DETERMINING SURFACTANT PRESENCE IN EMULSION POLYMER SAMPLES USING SINGLE-QUADRUPOLE MASS DETECTION

By

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ABSTRACT

Surfactant analysis is generally performed using liquid chromatography (LC) and a tandem triple-quadrupole mass spectrometer (MS-MS). This method provides the sensitivity and specificity required to resolve this type of molecule in a mixture. A less equipped laboratory may not have this option. Here we show a method developed to detect residual quantities of the surfactant Reasoap SR-10 in emulsion polymer samples. This was achieved using an Acclaim Surfactant Plus column from Thermo Scientific installed on a Waters 2695 liquid chromatograph connected to a Waters QDa single quadrupole mass detector set for single ion recording at m/z = 557, utilizing electrospray ionization. While specificity was achieved, the method had challenges with consistent sensitivity, precision and recovery which will lead to more effort in method refinement, namely sample preparation. Even though for now it is better classified as a qualitative method, a fully successful method would be a benefit for our laboratory. This would prevent causing delays for needed information and higher costs due to utilizing a reference laboratory, creating efficiencies for in-house product troubleshooting as well as research and development.

This thesis is dedicated to Krista, Claire, and Connor.
Thank you for unending support.

ACKNOWLEDGEMENTS

This would not have been possible without the support and encouragement of my family and colleagues. I greatly appreciate and am thankful for the College of Natural Science - Biomedical Laboratory Diagnostics Program, and the Department of Chemistry. I am forever grateful for the support and guidance of my committee comprised of Dr. Brennen, Dr. Hoag, and Dr. Blanchard from Michigan State University as well as Dr. Blevins from BASF. My sincere thanks to you all for helping me complete this project.

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INTRODUCTION

Surface active agents, or surfactants, have a wide range of compositions and uses. This is the most powerful substance that lowers the surface tension of the solvent, particularly water.[1] Surfactants are typically composed of a hydrophilic tail and hydrophobic head which defines their amphiphilic nature. Surfactants can be classified as ionic, non-ionic, or amphoteric according to the charge carried by the surface-active part of the molecule.[2] These materials can be synthetically produced and used in industry as well as occur naturally in biological systems. Examples in industry include dispersing agents such as emulsion polymerization and resin modifiers, wetting agents, and paints. They are also used in household items such as shampoo and toothpaste with the most popular being sodium lauryl sulfate.[3] In contrast, an example of a naturally occurring biological surfactant can be found in the lung surfactant system with lamellar bodies. These micelles contain both phospholipids and proteins, with lecithin being an example of a phospholipid and pulmonary associated surfactant protein-A as an example of a protein surfactant that lines the lungs and helps reduce surface tension. [4, 5, 6] Another example would be Ki-67. This marker protein is present during mitotic chromosome movement and prevents chromosomes from collapsing into a single chromatin mass after the nuclear envelope is disassembled, enabling independent chromosome movement and better interactions with the mitotic spindle.[7]

Surfactants are also essential in synthetic latex production. In order to create a synthetic latex, the main component of water-based paints and adhesives, the leading process is emulsion polymerization.[8] This involves the process of initiation, propagation and termination using the reaction of free radicals with monomer molecules in a very large number of discrete polymer particles dispersed in the continuous aqueous phase.[9,10,11] This mixture of surfactant, water, monomer and initiator can be controlled as needed to produce particles of a desired structure, size and distribution in line with the target structure-property relationship. In this mixture, the surfactant is used to create micelles dispersed throughout the continuous aqueous phase that have the hydrophobic portion oriented inward and the hydrophilic portion in contact with the aqueous environment. In the hydrophobic interior of the micelles, the monomer molecules reside and can interact with the initiator. The reaction occurs and chains together monomer to

make polymer particles. This reaction continues under controlled conditions until the monomer is consumed or termination occurs.[12] However, this process rarely converts all the monomer introduced and the residual monomer must be considered. Besides potentially affecting the properties of the dispersion, residual monomers, residual surfactant and initiator are also often toxic so a product with zero or very low levels of residual monomer, surfactant and initiator would have great appeal.[13]

More specific to this project, as a reactive surfactant where a large fraction of the surfactant molecule becomes irreversibly bound to the emulsion polymer chains and droplets,[14] Reasoap SR-10 is used as an emulsifying polymerization agent as well as a monomer for radical polymerization.

Along with the use of surfactants there is also a need to monitor these materials either for research and development, manufacturing, environmental or medical reasons. Typically, this can be done using liquid chromatography with tandem mass spectrometry (LC-MS) where the liquid chromatograph performs the separation of components and the detector is a mass spectrometer that has a triple quadrupole configuration to take advantage of mass to charge ratio (m/z) filtering (Q1 and Q3) and use of a collision cell (Q2). However, separation and detection can also be performed using ion-pair separation with gradient elution by ion chromatography.[15] Considering the liquid chromatograph portion there are multiple column possibilities. Surfactant analysis can be performed using hydrophilic interaction liquid chromatography (HILIC) columns for nonionic surfactants to identify alkylphenol ethoxylates (APEOs), which is one of the largest classes of non-ionic surfactants.[16] Other surfactants have been analyzed using carbon chain columns with different functional groups. This has been done by Harayama et al. with surfactant protein SP-C using a C4 column,[17] while two different columns such as weak anion exchange (WAX) and HILIC have been used in combination for both nonionic and anionic analysis.[18] Reverse phase C18 columns have also been utilized by Matsumoto, et al. for the simultaneous analysis of anionic, amphoteric, and non-ionic surfactants.[19] Mobile phase choices also vary, and analysis can be performed using buffered and non-buffered systems. The use of ammonium acetate may prove useful as it can be used as an ion-pairing agent and as a volatile electrolyte for thermospray LC-MS detection.[20] Concerning detection, mass

spectrometry in a triple quadrupole configuration is typically used, although suppressed conductivity has also been used with ion chromatography which in contrast to mass spectrometry is non-destructive.[15]

There are other methods that could detect surfactants, but there would be caveats. Considering spectroscopy, ultraviolet – visible light (UV-Vis) spectroscopy is dependent on structure and the sample would need to have conjugated pi-electrons that can be accessed. Not all surfactants have this. In addition, the signal would be non-specific in that there could be other materials present contributing to the target signal. This could create false positives or inaccurately quantify a target surfactant. Infrared (IR) spectroscopy could be helpful in identifying structure but would present difficulty for quantification due to the lack of specificity. Nuclear magnetic resonance (NMR) spectroscopy could be useful for identification and quantification but becomes clouded for systems that are distributions. Also, isolating in a mixture can be complicated due to similarities between surfactants and broadening due to these distributions.

Ion chromatography could also detect surfactant when it is the only counter ion source. The ion exchange columns could separate components, but like UV-Vis the signal is nonspecific and would not provide the specificity needed when working with a sample with multiple components.

Understanding how surfactants are structured and used, the ideal method for separation and detection remains LC-MS. This instrumentation provides the specificity and sensitivity needed to reliably identify surfactants in emulsion polymer samples using a carefully created method.

The objective of this project is to develop a reliable method that will detect and quantify free Reasoap SR-10 in emulsion polymer samples that uses high pressure liquid chromatography (HPLC) and a single-quadrupole mass spectrometer. The first task is to determine the ideal stationary phase and mobile phases, followed by optimizing the acquisition parameters. Once this is achieved sample preparation can be refined, a unique target for SR-10 can be identified, and a standard curve created. Using the verified standard curve, the method will be verified for the absence of carryover as well as establish a limit of detection (LoD) and limit of quantitation (LoQ).

The method will be further tested by evaluating percent recovery in a spiked sample, within run precision, between run precision, and inter-operator variance.

The surfactant target for this method is Reasoap SR-10. This is an ether sulfate surfactant that can be used as an emulsifying polymerization agent and other applications such as a resin modifier. This can be difficult to detect, identify, and analyze in a sample because there are no components that are UV active and because it is essentially a product of a distribution of distributions that can mask specificity. Under these conditions the challenge lies in identifying a unique signature that can be used to confidently identify the presence in a mixture that has multiple components including gel, other unknowns, and even additional surfactants. In this environment LC-MS is potentially a great tool when coupled with physical separation. Physical separation allows the removal of polymer and gels to focus on the water soluble portions such as free monomers, oligomers, and surfactants. Assuming that each surfactant fragments differently it could be possible to identify and analyze surfactants in a mixture whether a chromophore is present or not. This would provide identification and specificity, something that UV detection could not provide. Figure 1 presents the structure of Reasoap SR-10.

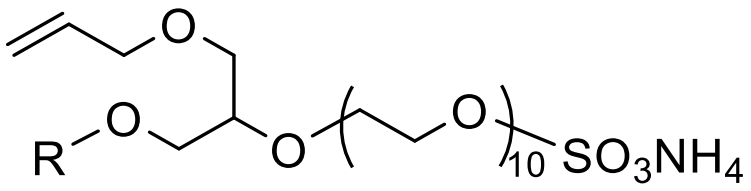


Figure 1 – Structure of Reasoap SR-10.

The structure of SR-10 presents that there are ten ethylene oxide groups before being terminated by a SO_3NH_4 group while R represents an alkyl group that can be specified to the user's application and need. This chain is often proprietary, and the specific structure is unknown.

MATERIALS AND METHODS

In the case of Reasoap SR-10, the expectation would be to see a distribution of fragments cleaved from the intact molecule. Figure 2 shows a few possibilities that could include pieces from the upper alkene chain (A), lower alkyl chains (B and C), and upper and lower chains with degrees of ethylene oxide. Should there be success in defining unique identifiers for surfactants it would be advantageous to curate a spectral library to facilitate the identification of surfactants in samples, namely samples with more than one surfactant present.

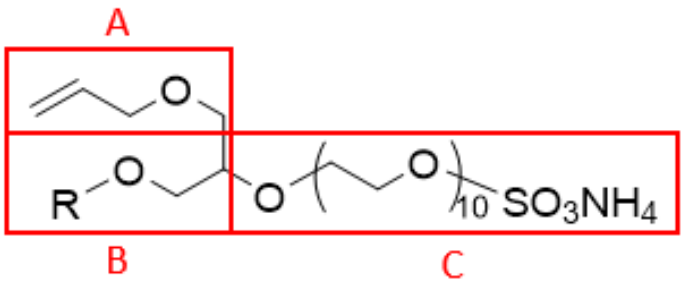


Figure 2 – Possible SR-10 fragment combinations that may be useful in mass spectrometry identification.

One limitation, though, is the quality of the mass spectrometer, which in an ideal case would be a tandem MS-MS configuration that would allow the use of three quadrupoles (Q1, Q2, and Q3). This would provide more selective scanning options, an example being that a sample could be filtered for a known mass in Q1, fragmented in Q2 and have Q3 perform a full range scan to reveal how the ion fragments. Multiple reaction monitoring (MRM) could also be an option where Q1 and Q3 are set to more than one mass which could provide increased specificity and sensitivity to the desired target. In this lab a single quadrupole mass detector is available for use and it will be determined if the specificity can be found to identify and quantify the SR-10 surfactant.

The separation and acquisition parameters were determined by exploratory experiments with the goal being to be able to have a simple and easily adjustable method that can provide sufficient separation and resolution of the water-soluble materials in a sample. Starting with column selection, the Acclaim Surfactant Plus

(reverse phase) from Thermo and a HILIC column from Agilent were considered. After initial injections the reverse-phase column provided better separation and overall performance. For the mobile phases, using a small concentration of ammonium acetate has been common in separations and this was utilized for this method similar to Matsumoto et al. (2012) by starting with an aqueous mobile phase of 10mM ammonium acetate in water and organic mobile phase of 90% acetonitrile/10% ammonium acetate in water.[19] Formic acid was added to the organic mobile phase at a concentration of 0.01% to provide a source of protons and improve peak shape, a common practice in reverse phase HPLC (Nunez et al., 2014).[21] A flow rate of 0.400 mL/min was selected because it was the highest flow rate that provided adequate resolution in a reasonable amount of time without creating an unmanageable amount of backpressure. Running at a rate above 0.400 mL/min took the backpressure above 1,000 PSI. Since the instrument does not utilize ultra-high pressure liquid chromatography (UHPLC) that can accommodate higher thresholds, it was desirable to keep the backpressure below 1,000 PSI to ensure that the stationary phase did not migrate over continued use.

