"A molecular-level approach for characterizing water-insoluble components of ambient organic aerosol particulates using ultra-high resolution mass spectrometry" by A.S. Willoughby et al.

Anonymous Referee #1

Received and published: 19 May 2014

Overview: This manuscript describes the molecular composition of ambient organic aerosol from a coastal Virginia site. The water soluble components were extracted in water and acetonitrile. The water-insoluble components were extracted in pyridine. Molecular composition was analyzed by both proton NMR and ESI-FT-ICR-MS. The results indicate water and acetonitrile extract chemically similar organic matter components. However, pyridine extracts a unique fraction of organic matter, containing less polar and more aliphatic components with a large fraction of sulfur containing compounds. The manuscript is well written and easy to follow. The use of pyridine to extract the water-insoluble portion of organic aerosol is a unique contribution to the ESI technique and provides a more complete view of aerosol chemical composition. The complementary use of NMR provides a basis for quantification of organic aerosol components not possible with ESI alone. I support publication in ACP after these comments are considered.

Specific Comments:
1) a. Page 10396 line 12: "…comprises up to 90 % of the OM." Please provide a reference for this statement. Is it because 10 – 70% of the OM is water-soluble as stated in the intro? If so, then 30-90% of the OM should be water-insoluble.
b. Page 10415 line 23: Similar comment as above.

We agree that the text is unclear about 90% of OM being water-insoluble. Yes, it is deduced from the idea that if 10-70% of OM is water-soluble, then 30-90% must be insoluble. We will change the text to include the range of 30-90% and provide the references.

2) Page 10405, line 5: Why not show the full spectrum? It would be interesting to see a figure of the full mass spectrum for each solvent type

In the case of our samples, and in the case of many NOM samples, the full spectrum does not provide sufficient useful information to discuss in a manuscript. The important molecular details are buried within each spectrum, and we believe that displaying representative expanded spectra provides a better opportunity to make meaningful comparisons of the different solvents. Thus, in the interest of a succinct manuscript (and lesser publication costs) we chose to omit the full spectra and provide representative expanded spectra. The full mass spectra for each of the solvents will be added to the supporting information for interested readers to view.

3) a. Pages 10405, line 12 – page 10406, line 2: How general is this trend? The authors show only information from one nominal mass? What are the results from all the peaks in each solvent? Perhaps a figure such as a Kendrick diagram could show families of major mass defects in each solvent, or a histogram for mass defect for all the peaks in each solvent.

We state (P10405, L7-8) that this peak distribution is representative of odd nominal masses across the spectral range. A histogram (see attached fig. 1) of distribution of the percentage of formulas found within specific mass defect ranges for each of the solvents demonstrates that most of the peaks appear between 0.1-0.2 mass defect. As described in the text, the PSOM clearly shows the highest percentage of peaks in the 0-0.1 mass defect region. This figure will be included in the supplementary information for readers interested in verifying this trend.

A KMD (CH_2) plot of every peak assigned a molecular formula proved difficult for a reader to digest and is not included here. A KMD plot using a subset of the data (see attached fig. 2) reveals that many of the peaks at m/z = 427 shown in *fig. 2 of the manuscript are part of large series (at least 10) unique to the solvent of interest suggesting that the results are representative of the entire sample. Collectively, these series span almost the entire mass range of molecular formulas identified in this study (200-800 m/z). This plot will not be included in the revised text but is included here for the reviewer.*

b. page 10405, line 19 –page 10406, line 2: This section ends with the authors informing us that information in the magnitude of peaks is unknown and not to be used. However, page 10405 lines 19 – 29, analyzes the differences in peak intensities. Please reconcile.

Yes, this discrepancy should be clarified. Our intention was not to give the impression that peak magnitudes do not provide useful information, rather that peak magnitude depends on the interrelated issues of compound ionizability, solvent, and sample composition and should be viewed cautiously. For simplicity, we do not explicitly consider peak magnitudes in the remaining data presentation. In the revised manuscript, the final sentence of this paragraph will be deleted and replaced with a sentence such as the following "While these differences in peak magnitude can provide useful information, the remainder of the discussion of the data focuses on differences in the presence and absence of peaks in water, pyridine, and acetonitrile extracts to provide a qualitative view of WIOM components detected by ESI-FTICR-MS."

