ not measured in the model substance samples? Amino acid standards can often have relatively high NH_4^+ concentrations depending on how the standards are prepared. If there is any sample left, I suggest checking the NH_4^+ concentration as it could explain some of the deviations in DON values.
11. Page 10, line 53 – How was 0.4 chosen as the DIN:TDN ratio used to calculate the recovery rate?

12. Page 11, line 27 – Start a new paragraph after "…..the standard approach, respectively." Throughout the paper there are paragraphs that go on and on, which makes it hard to follow.
13. Page 11, end of page – Strikes me that a summary table of the various treatments and sample sizes would be useful. As written it is this seemingly endless list of things that sound the same but are not.
14. Page 12, line 5 – Why use an n=6 for the standard approach but an n=5 for SEC?
15. Page 12, line 46 – Suggest starting a new paragraph after "….at high DIN:TDN ratios."
16. Page 18, line 8 – What were the problems with the DIN measurements?
17. Page 19, Table 1 – Here I am baffled. The point of the manuscript is to test the SEC-DON method and yet they only use an n=2 and so standard deviations can not be reported. What happened? Surely the study was not designed that way. The same problem is seen in Table 2.
18. Page 20, line 58 – The authors state that the main reason DON concentrations are overestimated was an overestimation of HTCO-DON. Is this a blank issue? There are no data provided for blanks and no discussion of how blank issues could impact the results. These data and such a discussion need to be added.
19. Page 21, line 12 – Why were NH_4^+ and NO_3^- /NO_2^- overestimated? What was the problem?
20. Page 22, Figure 5 – Maybe I'm just tired but something doesn't seem right. On page 21 it is noted that the inorganics were overestimated and yet the DON concentrations for these samples were also overestimated – almost doubled. I can believe this for deep ocean waters, for example, where DON concentrations are so low that accurate measurement is hard. In Figure 5, however, analyses were at the mg N/L level. How can the analysis possibly be off by that much while also overestimating the inorganics? Something seems very wrong to me.
21. Page 25, last line – I think it highly unlikely that size exclusion chromatography will work well, or at all, in full strength seawater. Unless the authors have some data to support their contention I think they should remove the sentence or they could be sending people on a wild goose chase. If it does work in seawater then I highly encourage them to show it and publish it because I for one would LOVE a better method!