The gradient was selected because it was a compromise of peak resolution and acquisition time, providing good separation in ten minutes. The initial gradient did not provide workable separation so the timing, rate, and duration of increase in organics were sped up and lengthened to better separate fragments. This was monitored by utilizing the total ion count (TIC) plots. Figure 3 shows the TIC plot for the first gradient that was used (A) followed by the gradient after first adjustment (B). The bottom plot (C) shows the final gradient after further refinement. The first gradient did not start to increase organics until 10 minutes and held at 10% for two minutes.

The final gradient increased organics by 20% at 1.5 minutes and held for three minutes. Moving this timing up and increasing the duration provided much better resolution and allowed the acquisition window to be brought down to 10 minutes. The injection volume of 10µL was found to provide a workable balance between sensitivity and the signal to noise ratio while the acquisition window of ten minutes proved large enough to capture all sample data and return to baseline.

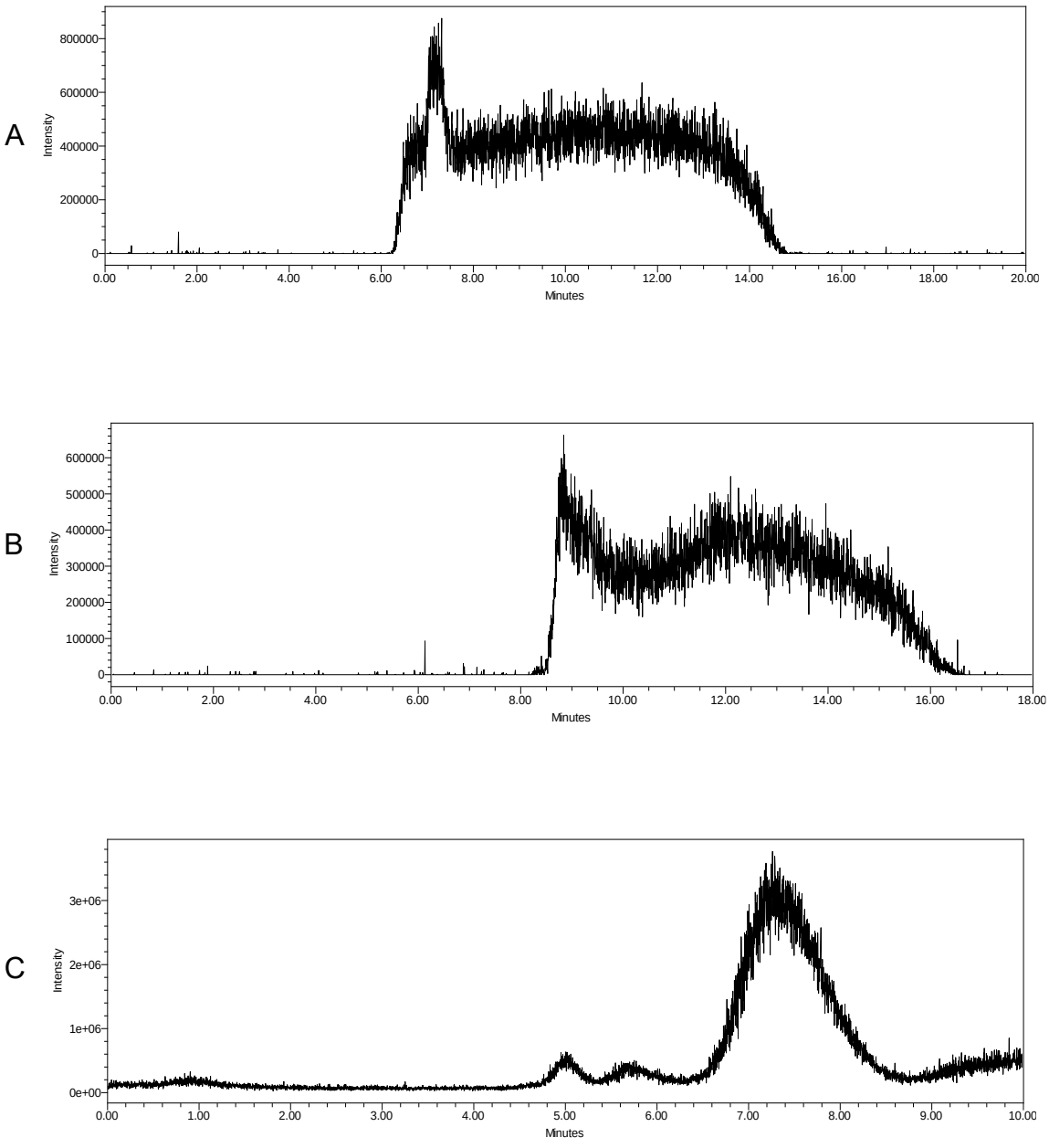


Figure 3 – Gradient comparison. Plot A increased %B by 10% at 10 minutes, plot B increased %B by 15% at 5 minutes, and plot C increased %B by 20% at 1.5 minutes.

Final Instrument Setup and Initial Conditions

Separations:

- Column – Acclaim Surfactant Plus (3um, 4.6 x 150mm), Thermo Scientific
- Mobile Phase A – 10mM ammonium acetate in water
- Mobile Phase B – 90/10 (acetonitrile/10mM ammonium acetate in water) with 0.01% formic acid.
- Flow rate – 0.400 mL/min
- Gradient – Initial: 25% A ramping down to 5% A at 1.5 minutes, holding until 3 minutes and then ramp back up to 25% A at five minutes.
- Injection volume: 10µL
- Acquisition time – 10 minutes with a 10 minute delay between injections

Detection:

- Ionization method – Electrospray ionization (ESI) in negative mode
- Single ion recording (SIR) – 557.58 Da
- Cone voltage – 15V
- Sampling rate – 10 points per second

Sample Preparation and Injection

Latex preparation: Coagulate emulsion with 30:70 (acetonitrile:HPLC water) 1:1 (2mL sample + 2 mL 30:70) and place on shaker for one hour. Centrifuge at 462000 x *g* for 15 minutes to stratify the sample. Draw the supernatant into a syringe and attach a 0.45 µm syringe filter. Pass the sample through the filter and directly into an autosampler vial. Cap the vial and label appropriately. The sample is ready for injection and acquisition. If the initial data shows a concentration above 1000ppm, a dilution using with 30:70 (acetonitrile:HPLC water) will need to be performed in order for the injection to generate a result within the established calibration curve and to prevent poor chromatography. Note the dilution factor and apply the multiplier when the data is quantitated.

Neat surfactant for calibration: Dilute surfactant to following concentrations using HPLC water: 5ppm, 10ppm, 100ppm, 250ppm, 500ppm, and 1000ppm. Next, dilute 1:1 with 30:70 (acetonitrile:HPLC water) to maintain the same treatment as the latex samples by shaking for one hour followed by centrifuging at 462000 x *g* for 15 minutes.

Afterwards, draw the sample into a syringe and attach a 0.45 µm syringe filter. Pass the sample through the filter and directly into an autosampler vial. Cap the vial and label appropriately.

RESULTS

Ion Selection and Specificity

Figure 4 presents the overall mass spectrometry profile of Reasoap SR-10. The plot A shows detected fragments which begin to be seen just before five minutes. Three distinct groups of bands can be seen at around five minutes, five and a half minutes, and seven and a half minutes. This is reflected in the total ion count (TIC) in plot B.

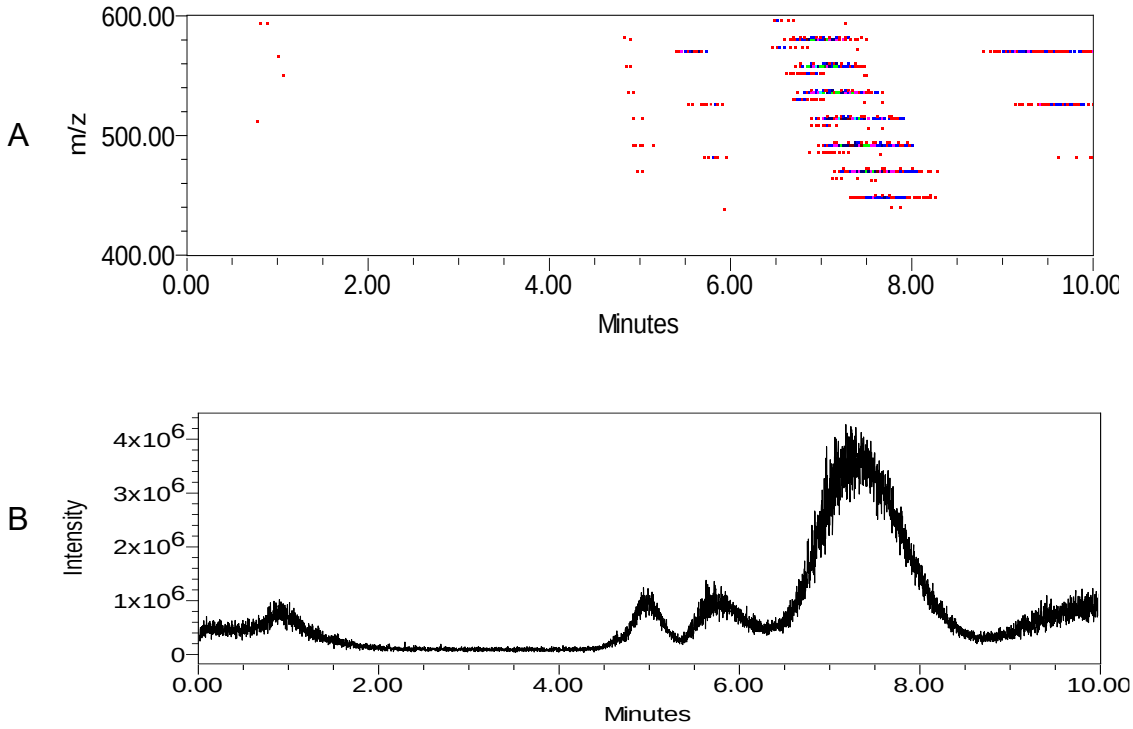


Figure 4 – Reasoap SR-10 profile with fragment plot (A) and TIC (B). This data provided the first glimpse into the SR-10 mass data using this method.

Looking at each peak of the separated distribution of the sample we can look at the mass spectrum of each peak. In Figure 5 the peak in the 4.6 – 5.4 minute range showed fragments ranging from m/z = 469 – 557, with m/z = 513 being the most abundant.