4) a. Page 10407, line 16 – 18: There needs to be more discussion of the fact that an ionizable functional group is required for detection in ESI. The authors have no knowledge of how much material extracted into pyridine was detected by ESI, meaning that there can be a large fraction of material that is water-insoluble and not detected by ESI-MS. Therefore, it should be stressed throughout the manuscript that it is the water insoluble fraction that can be detected by ESI-MS.
b. Page 10407, lines 13-14: again this sentence needs to mention that you are looking at the water-insoluble organic matter that can be detected through ESI-MS.

We agree that the ability to detect only ionizable compounds is an important limitation of the instrument. Reviewer #2 also mentioned that we need to articulate this more clearly. We will revise the text in this section to expand on this limitation and indicate that the compounds we are discussing throughout the manuscript are only those that can be efficiently ionized by ESI.

5) Page 10409, lines 11-13, also Page 10413 lines 8- 11, also Page 10416 lines 2-3: I do not think that the authors can prove that pyridine is preferably extracting sulfur containing compounds. It could also mean that the more aliphatic molecules, i.e. extracted in pyridine, are more prone to contain sulfur. This could be due to co-generation of SO2 and carbonaceous aerosols (soot), or burning of sulfur containing diesel.

The reviewer makes a good point that the selectivity may not be for the sulfur, rather the aliphatic material that may be prone to reactions with sulfur. We will revise the text to acknowledge this possibility.

6) page 10409 line 25 –page 1410 line 7-9: a. There could be a large quantity of aromatic compounds that are not detected by ESI-MS that would still be defined as water-insoluble. Similar to comment 4, the authors should draw attention to the measurement of insoluble OM that is detectable by ESI-MS. b. The authors do not have NMR data for the aromatic region extracted in pyridine to back up the statement that "…WIOM may not absorb light as efficiently as WSOM."

We agree with the reviewer in that we can only speculate about the presence of aromatic compounds in the sample. The revised text described in our response to comment #4, we will note that an unknown quantity of low O/C aromatic compounds are among those that would go undetected by this technique. We will add a qualifying statement such as "Unless the WIOM contains a significant portion of non-ionizable (by ESI) aromatic compounds, the WIOM analyzed in *this study may not absorb light as efficiently as the WSOM. We speculate that the aromatic character in these samples is low due to a lack of a strong combustion source. Unfortunately we cannot verify true aromatic content using these methods due to the signal from pyridine in the aromatic region of the* ^{1}*H NMR spectrum."*

Technical Comments:
Page 10394, Line 3: Change "...human emissions, and the effect…" to "human emissions. The effect"

The suggested change has been made.

Page 10394, Line 6: Delete comma after and

The correction has been made.

Page 10395, line 10: Abbreviation "OM" has not been defined.

The reviewer is correct, and it has now been appropriately defined in this location.

Page 10398, Line 16-17: Change "…(ThermoFinnigan), where quantification…" to "…(ThermoFinnigan). Quantification…"

The suggested change has been made.

Page 10399, Line 21-23: This sentence is very colloquial. Please explain why acetonitrile interfered. Something along the lines of, "Acetonitrile interferes greatly with our measurement strategy because…. . Due to… it was not possible to determine the extraction efficiency."

The problems with using acetonitrile as a solvent for ^{1}*H NMR are explained in the results section (P 10403, L 6-13).We agree that this explanation should be presented here in the methods and have revised the sentence to include this explanation and use more formal language. The revised sentence will read: "Acetonitrile interferes with our* ^{1}*H NMR quantification strategy due to a strong signal from acetonitrile hydrogen (occurring at ~2 ppm) that overlaps with the signal from organic matter (occurring between 0.1-4.4 ppm). This overlapping signal impedes our ability to determine the amount of proton signal derived from the sample and precludes a reliable calculation of extraction efficiency."*

Page 10403, line 25-26: Please provide a reference for shifting peaks due to solvent interactions.