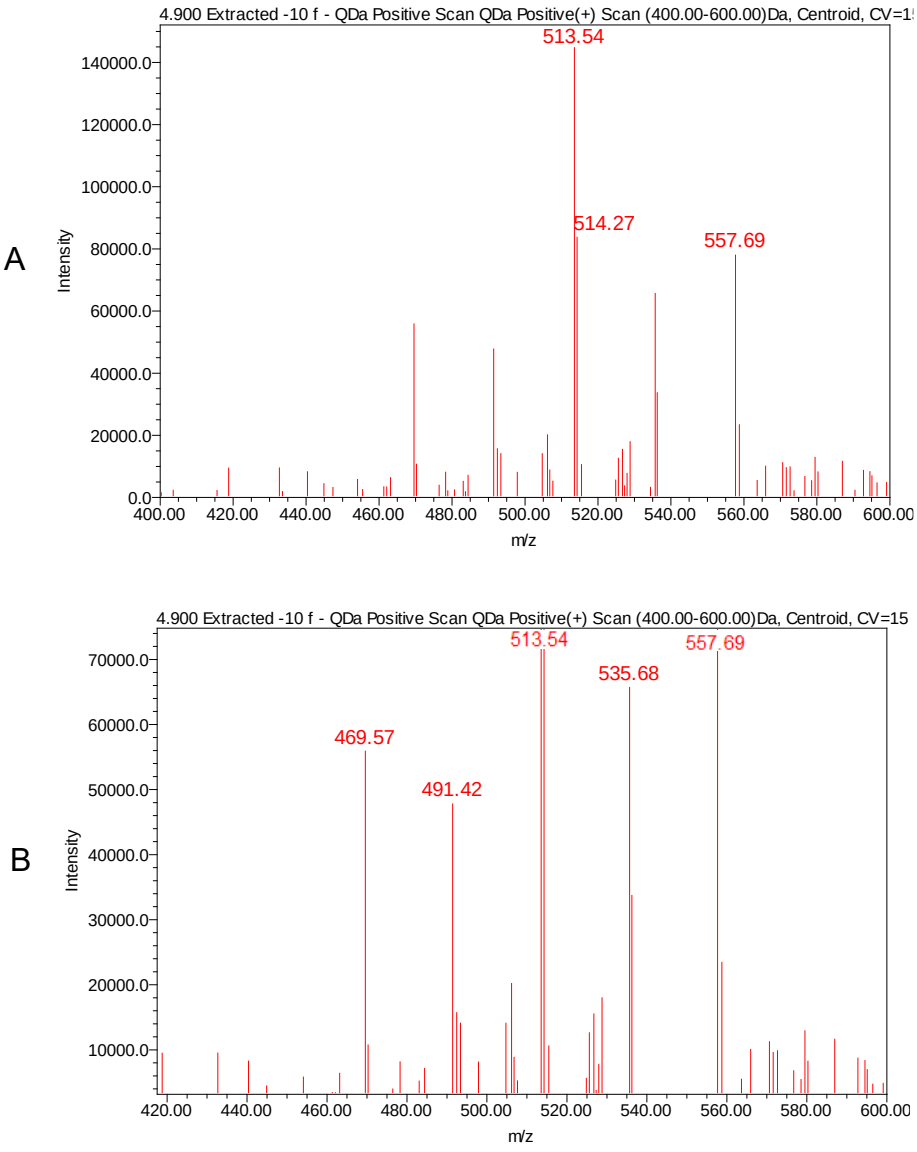
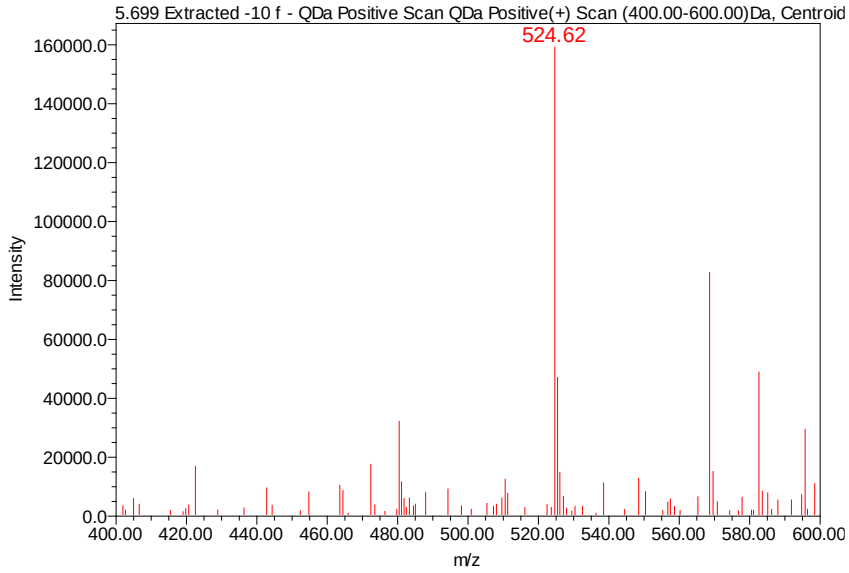


Figure 5 – Reasoap SR-10 mass data for the peak at 4.9 minutes. Spectrum A shows the distribution of most abundant species ranging from m/z = 469 to m/z = 557. Spectrum B is a magnified view of the top plot to show the m/z values of the less abundant species.

Looking to the next peak at 5.7 minutes, Figure 6 shows details for the 5.5 – 6.4 minute range with fragments ranging from m/z = 422 – 595 with m/z = 524 being the most abundant. There is adequate resolution from the peak at 4.9 minutes but does not reach baseline before the peak at 7.3 minutes.

A



B

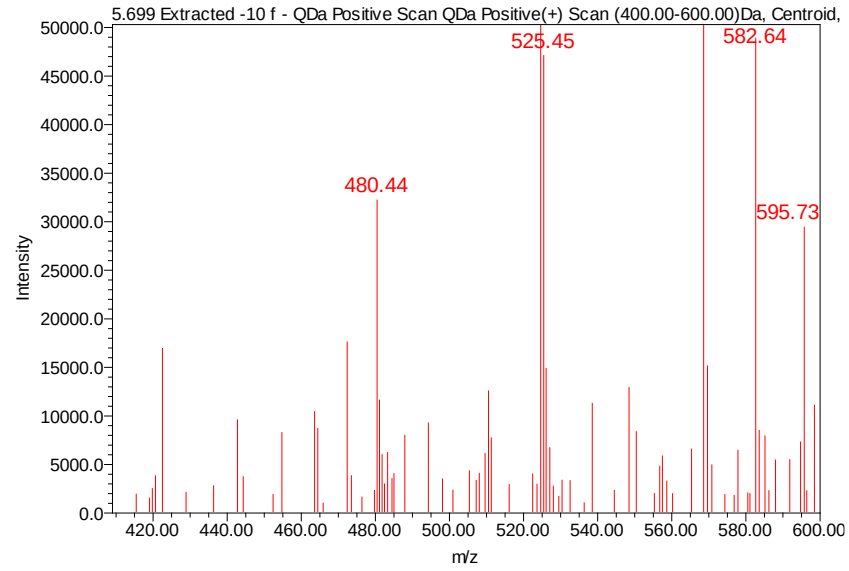
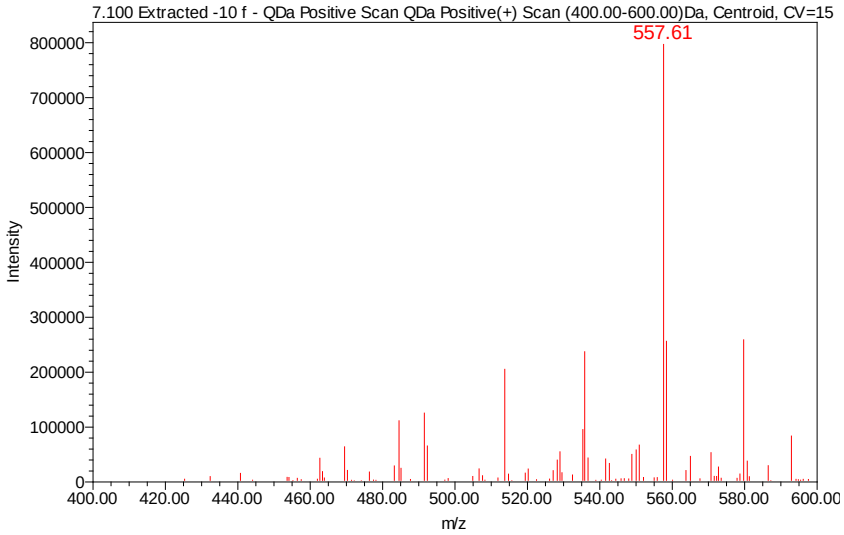


Figure 6 – Reasoap SR-10 mass data at 5.7 minutes. Spectrum A shows the distribution of most abundant species ranging from m/z = 422 to m/z = 595. Spectrum B is a magnified view of the top plot to show the m/z values of the less abundant species.

Moving on to the last and largest peak at 7.3 minutes, Figure 7 shows the detailed 6.5 – 8.5 minute range with fragments ranging from m/z = 462 – 579 with m/z = 557 being the most abundant. This peak likely represents the large amount of ethylene oxide groups present, noted by the m/z = 44 interval between the bands.

A



B

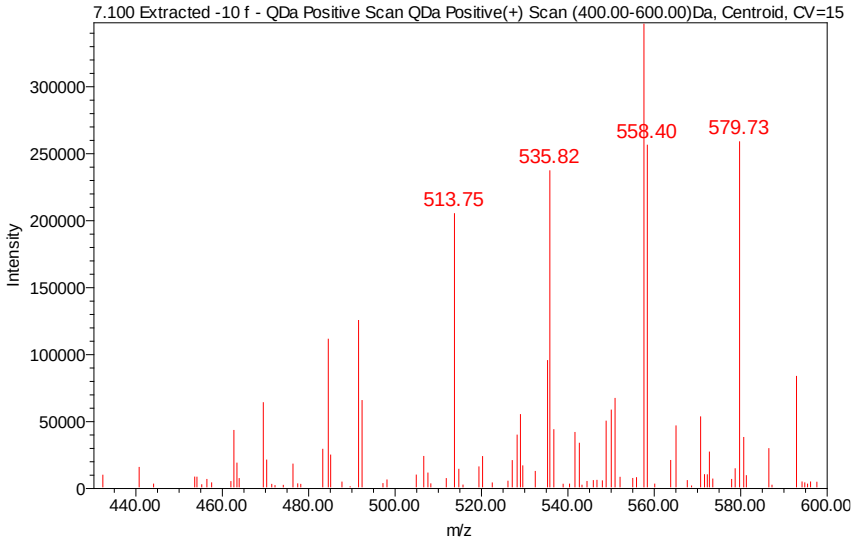


Figure 7 – Reasoap SR-10 mass data at 7.3 minutes. Spectrum A shows the distribution of most abundant species ranging from m/z = 462 to m/z = 579. Spectrum B is a magnified view of the top plot to show the m/z values of the less abundant species.

The ethylene oxide groups in SR-10 were identified by their predicted m/z of 44 and were observed to elute from about 6.5 to 8.5 minutes, previously illustrated in Figure 7. There were 44 m/z intervals between the bands, indicating either the loss of an ethylene oxide group from the chain of ten during fragmentation or the current method produced multiple fragment lengths of the original ethylene oxide chain, illustrated in Figures 8 – 10.

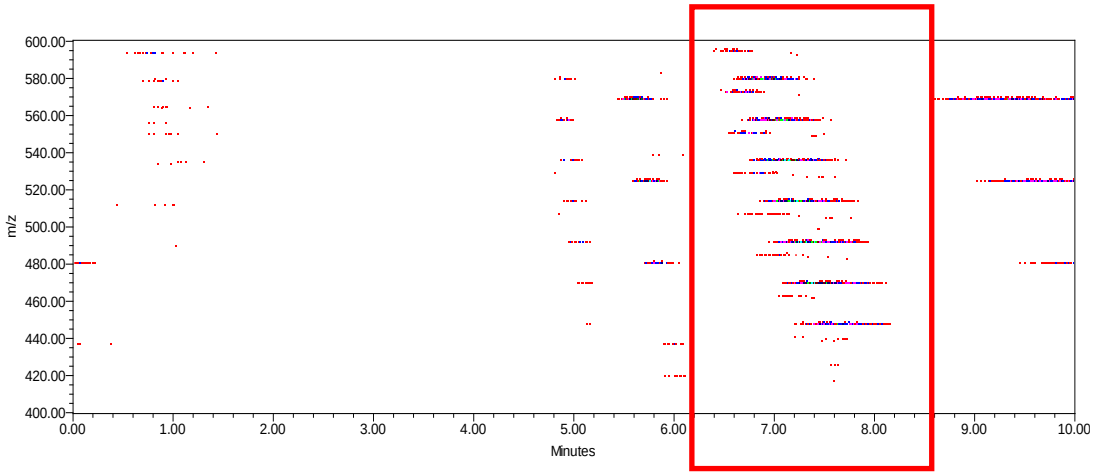


Figure 8 – Ethylene oxide region (outlined).

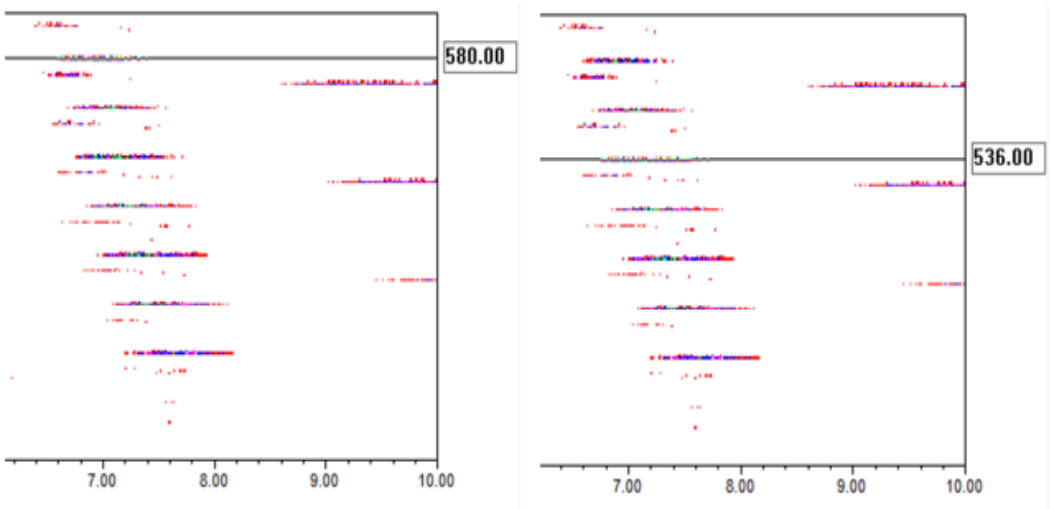


Figure 9 – Bands at m/z=580 and m/z=536.

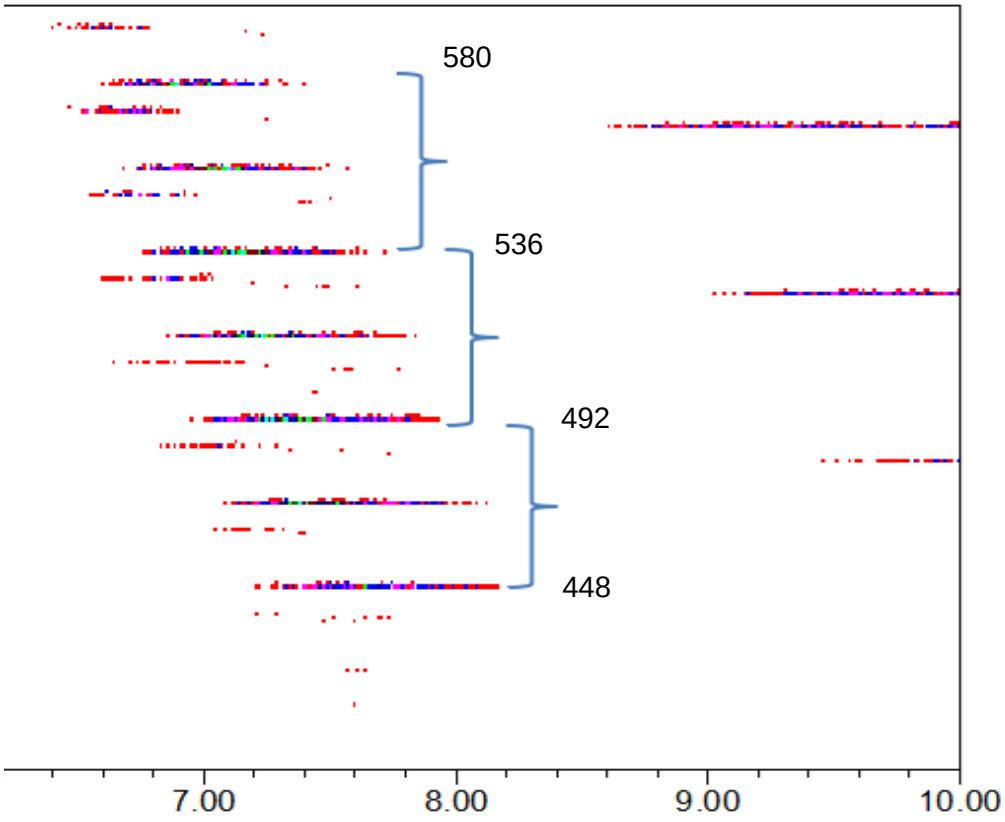


Figure 10 – Set of intervals of m/z=44 (580 – 536, 536 – 492, and 492 – 448).

The next step was to find a target ion away from the 6.5 - 8.5 minute range despite being the largest peak because the distribution of the surfactant observed here is dominated by the ethylene oxide fragments, masking other fragments in that window. Understanding that ethylene oxide groups are common in surfactants, it would not be a good candidate as a specific identifier for SR-10. Ethylene oxide fragments, while visible, should be ignored regarding targeted identification in a sample that could contain multiple surfactants. However, observing this can suggest the presence of a surfactant.

The target had to produce a large enough signal to detect, provide a usable analytical range, and be reproducible. Figure 11 shows a possible candidate by looking at the TIC previously mentioned showed that the 4.6 – 5.4 minute window or the 5.5 – 6.4 minute window may have viable candidates.

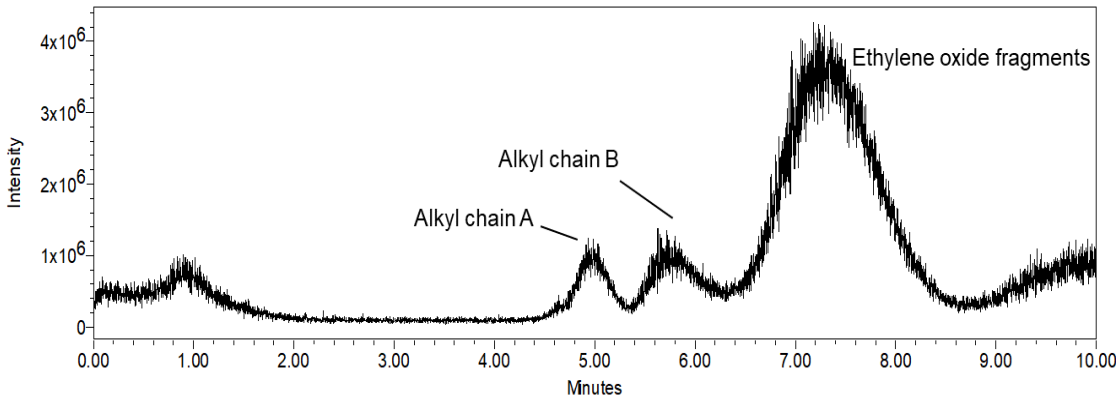


Figure 11 – TIC Chromatogram of Reasoap SR-10. Note the groupings identified and how alkyl chain A is comfortably far enough away from the ethylene oxide peak. Chain B also has workable resolution between the other two peaks and is of similar size, but Chain A has better peak shape than chain B.

A candidate was found around the 4.9 minute mark with m/z 557.58 which is likely part of the alkyl chain opposite the ethylene oxide group. Figure 12 shows the single ion recording (SIR) run set to this m/z = 557 which yielded a signal that provided the specificity and retention time combination as well as the reproducibility that was needed. It should be noted that the region around 7 minutes also shows a large signal for the m/z = 557 fragment but concern over the ethylene oxide precludes this as the optimum target.

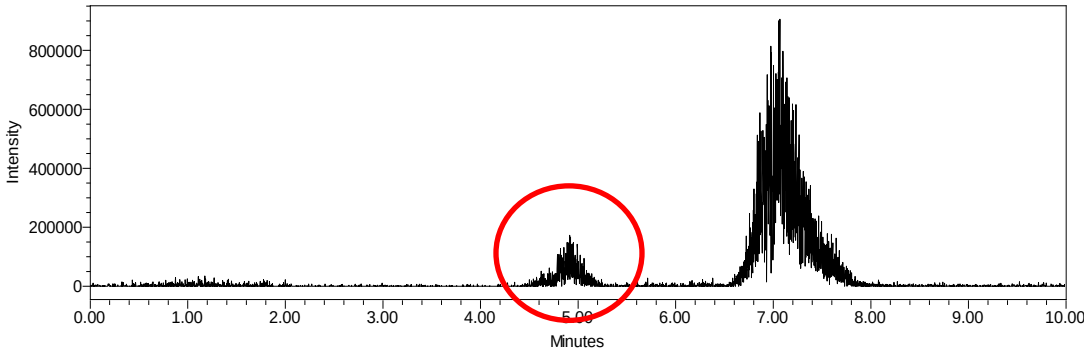


Figure 12 – SIR chromatograph of Reasoap SR-10 at m/z 557. The signal at around 4.9 minutes is highlighted to illustrate its difference from the ethylene oxide chain region. The mass spectra are the same as illustrated in Figure 5.

To understand the specificity of the observed target ion for SR-10 a different surfactant was also analyzed. Hitenol BC-2020 was selected because it also contains a significant amount of ethylene oxide but a much different hydrophobic portion, so the unique target ions should be different. Analyzing this against what had been seen with SR-10 will provide insight into the robustness of the method. Figures 13 - 17 show the structure and breakdown of Hitenol BC-2020 while Figures 18 and 19 presents a comparison to Reasoap SR-10. No interference with the SR-10 target ion from Hitenol BC-2020 was observed.

Figure 13 presents the structure of Hitenol BC-2020 with a comparison to Reasoap SR-10. Note that there are also two alkyl chains present and the ethylene oxide chain is also terminated by an SO_3NH_4 group. Note that Hitenol BC-2020 has twenty ethylene oxide groups in its chain.

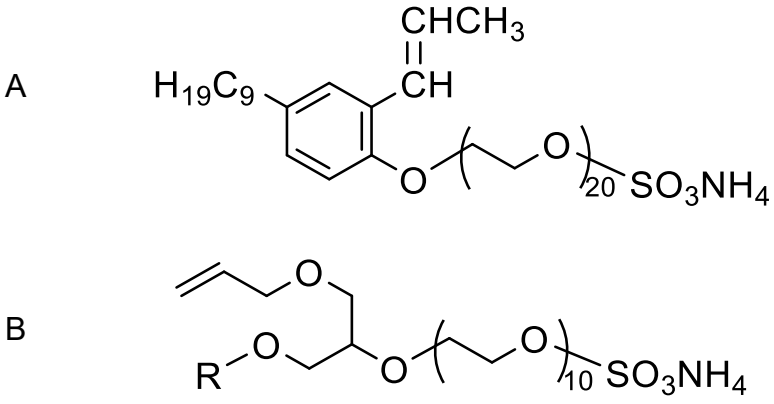
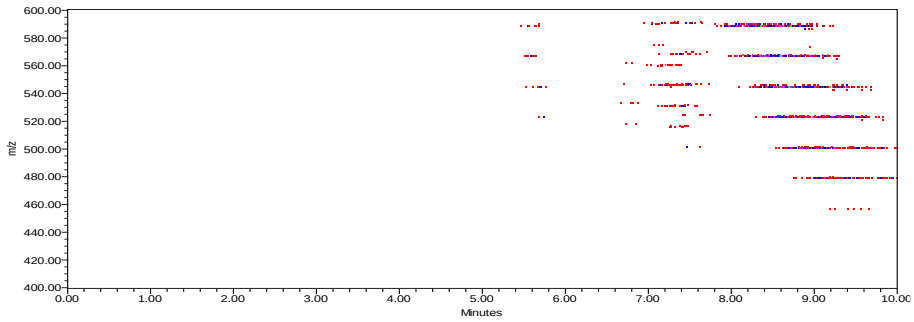


Figure 13 – A: Structure of Hitenol BC-2020. B: Structure of Reasoap SR-10. There are similarities to Reasoap SR-10 regarding side chains that can lend confidence to the comparison study.

Figure 14 presents the overall profile of Hitenol BC-2020 from the mass spectrometer. Plot A shows detected fragments which begin to be seen just before six minutes. Three groups of bands can be seen at around six minutes, seven minutes, and nine minutes. The low percent solids in the sample of Hitenol led to a lower overall signal which does not resolve as well when observing the TIC in plot B. The SIR at m/z = 557 in plot C visualizes the target m/z in order to compare to Reasoap SR-10. Referring back to Figure 12 that displays the SIR for Reasoap SR-10, it can be

observed that utilizing the SIR mode revealed a unique chromatogram for each surfactant by only selecting for m/z = 557.