We have added the following reference:
Sanders, J. K. and Hunter, B. K.: Modern NMR spectroscopy: a guide for chemists, Oxford University Press Inc., New York, NY, 1993.

Page 10404, line 2-3: It is not clear what the following refers to, "…(a ratio of 4 for both WSOM and PSOM)."

We agree that this can be clarified. We will change the text to read …"(a CH_2/CH_3 *ratio of 4 for both WSOM and PSOM)."*

Page 10404, line 5-7: What electron withdrawing functional groups would you be talking about that are likely in OM?

Many functional groups commonly found in OM would contain protons that appear in this region including alcohols, ethers, esters, carboxylic acids, aldehydes, ketones, amines, and amides. We will add text that gives examples of these functional groups.

Page 10406, line 24-28: Please provide references for both statements.

We have added the citation Koch and Dittmar (2006) for the statement regarding the aromaticity index values. We have added the citation Andreae and Gelencsér (2006) for the statement about the role of aromatic compounds in light-absorption.

Page 10408, line 11: Change "…sampling site, and they show…" to "…sampling site. They show…"

The suggested change has been made.

Page 10409, lines 5-8: How can they be outside the window of ESI-FTICR-MS if they are detected in the PSOM formulas?

We agree that the wording is unclear. We will remove the statement "...outside the analytical window of ESI-FTICR-MS..." for clarity. We will change the wording to read "...indicating that they are truly water-insoluble compounds or do not ionize well in water due to being suppressed by other WSOM components with higher ionization efficiencies."

Page 10409 lines 16 -14: What about compounds that are found in both PSOM and ASOM, any comments on the chemical composition of WIOM found in both organic solvents?

This comparison, while interesting, is of minor importance relative to the focus of the manuscript, identifying OM formulas that are uniquely WIOM. For simplicity, we focused the data discussion on the differences between the water and solvent and not the similarities/differences between the two solvents. 400 formulas were identified as common between ASOM and PSOM. The formula distribution is similar to that of PSOM-WIOM with more than 90% of the formulas being aliphatic or olefinic and CHOS formulas showing the highest contributions followed by CHO formulas and lesser contributions from the N and P-containing formulas. We will add a few sentences about this comparison in the revised manuscript.

Page 10409 line 20 -22: Similar atomic distribution to what?

The atomic distribution of the ASOM-WIOM formulas is similar to that of the WSOM formulas. We will clarify this in the revised text.

Page 10410 line 24: Has the abbreviation NOM been used or defined before? If not please define.

The abbreviation had not been defined. It was the only use of the abbreviation, so we will change it to read natural OM.

Page 10410 line 28-19: As mentioned in the preceding paragraphs, the authors suggest a lack of aromatic compounds. I am confused with the mention of aromatic rings.

We agree with the reviewer in that the mention of aromatic rings here is confusing. Many other FTICR-MS studies have described this region of the van Krevelen diagram as the region where lignin compounds would plot. However, as we *state, we don't expect there to be a significant amount of lignin in the sample. Other studies have classified this region as SOA compounds, lignin-like, or CRAM (carboxylic rich alicyclic molecules). We believe that CRAM may be a more representative classification of this OM, and will change the text to reflect this. Describing it as CRAM will not conflict with the lack of aromatic material in the samples, and is consistent with the fact that many of these compounds are classified as olefins. This region may contain SOA compounds, but these will be present throughout the van Krevelen diagram, and we do not have direct evidence for them being secondary in nature.*

Page 10411 line 6-9: Can NMR data help with determining N-H bonds versus C-H bonds near an NO3 functional group?