A



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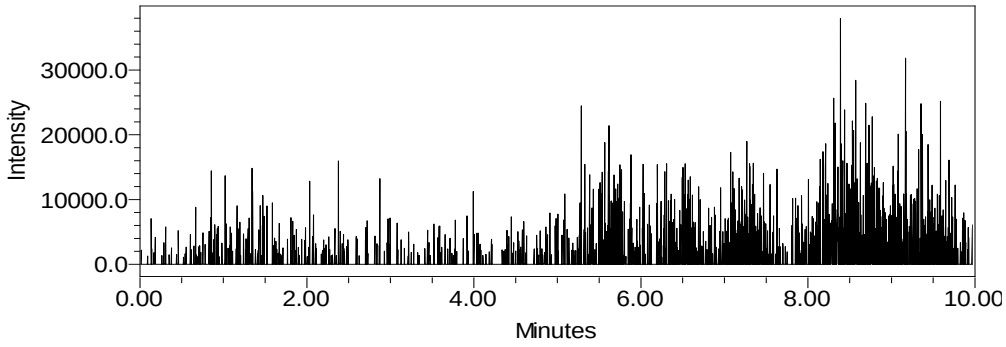


C

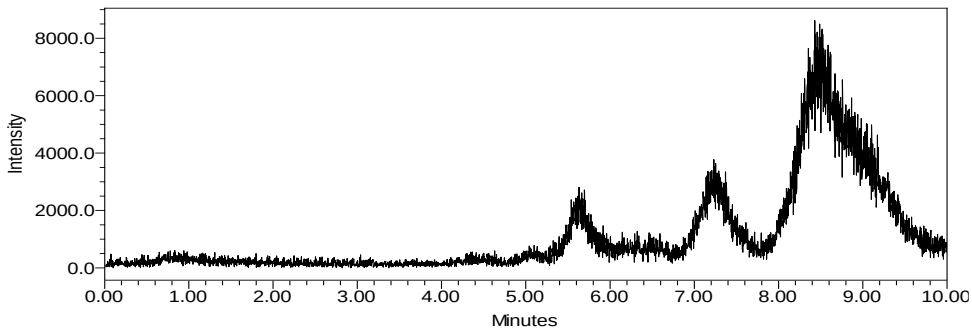


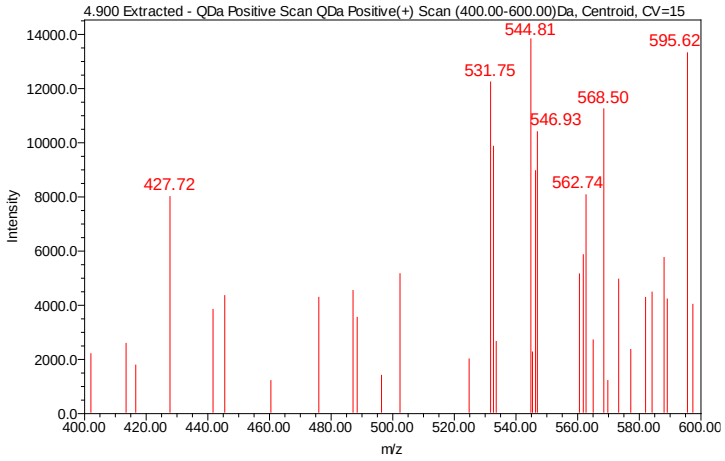
Figure 14 – Hitenol BC-2020 contour plot (A), TIC (B), and supplemented by a SIR plot at m/z = 557 (C).

Looking at each region in the separated distribution of BC-2020 we can examine the mass spectrum of each segment. This is presented in Figure 15, plots A, B and C. The 5.6 – 5.8 minute range showed fragments in plot A ranging from m/z = 427 – 595, with m/z = 544 being the most abundant. There is some evidence of ethylene oxide in the region from the m/z = 44 intervals between the bands, but the separation is different in comparison to SR-10. This relates to the first region seen in the TIC and is also the range in which our target ion of m/z = 557 and desired retention time is located.

The next region of BC-2020 in is the 6.8 – 7.8 minute range that showed fragments in plot B spanning from m/z = 502 – 589, with m/z = 574 being the most abundant. This relates to the middle section of the TIC where increased concentrations of ethylene oxide groups being to be observed.

The 7.9 – 9.9 minute range of BC-2020 showed fragments in plot C ranging from m/z = 478 – 602, with m/z = 522 being the most abundant. This relates to the third grouping in the TIC where the ethylene oxide groups seem to be the most concentrated.

A



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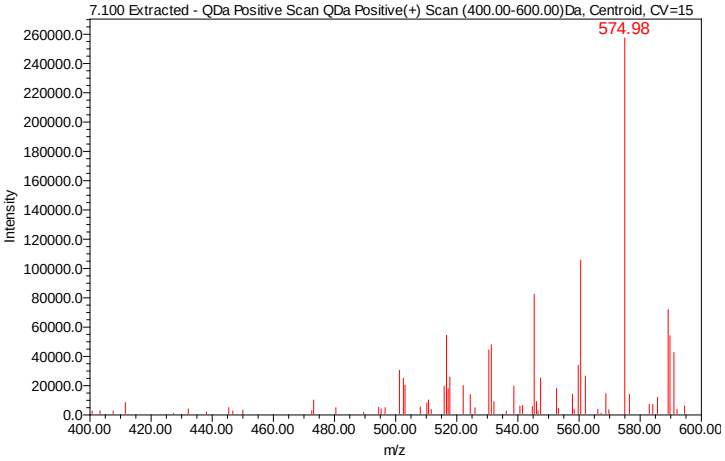


C

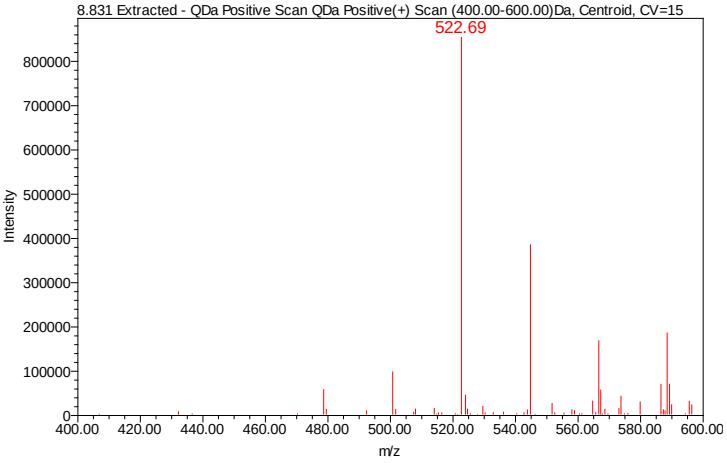


Figure 15 – Hitenol BC-2020 mass distributions in detail for each group observed in Figure 14, plot A.

Single ion recording at m/z = 557 would have greater resolution and the potential to quantify at lower concentrations. This target ion of SR-10 was evaluated and compared for both SR-10 and BC-2020. Figure 16 displays the SIR chromatographs of BC-2020 (A) and SR-10 (B).

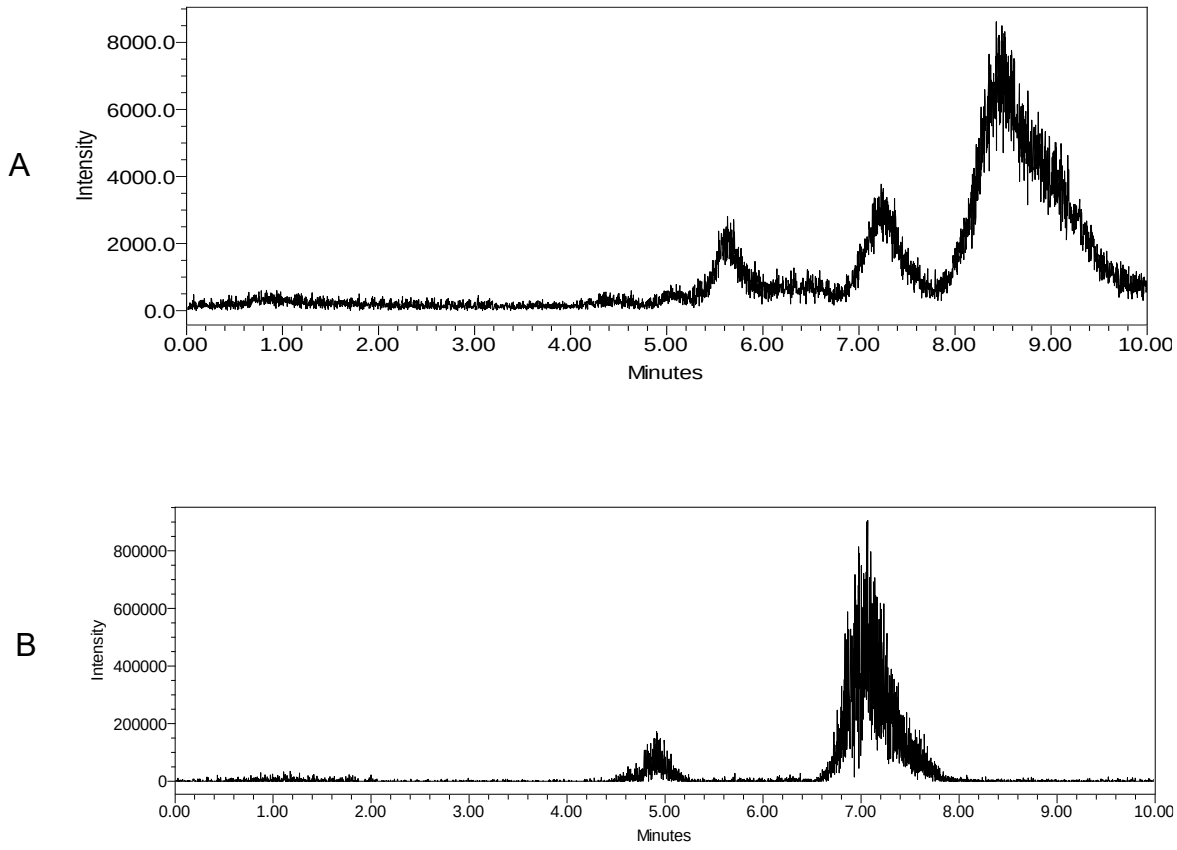
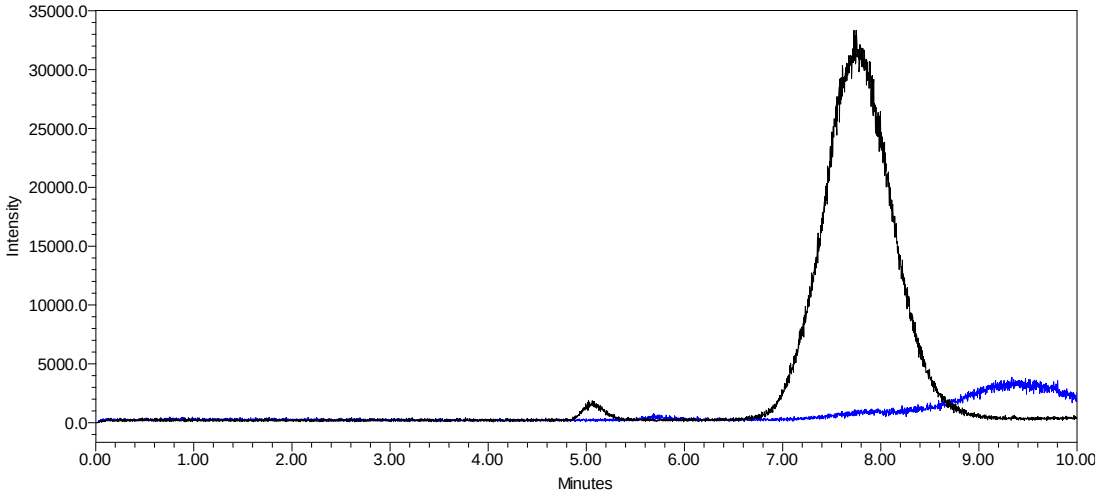


Figure 16 – SIR chromatograph of Hitenol BC-2020 at m/z = 557 (A), SIR chromatograph of Reasoap SR-10 at m/z = 557 (B).

Figure 17 provides an overlay of SR-10 and BC-2020 to illustrate how the target ion for SR-10 is not impacted by BC-2020. In Figure 19 the black trace is from SR-10 and the blue is from BC-2020. Figure 17 also includes a zoom of the baseline to better visualize the comparison and have confidence that there is no overlap in the target range.