C-H protons adjacent to a nitrate functional group and C-H protons adjacent to amine groups are expected to give signals with significantly different chemical shifts (at least 1 ppm), and should be easily distinguishable from one another. Unfortunately, C-H bonds near a nitrate group are expected to have a signal very close to that of the water signal (~ 4.8 ppm) and the signal for C-H bonds near an NO_3 *group are likely suppressed with the water-suppression pulse program used in this study.*

Anonymous Referee #2
Received and published: 25 May 2014

Overview: The manuscript presented by Willoughby et al. titled "A molecular-level approach for characterizing water-insoluble components of ambient organic aerosol particulates using ultra-high resolution mass spectrometry" describes the elemental formula compositions of three extractions of OA using water, acetonitrile and pyridine. The extractions were done in parallel and not in tandem, thus overlap of compounds that are soluble in multiple solvents or water is expected. The acetonitrile extracts were not obviously different than the water extracts and H-NMR quantitative data was not feasible, thus the manuscript describes most thoroughly the negative ion ESI-FT-ICR MS compositions of the water and pyridine extractions. They propose that the water-insoluble OM may contain most of the fossil material and possibly up to 90% of the OM, thus it's important to study. This statement is a bit of a stretch, since atmospheric oxidation will likely alter the fossil material rendering it more water-soluble with time. The cited studies used in this claim are also somewhat limited, thus this statement is too strong given the lack of concrete knowledge. Another key point of this paper is the presentation of a NMR method for quantification of the OM from different solvent extracts. The results indicate up to ~60% of the OM from these studies was water-soluble and pyridine soluble. It's not clear which samples had which values or what is the associated error on the measurement. As stated in the manuscript the focus is on determination of the water-insoluble OM, but the extracts were apparently only analyzed using negative ion electrospray after NH4OH and MeOH dilutions. Thus it is probable that a large fraction of the water-insoluble compounds did not ionize. This limitation is not obvious to non-experts. Overall, assuming these limitations are more clearly articulated, this manuscript presents substantially interesting results for the audience of Atmospheric Chemistry and Physics and should be published. A few additional comments are provided below for additional consideration.

Reviewer #1 made a similar comment that the discussion of the instrumental limitations should be more explicitly discussed. We agree that these limitations are important and we will thoroughly review the manuscript to be sure these limitations are more adequately conveyed.

P10416, L2-4: The sentence about PSOM is misleading. It is the solvent that extracts the CHOS compounds with greater efficiency. Why is this? Is it related to the fact that pyridine is an organic base and assumed organosulfates are an acid? Perhaps this point can be made clearly in the manuscript. The more efficient extraction of the assumed organosulfates is counterintuitive since as you indicate may be important for hygroscopicity and CCN.

Reviewer #1 also commented on our assertion that pyridine preferentially extracts sulfur containing compounds and suggested that pyridine could be selective for aliphatic compounds, and those aliphatic compounds may be prone to reactions with co-generated sulfur species. We believe that pyridine's affinity for aliphatic compounds as well as acid/base chemistry may be allowing for a greater detection of CHOS compounds in the PSOM. We will revise the text to acknowledge both possibilities.

TSP samples were collected here. Is it possible the larger PM is more biologically influenced?

It is possible that we are collecting more biologically influenced particles, but confirmation of this would require additional measurements (e.g., microscopic or biomarker analyses) and is outside of the scope of this manuscript. We collected at times where pollen counts were low, so we do not expect that there was an overwhelming amount of plant-derived biological matter in the samples.

The PPL extraction method was recently reported in Wozniak et al., ACPD 2014. In that paper, they reported the use of strong acids for a pH adjustment of the water used to rinse and extract the cartridges. Was the pH adjustment used here? In that paper, they also stated that the addition of NH4OH to the extracts lead to problems with the ESI. Thus, they reported results without pH adjustment. Please comment on the method differences.