A



B

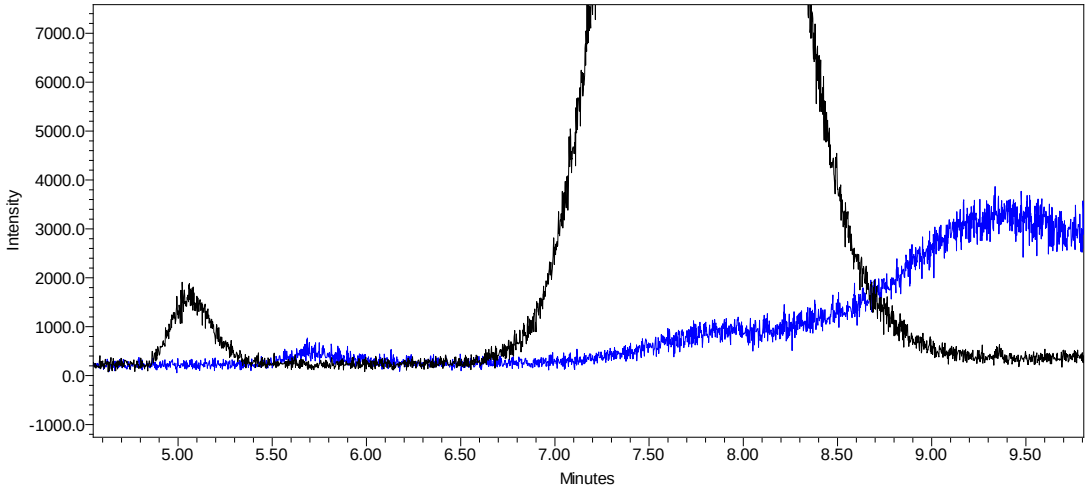


Figure 17 – Overlay of SIR chromatograms with SR-10 in black and BC-2020 in blue (A). Magnified baseline (B) for detail. Note that the target peak for SR-10 at 5.1 minutes is not impacted and there is no overlap with the BC-2020 peak at 5.8 minutes. Target ion of m/z = 557 remains a good candidate for SR-10.

Calibration

A calibration curve that spans 10 – 250ppm was able to be achieved with an R^2 value of 0.99. During experiments it was observed that saturation was beginning to occur at concentrations greater than 1000ppm where peak area began to level off and no longer scale, but since this method is seeking to measure residual surfactant concentrations the curve is not required to extend to higher concentrations. Figure 18 illustrates the curve that was more practical for use.

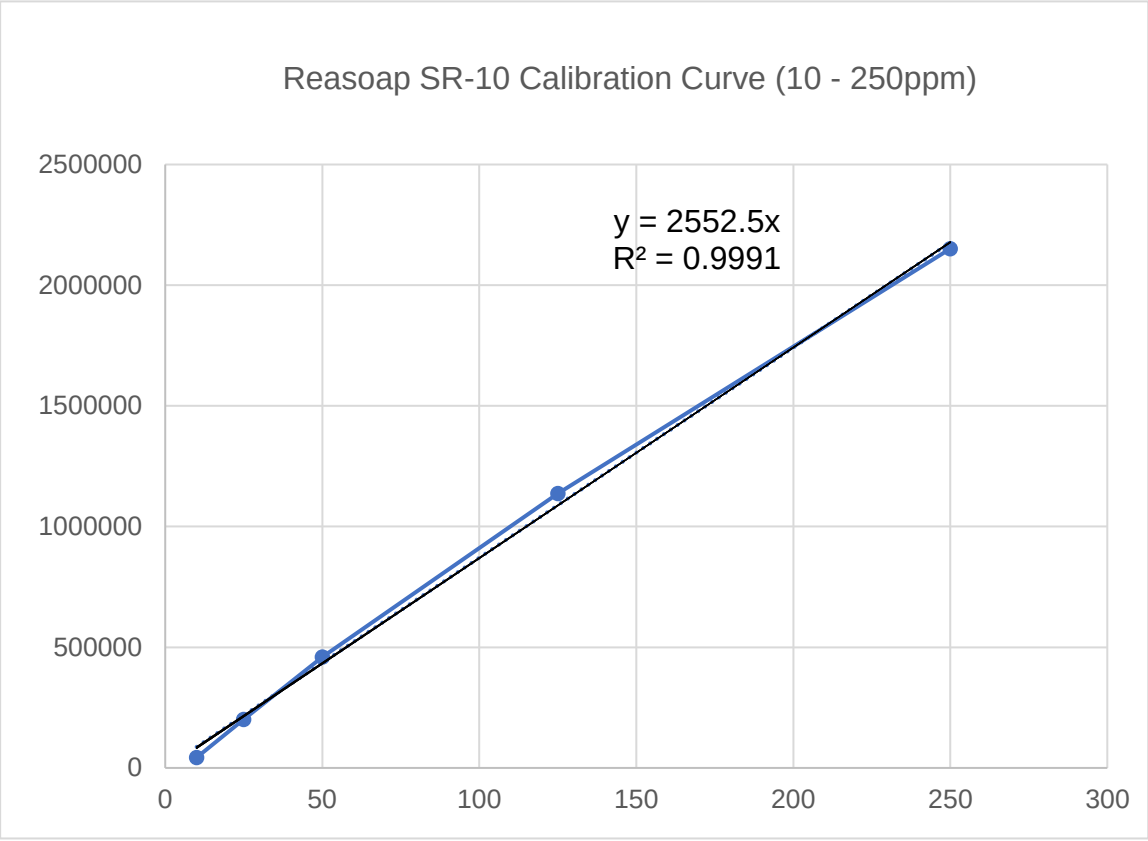


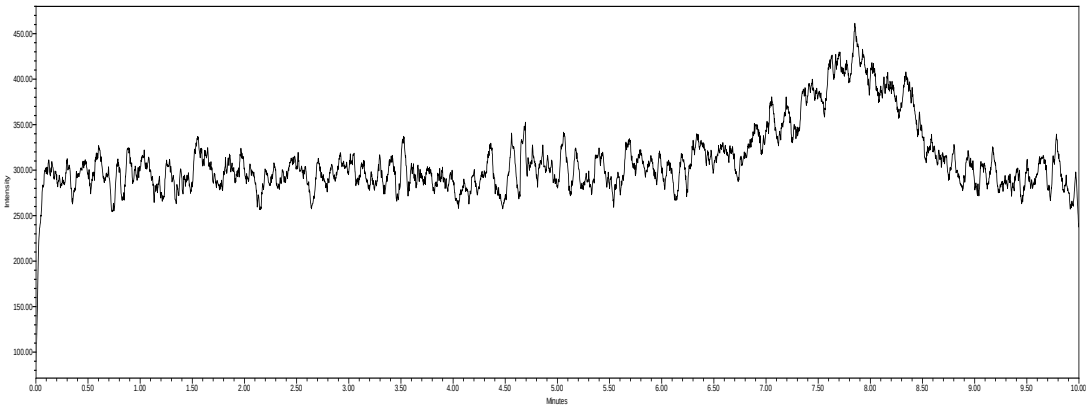
Figure 18 – Mass spectrometry calibration curve for Reasoap SR-10 at m/z = 557.

Limit of Detection and Quantitation

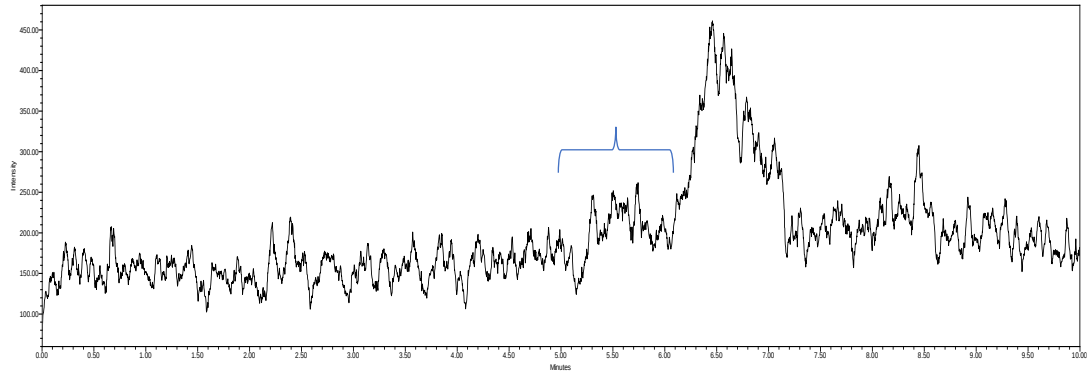
The generally accepted criteria for limit of detection (LoD) and limit of quantitation (LoQ) is based on the signal to noise ratio of the data, with the LoD usually lower than the LoQ. A ratio of three of signal to noise is acceptable for the LoD and a ratio of down to ten is acceptable for the LoQ. The signal to noise ratio was calculated by taking two times the peak height divided by the height of the baseline (2H/h).

The data for a 5ppm injection was examined and found to have a signal to noise ratio of 2.8 while the 10ppm had a ratio of 3.7. This would mean that the LoD could be set at 5ppm, but it may be more reliable and consistent if this were set at 10ppm. There is likely to be baseline variability with actual samples. Regarding the LoQ, an acceptable signal to noise ratio of 11.1 was found at the 25ppm concentration. Figure 19 illustrates the peak heights that were observed, including 1ppm and 5ppm plots for comparison. The peak height was measured starting at the baseline and was provided by the software. The baseline noise height was measured on each side of the peak and averaged using the height data provided by the software. The data from these measurements was used to calculate the signal to noise ratio using the formula 2H/h, where H is the height of the target peak while h represents the baseline height.

A



B



C

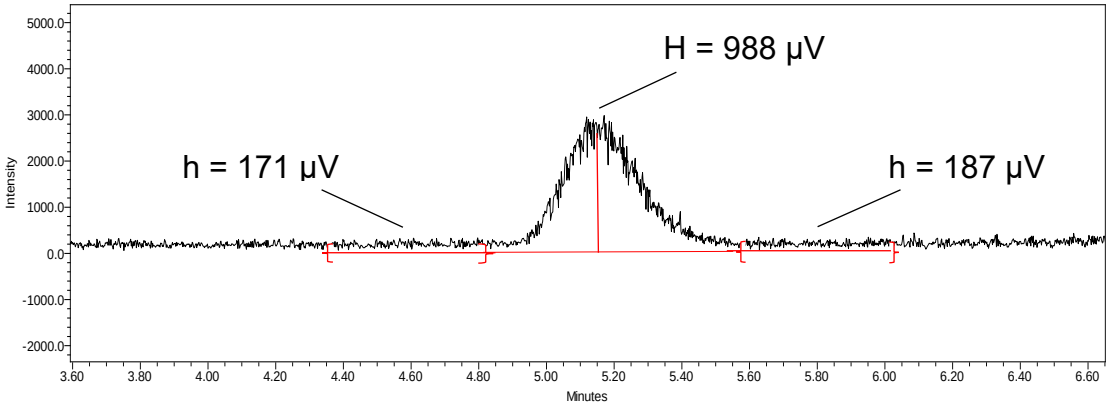


Figure 19 – 1ppm plot (A) followed by a 5ppm plot (B). In plot B notice the weak target signal starting just after the five-minute mark. The larger peak at 6.5 minutes could be a different fragment or impurity. There is no observable target signal in plot A, although a small peak at eight minutes in the ethylene oxide region is visible. Plot C shows an example signal to noise ratio calculation of Reasoap SR-10 using a 25ppm sample. Using the formula 2H/h, the heights found in the 25ppm sample provided a signal to noise ratio of 11.1.

Percent Recovery

Surfactant-free latex samples were prepared at concentrations of 110ppm and 55ppm to examine percent recovery in samples that this laboratory is likely to encounter. These two samples were prepared according to the established method and injected in triplicate. With at least 90% recovery being acceptable, the data showed that the percent recovery was unsatisfactory at the 110ppm and 55ppm concentration and is presented in Table 1.

Table 1 – Percent recovery in spiked latex-free samples.

Injection	Recovery at 55ppm	Recovery at 110ppm
1	17.85%	50.58%
2	24.28%	36.46%
3	20.93%	44.92%

Carryover

Carryover is a common problem that leads to erroneous results and can be present without careful method development. An evaluation was conducted to determine at what concentration did carryover become problematic. A small amount of carryover was observed after injecting higher concentrations, greater than 1000ppm. This was mitigated by adjusting the HPLC settings to perform an extended needle wash in between injections using 100% acetonitrile.

Figure 20 illustrates what was seen while investigating carryover and the result of action taken. It was observed (top) that there was a contamination peak at around 4.5 minutes, likely from the solvent, but the peak size was not significant and did not interfere with the target peak. A blank run after a 2500ppm sample of SR-10 (middle) showed a small amount of carryover at around 5.1 minutes as well as carryover from the large peak from around 7.8 minutes. In response, the needle wash parameter was adjusted in the method to perform an extended needle wash between injections with acetonitrile. After updating the needle wash parameter and injecting another blank after a 2500ppm SR-10 sample it was observed (bottom) that the carryover peak at around 5.1 minutes was no longer detected. The impurity peak at around 4.5 minutes was still

visible along with some carryover remaining from the large peak at around 7.8 minutes, but there was no overlap at the target window.

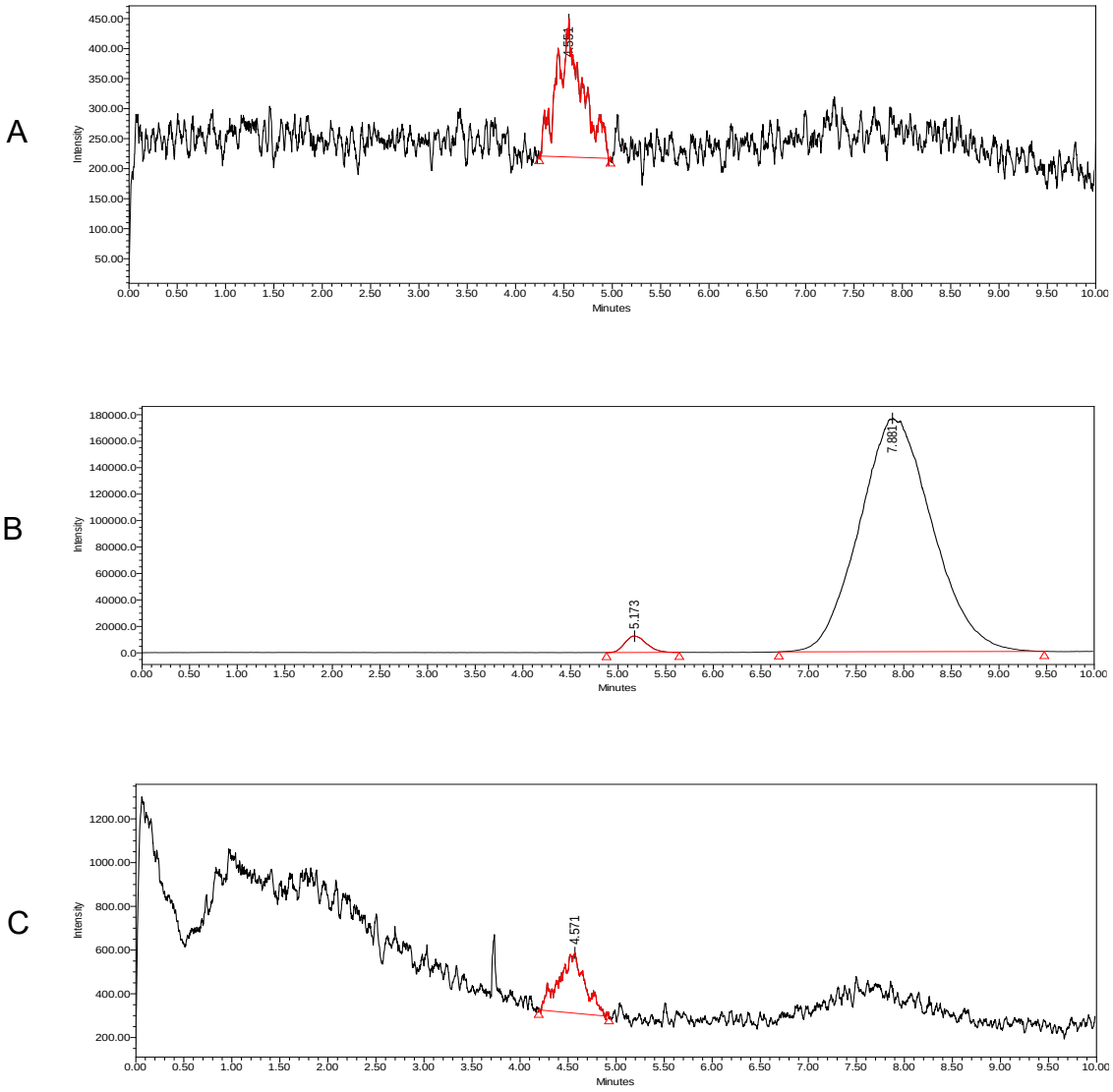


Figure 20 – Illustration of carryover mitigation with needle wash. Plot A is a blank injection that showed a peak at 4.5 minutes which may be a solvent impurity. Plot B shows a blank injection that directly followed an injection of 2500ppm SR-10. Note the peak of interest at 5.1 minutes and carryover from the large peak at 7.8 minutes. and then a final blank injection. Plot C is a blank injection following the 2500ppm SR-10 injection after wash adjustment. A peak remnant at 7.8 minutes from the previous injection can be observed but nothing was observed around 5.1 minutes. In addition, the baseline did not fully recover when acquisition started. The peak at 4.5 minutes is still present as a likely solvent impurity.

Between Run Precision (BRP)

The between run precision was performed by analyzing three samples once a day for three days. The samples were prepared by randomly spiking surfactant-free latex with Reasoap SR-10 and the same curve was used for the experiments. Table 2 shows the data along with its calculated %CV for each sample. With a %CV of 10% or less being the target, BRP 1 and BRP 2 were acceptable while BRP 3 was not acceptable.

Table 2 – Between run precision using randomly spiked latex. %CV was acceptable for BRP 1 and BRP 2, resulting at less than 10%. The %CV for BRP 3 was unacceptable as it was greater than 10%, but it also resulted less than the established LLOQ. This observation can be used to reinforce the decision to set the LLOQ at 25 ppm.

Sample	Day 1	Day 2	Day 3	%CV
BRP 1	79 ppm	82 ppm	77 ppm	2.75%
BRP 2	47 ppm	39 ppm	46 ppm	7.83%
BRP 3	11 ppm	15 ppm	12 ppm	14.36%

Within Run Precision (WRP)

The within run precision was performed by analyzing three samples five times in the same programmed run on the same day. The samples were prepared by randomly spiking surfactant-free latex with Reasoap SR-10. Table 3 shows the data along with its calculated %CV for each sample.

Table 3 – Within run precision using randomly spiked latex. %CV was acceptable for all samples, resulting at less than 10%.

	WRP 1	WRP 2	WRP 3
Replicate 1	14 ppm	57 ppm	35 ppm
Replicate 2	12 ppm	56 ppm	33 ppm
Replicate 3	12 ppm	52 ppm	33 ppm
Replicate 4	14 ppm	52 ppm	34 ppm
Replicate 5	14 ppm	46 ppm	30 ppm
%CV	6.19%	8.38%	5.03%

Inter-operator Variability

One surfactant-free latex sample was randomly spiked with SR-10 by adding Reasoap SR-10 to 20mL of latex and mixing for one hour. This was distributed to three peers in the laboratory and provided with the method. The sample was prepared by each individual and injected in triplicate with the peak around 5.1 minutes being the peak of interest. The samples and were run together on the same queue on the same day using the same calibration curve. Overall, the retention time was comparable across the three operators. The quality of the chromatography, though, was not as expected even though the target peak was present at around 5.1 minutes. This is likely due to this experiment being conducted after yearly preventative maintenance on the instrument had been performed, and sensitivity had not yet returned fully. The overlay in Figure 21 illustrates the chromatography and Table 4 provides the sample statistics, with the target peak within the red circle.

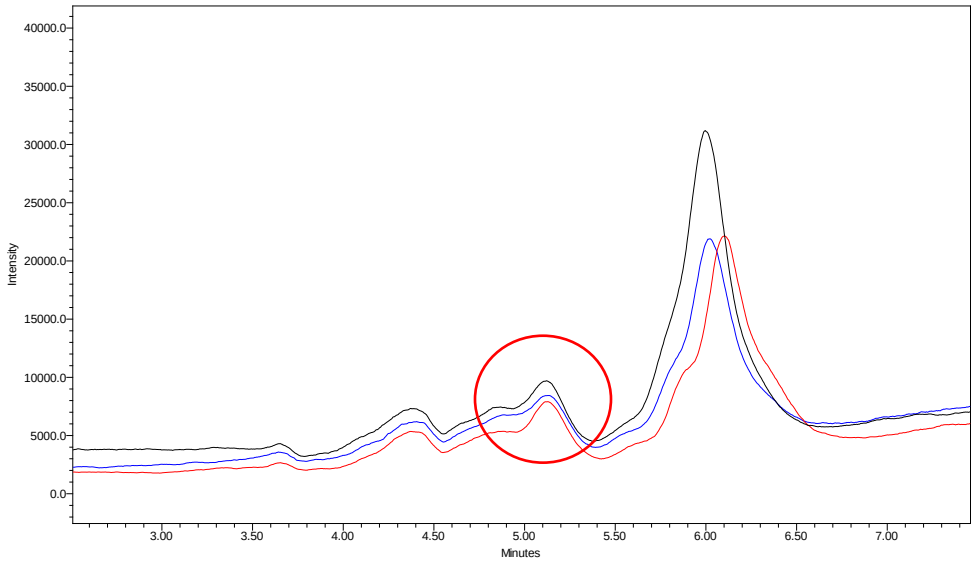


Figure 21 – Inter-operator variability chromatography.

Table 4 – Inter-operator variability data comparison.

Sample	Avg. Retention Time (min)	Avg. Peak Area	Concentration (ppm)	Color
S1	5.12	99218	39	Red
S2	5.12	118640	46	Black
S3	5.12	101696	40	Blue

DISCUSSION

Method and Target Ion Selection

The created instrument method has shown to be uncomplicated to run and train other operators to use. The mobile phases are simple to make as well as inexpensive, only needing HPLC grade water, acetonitrile, ammonium acetate and formic acid. The gradient is straightforward and provides good separation for mass spectrometry analysis in neat as well as latex samples. Sample preparation has relied on producing a clean supernatant to measure residual surfactant, accomplishing this by coagulating the latex using equal portions of a solution of 30% acetonitrile in water. An insufficient amount of acetonitrile would not coagulate the latex while too much acetonitrile could prematurely begin to break down the surfactant before analysis is carried out. Altogether, the simplicity of the method and sample preparation allows for adjustments to be made easily should sample chemistry require to achieve optimum resolution and response.

Reasoap SR-10 in neat, known samples were created with HPLC water by first making a stock solution then diluting to the desired working solutions. These samples were used to create a verifiable calibration curve with acceptable reliability, achieving an R^2 of 0.99. The target ion has initially been shown to remain unique when compared against another surfactant that also has an ethylene oxide chain (Hitenol BC-2020). However, whenever a surfactant that is new to the lab is analyzed for the first time it will need to be screened against other previously analyzed material to verify that there is not a species that is detected that elutes and fragments in the same manner. This is when a library would be useful. If a similar profile were to be encountered the method would need to be adjusted to be able to resolve this mixture. It is also possible that this method will not be able to screen for or uniquely identify every mixture of surfactant that may arrive, which may relegate this method to a general screening tool that would lead to a more specific method depending on the surfactant in question.

Percent Recovery in Latex Samples

While evaluating surfactant-free latex spiked with known concentrations of Reasoap SR-10 the percent recovery was poor and seems to be concentration dependent. Evaluating two concentrations showed that the 110ppm sample had an

average percent recovery of 44% after three injections, while the 55ppm sample showed an average percent recovery of 21% after three injections. Additionally, each triplicate study was not consistent from injection to injection. This may point to instrument resolution, sample preparation and overall robustness of the method. Being a reactive surfactant, spiking with Reasoap SR-10 may provide interactions within the emulsion that prevents some of the free surfactant to be available for detection. Also, sample preparation may not be optimal for extracting all free surfactant in a latex sample. It is likely that sample preparation will need to be examined and adjusted to find a sample preparation method that will improve recovery and increase confidence in sample analysis and quantitation. Since the calibration samples are prepared in the same manner as the latex samples any preparation changes would need to be made to the calibration samples as well and verify that the changes are compatible.

Precision

Between run precision (BRP) showed favorable %CV for two of the three samples, BRP1 with 2.75% and BRP2 with 7.83%. The third sample, BRP3, had a %CV of 14.36% which was not acceptable. It was noted that the %CV increased as the sample concentration decreased. This is again suggesting that the method is not recovering free surfactant consistently and completely. In addition, this inverse relationship supports the idea that something is happening within the sample matrix when latex is spiked with Reasoap SR-10. In contrast, within run precision was more stable and all %CV's were acceptable. However, the sample replicates did show some small variation in their respective groups which was not expected since the samples were all injected on the same day on the same run. The curve was verified daily to ensure no drift was occurring. A similar trend was observed in the percent recovery experiment, further suggesting another look at instrument sensitivity.