Both papers use the PPL extraction methods described in Dittmar et al., 2008. We do acidify the samples to a pH of 2 before PPL extraction as stated in Wozniak et al., ACPD 2014 and in Dittmar et al., 2008. The pH adjustment is required to optimize OM retention by PPL.
It is common practice in our laboratory to optimize instrumental response for each sample set and that includes testing a representative sample with and without the addition of NH_4OH*. In some cases, adding* NH_4OH *will enhance the ionization of peaks attributed to inorganic salts that suppress the ionization of organic matter peaks in ESI. Wozniak et al., ACPD 2014, which notably analyzed aerosol WSOM collected over the marine environment and is expected to have a different composition than the samples analyzed here, compared spectra obtained with* NH_4OH *and without and found that omitting* NH_4OH *gave better quality spectra, so they analyzed all samples without the pH adjustment. During optimization of the samples presented here we found that the addition of* NH_4OH *gave better quality spectra as evidenced by increased spectral signal for organic matter peaks.*

The reported method reproducibility is stated to be good with 67% of the assigned formulas in common between duplicate analyses. This seems very low to me. Higher reproducibility may be found with higher S/N thresholds. Thus, why do you consider 67% to be good or adequate?

The method reproducibility was stated to be greater than 67% in agreement with a study by Sleighter et al. (Analytical Chemistry 2012). That study evaluated the reproducibility of peaks detected by (-)ESI-FTICR-MS in 10 representative natural organic matter types and recommended that a minimum value of 67% be used to establish samples as reproducible at a S/N of 3. The corresponding reproducibilities are higher at higher S/N thresholds, but increasing the S/N threshold omit significant numbers of organic matter peaks thereby needlessly reducing the amount of molecular information that is obtained. The peak reproducibilities calculated for these samples were 84%, 71%, and 69% for WSOM, PSOM, and ASOM, respectively.

P10404: A higher relative abundance of unsaturated carbon was observed in the NMR of the water-soluble OM and this is expected to contribute to higher light absorption. Given the FT-ICR MS data, what is the more likely form of the carbon unsaturation?

Signals in that region of the NMR can be attributed to hydrogen that are attached to carbon adjacent to a C=C or in alpha or beta position to a C=O, and it is difficult to distinguish between the two. Certainly, much of the signal in this NMR region corresponds to hydrogen attached to carbon adjacent to C=C bonds (olefinic compounds). The FTICR-MS data show the majority of olefinic formulas to correspond to CHO and CHON formulas. Signal in this ^{1}*H NMR region could also include ketones and carboxylic acids which are known to be constituents of aerosol OM. LeClair et al. (2012) showed that olefinic compounds frequently show a neutral loss of a* CO_2 *group, indicating carboxyl groups, and we can speculate that much of the unsaturation is due to carboxylic groups on compounds with and without nitrogen containing functionalities. Unfortunately, we cannot be more specific without doing further studies.*

P10405: How were the more H deficient molecular formulas eliminated? The observation of more S-containing compounds is counterintuitive given the SP2 bounding of the pyridine.

H-deficient formulas were not specifically eliminated from consideration of formula assignment. As stated in the manuscript, peaks at low mass defects, as a general rule, typically correspond to formula assignments that are H-deficient or O-rich. In this instance, using the measured mass and the formula assignment rules described in the methods, the molecular formulas corresponded to CHOS formulas. No reasonable H-deficient formulas corresponded to the measured mass.

P10406: Molecular characteristics (of the OM extracted by) each solvent. Please clarify.
The labels for the subset of PSOM and ASOM corresponding to uniquely extracted compounds are confusing. Perhaps you can write PSOM-WI or WI-PSOM instead of
PSOM-WIOM. Or alternatively, one could write PSOM-WSOM indicating the PSOM minus the WSOM.

We understand that abbreviating it as PSOM-WIOM could be interpreted as PSOM minus WIOM rather than the PSOM that is water-insoluble. We thank the reviewer for the suggestion and will change the abbreviation to be more intuitive. The PSOM-WIOM and ASOM-WIOM, meant to represent the water insoluble OM unique to pyridine and acetonitrile extracts will now be referred to as $WIOM_{pyr}$ *and* $WIOM_{acn}$*, respectively.*

What are the molecular weight range differences between the extraction compositions?

The molecular weight ranges are 200-800 m/z to in each of the extracts, which is largely defined by the instrumental parameters and is stated on page 10402 line 10. Peaks are sparsely found above 800 m/z in the case of this sample set, so those peaks are not considered for formula assignments. The average m/z for all assigned formulas is very similar and is between 425 and 450 m/z in each solvent extract.

A well-known limitation of the solid phase extraction methods is the loss of highly water soluble compounds that are rinsed away with the salts. Thus, it would be interesting if the PSOM or ASOM molecular weight ranges were lower. Unless, the FT-ICR mass spectrometer does not trap the low MW ions well. In either case, these limitations should be stated.

There are no major differences between the observed masses. The instrument does not adequately resolve peaks below ~200 m/z, so we do not consider them for formula assignments. As stated in the previous comment, the average m/z for all of the solvents is between 425 and 450 m/z. We also looked at the total formula distribution over the mass range (see attached fig. 1), and there is no apparent dominance at lower masses by either of the solvents. I believe that the reviewer makes a good point, and it is possible and likely that lower molecular weight species are extracted by the organic solvents because of the lack of SPE. However, our FTICR does not detect those species effectively, so we were not able to assess *that. We will add text that states the molecular weight limitations of the instrument, and that the SPE could be removing those species.*

LeClair et al., 2012 provides an MS/MS evaluation of several CHOS, CHNO and CHNOS compounds. They did not find the associated nitrate and sulfate losses for all of the compounds thus they suggest the terms organosulfates and organonitrates are not wholly accurate.

We recognize that not all compounds containing N and/or S will be organonitrates or organosulfates, and state that more reduced forms of the atoms could be present. We use the O/N and O/S ratios to determine whether it is even possible for the compound to contain a nitrooxy or sulfate group, and state that it is a possible structural feature. The implication is that it is a possible structure and that there are other possible structures, but the reviewer's comment suggests clarification is warranted. The LeClair study referenced by the reviewer did indeed find that 15% of S containing formulas and 58% of N, S containing formulas did not show neutral losses consistent with sulfate functional groups. Similarly, 37% of CHON formulas did not show HNO_3 *losses consistent with organonitrates. This does support the idea that many CHOS, CHON, and CHONS formulas do not represent organosulfates, nitrooxyorganosulfates, and organonitrates. It should be noted, however, that the above percentages do suggest that very large percentages of these formulas do represent organosulfates, nitrooxyorganosulfates, and organonitrates. Taking this comment into account, the text will be revised to more clearly acknowledge that while not all of these compounds are actually organosulfates (etc.) but that their formulas are consistent with an abundance of these compounds. The LeClair reference will be cited in the text both as evidence for the probable abundance of these compounds and as evidence that the N and S formulas likely take additional forms as well.*

P10414: The discussion of aliphatic compounds in WSOM would be improved with comparison to other studies beyond Wozniak et al. Look at LeClair – they define aliphatic/olefinic/aromatic the same way.

This comment is general in nature and therefore somewhat difficult to address. The discussion on page 10414 concentrates on the WIOM having higher contributions from aliphatic compounds than WSOM and references the Wozniak radiocarbon paper as evidence for aliphatic compounds being largely insoluble. The LeClair paper examined filtered fogwater OM (equivalent to WSOM) components and doesn't provide a direct comparison. Nonetheless, we will review this section and the LeClair paper (among others) and edit it as necessary adding references.

Please enlarge Figure 2 in the ACP version of the manuscript.

We will enlarge the figure size in the publication.