Inter-operator Variability

The operators prepared the samples and were run together on the same run on the same day using the same calibration curve. The inter-operator variability was shown to be consistent across three operators with results within 20% of each other. The poor chromatography was likely due to this experiment being performed after a recent yearly service visit from the manufacturer despite an extended equilibration at starting

conditions. The annual preventative maintenance keeps the instrument setup in working order and includes a thorough inspection, scheduled consumable parts replacement, cleaning and verification that the instrument is operating according to the manufacturer's specifications. The source is removed, sample cones cleaned, and aperture seal replaced. During sample clone cleaning, a polish is often used as a final step. If this polish is not cleaned off completely afterwards, it can especially affect sensitivity. The ion block is serviced including removing and cleaning the ion guide assembly. Less intrusive maintenance includes emptying the nitrogen exhaust trap and replacing the rough pump oil. These events can often affect the instrument when it is returned to service, most notably the initial sensitivity. It can take a few runs for the instrument to gradually return to historical performance. An extended equilibration was performed after the maintenance to account for this, but the chromatography showed that more should have been done. Aside from this, the chromatography and peak area remained comparable but this experiment should be revisited once the method is revised.

Future

The overarching concern is the instrument's lack of overall robustness, which is influenced by the combination of the method and maximizing the sensitivity of the instrument. This was first hinted at examining the first spiked latex samples. The poor recovery and small variations in consecutive injections compel the sample preparation method be adjusted. The abrupt change in chromatography after maintenance adds to the idea of improving sensitivity and instrument stability. This instrument is used for assays other than surfactant analysis and there needs to be confidence that other methods can be used reliably after a reasonable equilibration time.

The result of a usable sample preparation method must produce a clear supernatant or one that will filter clear before being injected on the instrument, and the calibration sample must be prepared in the same manner. The separation of liquids and solids in a latex sample can be performed in more than one manner. The simplest way is to centrifuge the unaltered sample, but this is only useful if there is sufficient liquid content vs. the solids and gel content. Another possibility would be to coagulate the latex with solvent or salt solution. This project used a 30:70 (acetonitrile:HPLC water) to

accomplish this, however, different ratios could be investigated as well as a different solvent. Any changes here would need to be considered against the mobile phases to ensure that all liquids that would encounter each other are miscible. A final preparation option would be a series of washes of the coagulated latex followed by a reduction of the supernatant to a small volume that would be injected on the instrument. This manner of washing and concentrating a sample would need to be taken with care to calculate the dilution as well as be wary of handling loss.

It is also known that surfactants can possibly be suppressed to a degree when analyzed using electrospray ionization (ESI) which is how the Waters QDa is equipped. The initial method in this project did account for this by building in a ten-minute delay between injections to provide a chance to keep the source clean. However, switching the probe to atmospheric pressure chemical ionization (APCI) is not possible with this mass analyzer. APCI is a different ionization process that is suitable for small polar and nonpolar species. It works by introducing the protons to the analyte in the gas phase, utilizing corona discharge on the solvent spray. Any improvements to be made will have to work around this.

CONCLUSION

The method as it stands now can detect Reasoap SR-10 in emulsion polymer samples and using the current selected ion can be resolved when mixed with another surfactant. The later Appendix provides a standard operating procedure (SOP) of this method that can be followed. The lack of recovery and sensitivity, however, classifies this more as a qualitative method since it cannot quantitate accurately and with precision yet. Future efforts will lead to adjustments in sample preparation and instrument setup that will hopefully bring about improvement.

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APPENDIX A: STANDARD OPERATING PROCEDURE

SOP – Determination of Reasoap SR-10 in Emulsion Polymer Samples Using Single-Quadrupole Mass Detection

1. Purpose

   To prepare and analyze emulsion polymer samples to detect residual surfactant using High Performance Liquid Chromatography (HPLC) and a single-quadrupole mass detector.

2. Scope

   This method can be used for emulsion polymer samples containing Reasoap SR-10 but may be adjusted to investigate other surfactants in emulsion polymer samples.

3. Materials and Methods

   3.1. Required Reagents

      3.1.1. HPLC grade water

      3.1.2. LC-MS grade acetonitrile

      3.1.3. Ammonium acetate

      3.1.4. Formic acid

   3.2. Preparation

      3.2.1. Mobile phase

         3.2.1.1. Mobile Phase A (10mM ammonium acetate in water) – Create a stock solution by weighing 3.854 g of ammonium acetate into a 500mL volumetric flask. Add HPLC grade water to the line on the flask and mix well. Create the working mobile phase by transferring 100mL of stock solution to an empty 1L volumetric flask and then add HPLC grade water to the line on the flask and mix well. Transfer to a clean glass media bottle and load onto the HPLC.

         3.2.1.2. Mobile Phase B (90% acetonitrile : 10% 10mM ammonium acetate in water with 0.01% formic acid) – Add 900mL of LC-MS grade acetonitrile to clean glass media bottle along with 100mL of 10mM ammonium acetate in water from the mobile phase A preparation. Mix

well. Remove 100µL of preparation and then add back 100µL of formic acid. Mix well and load onto the HPLC.

3.2.2. Standard preparation

3.2.2.1. Dilute surfactant to following concentrations using HPLC water: 10ppm, 25ppm, 50ppm, 125ppm, and 250ppm. Add 30:70 (acetonitrile:HPLC water) 1:1 (2 mL standard + 2 mL 30:70) and place on shaker for one hour. After shaking, spin at 462000 x *g* for 15 minutes. Draw into a syringe and attach a 0.45 micron syringe filter with a PTFE membrane. Pass the sample through the filter and directly into an autosampler vial, collecting a minimum of 1mL. Cap the vial and label appropriately. Standard samples are prepared differently than emulsion samples due to the complex matrix of an emulsion sample and the desire to prevent any unknown interactions that may affect the accuracy of the standard samples.

3.2.3. Emulsion sample preparation

3.2.3.1. Coagulate emulsion with 30:70 (acetonitrile:HPLC water) 1:1 (2 mL sample + 2 mL 30:70) and place on shaker for one hour. After shaking, spin at 462000 x *g* for 15 minutes. Draw the supernatant into a syringe and attach a 0.45 micron syringe filter with a PTFE membrane. Pass the sample through the filter and directly into an autosampler vial, collecting a minimum of 1mL. Cap the vial and label appropriately.

3.3. Procedure

3.3.1. HPLC Conditions

- Column – Acclaim Surfactant Plus (3um, 4.6 x 150mm), Thermo Scientific
- Extended needle wash
- Flow rate: 0.400 mL/min with gradient
- Gradient: Initial: 25% A ramping down to 5% A at 1.5 minutes, holding until 3 minutes and then ramp back up to 25% A at five minutes

- Acquisition time – 10 minutes with a 10-minute delay between injections
- Injection volume - 10µL

3.3.2. Detection Parameters (Single quadrupole mass spectrometer)

- Ionization method – Electrospray ionization (ESI)
- Single ion recording (SIR) – 557.58 Da
- Cone voltage – 15V
- Sampling rate – 10 points per second

3.3.3. Data acquisition – Calibration

3.3.3.1. If Reasoap SR-10 is present and at a high enough concentration to be detected a peak should be able to be observed around 5.0 minutes, illustrated in Figure A1. The peak area will vary with concentration and used to plot the calibration curve using the samples created in step 3.2.2.. An example calibration curve is provided in Figure A2. If the linearity produces a R^2 value of at least 0.99 using zero at the intercept, verify the curve by creating another sample of known concentration. If the verification sample is withing 20% of the known value the curve may be used to calculate the concentration of the emulsion samples. A verification sample is to be run with each batch to monitor performance and a new calibration curve is to be created after any of the following events: column change, mobile phase change, verification sample failure or instrument service call.

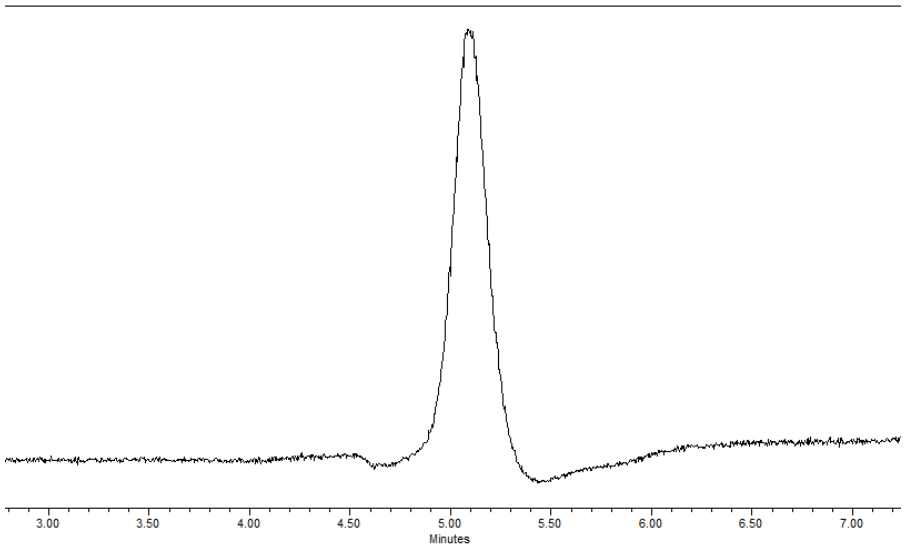

Figure A1. Example retention time and chromatography for Reasoap SR-10 at m/z = 557.

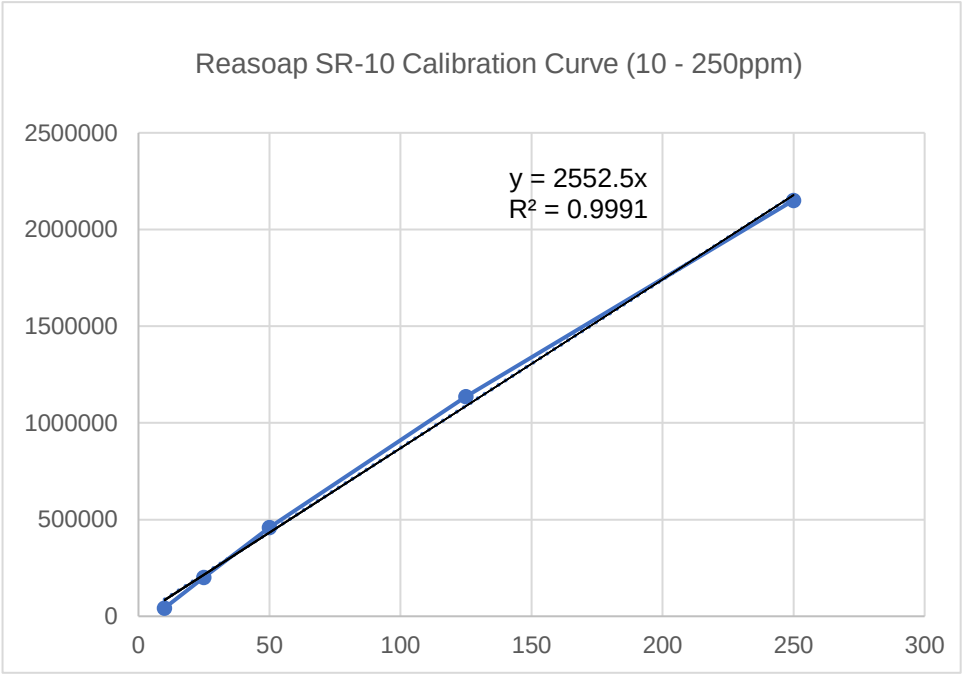

Figure A2. Example of a calibration curve for Reasoap SR-10 at m/z = 557.

3.3.4. Data acquisition and reporting – Emulsion samples

3.3.4.1. Prepare emulsion samples as outlined in step 3.2.3. and calculate the concentration using the verified curve. Any value below 10ppm should be reported as less than the lower limit of quantitation (<LLOQ), while a sample with no observable peak should be reported as below the limit of detection (<LOD). Any value above 250ppm requires the supernatant be diluted with 30:70 (acetonitrile:HPLC water) and rerun. Use the lowest dilution factor possible.