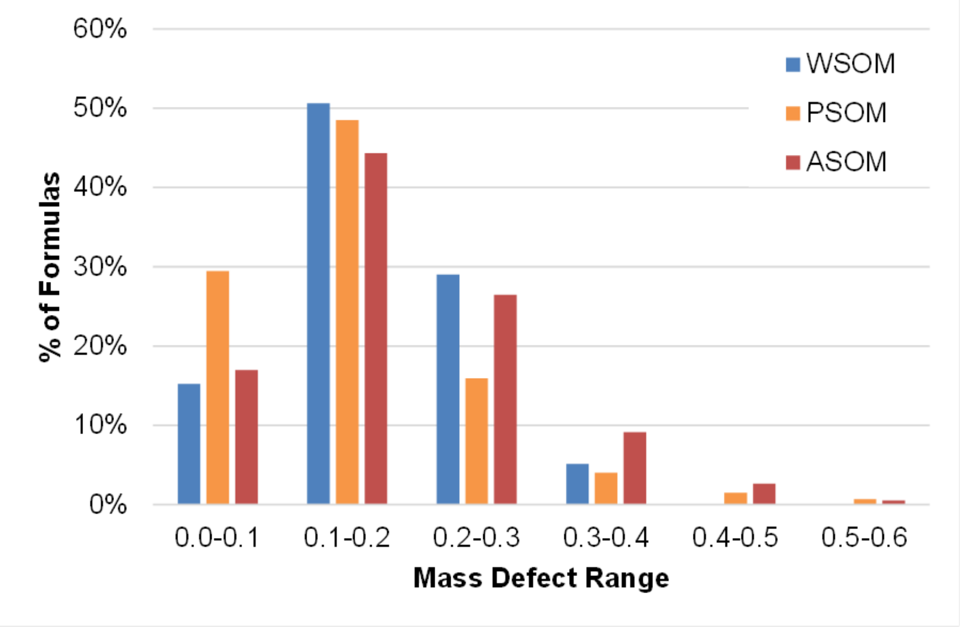

Fig. R1. The distribution of the percentage of formulas found within specific mass defect ranges for each of the solvents.

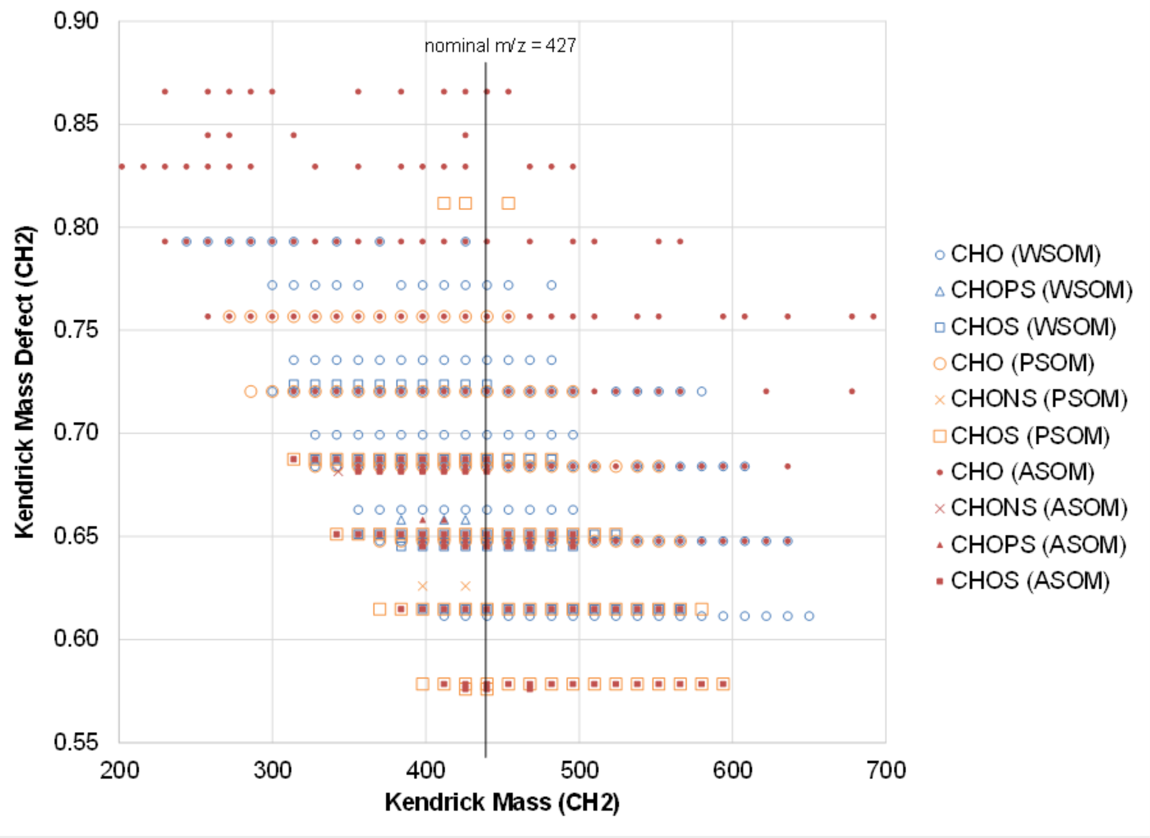

Fig. R2. A Kendrick Mass Defect (CH2) plot for the formulas identified for peaks shown in Fig. 2.

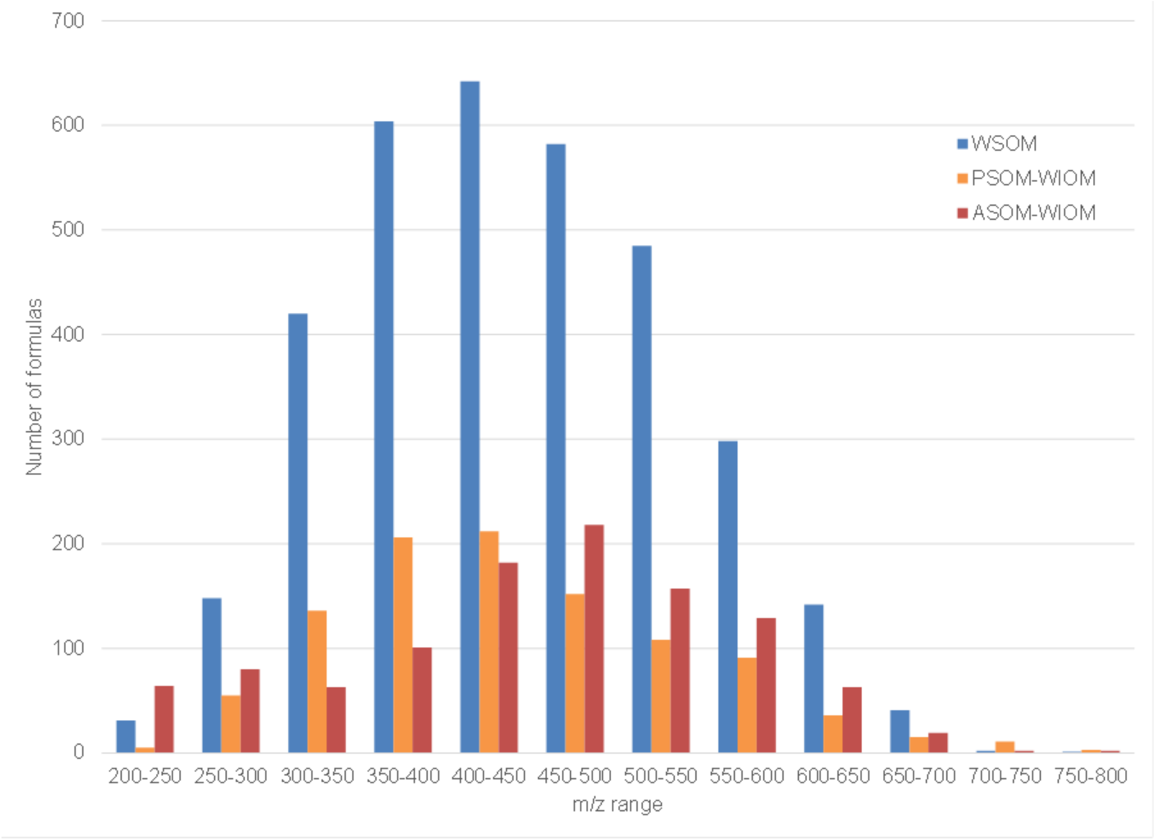

Fig. R3. A histogram of the number of formulas present across the entire mass range, split into 50 mass unit segments. The average mass for each solvent is found between 425-450 m/z and each gives nearly a Gaussian distribution of formulas.